Northeastern Minnesotans for Wilderness (Matt Norton)

September 22, 2025

Minnesota Pollution Control Agency
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https://mpca.commentinput.com/?id=BsGkbZQAt

Re: NPDES/SDS Permits MN0031879 & for U.S. Steel – Keetac (Mine/Mill & Tailings Basin); MPCA's Preliminary Denial of U.S. Steel Application for Variance From Wild Rice Sulfate Standard for Wild Rice Waters

Dear Ms. Stephanie M. Handeland:

On behalf of Northeastern Minnesotans for Wilderness, thank you for the opportunity to comment on the draft National Pollutant Discharge Elimination System ("NPDES")/State Discharge System ("SDS") permits for U.S. Steel Corporation's ("USS") Keetac operation, and USS' application for a variance from the sulfate standard for wild rice waters. Northeastern Minnesotans for Wilderness ("NMW") is a 501(c)(3) non-profit corporation founded in Ely by residents of northeastern Minnesota. Since 1996, NMW as worked to protect the Boundary Waters Canoe Area Wilderness ("Boundary Waters" or "the Wilderness"), Voyageurs National Park, and the larger Quetico-Superior region, and to foster broad appreciation of and support for the preservation of wilderness and wild places. Since 2013, NMW has focused substantial resources in efforts to achieve permanent protection of the Rainy River-Headwaters watershed – also known as the Boundary Waters watershed – from sulfide-ore copper mining and since 2020 has extended its efforts to documenting, tracking, and eliminating to the maximum extent possible industrial-sourced sulfate contamination of otherwise low sulfate waters in the Rainy River-Headwaters watershed.

NMW has a strong interest in seeing the MPCA use its ample authorities under the Federal Water Pollution Control Act and Minnesota law to control mining pollution including sulfate as vigorously as possible. The NPDES/SDS permits and variance application for USS' Keetac operation ("USS-Keetac") is a critical test case for the MPCA to ensure the decisive role of science, data, and reasoned decision-making in the issuance of NPDES/SDS permits and variances, and for the rule of law in Minnesota.

NMW applauds the Minnesota Pollution Control Agency's ("MPCA") action to enforce the sulfate standard for protection of wild rice waters (hereinafter, "sulfate standard") by including enforceable numeric limits surface discharges specified in the two NPDES/SDS permits (MN0031879, MN0055948) for USS-Keetac. NMW strongly supports the MPCA's preliminary determination to deny USS' application for a variance from the sulfate standard for wild rice waters, and urges MPCA to issue a final decision denying USS' application for a variance.

NMW encourages MPCA to review USS' SEC filings, such as its 2024 10-K, and include those filings in the decision record. The USS 2024 10-K's contents support the MPCA's determination that USS can afford construction and operation of water treatment necessary to comply with state and federal standards. In addition, the 10-K's lack of any assertion or disclosure to investors, along the lines that state requirement of USS to comply with the 14 mg/L (average) numeric limit on sulfate and denial of a variance would result in closure or long-term idling of USS-Keetac, likewise undercuts arguments that if USS is not given a variance but is forced to control sulfate, then USS-Keetac would be idled or closed, leading to substantial and widespread economic and social impacts. It is noteworthy that, at least in the hearing in Virginia on Sept. 3rd, NMW heard commenters of all stripes except USS itself make these fear-based arguments. SEC filings such as USS' 2024 10-K carry weight because material omissions and misstatements in companies' SEC filings are punishable as felonies for which top company executives are responsible, whereas NMW is not aware that misleading labor or local elected officials violates any state law or carries any penalties. MPCA should weigh the evidence before it accordingly.

1. **The 10 mg/L sulfate standard is supported by the findings of published, high-quality scientific studies conducted in Minnesota, and is reasonable, necessary, and rationally related to the protection of wild rice waters.**

The 10 mg/L sulfate standard for wild rice waters has been law in Minnesota since 1973. It has been federal law since it was approved by the U.S. EPA in 1975.

In 2011, the Minnesota legislature directed the MPCA to re-evaluate the 10 mg/L standard, and appropriated state funds for a multi-year high-quality scientific study, sometimes called the Wild Rice Sulfate Standard Study (hereinafter, "sulfate study"). The sulfate study's early and final results gave MPCA information necessary to form "a much better understanding of the relationship between wild rice presence and absence, sulfate in the water column, and sulfide and iron in sediment porewater." *See* **Exh. 1**. MPCA. Wild Rice Sulfate Standard – Summary of Findings and Preliminary Recommendations, Legislative Briefing Document; February, 2014 ("Feb. 2014 briefing document").

In February of 2014, the MPCA, based on preliminary findings from research conducted by research scientists at the University of Minnesota, the MPCA, and other institutions, formalized its preliminary conclusions and recommendations. The first preliminary conclusion listed in the Feb. 2014 briefing document is, "The 10 mg/L standard is needed and reasonable to protect wild rice production from sulfate-driven sulfide toxicity." The second preliminary conclusion listed is, "The 10 mg/L wild rice sulfate standard should continue to apply to Minnesota wild rice lakes and streams. *Id.*

After briefing legislators, and one day before the planned release of the MPCA research scientists' findings, political blowback from Iron Range legislators caused MPCA leadership at the time to pause its release of its research scientists' preliminary conclusions based on data gathered in the sulfate study research. Ample evidence of this as a political rather than a

scientific decision is compiled, explained, and available for review on the pages of WaterLegacy's website devoted to this topic (https://waterlegacy.org/wild-rice-and-sulfate-rulemaking/; *see* **Exh. 2**).

In June of 2014 the MPCA announced it would investigate an equation-based sulfate standard to replace the 10 mg/L numeric standard. The equation would dictate a standard allowing more – sometimes substantially more – than 10 mg/L sulfate in waters whose sediments were high in extractable iron, and a standard allowing less – sometimes substantially less – than 10 mg/L sulfate in waters whose sediments were low in extractable iron, on the basis of evidence that sediment iron scavenges sulfide. Implicit in MPCA's proposal was the theory that by scavenging sulfide as iron-sulfide in the sediment, extractable iron protected wild rice plants from the harmful effects of sulfide toxicity in the rooting zone. Note that this MPCA interpretation of extractable iron (sediment porewater iron) as protective of wild rice was disputed by various parties, including some of the key scientists involved in the sulfate study, as unsupported by the scientific evidence.

The extensive research conducted by the state supported sulfate study culminated in at least five peer-reviewed scientific papers addressing the bioactivity and aquatic chemistry dynamics of sulfate being published in high-impact scientific journals in 2017. *See*:

- Myrbo, A., Swain, E. B., Engstrom, D. R., Coleman Wasik, J., Brenner, J., Dykhuizen Shore, M., Peters, E. B., and G. Blaha. (2017). Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. *J. Geophys. Res.: Biogeosciences*, 122. https://doi.org/10.1002/2017JG003787 ("Myrbo A., et al. (2017a)"; **Exh. 3.**);
- Myrbo, A., Swain, E. B., Johnson, N. W., Engstrom, D. R., Pastor, J., Dewey, B., Monson, P., Brenner, J., Dykhuizen Shore, M, and E. B. Peters. (2017). Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. *J. Geophys. Res.: Biogeosciences*, 122. https://doi.org/10.1002/2017JG003788 ("Myrbo, et al. (2017b)"; **Exh. 4**);
- Pastor, J., Dewey, B., Johnson, N. W., Swain, E. B., Monson, P., Peters, E. B., and A. Myrbo. (2017). Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments. *J. Ecological Applications*: 27 (1) 321-336. https://doi.org/10.1002/eap.1452 ("Pastor, et al. (2017)"; **Exh. 5**);
- Pollman, C. D., Swain, E. B., Bael, D., Myrbo, A., Monson, P., and M. Dykhuizen Shore. (2017). The Evolution of Sulfide in Shallow Aquatic Ecosystem Sediments: An Analysis of the Roles of Sulfate, Organic Carbon, and Iron and Feedback Constraints Using Structural Equation Modeling. *J. Geophys. Res.: Biogeosciences*, 122. https://doi.org/10.1002/2017JG003785 ("Pollman, et al. (2017)"; **Exh. 6**); and
- Ng, G.-H. C., Yourd, A. R., Johnson, N. W., and A. E. Myrbo. (2017). Modeling hydrologic controls on sulfur processes in sulfate-impacted wetland and stream sediments. *J. Geophys. Res.: Biogeosciences*, 122. Https://doi.org/10.1002/2017JG003822 ("Ng, et al. (2017)"; **Exh. 7**).

These and other subsequent papers document sulfate-driven sulfide toxicity to wild rice, and support many of the MPCA's key understandings derived from the sulfate study's preliminary results as reflected in Feb. 2014 briefing document. These include the conclusions that: sulfide

is toxic to wild rice; sulfate is converted by sulfate-reducing bacteria in anaerobic sediment to sulfide in the rooting zone of wild rice; sulfide formation in the sediment is driven by the amount of sulfate in the water column; iron in sediment scavenges sulfide to form iron-sulfide, thus precipitating the affected pore water sulfide; and that pore water sulfide is a function of surface water sulfate, sediment iron and sediment organic matter.

In 2017 the MPCA proposed replacing the 10 mg/L standard with an equation-based standard. In the technical support document (*See* MPCA. August, 2017. TSD: Refinements to Minnesota's Sulfate Water Quality Standard to Protect Wild Rice. ("2017 TSD"); **Exh. 8**), MPCA explained that a comparison of the proposed equation-based standard to fixed standards based on ratio of Type 1 to Type 2 errors (Type 1 errors indicating instances in which the fixed standard was found to be overly protective, and Type 2 errors indicating instances in which the fixed standard was found to be under-protective of wild rice) showed that if there is to be a fixed standard, then fixing it at 10 mg/L would produce both the lowest error rate (the sum of Type 1 and Type 2 errors) possible, and that it would be most balanced (16% of instances in the dataset would show 10 mg/L to be overprotective (a Type 1 error) and 16% of instances in the dataset showed 10 mg/L to be under-protective (a Type 2 error). Thus, the MPCA asserted in 2017, based on the modern dataset produced by a highly-respected team of scientists conducting the multi-year sulfate study with state financial and other backing, that if there is to be a fixed concentration sulfate standard to protect wild rice, then the 10 mg/L sulfate standard is already correctly and reasonably set at the most ideal and balanced concentration level possible to achieve the standard's purpose. *Id.* at p. 48.

Nonetheless, MPCA proposed the equation-based standard as preferable to the fixed sulfate standard because the equation-based standard was predicted to achieve an overall error rate (a smaller summed % of Type 1 + Type 2 errors) about half the error rate of the fixed standard. This equation, however, was focused on calculated sediment pore water sulfide concentrations, which are shown to be lower in the presence of sufficient sediment iron. And the MPCA's choice of the equation-based standard was predicated on the theory that precipitation of porewater sulfide to iron-sulfide prevented harm to wild rice.

The equation-based standard was rejected by the Administrative Law Judge ("ALJ") and the rejection was affirmed by Minnesota's Chief ALJ, principally on the basis that because MPCA was proposing to rescind the 10 mg/L sulfate standard at a time when almost all of the state's wild rice waters lacked the surface water and sediment analytical data necessary to plug into the equation-based standard, and thus MPCA proposed rescinding a perfectly clear and understandable fixed standard with an unconstitutionally vague sulfate standard. In short, the MPCA's bid to replace the fixed standard with the equation-based standard was rejected because it was unclear to all concerned (permittees, interested citizens, and the MPCA) – and likely would remain unclear for at least a decade or more – what the sulfate standard would be for any given wild rice water, and what permit limits any given industrial discharger would have to meet.

Even had the sulfate standard rule revision proposed by MPCA not been rejected by the Minnesota Chief ALJ and then abandoned by the agency, additional peer-reviewed science published since 2017 has undercut the MPCA's hypothesis that high sediment iron is protective of wild rice in high-sulfate waters. Instead, mesocosm studies demonstrate that in high-sulfate

waters with high sediment iron levels, while precipitates sulfide as less reactive iron-sulfide, the iron-sulfide precipitating in the rooting zone forms a plaque on wild rice roots, inhibiting or halting uptake of nitrogen by the plants. Those plants then produced fewer and lighter seeds containing less nitrogen than plants growing in lower-sulfate water, and that continuation of high-sulfate conditions exerted a downward on wild rice across multi-year population cycles. At high-enough sulfate concentrations, the downward pressure led to local extirpation of wild rice populations within six multi-year population cycles. (*See, e.g.*, LaFond-Hudson, S., Johnson, N. W., Pastor, J., and B. Dewey. Sulfur Geochemistry Destabilizes Population Oscillations of Wild Rice (*Zizania palustris*). *J. Geophys. Res.- Biogeosciences*, 127 (2022) https://doi.org/10.1029/2022JG006809 ("LaFond-Hudson, et al. (2022)"; **Exh. 9**). In short, the MPCA's hypothesis that high sediment iron is protective of wild rice populations in high-sulfate waters, such as those downstream from taconite facilities, is not supported by scientific papers and data on the topic published since 2017.

2. **NMW supports issuance of NPDES/SDS Permits MN0031879 and MN0055948 with monitoring requirements and enforceable numeric limits on sulfate concentrations (average and maximum) at all documentable points of surface discharge and seepage from the USS-Keetac areas and facilities.**

As required by 40 CFR 122.44(l), MPCA has retained enforceable numeric limits on sulfate at the specified surface discharges in the NPDES/SDS permits for USS-Keetac.[1] These include MN0031879-SD-002, SD-003, and SD-012, and MN0055948-SD-001, SD-005, and SD-009. There is no sulfate limit proposed for MN0031879-SD-001. NMW encourages MPCA to include a numeric limit on sulfate at MN0031879-SD-001.

NMW supports the permits' requirement to monitor sulfate twice monthly at most surface discharges -- those designated as such in the draft permits. Twice-monthly monitoring is required at MN0031879-SD-002, SD-003, SD-012, and at MN0055948-SD-001, SD-005, and SD-009, but not at MN0031879-SD-001, where monitoring is required only on a quarterly basis. NMW encourages MPCA to apply consistent twice-monthly monitoring requirements to all designated surface discharges, including MN0031879-SD-001.

[1] Limits cannot be adjusted any higher proposed based on 40 CFR 122.44(l), nor should they be. Depending on biotic sampling for mercury in fish tissue ("Hg_F") there may be strong scientific cause for the numeric limits on sulfate to be adjusted lower. Published scientific journal articles and the best existing statistical analysis of surface water parameters (sulfate, DOC, Hg, and MeHg) shows that in waters with plenty of extractable iron in the sediment, methyl-mercury formation increases linearly with increases in sulfate, suggesting that by scavenging sulfide, high sediment iron leads to more Hg_F as surface water sulfate concentrations increase. *See* Myrbo, et al. (2017b), **Exh. 4**. Many waters downstream from USS-Keetac are listed as impaired for Hg_F, including O'Brien Lake (WID 31-0032-02) and Swan Lake (WID 31-0067-02), which also are wild rice waters. NMW encourages MPCA to conduct or require permittee to conduct a simultaneous annual sampling effort during each year of the permits' five-year term, including at a minimum water quality sampling for sulfate, dissolved organic carbon, filtered mercury and methylmercury, and unfiltered mercury and methylmercury; sediment sampling for total organic carbon, extractable iron, and acid-volatile sulfide; and biotic sampling of fish species for mercury loads in the following waters: O'Brien Creek, Welcome Creek, Hay Creek, West Swan River, Hay Lake, Little O'Brien Lake, Reservoir 2, Reservoir 2 North, and Reservoir 6.

There are at least two important ways in which the NPDES/SDS permits must be tightened. First, maximum permitted discharge flow volume limits should be included for all specified surface discharges. In the alternative, each specified surface discharge should include a maximum sulfate load limit. Second, the list of surface discharges subject to flow and sulfate monitoring must be expanded to encompass all obvious pools and seeps on the periphery of the USS-Keetac tailings pile.

A. The NPDES/SDS permits lack numeric limits on discharge flow volumes and hence lack computable pollutant load limits for all pollutants at all specified surface discharge locations. Without either load limits or maximum discharge volume limits, how can the NPDES/SDS permits serve as a basis for future determinations of compliance with antidegradation requirements, and isn't MPCA inviting water diversion manipulations rather than pollution reduction to achieve compliance with numeric concentration limits?

NMW understands that MPCA determined prior to issuance of the 2011 permits that these numeric limits are necessary to address the impairment of wild rice waters downstream, including in Hay Lake. MPCA has not, however, conducted a Total Maximum Daily Load ("TMDL") study for Hay Lake, and does not intend to prepare a TMDL study before the year 2033 for Hay Lake or any other wild rice water listed as impaired for sulfate. *See* MPCA. April 2024. EPA-Approved Final 2024 Impaired Waters List_wq-iw1-81, **Exh. 10**; *and see* MPCA. April 2024. Minnesota's TMDL Studies Prioritization Framework, 2022-2032, **Exh. 11**.

Without a TMDL calculation, MPCA has not apportioned the TMDL across all NPDES/SDS permitted discharges. NMW has not conducted an independent review of the assumptions and calculations upon which MPCA's determination is based. Until NMW has arranged an independent reviewed and understands those assumptions and calculations it reserves judgment as to whether the 14 mg/L calendar month average limit on specified surface discharges is sufficiently stringent, and whether the current list of specified discharges in sufficiently inclusive of total USS-Keetac discharge.

B. MPCA should strengthen the NPDES/SDS permits by ensuring that all documentable points of discharge and seepage from the mine properties and to waters of the state are subject to sulfate and flow volume monitoring requirements, and enforceable numeric limits on sulfate discharges.

NMW supports the permits' requirement to monitor sulfate twice monthly at most surface discharges -- those designated as such in the draft permits (MN0031879-SD-002, SD-003, SD-012, and at MN0055948-SD-001, SD-005, and SD-009). Yet there are many actual points of discharge that are not among the few surface discharges specified with an SD number in the permits as drafted by MPCA.

It is evident that the permits as written, particularly MN0055948, would allow MPCA and USS-Keetac to avoid the measurement of discharge volumes and pollutant concentrations, and therefore loads, from de facto surface discharges originating from the USS-Keetac properties

covered by the two permits. The Public Notice of intent to reissue the USS-Keetac Mining Area NPDES/SDS Wastewater Permit, MN0031879, states in pertinent part, on page 2:

> A 2020 Supreme Court Decision in County of Maui v. Hawaii Wildlife Fund, 140 S. CT. 1462 (2020), (Maui) requires that regulators consider whether discharges of wastewater from a facility to groundwater are functionally equivalent to direct discharges of wastewater to surface water. This permit does not authorize a functionally equivalent discharge from tailings basin seepage to surface water.

It appears evident that substantial areas of waste rock stockpiles or other mined materials abut much of the eastern margin of O'Brien Creek's headwaters reach. Surely by omitting any mention of or requirement to monitor and control sulfate and other pollutants entering O'Brien Creek from seeps and flowages emerging from the toe or margin of this heavily mining-influenced area, the permit MN0031879 is tacitly authorizing the functional equivalent of discharge from USS-Keetac's active wasterock disposal areas, even if not from the tailings basin. This omission should corrected, and permittee should be required to monitor flow volumes and pollutant concentrations in the pools and seeps entering O'Brien Creek. There are many such areas visible in satellite imagery along O'Brien Creek (reach 07010103-607).

The equivalent Public Notice of intent to reissue the USS-Keetac Tailings Basin Area NPDES/SDS Wastewater Permit, MN0055948, states in pertinent part, on page 7:

> A 2020 Supreme Court Decision in County of Maui v. Hawaii Wildlife Fund, 140 S. CT. 1462 (2020), (Maui) requires that regulators consider whether discharges of wastewater from a facility to groundwater are functionally equivalent to direct discharges of wastewater to surface water. At this time, there is not sufficient information to make a functional equivalent determination at the Keetac tailings basin. This permit requires the Permittee to conduct additional sampling at various surface water and internal waste stream monitoring locations to determine whether or not there is a functionally equivalent discharge from the tailings basin. This permit does not authorize a functionally equivalent discharge from tailings basin seepage to surface water. If it is determined a functionally equivalent discharge from the tailings basin exists, the permit will be modified or reissued and the functionally equivalent discharge will be regulated under the modified or reissued permit.

The five years since Maui was handed down is sufficient time to have required permittee to sample in all seeps, pools, and flowages ringing the Keetac tailings pile, and for MPCA to have sampled the same waters as well as the tailings basin pools, and yet a review of MPCA's surface water data collection makes clear that no such sampling has been required or conducted. This is a missed opportunity and five years of data collection wasted. While it is good that MPCA is requiring monthly monitoring at SW-005 and SW-006, both of which appear to be located within 100-200 yards of the Keetac tailings pile perimeter, those two monitoring sites are only a subset of the seeps, pools, and flowages visible in satellite imagery and traceable through wetlands to flowing waters of the state. Additional sites of pooling and flowing water at the toe of the Keetac tailings dam, which MPCA must require the permittee to monitor in the same fashion as SW-005 and SW-006, are visible at the following Lat./Lon. coordinates:

- 47.337248, -93.075418
- 47.346613, -93.094143 and
- 47.348477, 93.094386

NMW recommends that SW-006 be effectively paired with at least one additional surface water monitoring location at approximately 47.33642, -93.099983, a site located along the northeast side of the unnamed creek 07010103-706, which flows from reservoir 2 to Hay Creek, as it appears likely that the water backing up at those coordinates is directly downgradient from SW-006 and would help establish a connection to the unnamed creek (to Hay Creek). Likewise, NMW recommends that most or all of the excavated pools clustered around MN0055948-SD-006 (which surface discharge, oddly, though it appears clearly in the MPCA's surface water data access application, is not mentioned in the draft NPDES/SDS Permit MN0055948) be added to the list of waters where permittee must monitor and report on a monthly basis. These pools appear to be largely interconnected and it appears that flowages from these pools are traceable to Hay Creek's headwaters or to the unnamed tributary east of and tributary to Hay Creek. Sampling these pools will provide data for comparison to SW monitoring sites SW-008, SW-005, and SW-002.

NMW is of the view that these sites should be described with "local names" that are accurate to the setting of these seeps, pools, and flowages. NMW suggests "unnamed pool or flowage number [_X_] tributary to [__water X_] originating at toe of Keetac tailings dam," or some equivalent.

NMW urges that discharge from these pools and flowages be monitored, where possible by installation of weirs equipped with continuous flow monitoring dataloggers, as that will better capture flows over time and be less likely to underrepresent flows and loads emerging from the USS-Keetac tailings pile.

3. NMW agrees with and supports MPCA's preliminary determination requiring denial of USS' application for a variance from the sulfate standard.

A. The MPCA correctly determined that USS does not qualify for a variance under either of the grounds upon which it applied.

MPCA correctly determined that USS does not qualify for a variance under either of the grounds on which it applied. Since the MPCA's preliminary determination to deny USS' variance application, conditions for the U.S. steel industry and for USS, in particular, have improved markedly. U.S. steel makers are now benefitting from U.S. tariffs on imported steel, and USS has been taken over by Nippon Steel Corp., which has promised not to shutter plants but to invest some $11 Billion in USS facilities in coming years. Moreover, the federal government now holds a golden share in USS, entitling it to block the company from halting work at its facilities. In fact, it did so just last week, as reported in Bloomberg News. *See* 2025-09-19. Bloomberg News. Trump officials blocked US Steel from stopping work at plant; **Exh. 12**.

B. USS' SEC filings make clear that USS can afford to and does spend substantial amounts on environmental compliance; USS does not assert in its SEC filings that

enforcement of the sulfate standard through limits in its NPDES/SDS permits would result in Keetac being closed, which it likely would have to have disclosed, were it the case.

The USS 2024 10-K filing is relevant to the MPCA's determination to deny USS application for a variance for several reasons. See 2024 10-K of United States Steel Corp.; **Exh. 13**.

First, it includes boilerplate assertions of good environmental stewardship and corporate citizenship, following the law, and recitation of what USS spends and has spent in the three years leading up to the filing on environmental compliance and control. It also shows annual expenditures on other things, such as stock options, which presumably are reserved for upper management.

Second, it does mention at pp. 20-21 USS' Minnesota operations generally and Keetac specifically, including MPCA's denial of USS-Keetac's application for a site-specific standard to avoid the 14 mg/L numeric limit in its draft NPDES/SDS permit. It does state at the bottom of p. 32 that, "... the Company may need to make significant capital and operating expenditures to ... control water discharges or to perform certain corrective actions to meet the conditions of the permits issued pursuant to applicable environmental laws and regulations."

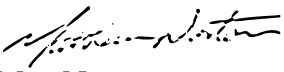
In the same section there is a selective recitation of the history of the sulfate standard. For example, the company notes that the sulfate standard has not been revised, but does not note that the mining industry generally (along with almost all commenting interest groups and others) opposed the MPCA's proposed revision of the numeric water quality standard to an equation-based standard. The company recites how EPA "recognized" the conflict between state laws forbidding application/enforcement of the sulfate standard, but does not state that the EPA disapproved the proposed changes to Minnesota's Clean Water Act implementation program that those state laws represented, and omits the obvious follow-up, which is that federal law cannot be nullified by state law.

NMW has found no statement in the 10-K where USS says there is a possibility of the mine and mill having to be shut down if existing standards were to be enforced, but U.S. Steel does say (p. 21): "U. S. Steel is continuing to work to determine the most efficient and effective options to meet the applicable sulfate standards. However, if MPCA does not revise the sulfate standard of 10 mg/L for the receiving waters of Keetac or approve the [site-specific standard], an impact on mining operations is likely as a result of extensive changes to water collection and treatment that will be required."

Perhaps the euphemistic phrase "an impact on mining operations is likely" is sufficient to cover mine closure, but NMW believes that it is not. This is supported by the rest of the sentence following that phrase, which spells out changes that would have to be made - which changes would only have to be made to enable continuation of operations in compliance with the applicable standard. The possibility that enforcement of standards would lead to mine shutdown or closure is the kind of risk the company would have to disclose to investors in an SEC 10-K filing. The omission of a clear statement threatening shut-down or closure is relevant to MPCA's assessment of the potential for widespread economic and social impact.

NMW's understanding is that a material omission or falsehood in an SEC filing is a felony for which the responsible executive can be charged, and the responsible executives generally take care to make sure that enough is said in SEC filings to satisfy the legal requirements.

Thank you again for the opportunity to comment.



Matt Norton
Northeastern Minnesotans for Wilderness.

NMW Exhibits

February, 2014

Wild Rice Sulfate Standard – Summary of Findings and Preliminary Recommendations
Legislative Briefing Document; February, 2014

Background:
In 2011, the Minnesota Legislature appropriated funding and directed the Minnesota Pollution Control Agency (MPCA) to conduct research on the effects of sulfate and other substances on the growth of wild rice. This research was intended to inform an evaluation of the existing wild rice sulfate standard. In 1973, the MPCA adopted and the U.S. Environmental Protection Agency (USEPA) approved that standard to protect the beneficial use of "water used for production of wild rice" during periods when the rice "may be susceptible to damage by high sulfate levels." (Minn. R. 7050.0224, subpart 2).

Following the development of a detailed research protocol in 2011, in 2012 the MPCA contracted with groups of scientists at the University of Minnesota Duluth and Twin Cities to undertake a Wild Rice Sulfate Standards Study. The Study's main hypothesis is that wild rice is impacted by sulfate via the conversion of sulfate to sulfide in the rooting zone of the plants. Data collection was completed in December 2013 and is documented in individual reports from the researchers (see Table 1 for a summary of Study components).

During January and February 2014, MPCA staff integrated the study results; analyzed the data as a whole; gained input from the Wild Rice Standards Study Advisory Committee; and reviewed existing monitoring data, other relevant scientific studies/information, and the original basis for the wild rice sulfate standard to develop findings and preliminary recommendations regarding the standard. In evaluating the existing sulfate standard, the MPCA has the responsibility of demonstrating that any recommended changes to the standard have a scientific basis and would protect the beneficial use of "water used for production of wild rice."

Findings and Preliminary Recommendations Regarding the Wild Rice Sulfate Standard

Key Findings:

1. **Sulfate is not directly toxic to wild rice**. Both the MPCA Study and the research commissioned by the Minnesota Chamber of Commerce support this conclusion. However, sulfate in the surface water can be converted by bacteria to sulfide in the rooting zone of wild rice (see Figure 1).
2. **Sulfide is toxic to wild rice.** The MPCA Study demonstrated that elevated sulfide concentrations were toxic to wild rice seedlings. Hydroponic experiment data showed deleterious effects of sulfide on seedling plant growth when sulfide exceeded the range of 150 to 300 µg/L.
3. **Sulfide in the sediment is affected by the amount of sulfate in the water column, and the amount of iron in the sediment**. Data from a majority of the field sampling sites show that the range of 150 to 300 µg/L sulfide in the sediment relates to a water column concentration of sulfate between 4.3 and 16.2 mg/L. This range illustrates that conditions at some of the field sites are more effective than others at converting sulfate to sulfide, in part due to the availability of iron in the sediment (see Figure 1).

Preliminary Conclusions and Recommendations:

1. **The 10 mg/L sulfate standard is needed and reasonable to protect wild rice production from sulfate-driven sulfide toxicity.** The MPCA will also consider including a sediment sulfide concentration as a component of this water quality standard, in the range of 150 to 300 µg/L sulfide.
2. **The 10 mg/L wild rice sulfate standard should continue to apply to both lakes and streams**. Analysis of the field data does not support placing lakes and streams into separate subclasses. Iron availability, not water body type, appears to be a key controlling factor in the concentration of sulfide.
3. **Site-specific standards are expected for some waters**. Considerable data suggest that in some cases the development of a site-specific standard would be protective of wild rice production. This is most likely to

occur in waters where the sediment iron is elevated and therefore a higher sulfate water column concentration may not result in a sulfide sediment concentration above 150 to 300 µg/L. There are also data to suggest that a site-specific standard lower than 10 mg/L may be needed for waters where sulfate is more efficiently converted to sulfide.

4. **MPCA will continue to explore if the sulfate standard is needed to protect paddy-grown wild rice production.** The Study data do not suggest that paddy-grown wild rice is less susceptible to impacts from elevated sulfide. However, the land- and water-management activities associated with paddy wild rice production likely reduce the potential for sulfide production in the sediment.
5. **MPCA does not currently have a recommendation regarding the "period of susceptibility" of wild rice to sulfate effects, but will continue to analyze data to further explore this question.** The sediment incubation experiment data show that sulfate can be converted to sulfide in both warm and cold conditions, and that sediment sulfide concentrations decrease once sulfate concentrations in the overlying water decrease. This is a complex interaction and more data analysis is needed before recommendations can be developed about this important question; any recommendation may also need to consider site-specific factors that affect this question.
6. **Consideration should be given to changing the use class of the wild rice sulfate standard:** The MPCA is considering moving the wild rice sulfate standard from Class 4 where it currently resides to Class 2 and creating a new subclass to clarify that the wild rice sulfate standard is designed to protect the growth of wild rice grains for consumption by humans and wildlife. The MPCA is also considering revising the term "water used for production of wild rice." The MPCA has received comments asserting this wording is not the best descriptor for natural stands of wild rice that provide benefits to humans and wildlife.

Next Steps

- In late March, MPCA will meet with the Wild Rice Sulfate Standards Advisory Committee and Minnesota Tribes in separate meetings to get their feedback on the MPCA's Findings and Preliminary Recommendations. MPCA will also continue to seek feedback from USEPA and the Study researchers.
- MPCA technical staff will continue to develop and assemble material for a technical support document that will be used in wild rice rulemaking. Further analysis of results and data from the outdoor container experiments and sediment incubation study will also continue. MPCA will, as appropriate, refine the recommendations based on the input received and this continued analysis.
- The MPCA is also contracting for expert scientific review of the wild rice study reports and specific aspects of the MPCA's preliminary recommendations and rationale about whether a change to the current 10 mg/L wild rice sulfate standard is warranted, and the nature of the change. The expert review panel will likely be convened in late spring 2014, and will include the opportunity for interested stakeholders/members of the public to address the panel.
- In a parallel effort MPCA is working to develop factors that will help identify specific waterbodies as "water used for production of wild rice." These factors will be used in case-by-case determinations and to inform rulemaking to identify specific waterbodies as "water used for production of wild rice" in Minnesota Rules Chapter 7050. The goal is to put these factors on public notice for a 30-day public comment period in March 2014. Comments received will be used to help refine the factors.
- Any proposed change to the wild rice standard would be adopted into Minnesota's water quality standard rule (Minnesota Rules Chapter 7050) in accordance with the requirements of the Minnesota Administrative Procedures Act and would require the approval of the USEPA. MPCA is targeting fall 2014 for having the rule package ready for public notice and comment.

Considerations

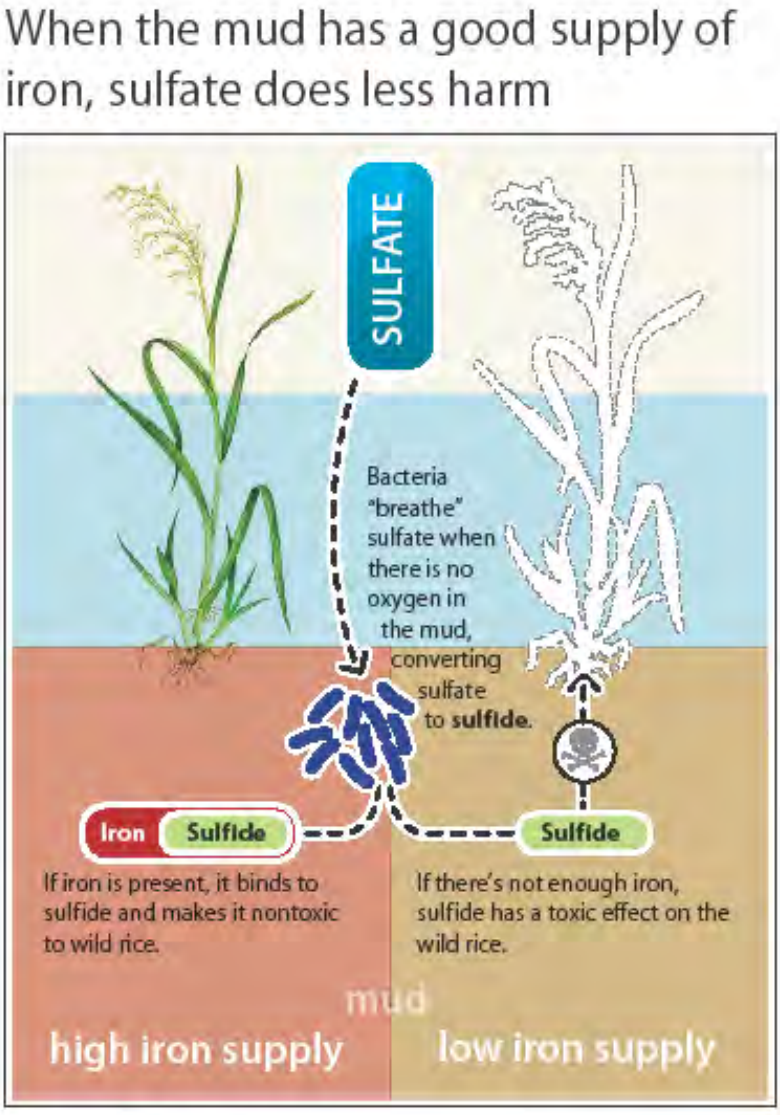
- As a result of the Wild Rice Sulfate Standard Study and other data collection efforts, MPCA now has a much better understanding of the relationship between wild rice presence and absence, sulfate in the water column, and sulfide and iron in sediment porewater. This includes a reinforcement of the hypothesis that sulfate is not directly toxic to wild rice, but is converted to sulfide, which is toxic to wild rice (this is supported by the hydroponics and field data).
    - This enhanced understanding is very important because it helps explain why a subset of waterbodies have elevated sulfate concentrations and apparently healthy wild rice stands, when most waterbodies that have successful wild rice beds have relatively low sulfate concentrations.
    - This enhanced understanding – which MPCA did not have prior to the study – will be invaluable in implementing the wild rice sulfate standard. This will be particularly valuable in evaluating the need for site-specific standards and developing such standards.
- Any changes to the current wild rice sulfate standard will take some time to implement. The Clean Water Act requires implementation of the existing standard while any proposed changes are going through the administrative process and USEPA approval.
- The MPCA wastewater permitting approach for the current wild rice sulfate standard is as follows:
    - Where elevated discharge sulfate levels are suspected but no sulfate data are available, discharge monitoring requirements are being added to NPDES permits as they come up for reissuance every five years.
    - If discharge data collected during the five year permit cycle indicate a potential to violate the sulfate standard for wild rice, and it is determined that a water used for production of wild rice may be affected by the discharge, a discharge limit will be added to the permit upon reissuance.
    - If discharge limits cannot be met immediately a schedule of compliance will be included in the permit to allow for steps such as evaluation of treatment technologies, design, procurement of funds, and construction.
    - The compliance schedule will contain a requirement that the facility either demonstrate compliance with the limit as soon as possible or submit a variance request with the application for permit reissuance.
        - It is important to note that the sulfate discharge limit included in a permit may not be identical to the wild rice sulfate standard. When setting discharge limits, MPCA factors in variables such as dilution in the receiving water and distance between the discharge point and the water used for production of wild rice. The discharge limit is set such that the standard is achieved at the water used for production.
    - Note that in accordance with federal law, compliance schedules and variances are typically not allowed for new dischargers, which must meet the effluent limit upon commencement of operations.

- Implementation of the existing standard does not preclude permitted facilities from requesting a site specific wild rice sulfate standard. The MPCA can employ the knowledge gained about site-specific standards immediately, where conditions indicate that such a standard likely is needed. MPCA is exploring options for addressing site-specific standards requests efficiently.

Table 1. Purpose, strengths, and limitations of Study components.

	Field Survey	Laboratory Hydroponic Experiments		Outdoor Container Experiments	Collection and Analysis of Rooting Zone Depth Profiles	Sediment Incubation Laboratory Experiments
		Sulfate (SO_4)	Sulfide (H_2S)			
Main Purpose	Expand understanding of environmental conditions correlated with presence/ absence of wild rice.	Evaluate effects of sulfate on wild rice seed germination and growth of sprouts.	Evaluate effects of sulfide on wild rice seed germination and growth of sprouts.	Evaluate effects of sulfate on wild rice plants over full life cycle, and multiple years.	Characterize sulfate, sulfide, and iron in the rooting zone of wild rice container experiments and field sites.	Evaluate effect of temperature on movement of sulfate into and out of underlying sediment.
Endpoints	Concentrations of chemicals in surface water & rooting zone (e.g. SO_4 & H_2S vs. wild rice occurrence).	Growth of wild rice sprouts (biomass, root & shoot elongation). Germination rate of seeds.	Growth of wild rice sprouts (biomass, root & shoot elongation). Germination rate of seeds.	Growth of wild rice (biomass, plus number & weight of seeds). Sulfide concentrations in rooting zone.	Concentrations of sulfate, sulfide and iron in porewater.	Sulfate concentrations in overlying water over time; SO_4, iron, H_2S, & anion tracers in sediment porewater. Simple model.
Key Strengths	Most reflective of actual environmental conditions. Multiple wild rice stands and breadth of characteristics sampled.	Controlled dose-response experiment. Controlled exposure to known concentrations of SO_4.	Controlled dose-response experiment. Controlled exposure to known concentrations of H_2S.	Controlled dose-response experiment. Includes natural sediment matrix as rooting environment. Involves entire growth cycle, multiple years.	Provides additional data to understand and interpret container experiments and field sites.	Controlled experiment with natural sediment and water.
Key Limitations	Least controlled. Annual visit for most sites, 3x/year for a subset. Not definitive on cause and effect.	Only evaluates early growth stages. Leading hypothesis is that sulfate is converted to sulfide, which is directly toxic.	Only evaluates early growth stages. Unable to simultaneously keep roots anaerobic & shoots aerobic.	Full effect of sulfate may take longer than several years to realize. No groundwater movement.	Utility lies in the integration of this data with the other Study components, not in this data set alone.	Provides preliminary assessment of sediment from two sites that may inform but is not fully transferrable to other sites. No groundwater movement. No wild rice plants grown.

Figure 1. The relationship of sulfate, sulfide and iron in surface water and sediment.



The iron-sulfide battle
The amount of iron and sulfide are dynamic and one affects the other. If enough new iron is flowing into the mud (e.g. via groundwater), then even a lake or stream with high sulfate levels can support wild rice. On the other hand, enough sulfate can overwhelm the supply of iron and make sulfide levels toxic.

Rulemaking: Wild Rice Sulfate Standard

What is the wild rice sulfate standard?

In 1973, Minnesota's wild rice sulfate standard was adopted by the Minnesota Pollution Control Agency (MPCA) and approved by the U.S. Environmental Protection Agency (EPA) under the Clean Water Act. Minnesota's wild rice sulfate standard limits sulfate to 10 parts per million (mg/L) in wild rice waters.



Images used in community organizing to preserve wild rice sulfate standard, WaterLegacy.

MPCA's enforcement of the wild rice sulfate standard to control sulfate discharge was weak and sporadic for decades. In 2010, when EPA told MPCA that the standard must be enforced under the Clean Water Act, mining interests and their supporters attacked the standard. WaterLegacy engaged in an eight-year advocacy and organizing campaign to preserve the wild rice sulfate standard.

What efforts were made to weaken or eliminate the standard through rulemaking?



Alice White Cadotte, LCO, 1941, "Knocking Rice."

In 2011, the Minnesota Legislature passed a session law directing the MPCA to engage in rulemaking to amend the wild rice sulfate standard. This session law appropriated $1.5 million for new scientific research and a task force run by the MPCA, anticipating that the rule would be amended to repeal or weaken the wild rice sulfate standard. This rulemaking process took seven years – from 2011 through 2018.

Learn More: Legislation

What was the result of the rulemaking process?

Minnesota's wild rice rulemaking process funded scientific research that confirmed the wild rice sulfate rule is reasonable and necessary to protect wild rice.

Learn More: Science

In 2017, MPCA proposed an amended rule to repeal the wild rice sulfate standard and replace it with an equation that allowed more sulfate pollution if there was a high level of iron in the affected waters. MPCA also proposed to identify approximately 1,300 waters as wild rice waters, but exclude some of the wild rice waters identified by tribes and the Minnesota Department of Natural Resources scientists, including waters downstream of existing and proposed mines.

WaterLegacy, tribes, and independent scientists opposed MPCA's proposed rule on several important grounds:

- MPCA's proposed equation would fail to protect wild rice, especially in mining regions.
- Additional sulfate allowed by MPCA's proposed rule would increase mercury methylation and contamination of fish.
- Excluding some Minnesota wild rice waters from protection would violate the federal Clean Water Act.

In January 2018, after contested case hearings, community testimony, written comments, and expert opinions, MPCA's proposed rule was rejected by Administrative Law Judge LauraSue Schlatter, with the approval of the Chief Administrative Law Judge in January 2018. MPCA then attempted to challenge the Administrative Law Judge's decision, but its challenge failed. MPCA withdrew its proposed rule to repeal the wild rice sulfate standard on May 7, 2018.

The wild rice rule adopted and approved in 1973 to limit sulfate to 10 parts per million (mg/L) remains in effect.

> **Read** the complete Report of the Administrative Law Judge and its approval by the Chief Administrative Law Judge (Jan. 9, 2018 and Jan. 11, 2018).
>
> **Read** the Chief Administrative Law Judge Order rejecting MPCA's request for a change in the decision to disapprove repeal and changes to the wild rice sulfate rule (Apr. 12, 2018).

What did the Administrative Law Judge decide?

The Administrative Law Judge agreed with WaterLegacy and tribes that the MPCA's proposed rule would fail to protect wild rice and improperly restrict wild rice waters, violating the Clean Water Act. The Administrative Law Judge:

> **DISAPPROVED** repeal of Minnesota's existing wild rice sulfate standard of 10 mg/L, stating:
>
> > The Administrative Law Judge DISAPPROVES the proposed repeal of the 10 mg/L sulfate standard at Minn. R. 7050.0220, subps. 3a, 4a, 5a, 6a and Minn. R. 7050.0224, subp. 2, due to the Agency's failure to establish the reasonableness of the repeal, and because the repeal conflicts with the requirements 33 U.S.C. § 1313(c), 40 C.F.R. § 131.10(b) (2015) and Minn. R. 7050.0155 (2017) [Clean Water Act and its regulations].
>
> **DISAPPROVED** restricting the list of wild rice waters. The MPCA could list 1300 wild rice waters only if other waters where wild rice has been an existing use since November 1975 were also protected.
>
> **DISAPPROVED** changing the narrative standard that protects wild rice from harm due to changes in human-made conditions, such as flooding.
>
> **DESCRIBED** concerns about preventing increased mercury methylation as well as further harm to wild rice and recognized the burdens that would be placed on Native

American communities if the wild rice sulfate rule were changed.

Wild Rice Sulfate Rule Timeline

2011

From 2011 through 2017, the Wild Rice Sulfate Standards Study Advisory Committee met at least once a month, and WaterLegacy attended every meeting and conference.

Feb. 2014

After the scientists had made their reports, the MPCA drafted Findings and Preliminary Recommendations that proposed preserving Minnesota's wild rice sulfate standard as needed and reasonable. Excerpts of MPCA's Findings and Recommendations are quoted below:

> **Sulfide is toxic to wild rice**. The MPCA Study demonstrated that elevated sulfide concentrations were toxic to wild rice seedlings. Hydroponic experiment data showed deleterious effects of sulfide on seedling plant growth when sulfide exceeded the range of 150 to 300 µg/L.
>
> **Sulfide in the sediment is affected by the amount of sulfate in the water column, and the amount of iron in the sediment.**
>
> **The 10 mg/L sulfate standard is needed and reasonable to protect wild rice production from sulfate- driven sulfide toxicity.** The MPCA will also consider including a sediment sulfide concentration as a component of this water quality standard, in the range of 150 to 300 µg/L sulfide.
>
> **The 10 mg/L wild rice sulfate standard should continue to apply to both lakes and streams.**

MPCA internal emails reveal that MPCA presented these findings and recommendations to a group of Iron Range legislators prior to their planned release. The day before the findings were set for release to the public, the Governor's staff wrote, "This is a big deal and it is blowing up this morning." MPCA's Commissioner agreed, "the meeting with range legislators went poorly." According to MPCA staff, the

"plug got pulled" on support of the existing standard. After February 2014, MPCA no longer supported Minnesota's existing rule limiting sulfate in wild rice waters to 10 mg/L.

June 2014

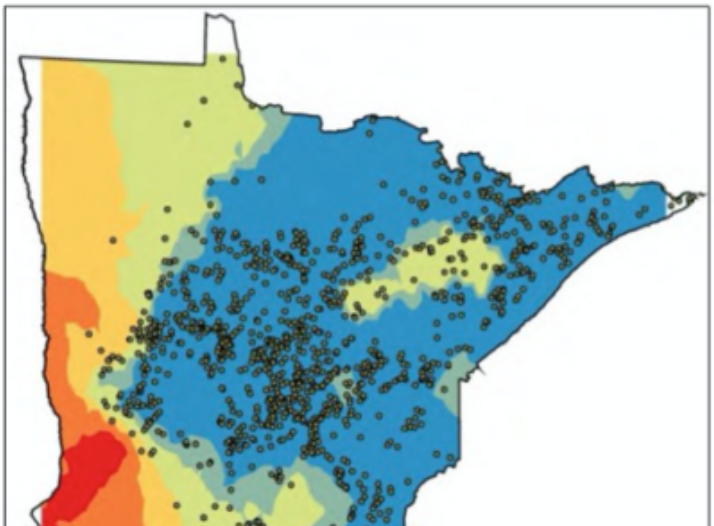
MPCA first proposed an equation that would allow more sulfate pollution in waters that had high levels of iron in their sediment. MPCA also proposed to set arbitrary acreage and "density" limits on the wild rice waters that would be protected from sulfate pollution. WaterLegacy opposed MPCA's proposal and MPCA's efforts to bias peer review. The documents below include MPCA initial rule proposal and WaterLegacy's responses:

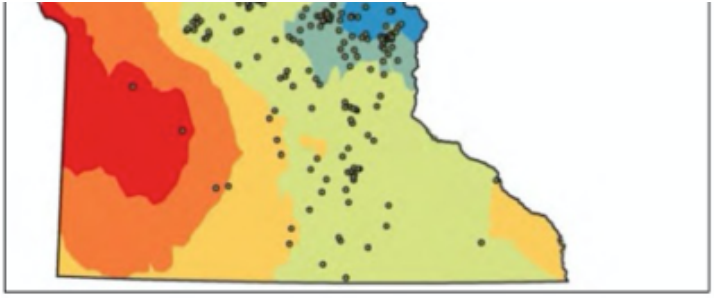
Read MPCA Preliminary Analysis of the Wild Rice Sulfate Standard Study (June 2, 2014).

Read WaterLegacy Comments on Proposed MPCA Charge Questions on the Wild Rice Sulfate Standard Studies (July 8, 2014).

Read WaterLegacy Comments on Wild Rice Sulfate Standard Studies and MPCA Preliminary Analysis (July 8, 2014).

Read WaterLegacy Presentation to Peer Review Panel for the Wild Rice Sulfate Standard Studies (August 13, 2014).





Dots show wild rice waters. Blue and green areas have less than 10 mg/L sulfate – within the range of the existing standard. Yellow-orange, orange, and red areas, respectively, have above 30 mg/L, 100 mg/L and 250 mg/L of sulfate. (MPCA map)

2015 WaterLegacy, tribes, wild rice task force participants, and experts challenged MPCA's proposed changes to weaken wild rice sulfate standard.

Read WaterLegacy Summary of Wild Rice Advisory Committee Concerns (May 9, 2015)

Read WaterLegacy Comment on MPCA Planned Wild Rice Sulfate Standard Rule Change (Dec. 18, 2015)

2016 WaterLegacy continued to submit legal and scientific analysis opposing MPCA proposed rule amendment as ineffective to protect wild rice and improper under the Clean Water Act.

Read WaterLegacy Comment on MPCA Draft Technical Support Document with Exhibits (Sept. 2, 2016)

2017 MPCA's proposed rule did not resolve the concerns raised by WaterLegacy, tribes, and independent scientists. WaterLegacy submitted a total of 2,406 citizen petitions, letters, and postcards opposing the MPCA's proposal to eliminate the 10 mg/L wild rice sulfate standard and arbitrarily restrict wild rice waters.

WaterLegacy also testified and submitted 1400 pages of comments, exhibits, and expert opinions opposing MPCA's proposed rule as unreasonable, arbitrary, inconsistent with science, unprotective of wild rice, and in violation of the Clean Water Act.

Read [WaterLegacy's Comments Opposing the MPCA's Proposed Rule to repeal Minnesota's wild rice sulfate standard, Nov. 22, 2017.]

(Exhibits available on request)

2018 The Administrative Law Judge rejected MPCA's proposed rule to repeal the wild rice waters.

The Timberjay reported:

"This is a really strong victory for clean water and the rule of law," said attorney Paula Maccabee of Duluth-based Water Legacy, who had submitted over 1,400 pages of expert testimony and comments in the case, some of which proved critical to the judge's decision. Water Legacy's Maccabee said that's the best part about the latest decision. "The existing standard is a whole lot more protective of wild rice in the case of mining pollution than what the MPCA would have given to them [the industry]," she said.



Photo by Eli Sagor.

"If the Legislature decides to get rid of the [existing] standard, we now have a detailed hearing record and an 82-page decision saying that the standard is needed to protect wild rice," she said. "We'd have a pretty decent record to challenge such an action by the Legislature. What this careful and rigorous opinion says above all is that the law matters."

Read [Judge invalidates MPCA sulfate proposal, Timberjay (Jan. 14, 2018)]

The Star Tribune reported:

"It has been a long and difficult road," said Paula Maccabee, head of Water Legacy, an environmental group that has fought the proposed rule since the beginning. "For the first time in a long time, it feels as if the rule of law matters."

Read [Judge throws out proposed Minnesota rule to protect wild rice, Star Tribune (Jan. 11, 2018)]

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Journal of Geophysical Research: Biogeosciences

RESEARCH ARTICLE
10.1002/2017JG003787

This article is a companion to Myrbo et al. (2017), https://doi.org/10.1002/2017JG003788 and Pollman et al. (2017), https://doi.org/10.1002/2017JG003785.

Key Points:
- Sulfate loading to freshwater ecosystems may alter aquatic plant communities when sulfate is reduced to sulfide in the anoxic rooting zone
- The occurrence of self-sustaining wild rice populations is mainly controlled by pore water sulfide concentrations
- Even if pore water sulfide is low, wild rice is less likely to be found if the surface water is turbid or warm

Supporting Information:
- Table S1
- Table S2
- Table S3
- Data Set S1
- Figure S1

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Sulfide Generated by Sulfate Reduction Is a Primary Controller of the Occurrence of Wild Rice (*Zizania palustris*) in Shallow Aquatic Ecosystems

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Abstract Field observations suggest that surface water sulfate concentrations control the distribution of wild rice, an aquatic grass (*Zizania palustris*). However, hydroponic studies show that sulfate is not toxic to wild rice at even unrealistically high concentrations. To determine how sulfate might directly or indirectly affect wild rice, potential wild rice habitat was characterized for 64 chemical and physical variables in over 100 sites spanning a relatively steep climatic and geological gradient in Minnesota. Habitat suitability was assessed by comparing the occurrence of wild rice with the field variables, through binary logistic regression. This analysis demonstrated that sulfide in sediment pore water, generated by the microbial reduction of sulfate that diffuses or advects into the sediment, is the primary control of wild rice occurrence. Water temperature and water transparency independently control the suitability of habitat for wild rice. In addition to generating phytotoxic sulfide, sulfate reduction also supports anaerobic decomposition of organic matter, releasing nutrients that can compound the harm of direct sulfide toxicity. These results are important because they show that increases in sulfate loading to surface water can have multiple negative consequences for ecosystems, even though sulfate itself is relatively benign.

Plain Language Summary Research in the 1940s and 1950s found that wild rice grew best in low-sulfate Minnesota lakes, but it was not known why. The correlation was a puzzle, since sulfate is not very toxic to plants or animals. This study found that the problem is sulfide, not sulfate. Sulfate can be converted into toxic levels of sulfide in the soil in which wild rice germinates and roots. Wild rice is an annual plant that must sprout each spring from seed that was dropped the previous fall into wet soil. Anaerobic microbes in the soil make sulfide from sulfate in the overlying water. Lakes, streams, and wetlands that have high concentrations of dissolved sulfide in the sediment therefore have a low probability of hosting wild rice. The study also found that wild rice prefers high-transparency water and cold winters.

1. Introduction

Minnesota is unique among U.S. states and Canadian provinces in having a water quality standard that regulates sulfate (SO_4) to protect wild rice, *Zizania palustris* and *Zizania aquatica*. The more common wild rice species in Minnesota, *Z. palustris* (northern wild rice), is an annual emergent aquatic grass that forms monocultures in shallow freshwaters (wetlands, lakes, and rivers) in the area of the Laurentian Great Lakes (Minnesota, Wisconsin, Michigan, Ontario, and Manitoba). Wild rice is culturally important to multiple groups in Minnesota, especially Ojibwe, Dakota, and other Native Americans, and also provides habitat and food for waterfowl and other wildlife (Vennum, 1988). In 1977, the Minnesota legislature voted to make wild rice the Minnesota state grain. Minnesota Rule 7050.0224, promulgated in 1973, seeks to limit the exposure of wild rice to SO_4 concentrations exceeding 10 mg L^{-1} (0.1 mmol L^{-1}). This value was based on empirical research that correlated water chemistry to aquatic plant assemblages and included the observations that no large populations of *Z. palustris* occur in waters exceeding 10 mg L^{-1} SO_4 and that stands are uncommon where SO_4 concentrations exceed 50 mg L^{-1} (Moyle, 1944, 1945). In addition, Moyle (1956) noted that plantings of wild rice seed in high-SO_4 regions generally failed. A larger unpublished Minnesota Department of Natural Resources data set also shows that sites with reported wild rice

presence (DNR, 2008) are generally correlated with surface water SO_4 below 10 mg L^{-1} (Figure S1 in the supporting information).

We report here on a multiyear field survey that was part of a larger study (Myrbo et al., 2017; Pastor et al., 2017; Pollman et al., 2017) designed to reevaluate the 10 mg L^{-1} SO_4 standard by testing potential mechanisms by which SO_4 might be harmful to wild rice. SO_4 is a relatively nontoxic and unreactive compound under aerobic conditions. Pastor et al. (2017) and Fort et al. (2014) have shown that SO_4 is not directly toxic to wild rice at concentrations up to 1,600 mg L^{-1}, which exceeds concentrations in virtually all natural surface waters of the upper Midwest (Gorham, Dean, & Sanger, 1983). The U.S. Environmental Protection Agency's nonmandatory drinking water standard of 250 mg L^{-1} SO_4 (2.6 mmol L^{-1}) is based on taste rather than toxicity (EPA, 2010).

1.1. Potential Effects of Elevated Sulfate and Sulfide in Freshwater Systems

SO_4 concentrations in most freshwaters are less than a few percent of the mean concentration in seawater (2,800 mg L^{-1} (29.1 mmol L^{-1})). We surveyed 108 different lakes, streams, and wetlands across Minnesota, where the median SO_4 concentration in lakes is 10 mg L^{-1} (10th and 90th percentiles of 0.2 and 285 mg L^{-1} (MPCA, 2016)). In the much higher SO_4 concentrations of marine waters, it is well established that SO_4 can diffuse into sediment and be converted by microbial sulfate reduction (MSR) to potentially toxic sulfide that influences the presence and absence of rooted macrophytes, such as seagrasses (Borum et al., 2005; Ingold & Havill, 1984; Koch & Erskine, 2001; Lamers et al., 2013).

Despite a long-standing assumption that SO_4 is benign (Pester et al., 2012; Schindler, 1986; Urban et al., 1994) and plays a negligible role in freshwater biogeochemistry (e.g., Capone & Kiene, 1988), there is evidence that SO_4 availability in freshwaters can control the concentration and therefore the toxicity of hydrogen sulfide (H_2S) in sediment pore water to plants and animals (Bagarinao, 1992; Kinsman-Costello, O'Brien, & Hamilton, 2015; Lamers et al., 2013; Wang & Chapman, 1999). The chemical species of H_2S varies with pH; below pH 7, H_2S dominates, and above pH 7, the bisulfide ion (HS^-) dominates. For simplicity in this discussion we refer to the sum of the two species as sulfide.

1.1.1. Sulfide Toxicity to Freshwater Plants

Remarkably little attention has been given to the potential toxicity of sulfide in sediment pore water, even after Bagarinao (1992) concluded in a major review that sulfide had been "largely overlooked as an environmental factor for aquatic organisms." In a discussion of sediment toxicity testing, Wang and Chapman (1999) also observed that the biological implications of sulfide in sediments are poorly understood and "all too often ignored." They suggested that sulfide may be more important than ammonia in determining sediment toxicity to organisms and made a suite of recommendations to fill the knowledge gap, including the measurement of sulfide in undisturbed sediments. Kinsman-Costello et al. (2015) measured sulfide in undisturbed sediments and concluded that the potential toxicity of pore water sulfide is likely shaping the plant and animal communities of freshwater ecosystems. Lamers et al. (2013), in a review of sulfide toxicity to aquatic plants, pointed out that traditional toxicity testing generally neglects the chemistry of the rooting zone. Simkin, Bedford, and Weathers (2013) showed that pore water sulfide in a wetland controlled the distribution of plants more than did nutrients.

The toxicity of elevated sulfide to freshwater plants was first recognized in paddy-grown white rice (*Oryza sativa*) in the 1950s (Lamers et al., 2013; Pearsall, 1950). Rice paddies and other water-saturated soils present a profound challenge for rooted plants because of the chemical changes caused by the absence of oxygen and resulting potential toxicity of the pore water (Ponnamperuma, 1972). Anaerobic decomposition of organic matter results in elevated pore water concentrations of ammonia, organic acids, and variable concentrations of sulfide and ferrous Fe, depending on the availability of SO_4 and Fe. The interaction of Fe and the S cycle is complicated (Hansel et al., 2015), but because under anoxic conditions sulfide forms an insoluble precipitate with Fe, elevated pore water sulfide concentrations occur when Fe availability is relatively low (Ponnamperuma, 1972; van der Welle et al., 2006). It is thought that the iron sulfide precipitates are relatively inert and that only sulfide dissolved in pore water is potentially toxic. The concentration of sulfide in pore water is the balance between production and competing fates of sulfide, including precipitation with metals such as Fe, oxidation by oxygen introduced by bioturbation or by release from the roots of macrophytes (Armstrong & Armstrong, 2005), and by downward advection of surface water due to groundwater movement or transpiration by dense macrophyte stands (Bachand et al., 2014).

1.1.2. Geochemical Consequences of Enhanced Microbial Sulfate Reduction

Increased SO_4 availability can allow increased anaerobic decomposition of organic matter, releasing the inorganic nutrients that generally limit growth of higher plants (N, P, and K) (Lamers, Tomassen, & Roelofs, 1998; Myrbo et al., 2017; Weston et al., 2006, 2011). Enhanced decomposition breaks down particulate and dissolved organic carbon (DOC) in the sediment, which can increase DOC and dissolved inorganic carbon (DIC) in the overlying water (Myrbo et al., 2017).

In addition to supporting organic matter mineralization, MSR production of sulfide causes a cascade of reactions that can alter ecosystem functioning independent of any toxicity to plants and animals. First, sulfide can participate in redox reactions, chemically reducing Fe, which converts Fe from solid phase Fe(III) oxyhydroxides to water-soluble Fe(II) (Hansel et al., 2015). The dissolution of the Fe oxyhydroxides releases sorbed ions into solution, including phosphate and trace metals (Caraco, Cole, & Likens, 1993; Davranche & Bollinger, 2000; Søndergaard, Jensen, & Jeppesen, 2003). Second, sulfide can precipitate dissolved metals, including essential plant nutrients Fe, Cu, and Zn, decreasing their bioavailability and leading to nutrient deficiency (Kirk, 2004; Lamers et al., 1998; Neue & Bloom, 1989; Neue & Lantin, 1994). In systems unpolluted with heavy metals, the sulfide precipitate is overwhelmingly dominated by iron sulfide compounds, consisting of a range of stoichiometries and minerals (Schoonen, 2004), which we here term "FeS compounds." Third, the conversion of SO_4 to sulfide entails the production of DIC, or alkalinity, an effect not fully appreciated in freshwater systems until the mechanistic consequences of acid rain were investigated in the 1980s (Baker, Brezonik, & Pollman, 1986; Cook et al., 1986; Schindler, 1986). Alkalinity is thought to be a major factor influencing the distribution of aquatic species, including macrophyte species (Moyle, 1945; Vestergaard & Sand-Jensen, 2000). In addition, elevated alkalinity may further enhance decomposition, producing a positive feedback to the effects of SO_4-driven mineralization (Geurts et al., 2009; Roelofs, 1991).

1.2. Multiple Plausible Negative Effects of Elevated Sulfate and Sulfide Production on Wild Rice

The purpose of this study was to examine the multiple ways that increases in SO_4 concentration and sulfide production can change the biogeochemical functioning of freshwater ecosystems and potentially negatively affect the growth and reproduction of wild rice populations. Before any field data were collected for this study, alternative hypotheses regarding the most likely negative effects were identified (MPCA, 2011), so that appropriate data would be available to test them. The primary hypotheses of how increases in surface water concentrations of SO_4 could harm wild rice populations include direct toxicity by elevated pore water sulfide; reduced bioavailability of Fe, Cu, or Zn; and increased P bioavailability promoting the growth of phytoplankton and macrophytes that compete for light and space. Additional variables were quantified so that exploratory data analysis could be pursued in addition to evaluation of the primary hypotheses. Surface water, pore water, and sediment physical and chemical properties were collected from 108 different sites, both with and without wild rice present, to inform evaluation of these multiple interrelated hypotheses.

2. Methods

2.1. Study Area

2.1.1. Physical Environment

SO_4 concentrations in surface water vary with geology and climate (Gorham et al., 1983) in a northeast-southwest gradient across Minnesota. Bedrock and bedrock-derived glacial deposits of northeastern Minnesota (the "arrowhead") comprise slowly weathered crystalline materials, generally low in S, and surface waters are dilute (specific conductance of $<$140 and often $<$30 μmho cm^{-1}; Gorham et al., 1983). Within this area of naturally low SO_4, iron-mining activities in the "Iron Range" district of northeastern Minnesota have created an "island" of lakes and streams elevated in SO_4 (Figure 1), a result of the weathering of sulfide minerals in waste rock piles and tailing basins. The surficial geology of southwestern Minnesota, in contrast, is derived from marine shales and carbonates that are relatively S-rich and readily weathered: surface waters often exceed 500 μmho cm^{-1} and may exceed 7,000 μmho cm^{-1} (Gorham et al., 1983). SO_4 concentration is positively correlated with conductivity above about 200 μmho cm^{-1} and 10 mg L^{-1} or 0.1 mmol L^{-1} SO_4 (Figure 7B in Gorham et al., 1983). Overprinting this geological pattern is a strong climatic gradient with warmer, drier conditions toward the southwest (toward the northern Great Plains) and colder, more humid conditions to the northeast (toward the Laurentian Great Lakes), which enhances the conductivity gradient: more evaporative conditions in the southwest serve to concentrate surface waters and further increase ionic

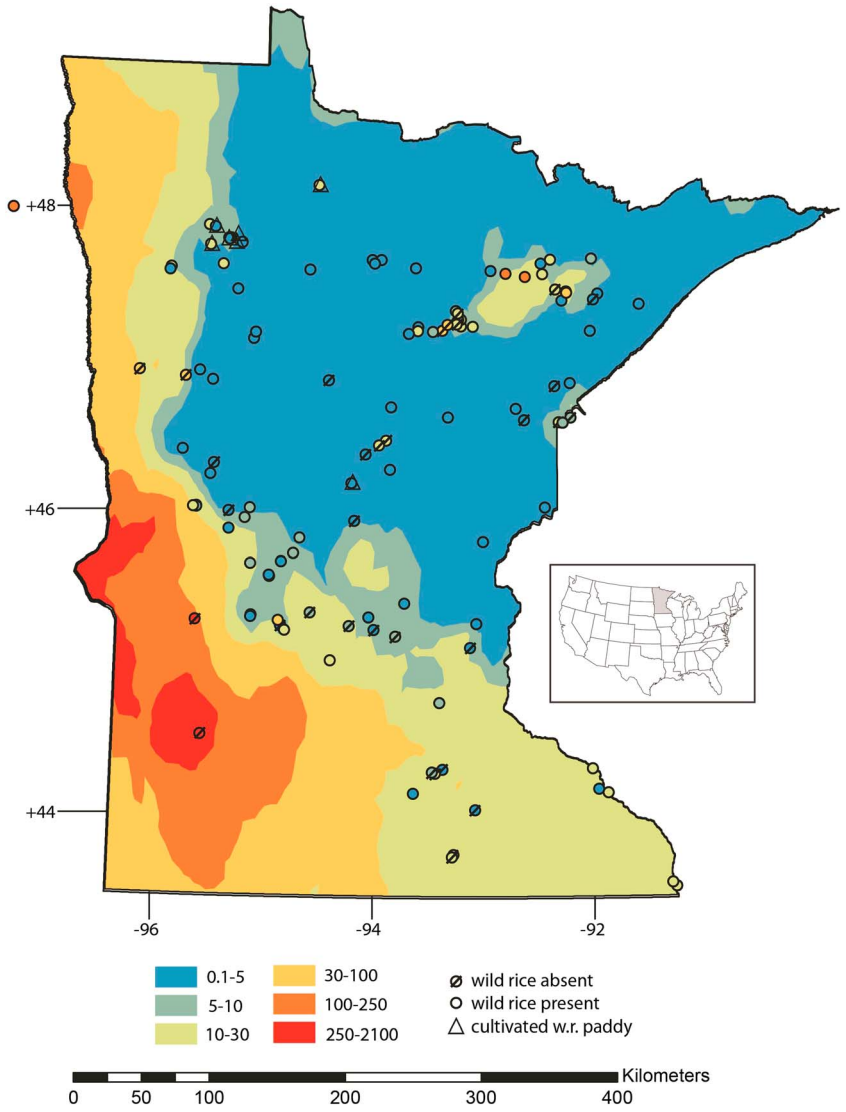


Figure 1. Map of Minnesota showing field sites overlain on kriged contours of average surface water SO_4 concentrations from 4,998 waterbodies (data from MPCA and DNR databases). The symbols are filled with the color corresponding to the site's surface water sulfate concentration. Site to the northwest of the Minnesota map is within the state of North Dakota, 40 km west of the border with Minnesota (see text). Sites where wild rice was not found have a diagonal line through the symbol.

strength, while moister conditions in the northeast maintain low ionic strength in surface waters (Gorham et al., 1983).

2.1.2. Habitat Preferences of Wild Rice

Although the scientific literature contains many assertions concerning the environmental preferences of wild rice regarding water and sediment quality (e.g., DNR, 2008, 2016; Lee, 2002; Moyle, 1944; Moyle & Krueger, 1964; Pillsbury & McGuire, 2009), sometimes little evidence was presented to support the putative preferences. The shallow freshwaters in which wild rice is found are usually relatively transparent because wild rice and other rooted macrophytes do best if they can photosynthesize as they grow each spring from the sediment to the water surface (Scheffer, 1998). Otherwise, any particular habitat preference of wild rice, such as specific chemical ranges of the sediment or surface water, is challenging to identify. Since wild rice is an opportunistic annual plant, a primary habitat requirement is periodic environmental disturbance (Grime, 1977) that keeps perennial plants such as water lilies from controlling the space and light of the shallow waters in which these species cooccur (Pillsbury & McGuire, 2009). Wild rice does not seem to have specific sediment requirements, as it has been observed growing in a variety of substrates (Aiken et al., 1988; Lee,

1986) within its range. Annual plants are adapted to exploit environments intermittently favorable for rapid plant growth and to maximize seed production (Grime, 1977). Wild rice produces between 25 and 150 relatively large seeds per stem (Eule-Nashoba, Biesboer, & Newman, 2012). Seeds buried in the sediment can survive for up to several decades until conditions are again favorable for germination and growth (DNR, 2008). *Zizania palustris* seeds germinate at low rates unless they have been exposed to near-freezing temperatures for at least 3 months (Kovach & Bradford, 1992), although the environmental cues for subsequent germination are poorly understood, aside from elevated temperature.

Because *Z. palustris* does not normally self-pollinate and is wind-pollinated (which implies that the pollen source must be relatively nearby; Friedman & Barrett, 2009), does not reproduce asexually, and has an annual life cycle (Aiken et al., 1988), in this analysis the presence of wild rice plants is taken to mean that the waterbody hosts a successfully reproducing and self-sustaining population. A few of the sites in this study may have experienced recent watershed changes such that steady state has not yet been reached among environmental variables and wild rice reproduction, but the balance of the 108 sites should have been in steady state at the time of sampling. The population of wild rice in a given lake can exhibit large fluctuations from year to year, which has been attributed to disturbances such as abrupt increases in water level (DNR, 2008) and to cyclical changes in N availability (Walker et al., 2010). Wild rice fills a unique ecological niche, in that there are virtually no other annual aquatic macrophytes across the Great Lakes region (Eggers & Reed, 2011).

2.1.3. Site Selection

The goal of the field survey was to identify and sample potential wild rice habitat across a gradient of SO_4 concentrations. Potential wild rice habitat was defined simply as shallow water (30–120 cm deep, though some sampled sites had water as shallow as 5 cm) that was sufficiently transparent to support rooted aquatic macrophytes. Turbidity from dense phytoplankton blooms or suspended solids may effectively exclude rooted macrophytes due to light limitation (Scheffer, 1998).

Most Minnesota lakes that host wild rice populations have low SO_4 concentrations; the median concentration of wild rice lakes is 1.8 mg L^{-1}, and the 75th percentile is 3.6 mg L^{-1} (MPCA, 2014). If only those waters hosting abundant wild rice were sampled, most of those would be low in SO_4, and little would be learned about how elevated SO_4 concentrations (or other important variables) might affect wild rice. Conservation biologists commonly identify important habitat variables through binary (i.e., is the species present or absent at a given site?) logistic regression, which requires the sampling of sites that do not support the species of interest (e.g., Carroll, Zielinski, & Noss, 1999; Peeters & Gardeniers, 1998; van der Heide et al., 2009). Consequently, potential wild rice habitats with a range in SO_4 concentrations were sampled without regard to whether the waterbody hosted or was known to host wild rice. An effort was made to sample waterbodies that covered the range of SO_4 concentrations across Minnesota and especially to sample sites in high-SO_4 regions that had reports of recent or historical presence of wild rice. This strategy also resulted in the sampling of a high-SO_4 wild rice site in the North Branch of the Turtle River (198 mg SO_4 L^{-1}), 40 km into North Dakota from northwestern Minnesota (Figure 1). A gradient of SO_4 concentrations was sampled by identifying potential wild rice habitat in two areas of elevated SO_4, the "island" of elevated SO_4 in the Iron Range region of northeastern Minnesota and waters naturally elevated in SO_4 to the west and south of the known range of wild rice (Figures 1 and S1). Nutrient (N and P) availability (reflecting natural soil fertility as well as agricultural runoff) also increases to the west and south, potentially supporting phytoplankton growth sufficient to exclude macrophytes, including wild rice. Consequently, in western and southern Minnesota, DNR lake databases were screened for the presence of water lilies (*Nuphar variegata* or *Nymphaea odorata*), the occurrence of which indicates transparency sufficient to support rooted macrophytes that also often cooccur with wild rice. In an analysis of DNR aquatic plant surveys from 1,753 shallow lakes, we found that the odds of finding wild rice where there are water lilies are 26 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20–36 times (neither wild rice nor water lilies = 968; wild rice but not water lilies = 60; no wild rice but water lilies = 272; both wild rice and water lilies = 453).

With these considerations, water bodies were selected for sampling based on preliminary data including average water depth, presence of water lilies, conductivity or SO_4 concentration, and geographic distribution (i.e., to sample widely across the state). Both rivers and lakes were sampled, as were seven different cultivated wild rice paddies. Within a given water body, the field team chose a location for sampling based on a decision tree (Table S1 in the supporting information).

2.2. Field Methods

Sampling occurred in August–September 2011 and June–September 2012 and 2013. Sampling efforts were focused in late summer to capture physical and chemical conditions when wild rice plants were maturing and when identification of wild rice is most certain; voucher specimens of wild rice were always taken. Samples were collected from an anchored canoe or small boat, except in the cultivated wild rice paddies when the water was too shallow to float a canoe, and samples were collected on foot. A Hach model HQ40d or Hydrolab Quanta sonde, calibrated daily, was used to measure temperature, specific conductance, dissolved oxygen, and pH in the surface water. Water transparency was determined with a 1 m long Secchi-tube (Water Monitoring Equipment & Supply, USA), and apparent color was measured using a Hach model CO-1 color test kit. Surface water samples (later split into separate subsamples; see below) were collected by a technician wearing long nitrile gloves in two 2 L amber Nalgene bottles that had been previously triple rinsed with deionized water and rinsed 3 times with water from the site before filling. Water samples were stored on ice.

Short (~50 cm) sediment cores with ~10–20 cm of overlying water were collected at eight undisturbed locations at least 1 m apart around the boat using an HTH corer (Pylonex, Sweden) with a 7 cm diameter polycarbonate barrel. A piston was inserted in the bottom end of each core as it was retrieved. Cores were kept upright and shaded prior to sample processing.

Aquatic macrophytes were identified and percent cover estimated within a plastic hoop 1 m in diameter placed at 4 locations around the boat. In 2012 and 2013, the number of stems of wild rice inside each hoop was also quantified.

Pore water samples were obtained from cores processed on shore using 10 cm Rhizon™ filters (pore size 0.12–0.18 μm) (Rhizosphere.com, Netherlands; Shotbolt, 2010) inserted vertically into the core tops, following extrusion of overlying water, and connected to evacuated serum bottles with PVC/polyethylene tubing and a stainless steel needle. Three separate cores were sampled, one for nutrients (nitrate + nitrite, TP, and TN) and DOC (70 mL), a second for metals (50 mL), and a third for dissolved silica, Cl, and SO_4 (30 mL). A fourth core was sampled for pore water sulfide, but in this case, the serum bottle was preloaded with 0.2 mL of 2.0 N zinc acetate, 0.5 mL of 15 M sodium hydroxide, and a stir bar, flushed with a nitrogen atmosphere, evacuated, and preweighed. Air was flushed from the Rhizon-tubing assembly with sample pore water using a second evacuated bottle before the needle was inserted into the sulfide sample bottle. In 2011 only pore water samples for sulfide and metals were collected. Pore water pH was measured on a fifth core by inserting the probe of a Hach model HQ40d pH meter into the sediment to a depth of 5 cm and allowing the reading to stabilize.

A composite sediment sample was collected from the uppermost 10 cm of the sixth, seventh, and eighth cores, placed into a stainless steel bowl, and stirred under nitrogen atmosphere to homogenize. A 50-mL subsample was placed in a polypropylene sample bottle along with 1.0 mL of 1.0 N zinc acetate for analysis of acid-volatile sulfide (AVS). The headspace of the AVS sample bottle was flushed with N_2 and the bottle capped; that bottle was placed in a larger glass jar and that jar flushed with N_2 and tightly sealed. The sample was immediately placed in a cooler with dry ice to freeze. The remaining composited sediment was placed into a polycarbonate container and stored on ice for later analysis.

Water subsamples were taken from the large amber Nalgene bottles by a technician wearing nitrile gloves. Sulfuric acid (5 mL of 10%) was immediately added to subsamples for the analysis of P, TKN (total Kjeldahl nitrogen), ammonia, and nitrate + nitrite in 250 mL polyethylene bottles. Nitric acid (2.5 mL of 20%, to acidify to pH < 2) was immediately added to subsamples for the analysis of total metals in 250 mL polyethylene bottles. Samples for dissolved metals were subsampled in 250 polyethylene bottles and subsequently filtered using a 0.45 μm filter and preserved with nitric acid in the laboratory. Samples were stored on ice.

2.3. Laboratory Methods

Surface water, pore water, and most sediment analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL) in 2012 and 2013, following standard methods. In 2011 other laboratories conducted the analyses (University of Minnesota Soils Laboratory (UMNSL), St. Croix Watershed Research Station (SCWRS), and Gustavus Adolphus College (GAC)); methodological differences are noted, and the laboratory is identified where relevant.

Surface and pore water samples were analyzed for anions (Cl and SO_4) by ion chromatography on a Dionex ICS-3000 (MDHEL), Fe, Ca, Na, Mg, and K by inductively coupled optical emission spectrometry on a Varian 715-ES (MDHEL), Mn, Cu, Zn, Co, Ni, Al, As, and Se by inductively coupled mass spectrometry on a Perkin Elmer Elan DRCE (MDHEL), and DOC by UV-persulfate oxidation on a Tekmar-Dohrmann Phoenix 8000 (SCWRS and MDHEL). N and P were measured by colorimetric methods (on a Lachat Quikchem Flow-Injection Autoanalyzer) at SCWRS and MDHEL following cadmium-reduction (nitrate and ammonia) or dual alkaline-persulfate digestions (TP and TN). Silica was measured colorimetrically at SCWRS on the Lachat Autoanalyzer and at MDHEL on a Beckman Coulter DU 800 UV/VIS spectrophotometer. Pore water sulfide was analyzed colorimetrically on each lab's Lachat Autoanalyzer following in-line acid distillation and NaOH trapping (SM 4500-S2). Alkalinity was measured by potentiometric acid titration (MDHEL) or as DIC by acid digestion and IR detection (SCWRS). DIC was converted to alkalinity using pH, temperature, and specific conductance of the surface water.

Sediment samples from 2011 were analyzed by combustion for total carbon (TC), total nitrogen (TN), and total sulfur (TS) using, respectively, a Tekmar Phoenix 8000 CO_2 analyzer, an Elementar Vario Max N analyzer, and a LECO sulfur analyzer (UMNSL). Samples from 2012 and 2013 were analyzed for CHN on a Costech 4010 Elemental Analyzer (UMN Stable Isotope Laboratory). Total inorganic carbon (carbonate) was analyzed by coulometric titration on a UIC CM5015 CO_2 coulometer, while water and organic matter content were determined by loss-on-ignition methods (Heiri, Lotter, & Lemcke, 2001) in the UMN LacCore facilities. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid-distillation and in-line alkaline trapping (SM 4500-S2; Hsieh & Shieh, 1997).

Sediment phosphorus was extracted from freeze-dried sediments following methods of Engstrom and Wright (1984) for total-P and Hieltjes and Lijklema (1980) for P fractions (NH_4Cl-extractable, NaOH-extractable, HCl-extractable, and residual (organic)-P). The P extracts were measured colorimetrically by flow-injection autoanalyzer (SCWRS). Extractable iron and trace metals were quantified from a 0.25 g homogenized freeze-dried sediment subsample incubated in 0.5 M HCl for 30 min at 80°C. The samples were centrifuged, decanted, and analyzed by ICP-MS at GAC. This extraction releases metal oxyhydroxides, sulfides, and loosely bound phases from the sediment without appreciably attacking the silicate matrix (Balogh et al., 2009).

2.4. Data Subsets and Statistical Analysis

From 2011 to 2013, 260 site visits were conducted in 108 different natural waterbodies, including lakes, small streams, backwaters of the Mississippi River, and wetlands, plus 7 different cultivated wild rice paddies. For a variety of logistical reasons, the full suite of samples could not be collected on some site visits. Three subsets of the field data were identified for the analyses reported here. A subset that consists of all of the samples from natural waterbodies (excluding the cultivated paddies) with virtually complete analyses (surface water, pore water, and bulk sediment; $n = 194$) was termed for internal purposes Class D. Pollman et al. (2017) used Class D to develop a structural equation model to elucidate key variables that govern the concentration of sulfide in sediment pore water. A second subset, Class S, of 51 samples from 7 stream and 8 lake sites was each sampled 3 to 5 times from 27 May to 19 September 2013, to provide a data set to assess seasonality in variables. A third subset, Class B, was used for conducting probabilistic analyses to identify the most likely parameters controlling the presence and absence of wild rice and to examine Spearman nonparametric correlations among field variables. Class B consists of one sample from each site ($n = 108$) and excludes samples collected in 2011, which were analyzed with slightly different lab methods. Although Class B was not created as a random sample of Minnesota waterbodies, the frequency distribution of SO_4 concentrations is intermediate between a probability-based survey conducted by USEPA (MPCA, 2016) and a list of known wild rice water bodies, which are overwhelmingly low-SO_4 lakes (MPCA, 2014). Therefore, the Class B data set is reasonably representative of potential wild rice habitat, and binary logistic regressions can be used to approximate the probability of wild rice occurrence as a function of field variables such as pore water sulfide. The Class B data set is used in all analyses presented here, with two exceptions: (1) Figures 3a–3c present the full Class D data set, plus for comparison, data from the cultivated wild rice paddies; (2) Statistical analysis was performed with the software package R version 3.2.3.

To identify variables associated with the presence and absence of wild rice, we relied on binary logistic regressions (BLR), using the glm function in R. BLR does not require normally distributed data, but

Table 1
Field Variable Correlation With the Presence-Absence of Wild Rice, Assessed Through Binary Logistic Regression (BLR), Plus Spearman Correlations Between Variables[a]

	Binary logistic regression (BLR) correlation with field variable			Spearman correlation with field variable (rho)				
Field variable	Log transformed?	*p* value	Correlation direction	Wild rice density (only wild rice sites) *n* = 67	Pore water sulfide	Sediment AVS	Water transparency	Water temperature
pw K	Y	0.0008***	Negative	−0.27*	0.46***	0.11	−0.10	0.33***
pw sulfide	Y	0.0012***	Negative	−0.31**	1.00	0.29**	−0.07	0.17
Water depth (m)	N	0.0028***	Negative	0.07	0.11	0.08	0.22*	0.19*
Transparency (cm)	N	0.0031***	Positive	0.11	−0.07	−0.13	1.00	−0.08
sw TN	Y	0.0054**	Negative	−0.12	0.22*	0.08	−0.61***	0.23*
sed Se % dry	N	0.0059**	Negative	0.12	0.08	0.27**	−0.21*	0.13
sw Temp	N	0.0077**	Negative	0.08	0.17	0.11	−0.08	1.00
pw Fe	Y	0.0109*	Positive	0.20	−0.58***	0.00	0.04	0.09
sw pH	N	0.0200*	Negative	−0.14	0.28**	0.08	−0.05	0.35***
sw TP	Y	0.0353*	Negative	0.15	0.05	0.29**	−0.58***	0.27**
Latitude	N	0.0376*	Positive	−0.04	−0.06	−0.09	0.13	−0.51***
sed TS % dry	Y	0.0483*	Negative	0.20	0.40***	0.42***	0.03	−0.08
sw K	Y	0.0922		−0.03	0.29**	0.21*	−0.18	−0.08
sed AVS % dry	Y	0.1317		0.02	0.29**	1.00	−0.13	0.11
sw sulfate	Y	0.1475		−0.10	0.44***	0.45***	−0.07	0.04
sed TP % dry	N	0.2697		−010	0.07	0.30**	−0.14	0.14
sw alkalinity	Y	0.2786		0.25*	0.22*	0.26**	0.11	0.17
pw TN	Y	0.2963		−0.30	0.31***	0.14	−0.20*	0.34***
pw NH4	Y	0.4505		−0.33**	0.33***	0.22*	−0.17	0.26**
sed Fe % dry	Y	0.4795		0.16	−0.35***	0.38***	−0.10	−0.06
pw DOC	Y	0.4865		0.08	−0.05	−0.1	−0.21*	0.09
pw Si	N	0.5548		0.03	0.33***	0.18	0.07	0.29**
pw TP	Y	0.6341		0.02	0.12	0.30**	−0.26**	0.26**
sed TN % dry	N	0.6807		−0.06	0.14	0.04	0.06	−0.06
sed water content	N	0.7274		−0.07	0.15	0.10	0.10	0.02
sed TOC % dry	N	0.7854		−0.10	0.10	0.02	0.10	−0.06

[a]Note. The variables are ordered by the significance of the BLR. The first 12 variables have BLR significance of $p < 0.05$. The additional 14 variables are listed because of their correlation with pore water sulfide, sediment AVS, or surface water transparency or temperature—or their notable lack of correlation with wild rice presence-absence (pw = pore water; sw = surface water; sed = sediment).
*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

nevertheless, we transformed some variables (Table 1) to achieve approximately normal distributions. BLR also yields the probability of occurrence at a given value of the variable. Spearman nonparametric correlations (rho) between field variables in Class B were examined as part of the effort to identify the major biogeochemical interactions in these shallow-water systems. Seasonality in a variable was assessed using linear mixed effects models, with time (fraction of a year) as a fixed variable and site as a random factor (to account for multiple samples per site). In the model developed for each field variable, we accounted for the seasonal cycle using the following equation (Crawley, 2007), where *A*, *B*, and *C* are fitted model coefficients:

$$y = A + B\ \sin\ (2\pi\ \text{time}) + C\ \cos\ (2\pi\ \text{time}) \quad (1)$$

3. Results and Discussion

3.1. Field Variables Associated With Wild Rice Presence and Absence

Of the 64 field quantified variables, BLR identified 12 that are associated with the presence/absence of wild rice at the 0.05 probability level or better (Table 1): pore water sulfide, K, and Fe (Figures 2a, 2b, and 2f); surface water temperature, TP, TN, and pH (Figures 2d, 2m, 2n, and 2o); sediment Se and TS (Figures 2c and 2p); water depth and transparency (not shown; Figure 2e); and latitude of the site (Figure 2g). These variables may be important in controlling the presence or absence of wild rice or may merely be correlated with one or more actual causative factors. Because we are primarily interested in factors that control presence or absence of wild rice, in contrast to the density of wild rice, we place primacy on the BLR results and use the Spearman

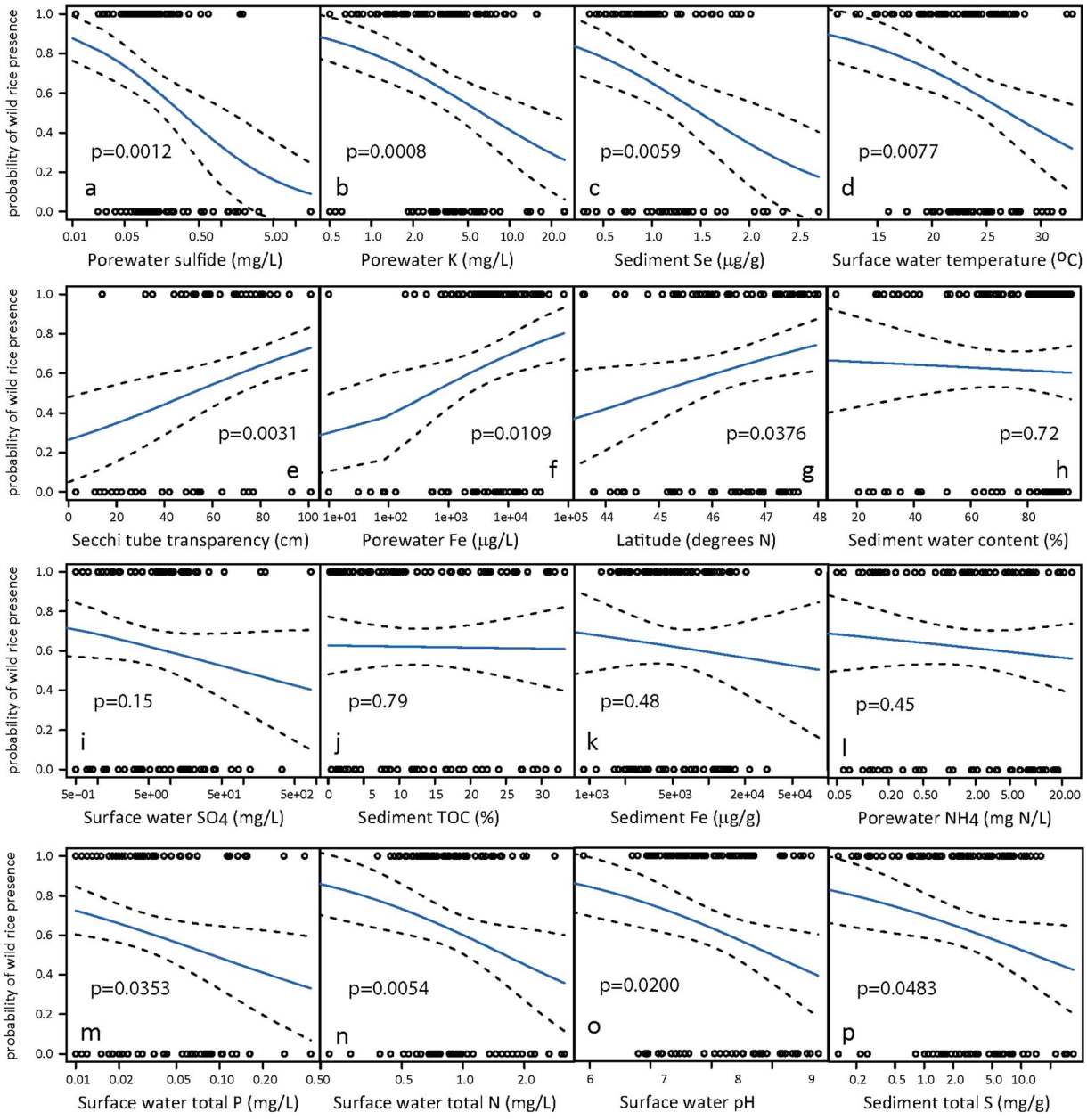


Figure 2. Binary logistic regressions for 16 variables found to be statistically significant in this study or indicated in the literature as important for wild rice habitat. The open circles indicate the value for a given parameter of each site used in the analysis; the circles at the top of a plot indicate sites with wild rice present; the circles at the bottom of a plot indicate sites with wild rice absent. The span of the dashed lines around the solid line indicates the 95% confidence interval.

correlations to help understand the relationships between environmental factors of interest. Wild rice density is negatively correlated with pore water sulfide, potassium (K), and NH_4 and positively correlated with surface water alkalinity (Table 1). There are likely factors controlling the density of wild rice at any particular location in addition to the variables measured, such as herbivory and hydrological disturbances.

One should be cautious in the interpretation of statistically significant associations between wild rice and field variables, in that true cause and effect are not necessarily obvious. For instance, is the toxic quality of pore water sulfide sufficient explanation for its negative associations with both the presence and density of wild rice? Do sites with greater density of wild rice have lower pore water sulfide because low sulfide allows wild rice to grow, or because plants release oxygen from their roots, oxidizing sulfide? The true explanation is likely a combination of the two mechanisms: elevated pore water sulfide can eventually extirpate a wild rice

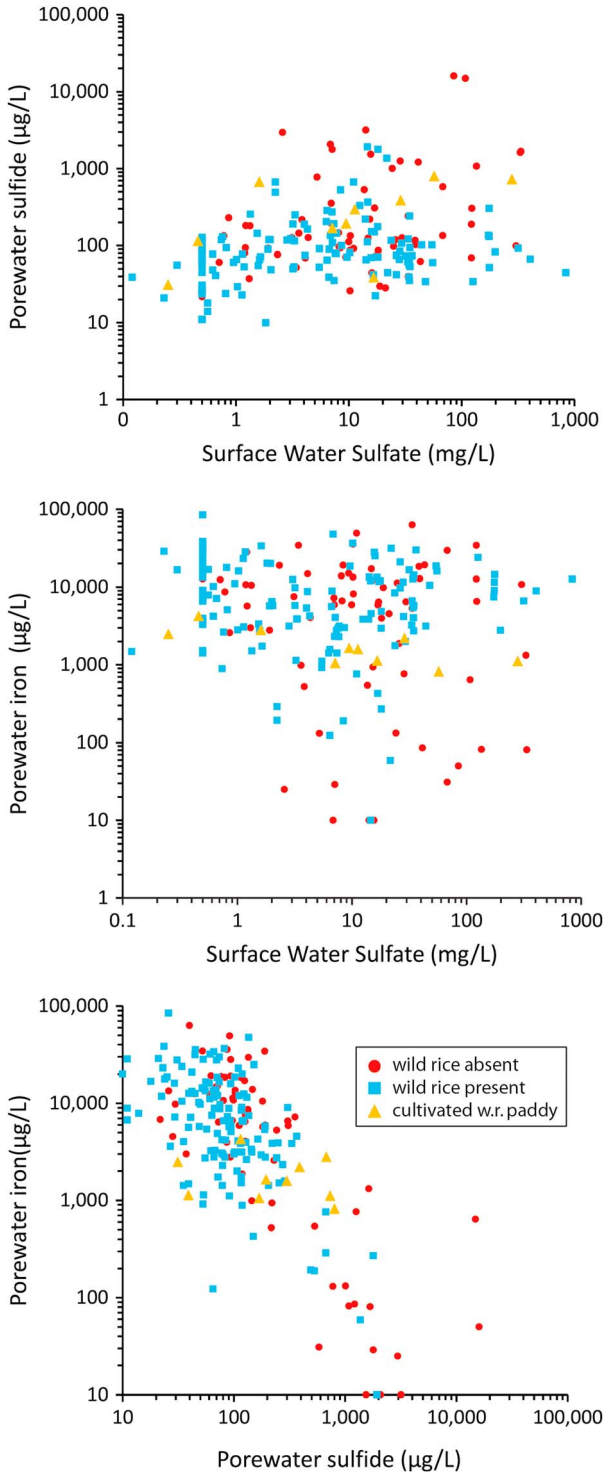


Figure 3. Class D data plus cultivated wild rice paddies (yellow triangles) showing sites with wild rice present (blue squares) and absent (red circles). Note that (a) pore water sulfide is not linearly related to surface water SO_4, indicating that additional factors besides SO_4 concentration contribute to the sulfide concentration in pore water; (b) wild rice can occur in sites over a wide range of surface water SO_4 values as long as pore water Fe is relatively high; and (c) pore water Fe and sulfide have a strong negative relationship, due to the energetically favorable formation of FeS compounds, and that wild rice mainly occurs where pore water sulfide is $<1{,}000\ \mu g\ L^{-1}$.

population by reducing seedling survival and seed production, weight, and viability (Pastor et al., 2017), but oxygen release from roots when sulfide concentrations are low enough to allow wild rice to successfully reproduce drives sulfide to even lower concentrations than would have occurred without the plants (Myrbo et al., 2017). Although the negative correlation between pore water sulfide and wild rice density (rho = -0.31, $p < 0.01$, Table 1) could be interpreted to mean that lower sulfide allows a larger population to grow, it is also possible the correlation is partly caused by oxygen release from the wild rice roots, decreasing sulfide concentrations. This hypothesis is supported by the observation from a controlled experiment that acid-volatile sulfide (AVS) sediment concentrations were 30% lower when wild rice was present (Myrbo et al., 2017).

Of the 12 variables significantly associated with the occurrence of wild rice, we propose that nine are noncausal and merely related to one or more of three truly causative factors: (1) pore water sulfide, (2) water transparency, and (3) water temperature. These three variables are not significantly correlated with one another (Table 1), and there are plausible mechanisms for each to independently exclude wild rice from otherwise suitable habitat: (1) elevated sulfide reduces the growth of wild rice (Pastor et al., 2017); (2) low water transparency plausibly constrains the ability of a germinated wild rice seed to reach sufficient light before it runs out of endosperm energy (Aiken et al., 1988; DNR, 2008); and (3) warmer winter water temperature at lower latitudes may limit seed germination, as the seeds of *Z. palustris* must experience at least 3 months in cold water to break seed dormancy (Kovach & Bradford, 1992) (although summer temperatures were measured in this study, winter and summer temperatures would be correlated).

3.2. Pore Water Sulfide as a Causal Variable and Associated Correlated Variables

Our primary a priori hypothesis was that elevated surface water SO_4 can produce elevated pore water sulfide concentrations which, in turn, negatively affect potential wild rice habitat. A plot of pore water sulfide against SO_4, with wild rice presence noted (Figure 3a), suggests that wild rice is generally not found at sites with elevated sulfide. Of the sites with pore water sulfide greater than $1{,}000\ \mu g\ L^{-1}$, wild rice was present in 23% (3 of 13, with only a few plants found at 2 of those 3 sites). In contrast, wild rice was present at 74% of the sites where sulfide was less than $1{,}000\ \mu g\ L^{-1}$. Despite historical reports of *Zizania aquatica* in Minnesota, inspection of voucher specimens, following the taxonomy of Terrell et al. (1997), found only *Z. palustris* at all sites sampled. *Zizania aquatica* (Southern wild rice) is rare in Minnesota, but its range extends east through Wisconsin and the southern part of the Great Lakes region to New England and occurs in freshwater tidal marshes south to Florida and west to Louisiana (Terrell et al., 1997). The findings of this study, especially those related to temperature, may not apply to *Z. aquatica*.

Despite a strong relationship between sulfide and wild rice, the relationship between SO_4 and pore water sulfide among the 108 field sites was not a simple linear function. High surface water SO_4 did not always result in high pore water sulfide (above $1{,}000\ \mu g\ L^{-1}$), and some samples with surface water SO_4 less than 10 mg L^{-1} had pore water sulfide greater

than 1,000 μg L^{-1} (Figure 3a). The 33 samples with surface water SO_4 below 1.0 mg L^{-1} all had pore water sulfide less than 200 μg L^{-1}, suggesting that surface water SO_4 might limit maximum sulfide production.

Wild rice was present at high and low SO_4 concentrations as long as pore water Fe was high and sulfide was low (Figures 3b and 3c): pore water Fe and sulfide each exert antipathetic control over the concentration of the other (Figure 3c; Pollman et al., 2017), and wild rice mainly persists in sites where pore waters are low in sulfide and high in Fe. Pollman et al. (2017) developed a structural equation model showing that variations in three variables (SO_4, sediment TOC, and sediment extractable Fe) contribute nearly equally to the observed variations in pore water sulfide among the sites in this study. Sulfate-reducing bacteria are simultaneously limited in their production of pore water sulfide by surface water SO_4 and sediment TOC (which are roughly proportional to available SO_4 and labile organic matter, respectively). Concentrations of sulfide in pore water are then constrained by the availability of pore water Fe, which is in turn controlled by the overall supply of Fe in the sediment (Pollman et al., 2017).

If pore water sulfide concentration is a causative factor controlling the presence of wild rice, as hypothesized, then other variables may be statistically significant because they either vary with the process of SO_4 reduction or with the sulfide itself. Pastor et al. (2017) showed that wild rice growth declines in proportion to sulfide concentrations in both hydroponic and outdoor mesocosm experiments. In a study of the Pastor et al. mesocosms, Myrbo et al. (2017) showed that the enhanced mineralization of plant litter associated with SO_4 reduction produced increases in surface water P and N, and increases in pore water sulfide caused decreases in pore water Fe. These effects from the controlled mesocosm experiment are consistent with correlations observed among sites in the field data between pore water sulfide and surface water N and pore water Fe (Table 1). Surface water P is not significantly correlated with pore water sulfide in the field data, despite dual mechanisms of P mobilization from sediment, (1) the interaction between sulfide and Fe (Caraco et al., 1993; Maynard, Dahlgren, & O'Geen, 2011; Smolders & Roelofs, 1993) and (2) mineralization of organic matter. The lack of correlation of surface water P with pore water sulfide in this field study is not surprising because given the wide variety of landscapes sampled, there is no reason that pore water sulfide would be proportional to the mass of Fe that has reacted with sulfide and released sorbed P. In SO_4 addition experiments where the P and Fe content of the sediment is held constant, there is often a significant correlation between pore water sulfide and mobilized P (Myrbo et al., 2017). In contrast to the lack of correlation of pore water sulfide with surface water P in this field study, AVS is significantly correlated with surface water P (rho = 0.30, $p < 0.01$, Table 1), presumably because AVS is proportional to the iron that has reacted with sulfide.

Elevated pore water sulfide is a product of SO_4 reduction-driven mineralization of sediment organic matter, which also releases the constituents of the decaying plant material, N, P, K, silica, and C (either as DIC, which increases alkalinity, or DOC) into sediment pore water and the overlying surface water (Myrbo et al., 2017). Consequently, the occurrence of wild rice is negatively associated not only with pore water sulfide ($p = 0.001$) but also with elevated pore water K ($p = 0.0008$) and surface water TN ($p = 0.005$, Table 1). Median concentrations of these variables are lower in wild rice waters compared to waters with no observed wild rice (88 versus 126 μg L^{-1} sulfide, 2.4 versus 4.3 mg L^{-1} pore water K, and 0.74 versus 0.95 mg L^{-1} TN, Table S3). Pore water sulfide is itself positively correlated with pore water K, TN, NH_4, and silica, surface water K, alkalinity, and pH, and sediment TS and AVS (Tables 1 and S4).

The strong evidence for the association of elevated pore water K with the absence of wild rice (Table 1) is interesting, as K is an essential plant nutrient, and therefore, it is unlikely that the association is based on toxicity to wild rice. Rather, it is likely that the association is a result of the simultaneous mobilization of K with the production of sulfide as plant matter is mineralized. Potassium does not bond covalently with organic compounds and is readily leached out of dead organic matter (Troeh & Thompson, 2005). Silica phytoliths dissolve as plant matter is mineralized, allowing additional K that had been trapped within the phytoliths to be released into sediment pore water (Nguyen et al., 2015). Wild rice and other wetland macrophytes develop abundant phytoliths that release dissolved silica upon decomposition (Struyf & Conley, 2009). Additional dissolved silica is likely released to pore water as epiphytic diatoms are mineralized. Pore water silica, K, and sulfide are all significantly correlated with each other (Tables 1 and S5). The negative correlation of pore water K with wild rice may be magnified by its additional positive correlation with elevated water temperature (Table 1), which plausibly accelerates dissolution of silica in organic matter (Gudasz et al., 2010; Kamatani, 1982).

AVS largely consists of solid-phase sulfide, which is not available to plants and therefore is not significantly associated with wild rice presence/absence ($p > 0.10$, Table 1). AVS is a measure of cumulative sulfide production, which is proportional to past mineralization of organic matter, consistent with significant positive correlations between AVS and pore water TP and NH_4, and surface water TP and alkalinity ($p < 0.01$, 0.05, 0.01, and 0.01, respectively, Table 1).

The negative association of sediment total-S (TS) with wild rice presence (BLR $p = 0.048$, Table 1) is likely the result of both TS and wild rice being controlled by sulfide production; the median TS concentration at sites with wild rice is 2.6 mg g^{-1}, compared to 4.1 mg g^{-1} at sites without wild rice (Table S3). Sediment TS is correlated with both pore water sulfide and AVS. The negative association of sediment Se with wild rice (BLR $p = 0.006$) is surprising, given that median Se concentrations are very low (0.9 and 1.2 μg g^{-1} in sites with and without wild rice, respectively). The slightly higher Se at sites without wild rice may be caused by the coprecipitation of Se and S by SRB, as shown by Hockin and Gadd (2003). Selenium is correlated with sediment total S (rho = 0.35; $p < 0.001$).

3.3. Water Transparency as a Causal Variable and Associated Correlated Variables

Lower water transparency is associated with a lower probability of wild rice occurrence (BLR $p = 0.003$). Transparency is not related to wild rice density (rho = 0.11, $p > 0.20$, Table 1); however, low transparency can apparently exclude wild rice (11 of the 12 sites with transparency <30 cm had no wild rice), but above that threshold, other variables control wild rice density. Low water transparency decreases photosynthesis of wild rice seedlings while growing to the water surface, which (a) decreases oxygen production that could otherwise be used to detoxify sulfide internally (Krüssel et al., 2014), or externally if released into pore water from the roots (Colmer, 2003); and (b) decreases the energy available for root development, enhancing vulnerability to a sudden increase in water depth, which can uproot an entire year's cohort (DNR, 2008). Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow over 50 cm before reaching the water surface, at which time floating leaves are developed that can supply energy for root development (Aiken, 1986). If transparency is too low, or the water too deep, the energy stored in the seed can be insufficient for the seedling to reach the water surface.

Water transparency can be controlled by the density of phytoplankton, which was not measured in this study. However, transparency is highly correlated with the nutrient concentrations that control phytoplankton growth, total phosphorus (rho = −0.58, $p < 0.001$), and total nitrogen (rho = −0.61, $p < 0.001$). Transparency can also be controlled by colored dissolved organic matter, consistent with the observed significant correlation between transparency and water color (rho = −0.68, $p < 0.001$, Table S4).

3.4. Temperature as a Causal Variable and Associated Correlated Variables

Wild rice germinates at higher rates following longer exposure at temperatures closer to freezing (Kovach & Bradford, 1992), consistent with the significant association of wild rice absence with lower latitudes (BLR $p = 0.04$) and warmer summer water temperature (BLR $p = 0.008$; medians of 21.9° and 23.7°C with and without wild rice, respectively). Sites with warmer summer water temperatures generally also have warmer winter temperatures. Warmer and shorter winters would cause lower germination rates, ultimately reducing the probability that a population could persist over the long term. For this reason, the Minnesota Department of Natural Resources hypothesizes that climate change may push the range of wild rice farther north (DNR, 2008, 2016). However, the temperature control of wild rice germination has not been adequately investigated to rigorously assess this hypothesis. The observed association of wild rice with lower pH surface waters (BLR $p = 0.04$; median pH values of 7.8 and 8.5 at sites with and without wild rice, respectively) may be due simply to the correlation of wild rice with cooler surface waters: the solubility of CO_2 is higher in colder water, leading to lower pH (Spearman correlation between temperature and pH = 0.35, $p < 0.0001$).

Lower latitude sites are negatively associated with wild rice presence and density (Table 1). Lower latitude sites are correlated with higher water temperature (rho = −0.51, $p < 0.001$). Geological and land use gradients also correlate with latitude, producing correlations with surface water N and P (rho = −0.25 and −0.31, $p < 0.01$). Despite these correlations with nutrients, latitude is not significantly correlated with water transparency (rho = 0.13), so the general unsuitability of lower latitudes for wild rice may be a combination of warmer winters and some reduced transparency as a result of nutrient enrichment. Latitude is not significantly correlated with pore water sulfide (rho = −0.06).

3.5. Synergy Among the Three Causal Variables of Sulfide, Temperature, and Transparency

While temperature may control wild rice presence/absence through winter temperatures too warm to achieve high rates of seed germination in the spring, higher summer temperatures may act synergistically on variables correlated with pore water sulfide. Elevated summer temperatures likely enhance microbial activity, no matter which electron acceptor is respired by the dominant microbes (Gudasz et al., 2010). Water temperature is indeed correlated with pore water concentrations of the plant nutrients N, P, and K released by decomposition, which are also significantly correlated with either pore water sulfide or AVS (Table 1). Although sulfide production may be enhanced by elevated temperature, the correlation between sulfide and temperature is weak (rho = 0.17; $p > 0.05$).

The production of sulfide plausibly contributes to the significant correlation of six of the nine variables with wild rice occurrence: P and N in surface water, Fe and K in pore water, and S and Se in sediment. Water temperature is significantly correlated with three of these variables (P, N, and K), and therefore, synergistically reinforces their associations with the absence of wild rice. Elevated surface water P and N are further synergistically associated with the absence of wild rice because their release via MSR-driven mineralization also enhances phytoplankton growth, reducing light available to wild rice seedlings; P and N are highly correlated with reduced transparency ($p < 0.001$, Table 1).

In this data set greater water depth is negatively associated with the occurrence of wild rice, not because wild rice grows better in shallower water, which it may, but because of where field crews took samples when wild rice was not present. Following a decision tree (Table S1), when wild rice was not present, field crews usually sampled among water lilies, which, on average, were observed in slightly deeper water than wild rice (67 cm compared to 52 cm).

Finally, *multiple* BLR was employed to investigate the question of whether any of the correlated variables provided additional explanatory power for the occurrence of wild rice beyond the models with sulfide, transparency, or temperature as base predictors. Surface water temperature is predictive of wild rice presence independent of sulfide ($p = 0.03$). Pore water K improves models based on sulfide ($p = 0.02$) or temperature ($p = 0.004$) alone but provides no significant additional explanatory power beyond a model built on both sulfide and temperature ($p = 0.07$). Overall, multiple BLR analysis confirms that other variables provide no additional explanatory power and that the three base predictors are independent of each other.

Multiple BLR was used to model the probability of wild rice occurrence (WR presence) as a function of pore water sulfide (pw sulfide, in mg L^{-1}), water transparency as measured by the Secchi tube (trans, in cm), and water temperature (Temp, in °C):

$$\text{Logit}\,(\text{WR presence}) = 0.532 + 0.0183\,(\text{trans}) - 1.169\,(\,\text{log10 pw sulfide}) - 0.107\,(\text{Temp}) \quad (2)$$

where odds = exp(logit) and probability = (odds/(1 + odds)).

Pore water sulfide and water transparency are significant variables in the multiple BLR ($p = 0.012$ and $p = 0.016$, respectively), whereas water temperature is only marginally significant ($p = 0.056$). To visualize the interaction of the variables, a 3-D plot was constructed that predicts the probability of wild rice occurrence as a function of sulfide and transparency, while holding constant the marginally significant variable, temperature, at the median value of the Class B data set (23.2 C; Figure 4). Within the range of variables in Class B, modeled probabilities of wild rice occurrence range from a high of 89.8% (trans = 100 cm, which was the maximum of the measurement device, and pw sulfide = 11 $\mu g\ L^{-1}$, which was the reporting limit) to a low of 9.6% (trans = 3 cm, which was the minimum observed, and pw sulfide = 2,000 $\mu g\ L^{-1}$). Modeling was cut off at a sulfide concentration of 2,000 $\mu g\ L^{-1}$ because only three Class B sites had pore water sulfide greater than 2,000 $\mu g\ L^{-1}$ and none of the three hosted wild rice.

3.6. Field Variables not Evidently Associated With Wild Rice Presence and Absence

Some variables included among the initial hypotheses, or in the past cited as important attributes of wild rice habitat, were not found to be significantly associated with wild rice presence and absence. These include sediment TOC ($p = 0.79$, Figure 2j; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), flocculent sediment (quantified here as the sediment water content; $p = 0.72$, Figure 2h; DNR, 2008; Lee, 1986; Moyle & Krueger, 1964), sediment N ($p = 0.68$, Table 1; Carson, 2002; Walker et al., 2010), sediment P ($p = 0.27$, Table 1; Carson, 2002; DNR, 2008), and surface water alkalinity ($p = 0.28$, Table 1; Moyle, 1944).

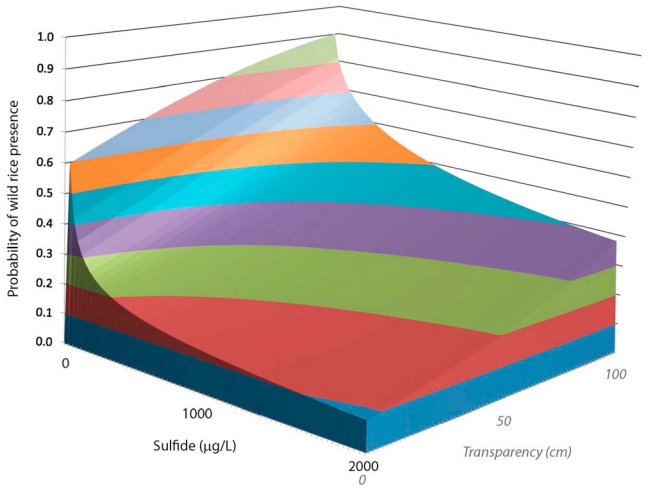


Figure 4. Visualization of the relationship, determined by multiple binary logistic regression, between water transparency and pore water sulfide in controlling the probability of wild rice occurrence at 23.2°C, the median temperature for all sites in the class B data set.

Reduced sediments are generally regarded as producing pore water constituents that are potentially toxic to rooted plants; potentially toxic agents, in addition to sulfide, include ammonia and Fe (Pezeshki & DeLaune, 2012). Analysis of the binary logistic regressions showed no evidence for ammonia toxicity (BLR $p = 0.45$, Figure 2l) or Fe toxicity (wild rice presence is positively correlated with pore water Fe, BLR $p = 0.01$, Figure 2f). As a product of decomposition, ammonia is correlated with pore water sulfide, K, and silica (Spearman correlations $p < 0.001$, Table S4).

The field data provide little evidence that elevated sulfide production excludes wild rice by producing Fe, Cu, or Zn deficiency (Table S3). The possibility that *low* pore water Fe limits wild rice growth is difficult to distinguish from sulfide toxicity because of the strong negative correlation between pore water Fe and pore water sulfide (rho = −0.58, $p < 0.0001$, Table 1). Hydroponic studies using chelated Fe in combination with elevated sulfide (Pastor et al., 2017) show reduced growth, implying that the mechanism is sulfide toxicity, rather than Fe limitation, assuming that the chelated Fe remains bioavailable in the presence of sulfide (Li et al., 2009). The median pore water Fe levels at sites with and without wild rice are similar, 5.7 and 5.9 mg L^{-1}, respectively; average levels are 11.0 and 7.0 mg L^{-1}, respectively, which produced the significant BLR positive correlation between pore water Fe and wild rice presence. On balance, there is little evidence to support the hypothesis that elevated sulfide reduces wild rice growth through Fe deficiency rather than by direct sulfide toxicity. Pore water Cu concentrations were often below the detection limit in the field sites sampled, precluding analysis of their correlation with wild rice presence and absence. The BLR for pore water Zn concentrations with wild rice presence and absence was not statistically significant (data not shown). Neither Cu nor Zn had a significant Spearman correlation with pore water sulfide (data not shown), unlike Fe.

3.7. Seasonality in Field Variables

Fifteen natural wild rice sites were sampled 3 to 5 times in 2013 to determine which field variables exhibit statistically significant seasonal trends that might be important to consider in the overall analysis, given that different sites were sampled in different months. Some variables conformed to expected seasonal trends, such as water temperature ($p = 0.001$), which increased in early summer, peaked about 1 August, and then declined. Some of the surface water variables exhibited monotonic increased concentrations over the summer (alkalinity, Na, Mg, and SO_4; $p = 0.0009$, 0.008, 0.021, and 0.05, respectively). One hypothesis that explains this observation is that waterbodies in Minnesota, after being diluted by spring snowmelt, in general become more concentrated as the summer progresses due to evaporation and, for waterbodies with shorter residence times, greater influence of groundwater flux. Cl and Ca had nonsignificant positive slopes ($p = 0.29$ and 0.37, respectively), whereas K exhibited no trend ($p = 0.48$). As expected, none of the solid-phase variables of the homogenized 10 cm long sediment cores showed any significant seasonal trends (e.g., sediment extractable Fe $p = 0.93$, sediment TOC $p = 0.96$). Among the pore water variables, only K and pH exhibited significant trends. Pore water K values decreased over the summer ($p = 0.006$), likely as a result of diffusion into surface water or uptake by growing rooted macrophytes. Pore water pH generally increased over the summer ($p = 0.003$), which may reflect loss of CO_2 as the sediment warmed over the summer, reducing the solubility of gases. The lack of seasonality in pore water sulfide ($p = 0.62$) indicates that sulfide concentrations were in steady state with the variables that exert primary control over its concentration, surface water SO_4, extractable Fe, and sediment TOC (Pollman et al., 2017). Of the variables controlling pore water sulfide, only SO_4 exhibited even marginally significant seasonal trends ($p = 0.05$, 0.93, and 0.97, for SO_4, extractable Fe, and sediment TOC, respectively). Despite the finding of seasonal variation in SO_4, Pollman et al. (2017) found that SO_4 is one of the primary variables that control pore water sulfide concentrations. Seasonal variation in SO_4 concentrations undoubtedly contributes to noise in the statistical relationship documented by Pollman et al. (2017). Myrbo et al. (2017) found that the microbial production of pore water sulfide in a wild rice sediment is proportional to the long-term average SO_4 surface water concentration.

3.8. Cultivated Wild Rice Paddies

Commercial wild rice paddies have been reported with healthy stands of wild rice growing under surface water SO_4 levels as high as 170 mg L^{-1} (Aiken et al., 1988). Our limited measurements in seven different paddies ranged from 0.3 mg L^{-1} to 279 mg L^{-1} SO_4, with a median of 8.3 mg L^{-1} (n = 7, Table S3). Natural wild rice waters in our study had a median SO_4 concentration of 4.1 mg L^{-1}. Surprisingly, median pore water sulfide in paddies was 182 μg L^{-1}, greater than the medians of natural wild rice waters (88 μg L^{-1}; n = 67) and waters without wild rice (126 μg L^{-1}; n = 41). The median sediment extractable Fe concentration of paddies (4.5 mg g^{-1}) is similar to that of natural wild rice waters (4.8 mg g^{-1}). In contrast, median sediment TOC is much higher in paddies (25.2%) than natural wild rice waters (9.1%). It thus appears that the greater TOC driving enhanced SO_4 reduction (Pollman et al., 2017) and driving median pore water Fe lower than in natural wild rice sediments (1.6 and 5.7 mg L^{-1}, respectively), despite the similar reservoirs of Fe in the sediment. Elevated production of sulfide coupled with the consumption of the available Fe may put some paddies on the brink of sulfide toxicity to wild rice.

However, the physical setting of cultivated wild rice paddies differs from that of natural lakes and streams in a number of important ways. Often paddies and their surface sediments are dewatered during the growing season through use of buried drainage tiles, allowing tillage after harvesting and enhanced aerobic decomposition of rice straw and roots, and possibly reoxidation of sulfide and reduced Fe. Prior to drainage tile installation in the 1980s, failure of wild rice crops was sometimes attributed to elevated sulfide (Grava & Rose, 1975; Gunvalson, personal communication, 2016). Water depth in paddies is also typically shallower (median of 30 cm) than wild rice habitat in natural lakes and rivers (median of 52 cm), allowing seedlings to emerge above water with less energy expended, photosynthesize sooner, and release oxygen from roots to oxidize sulfide. The use of nitrogen fertilizers may further enable seedlings to elongate more quickly through the water and into the air. Fertilized plants have been shown to be more resistant to sulfide toxicity than are control plants (Geurts et al., 2009). The production of sulfide would be inhibited if N fertilizers were applied as nitrate—but N fertilizers are applied as ammonia or urea (Oelke et al., 1997).

3.9. Conclusions and Implications

Analysis of an extensive suite of physical and chemical parameters from 108 different sites with potential wild rice habitat shows that pore water sulfide toxicity is a primary biogeochemical factor controlling the occurrence of wild rice populations in otherwise favorable habitat. High concentrations of pore water sulfide greatly decrease the probability that a wild rice population will be found in a waterbody. When pore water sulfide is low enough to support reproducing wild rice populations, however, it is likely that the relationship between sulfide and wild rice becomes more complicated and analysis of cause and effect more ambiguous. The variation in sulfide concentration is correlated to the density of wild rice, at least partially as a result of oxygen release from roots, and thus, not only does sulfide affect wild rice but wild rice affects sulfide.

Aside from low pore water sulfide, favorable wild rice habitat has long, cold winters and transparent surface water in the ice-free season. *Zizania palustris* seeds germinate at low rates if the winter is too warm or too short. The probability that wild rice seedlings successfully grow to maturity is reduced if photosynthesis is inhibited by low water clarity. Thus, pore water sulfide (itself a function of surface water SO_4, sediment Fe, and sediment organic matter (Pollman et al., 2017)), water temperature, and water transparency together largely determine wild rice presence and absence. These three factors are independent of one another but may act synergistically on other related processes.

In addition to generating sulfide, SO_4 reduction supports organic matter mineralization that releases nutrients and alkalinity to the surface water, which has the potential to change plant community structure even if Fe is high enough to keep pore water sulfide from reaching phytotoxic levels (Myrbo et al., 2017). Natural and anthropogenic SO_4 loading to freshwaters may thus strongly affect ecosystem composition and function, despite the low direct toxicity of SO_4 under oxic conditions.

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RESEARCH ARTICLE
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This article is a companion to Myrbo et al. (2017), https://doi.org/10.10022017JG003787 and Pollman et al. (2017), https://doi.org/10.10022017JG003785.

Key Points:
- Sulfate addition increased organic matter mineralization in wetland sediment, releasing C, N, P, and Hg to the water column
- Sulfate reduction caused not only higher methylmercury concentrations but higher total mercury concentrations in the surface water
- Increased sulfate loading to freshwaters can cause deleterious effects separate from direct sulfide toxicity to organisms

Supporting Information:
- Supporting Information S1
- Figure S1
- Data Set S1

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Increase in Nutrients, Mercury, and Methylmercury as a Consequence of Elevated Sulfate Reduction to Sulfide in Experimental Wetland Mesocosms

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Abstract Microbial sulfate reduction (MSR) in both freshwater and marine ecosystems is a pathway for the decomposition of sedimentary organic matter (OM) after oxygen has been consumed. In experimental freshwater wetland mesocosms, sulfate additions allowed MSR to mineralize OM that would not otherwise have been decomposed. The mineralization of OM by MSR increased surface water concentrations of ecologically important constituents of OM: dissolved inorganic carbon, dissolved organic carbon, phosphorus, nitrogen, total mercury, and methylmercury. Increases in surface water concentrations, except for methylmercury, were in proportion to cumulative sulfate reduction, which was estimated by sulfate loss from the surface water into the sediments. Stoichiometric analysis shows that the increases were less than would be predicted from ratios with carbon in sediment, indicating that there are processes that limit P, N, and Hg mobilization to, or retention in, surface water. The highest sulfate treatment produced high levels of sulfide that retarded the methylation of mercury but simultaneously mobilized sedimentary inorganic mercury into surface water. As a result, the proportion of mercury in the surface water as methylmercury peaked at intermediate pore water sulfide concentrations. The mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, perhaps enhancing the removal of nutrients and mercury to periphyton. The presence of wild rice decreased sediment sulfide concentrations by 30%, which was most likely a result of oxygen release from the wild rice roots. An additional consequence of the enhanced MSR was that sulfate additions produced phytotoxic levels of sulfide in sediment pore water.

Plain Language Summary In the water-saturated soils of wetlands, which are usually anoxic, decomposition of dead plants and other organic matter is greatly retarded by the absence of oxygen. However, the addition of sulfate can allow bacteria that respire sulfate, instead of oxygen, to decompose organic matter that would not otherwise decay. The accelerated decay has multiple consequences that are concerning. The bacteria that respire sulfate "breathe out" hydrogen sulfide (also called sulfide), analogous to the conversion or respiration of oxygen to CO_2. Sulfide is very reactive with metals, which makes it toxic at higher concentrations. In addition to the release of sulfide, the sulfate-accelerated decomposition of plants releases phosphorus and nitrogen, fertilizing the waterbody. Decomposition also mobilizes mercury (which is everywhere, thanks to atmospheric transport) into the surface water. The microbes that convert sulfate to sulfide also methylate mercury, producing methylmercury, the only form of mercury that contaminates fish. This study demonstrates that adding sulfate to a wetland can not only produce toxic levels of sulfide but also increase the surface water concentrations of nitrogen, phosphorus, mercury, and methylmercury.

1. Introduction

Organic matter (OM) accumulates in the sediments of aquatic systems when sediment concentrations of terminal electron acceptors (TEAs) are too low for microbes to completely decompose OM, especially when the supply of the most energy-efficient TEA, oxygen, is low. In water-saturated, organic-rich sediment, microbial sulfate reduction (MSR) can be a dominant pathway for the respiration of OM because oxygen is depleted in the uppermost sediment (Boye et al., 2017). Dissolved sulfate (SO_4) concentrations in continental surface

waters are often low (less than 50 mgL^{-1} or 0.5 mmol L^{-1}) (e.g., Gorham et al., 1983) compared to ocean concentrations (2,800 mg L^{-1} or 29 mmol L^{-1}). Because of lower SO_4 concentrations, and because MSR rates can be limited by SO_4 concentrations (Holmer & Storkholm, 2001), the biogeochemical significance of MSR is often considered minimal in freshwater and low-salinity systems (e.g., Capone & Kiene, 1988; Nielsen et al., 2003; Stagg et al., 2017). However, absolute rates of MSR are not clearly lower in freshwater systems than in marine systems (Pallud & Van Cappellen, 2006), and in some cases, rapid cycling between oxidized and reduced forms of S can occur (Hansel et al., 2015).

In this study, we investigated the cascade of biogeochemical effects associated with increased MSR that result from increased surface water SO_4. We simultaneously quantified three different categories of biogeochemical responses related to MSR: (1) mineralization of organic matter and associated release of dissolved C, N, P, and Hg; (2) methylation of Hg; and (3) production of sulfide.

The stoichiometric release of the constituents of OM during MSR, notably C, N, and P, is a phenomenon long recognized by marine scientists. For instance, Boudreau and Westrich (1984) constructed a model of the MSR-mediated decomposition of marine sediment. They showed that SO_4 is reduced to sulfide (H_2S) in stoichiometric proportion to the mineralization of C, N, and P according to the reaction

$$2(CH_2O)_x(NH_3)_y(H_3PO_4)_z + xSO_4^{2-} \rightarrow 2xHCO_3^- + xH_2S + 2yNH_3 + 2zH_3PO_4 \quad (1)$$

C is released as both dissolved inorganic carbon (DIC, from complete oxidation, produced as bicarbonate alkalinity in stoichiometric proportion to sulfide (reaction (1); Boudreau & Westrich, 1984)) and dissolved organic carbon (DOC, from partial oxidation). The nutrients N and P are released in forms that are readily taken up by plants; N is released as ammonia, and P as phosphate. The mineralization of sediment organic matter associated with MSR releases sulfide (S^{2-}) into sediment pore water, which speciates, depending on the pH, into hydrogen sulfide (H_2S) and bisulfide (HS^-), henceforth collectively termed sulfide. If reduced S compounds accumulate in the sediment, there may be additional consequences to an aquatic system, such as toxic concentrations of sulfide in pore water (Lamers et al., 2013; Pastor et al., 2017; Myrbo et al., 2017) or conversion of sediment Fe(III) to FeS compounds, which enhances the mobilization of P (Curtis, 1989; Maynard et al., 2011).

The multiple biogeochemical consequences of MSR in freshwater systems have been investigated and documented in more than two dozen publications (Table S1 in the supporting information), which typically address a single issue, such as the production of alkalinity that neutralizes atmospherically deposited H_2SO_4 (Baker et al., 1986; Cook et al., 1986; and others) or the methylation of Hg (Gilmour et al., 1992; Branfireun et al., 1999, 2001; and others). Experimental studies addressing SO_4 reduction, sulfide production, associated OM mineralization, and release of nutrients have been broader (Lamers et al., 2001, 2002; Weston et al., 2006, 2011; and others), but aside from the results reported in this paper, only the experiments of Gilmour, Krabbenhoft, et al. (2007) and Gilmour, Orem, et al. (2007) have investigated all three categories of biogeochemical consequences of SO_4 reduction: OM mineralization, Hg methylation, and sulfide accumulation (Table S1). We also investigated the potential for Hg to be released by mineralization, a phenomenon proposed by Regnell and Hammar (2004).

Sulfate-driven enhanced mineralization of sediment OM and release of dissolved sulfide, N, P, DOC, DIC, and associated increases in alkalinity and pH have the potential to change the nature of an aquatic ecosystem. The immediate release is to the sediment pore water, but these dissolved materials can diffuse into the surface water. Increased internal loading of N and P can drive a system toward eutrophy, which can increase carbon fixation and amplify the cascade of biogeochemical effects associated with increased MSR. Increases in DOC also have the potential to fundamentally change the nature of a waterbody. DOC influences many processes in freshwater ecosystems, including light availability for macrophyte growth, thermal stratification, and bioavailability of metals, P, and C. In addition, DOC interferes with drinking water purification (Williamson et al., 1999). Increases in DIC, alkalinity, and pH can also change the nature of a system. Aquatic macrophyte and algal species often have different optimal alkalinity concentrations (e.g., Moyle, 1945; Vestergaard & Sand-Jensen, 2000), so increases in alkalinity may change aquatic community composition. Because pH is a master variable in aquatic systems (Stumm & Morgan, 2012), increases in pH can cause changes in both aquatic chemistry and the biota that dominate a system, as best documented by changes in diatom assemblages (Patrick et al., 1968).

The release of sulfide into sediment pore water has multiple biological and geochemical consequences, several of which are related to the reactivity of sulfide with metals. If dissolved sulfide accumulates in pore water, it can negatively affect multicellular organisms inhabiting the sediment because sulfide can denature a range of metal-containing biomolecules, including cytochrome C oxidase, which is essential for respiration by both animals and plants (Bagarinao, 1992). Because aquatic sediment is a primary site of sulfide production, plants that root in sediment are vulnerable to toxic sulfide concentrations (Lamers et al., 2013; Pastor et al., 2017). However, if the watershed supplies sufficiently high loading of reactive Fe or other metals to the sediment, pore water sulfide concentrations may stay below toxic levels even while MSR proceeds as an important mineralization process (Pollman et al., 2017). The formation of FeS compounds effectively detoxifies sulfide (e.g., Marbà et al., 2007; Van der Welle et al., 2007). When Fe availability exceeds the production of sulfide, the accumulation of FeS is a measure of cumulative SO_4 reduction, which can be quantified as acid-volatile sulfide (AVS) (Heijs & van Gemerden, 2000). In addition, phosphorus is mobilized when oxidized Fe compounds with significant capacity to bind phosphate are converted to FeS compounds, which are incapable of binding phosphate (Lamers et al., 1998; Maynard et al., 2011). Thus, MSR mobilizes P both by mineralization of P-containing OM and by changing the form of Fe in sediment.

In addition to releasing C, N, and P, producing potentially toxic concentrations of sulfide, and reducing the solubility of metals, MSR is a primary process leading to the formation of MeHg, the bioaccumulative form of Hg (Gilmour et al., 1992; Hsu-Kim et al., 2013), although other microbial groups can also methylate Hg (Podar et al., 2015). In some cases, MSR can lead to toxic levels of MeHg higher in the food chain. The relationship between SO_4 concentrations and MeHg production is complex, however, and both field and laboratory studies in freshwater and saline ecosystems suggest that there is a dual effect of S on Hg methylation. At low SO_4 concentrations, the addition of SO_4 can stimulate MSR and Hg methylation (Jeremiason et al., 2006). At higher SO_4 concentrations, a greater abundance of inorganic sulfide appears to decrease the availability of inorganic Hg for Hg methylation (Hsu-Kim et al., 2013; Johnson et al., 2016). Because it has been observed that low SO_4 additions often increase Hg methylation and higher SO_4 concentrations decrease methylation, it has been proposed that there is a range of SO_4 and sulfide concentrations are optimal for Hg methylation, above which methylation is inhibited (Hsu-Kim et al., 2013). There is some debate regarding the underlying mechanism, but there is substantial evidence suggesting that dissolved inorganic sulfide above concentrations of 300–3,000 $\mu g\ L^{-1}$ has an inhibitory effect on Hg methylation (Bailey et al., 2017).

This study presents results from 30 wetland mesocosms in which the surface waters were treated to maintain a wide range of SO_4 concentrations over the course of 5 years (2011–2015) to assess the impact on wild rice, *Zizania palustris* (Pastor et al., 2017). We took advantage of this experiment to analyze the geochemical conditions in surface and pore water in the mesocosms during late summer 2013, 3 years into the experiment. Pastor et al. (2017) specifically examined the effect of increased SO_4 loading on wild rice, whereas this paper examines the broader biogeochemical impact of augmenting SO_4 to a low-SO_4 system.

2. Materials and Methods

2.1. Experimental Design

The experimental setup (Figure S1 in the supporting information), described in detail by Pastor et al. (2017), consisted of thirty 375 L polyethylene stock tanks containing sediment from a wild rice lake (Rice Portage Lake; +46.6987°, −92.6886°) in which wild rice was grown in self-perpetuating populations at five SO_4 treatment levels (control, 50, 100, 150, and 300 $mg\ L^{-1}$). SO_4 concentrations in six replicate mesocosms were routinely monitored, and amendments of SO_4 were added as Na_2SO_4 during the growing season as SO_4 was removed by MSR (Figure 1). Due to MSR, the mesocosm surface waters actually had time-weighted average concentrations of 7, 27, 59, 93, and 207 $mg\ L^{-1}$, respectively. Local well water containing an average of 10.6 $mg\ L^{-1}$ SO_4 was added as needed to compensate for evapotranspiration. Precipitation in the region contains an average of 2.1 $mg\ L^{-1}$ SO_4, and Rice Portage Lake has an average SO_4 concentration of 2.2 $mg\ L^{-1}$ (Fond du Lac Band, 2016), so the control was slightly elevated above the ambient SO_4 concentration of the sediment source for the experiment. During the ice-free period (generally May through October), the surface water temperature (T) measured in the morning was correlated with the previous day's mean air temperature (mesocosm T = 0.72 air T + 4.4 °C; R^2 = 0.65). Peak air temperature is reached in July, when the average

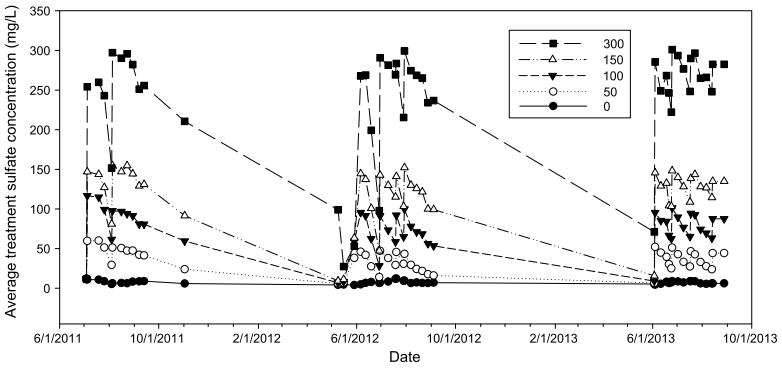


Figure 1. SO_4 concentrations in surface waters of each treatment, showing repetitive depletion and periodic amendment with Na_2SO_4 (average of six mesocosms per treatment on each sampling date).

temperature is 18.8°C (based on 1981–2010 air temperatures measured at the Duluth, Minnesota, airport, 10 km from the experimental site).

The experiments had been in progress for three growing seasons at the time of the sampling for this study, 27 and 28 August 2013, and for five growing seasons at the time of the second, less intensive, sampling (August 2015). The sediment of each mesocosm was divided into two parts for the 2013 growing season by a clear acrylic plate and all wild rice plants removed from one side in order to evaluate the effects of plant root presence on the geochemistry of the sediments. The plate was situated near one end of each mesocosm, such that about 10% of the surface area of 0.6 m^2 was plant-free (Figure S1). The plate was positioned to segregate the sediment without impeding the circulation of the surface water above all of the sediment. Sediment chemistry results presented here are from the side with wild rice plants present, except when analyzing the difference in AVS between the two sides.

2.2. Methods

2.2.1. Sample Collection

Rhizon™ samplers with a 10 cm long, 2.5 mm diameter, cylindrical porous tip (hydrophilic membrane pore size 0.12–0.18 μm (Rhizosphere.com, Netherlands; Shotbolt, 2010)), were connected by Teflon-taped Luer-Lok connectors and silicone tubing to a syringe needle. The sampler was inserted into the sediment, and the needle was then inserted through the 20 mm thick butyl rubber septum of an evacuated serum bottle (Bellco Glass) to initiate pore water draw through the tubing and displace air. After water was observed entering the serum bottle, the needle was removed from the first sacrificial bottle and inserted through the septum of a second evacuated serum bottle to collect the sample. One Rhizon and bottle were used to collect a sample for dissolved iron, preserved with 20% nitric acid. A second Rhizon and evacuated, N_2 gas-flushed sealed bottle, preloaded with 0.2 mL 2 N zinc acetate, 0.5 mL 15 M NaOH, and a stir bar, was used to collect a sample for dissolved sulfide analysis. Each Rhizon was positioned to sample pore water from the top 10 cm of sediment and to avoid collecting water from above the sediment surface. However, it is conceivable that some surface water was able to follow the path of the Rhizon into the sediment and dilute or partially oxidize the pore water sample.

Surface water in each mesocosm was collected for analysis of nitrate + nitrite, TP, TN, DOC, pH, temperature, and alkalinity from 5 cm below the surface of the water. Surface water samples for analysis of total Hg (THg) and MeHg were collected using clean hands/dirty hands protocols in September 2013, filtered through 0.45 μm glass fiber filters, and immediately acidified with 0.5% (by volume) trace metal hydrochloric acid. Samples were stored on ice during transport and at 4°C until analysis.

Pore water P availability was measured with three mixed bed ion exchange bags (Fisher Rexyn 300 resin) placed in the sediment of each tank in spring and harvested at the end of the growing season in 2013. A 3.8 cm diameter piston corer was used to obtain 10 cm long sediment samples for various analyses. Sediment samples for the analysis of AVS were taken monthly from June to October 2013 from replicate mesocosms of four SO_4 treatments (control, 50 150, and 300 mg L^{-1}; no mesocosm was sampled more

than once). Sediment samples were also taken on 8 October 2013 for the analysis of THg in bulk sediment and on 6 October 2015 for the analysis of total organic carbon (TOC).

2.2.2. Laboratory Analyses

Surface water and pore water analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL). Total P was measured by in-line ultraviolet/persulfate digestion and flow injection (APHA, 2005, 4500 P-I), DOC by persulfate-ultraviolet oxidation and IR CO_2 detection (APHA, 2005, 5310-C), and alkalinity by automated titration (APHA, 2005, 2320-B). Pore water sulfide samples were prepared for inline distillation and flow injection colorimetric analysis using procedures that avoided exposure to oxygen. The sulfide serum bottle was weighed to determine the amount of sample collected and to adjust for the slight dilution factor of an alkaline antioxidant that was added by injection through the stoppers. The sealed samples were then placed on a stir plate for at least 1 h and subsamples withdrawn for analysis through a needle. Reanalysis of sealed, processed samples 12 months later shows no significant difference in sulfide concentrations, indicating that the sulfide samples were stable prior to analysis (data not shown). SO_4 concentration was measured using a Lachat QuikChem 8000 Autoanalyzer (Lachat Method 10-116-10-1-A). The resin was eluted using a KCl solution and analyzed for PO_4 using a Lachat Autoanalyzer, following the methods of Walker et al. (2006).

An aliquot of the nitrate + nitrite/TP/TN/DOC serum bottle was filtered in the lab within 10 days of sampling using a 0.45 μm filter, preserved to a pH $<$ 2 with 10% sulfuric acid, and transferred to a 250 mL polyethylene bottle for DOC analysis. The remaining sample was preserved to a pH $<$ 2, with 10% sulfuric acid and transferred to 250 mL polyethylene bottle for nitrate + nitrite/TP/TN analysis. The contents of the metal serum bottle were transferred to a 250 mL polyethylene bottle and preserved to a pH $<$ 2 with 10% nitric acid. Analyses were conducted within 30 days of sampling.

THg in surface water and bulk sediment were analyzed with EPA method 1631 by MDHEL, and surface water MeHg was analyzed with EPA method 1630 by Frontier Global Sciences (Bothell, Washington). Inorganic Hg (iHg) was calculated as the difference between THg and MeHg. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid distillation and in-line alkaline trapping (APHA, 2005; SM 4500-S2). Sediment TOC was analyzed following SM5310C (APHA, 2005), using an OI Analytical Aurora 1030 at Pace Analytical Services, Virginia, Minnesota.

3. Data Analysis

3.1. Sulfate Depletion as the Independent Variable

Because SO_4 is relatively unreactive under oxidized conditions, its loss is attributable to diffusion or transpiration-driven advection (Bachand et al., 2014) into sediment and conversion to sulfide by bacteria. Surface water SO_4 concentrations decreased partly due to dilution by precipitation but largely from loss after movement into the sediment and reduction to sulfide. Sulfide would largely be retained in the sediment as FeS compounds, although some could be lost to the atmosphere as H_2S gas (Bagarinao, 1992) or as volatile organic sulfur compounds (Lomans et al., 2002). The cumulative SO_4 lost from surface water was calculated from a mass balance for each mesocosm from the inception of the experiment in spring 2011 through fall 2013; this quantity, termed here SO_4 depletion, $(SO_4)_{Depl}$, is used as a proxy for net MSR, following Weston et al. (2006). The surface water remained frozen from approximately 1 December to 1 April each winter, and the mesocosms were covered with plastic from November to late April each year and not amended with SO_4. SO_4 reduction was the major biogeochemical process altered by the experimental treatments, and therefore, $(SO_4)_{Depl}$ is the independent variable used in subsequent data analyses. It was only possible to perform a complete mass balance for SO_4, the only parameter consistently quantified in source water, precipitation, and overflow water.

3.2. Calculation of DIC From Measured Alkalinity

Dissolved inorganic carbon (DIC $\equiv [CO_3^{2-}] + [HCO_3^-] + [CO_2^*]$, where $[CO_2^*] = [CO_{2(g)}] + [H_2CO_3]$) was calculated from measured alkalinity and speciated using pH, temperature, and specific conductance of the surface water. At the pH range of the mesocosms (7.60–8.84), 95–98% of DIC is in the form of HCO_3^-, so DIC concentration on a molar basis is nearly the same as alkalinity (ALK) on an equivalent basis (DIC = 0.988 ALK + 0.077, R^2 = 0.995). In studies of freshwater, most inorganic carbon data are presented in terms of alkalinity because

alkalinity is a familiar metric; however, in comparisons with DOC, inorganic carbon data are presented as DIC so that the units are directly comparable. PHREEQC version 3 geochemical modeling software (Parkhurst & Appelo, 2013) was used to calculate saturation indices for carbonate minerals.

3.3. Statistical Analysis

Statistical analysis was conducted with R version 3.2.3 and STATA (StataCorp, 2015). The effect of increased sulfate availability was assessed through both categorical analysis of the sulfate treatments (Kruskal-Wallis ANOVA test, followed by Dunn's test for multiple comparisons with Holm-Sidak corrections) and through linear regression and nonparametric Spearman rank correlations. We rely primarily on regressions against SO_4 depletion to detect the effects of enhanced sulfate-reduction driven mineralization, rather than categorical analysis of the sulfate treatment results, because (a) biogeochemical changes are not driven directly by SO_4 concentration, but rather by MSR, quantified as SO_4 depletion; (b) although SO_4 depletion may be highly correlated to SO_4 concentration, deviations between experimental mesocosms develop over time, so cumulative SO_4 depletion values eventually no longer align exactly with treatment categories, but rather become continuous variables; and (c) regression provides more statistical power than ANOVA and builds models that allowed us to describe the relationships between SO_4 depletion and response variables (Cottingham et al., 2005). However, when the relationship is not linear, ANOVA and comparison of treatments through Dunn's analysis can help describe the nature of a relationship.

4. Results and Discussion

4.1. The Impact of SO_4 Reduction on Mineralization of Sediment Organic Matter

Increased concentrations of surface water SO_4 resulted in increased sulfate reduction, which necessarily increased the mineralization of organic carbon, as described by reaction (1). Concentrations of surface water DOC and DIC increased in proportion to sulfate reduction, as measured by $(SO_4)_{Depl}$ (Table 1 and Figure 2). The marine literature generally assumes complete mineralization of particulate organic carbon (POC) to DIC in the water column (e.g., Boudreau & Westrich, 1984) (reaction 1), but in freshwater systems and especially wetlands, not all carbon is completely oxidized during decomposition, and a portion of POC may be mobilized as DOC (Howes et al., 1985; Selvendiran et al., 2008). In principle, the constituents of organic matter, such as the nutrients N and P, are mobilized in proportion to the mass of carbon mineralized as a result of MSR-driven decomposition. Surface water DOC and DIC, and the sum DOC + DIC, are therefore used as indicators of OM mineralization in interpreting the mobilization of N, P, and Hg to surface waters (Figure 2 and Tables 2 and 3).

In contrast to many marine systems, it is likely that SO_4 reduction in these sediments was limited more by SO_4 than by organic carbon, given that $(SO_4)_{Depl}$ was linearly proportional to the average SO_4 concentration (Figure S2a; $R^2 = 0.87$), without any obvious curvature to the relationship that would indicate saturation of MSR.

Regressions of surface water DOC and DIC against SO_4 depletion demonstrate that, on a net basis, about 60% more DIC than DOC was mobilized to the surface water as a result of MSR-driven mineralization (slope of 0.235 mM C per unit SO_4 depletion compared to 0.148; Table 2). The significantly positive slope of the DIC:DOC ratio against SO_4 depletion (Table 2) indicates that increasingly more DIC than DOC was observed in the surface water as sulfate depletion increased. Some mineralization of DOC to DIC likely occurs in the surface water as a result of exposure to oxygen, aerobic bacteria, and sunlight, processes that could have a larger effect as DOC increases.

Not only did surface water DIC and DOC increase in concert with sulfate reduction, but parallel increases occurred in surface water concentrations of constituents of organic matter: N, P, and Hg (Table 1 and Figure 2). DIC, DOC, total P, total N, ammonia, and total Hg in surface water all had increases from the control to the highest SO_4 addition of about twofold, (2.3, 1.7, 1.9, 1.8, 1.7, and 2.6-fold, respectively, Table 1). However, available phosphate in the sediment, an estimate of P availability in pore water, had a larger increase (7.5-fold). MSR consumes acidity as the DIC-based alkalinity is produced (Baker et al., 1986), which increased the average pH from 7.57 to 7.81, a 44% decrease in hydrogen ion concentration (Table 1). If the sulfide subsequently oxidizes (which could happen in a natural system during drought (Laudon et al., 2004) or intentional dewatering), a proportional quantity of alkalinity is consumed as acid is produced

Table 1
Summary of Effects of Experimentally Increased SO_4 Concentrations on SO_4 Reduction (Quantified as SO_4 Depletion), Organic Matter Mineralization, and Mercury Methylation

		Average of each sulfate treatment (n = 6 for each treatment)						Correlation with SO_4 depletion (Spearman)	
Variable	Matrix	Control	50	100	150	300	Max/Min	Rho	p value
Variables mainly associated with SO_4 reduction									
SO_4 (T-W mean mg SO_4 L^{-1})	sw	6.7^{a}	26.9^{ab}	58.5^{abc}	93.2^{BC}	206.5^{c}	31.0	0.93	<0.0001
SO_4 depletion (mg S cm^{-2})	sw	0.14^{a}	2.52^{ab}	3.63^{abc}	4.28^{BC}	6.90^{c}	48.5	1	
Pore water sulfide (μg S L^{-1})	pw	69^{a}	184^{a}	224^{a}	393^{b}	728^{b}	10.5	0.81	<0.0001
Pore water iron (μg L^{-1})	pw	$12{,}883^{a}$	$11{,}122^{ab}$	$6{,}808^{abc}$	$4{,}483^{BC}$	$3{,}032^{c}$	4.25	−0.82	<0.0001
AVS (mg S kg^{-1})	sed	102^{a}	483^{ab}	NA	826^{ab}	$1{,}413^{b}$	13.8	0.77	<0.0001
pH	pw	7.57^{a}	7.52^{a}	7.55^{a}	7.75^{a}	7.81^{a}	1.03	0.39	=0.03
H^+ ion (μmol L^{-1})	pw	0.027	0.030	0.028	0.018	0.015	1.72	0.39	=0.03
Variables mainly associated with mineralization of organic matter									
TOC (% dry mass)	sed	9.26^{a}	7.90^{a}	8.18^{a}	7.17^{a}	8.22^{a}	1.29	−0.34	=0.065
DIC (mg C L^{-1})	sw	28.9^{a}	47.2^{ab}	56.3^{BC}	56.7^{BC}	66.3^{c}	2.30	0.94	<0.0001
DOC (mg C L^{-1})	sw	16.3^{a}	21.4^{a}	26.8^{BC}	24.0^{abc}	28.3^{bc}	1.74	0.79	<0.0001
Total N (mg N L^{-1})	sw	1.42^{a}	1.75^{a}	2.35^{BC}	2.03^{abc}	2.57^{BC}	1.81	0.77	<0.0001
Ammonia (mg N L^{-1})	sw	0.09^{a}	0.09^{a}	0.10^{a}	0.10^{a}	0.16^{a}	1.70	0.38	=0.04
Total P (μg P L^{-1})	sw	13^{a}	16^{ab}	22^{ab}	21^{ab}	25^{b}	1.92	0.73	<0.0001
Available P (μg P g^{-1} resin)	Resin in sed	0.34^{a}	0.40^{a}	0.59^{ab}	0.92^{ab}	2.56^{b}	7.45	0.86	<0.0001
Total Hg (ng L^{-1})	sw	1.83^{a}	2.09^{a}	3.61^{ab}	3.25^{ab}	4.80^{b}	2.63	0.82	<0.0001
Variables mainly associated with Hg methylation									
Methylmercury (ng Hg L^{-1})	sw	0.20^{a}	0.49^{ab}	1.21^{b}	1.08^{b}	1.18^{b}	5.91	0.66	<0.0001
Inorganic Hg (ng L^{-1})	sw	1.63^{a}	1.60^{ab}	2.40^{abc}	2.17^{BC}	3.62^{c}	2.22	0.80	<0.0001
Percent methylmercury	sw	$11\%^{a}$	$23\%^{ab}$	$30\%^{b}$	$32\%^{b}$	$23\%^{ab}$	2.90	0.45	=0.02

Note. Matrix abbreviations: sw = surface water, pw = pore water, sed = bulk sediment. Averages with superscript letters in common are not significantly different at the 0.05 level.

(Hall et al., 2006). However, the sulfide reoxidation does not reverse the mobilization of the constituents of organic matter (C, N, P, and Hg) or the production of methylmercury (MeHg; see below). Rather, any production of SO_4 from sulfide oxidation creates the potential for additional MSR-driven OM mineralization and Hg methylation (Coleman Wasik et al., 2015; Hansel et al., 2015).

The slope of linear regressions of the C, N, and P in surface water against $(SO_4)_{Depl}$ is an estimate of the increase of that variable in mesocosm surface waters per unit SO_4 reduction (Table 2). The regression slopes provide a basis for estimates of stoichiometric ratios of the constituents mobilized from the sediment solid phase, similar to the calculation that Weston et al. (2006) performed for pore water. The calculation of stoichiometric ratios from the slopes of regressions with $(SO_4)_{Depl}$ is more accurate than calculating ratios from surface water concentrations alone, as the use of slopes accounts for the concentrations of the control (the intercept of the linear regression).

The regression slopes of surface water C versus surface water N, P, and Hg in mesocosms are estimates of the net release of each element relative to that of C (Table 3). These estimates can then be compared to the ratio of these constituents in the primary source material—the sediment—to determine the efficiency of mobilization of sediment N, P, and Hg to surface water, compared to C (Table 3). Although we present efficiency relative to only DOC and only DIC, calculating efficiency relative to the sum of mineralized OM (DOC + DIC) represents the overall net efficiency of mineralization, which ranges from 8% to 38% for the three constituents (Table 3). Although the increases in surface water N, P, and Hg are consistent with the hypothesis that those elements were released to the surface water through sulfate-enhanced mineralization of sediment OM, their lower mobilization efficiencies relative to carbon suggest that other processes were operating to either increase carbon, decrease N, P, and Hg mobilization relative to carbon, and/or increase N, P, and Hg losses. It is likely that some carbon was introduced to the surface waters from sources other than the sediment (e.g., photosynthetic fixation of atmospheric carbon) and that there were losses for N, P, and Hg from the surface water (though adsorption, settling, biological uptake, or atmospheric evasion of N and Hg).

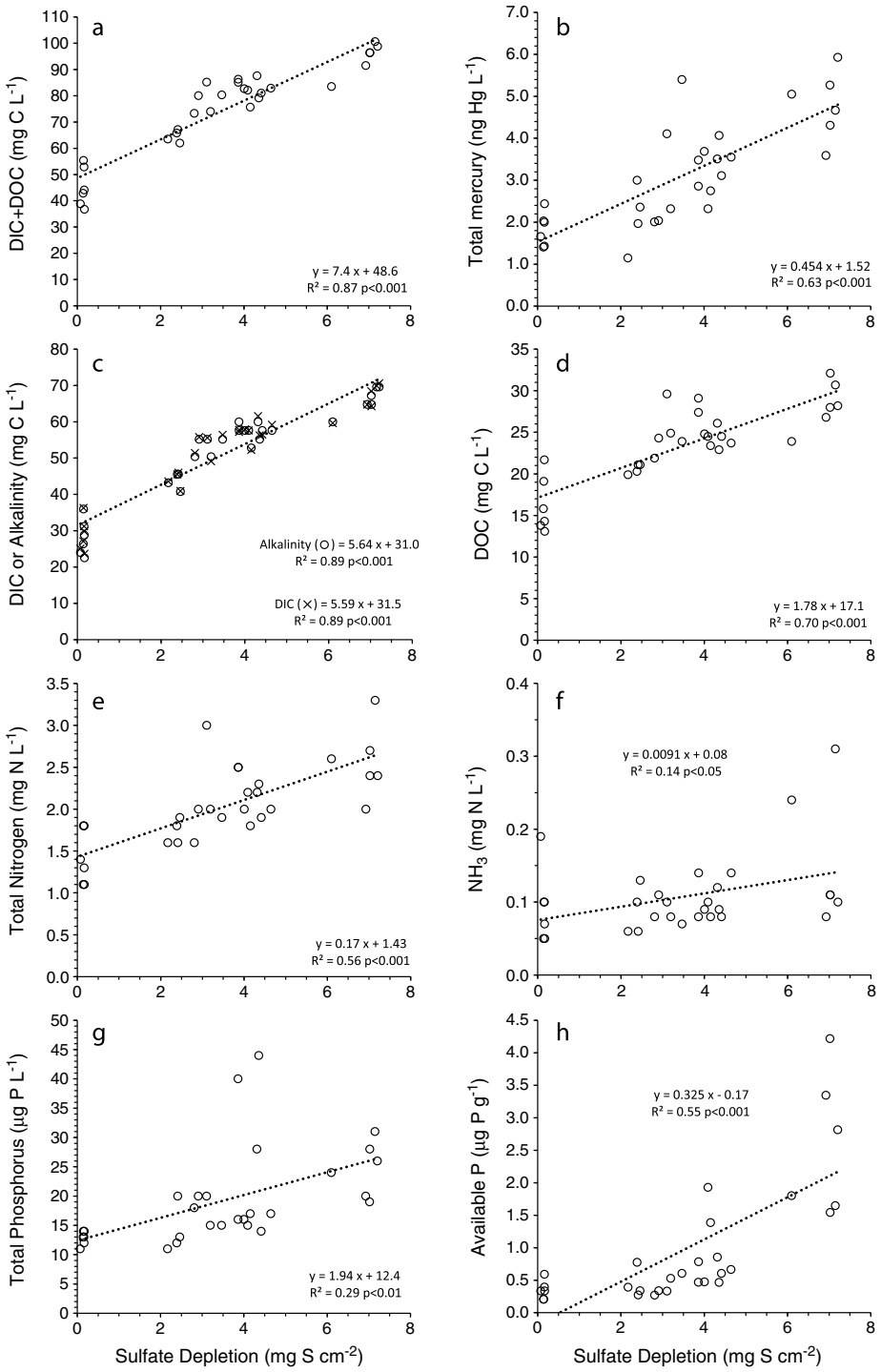


Figure 2. The release of constituents of sedimentary organic matter as a function of SO_4 depletion, showing linear regressions (dotted lines). (a) Sum of surface water DIC and DOC; (b) surface water total mercury; (c) surface water alkalinity and DIC (symbols ○ and ×, respectively; the two regressions are superimposed); (d) surface water DOC; (e) surface water total nitrogen; (f) surface water ammonia; (g) surface water total phosphorus; (h) available phosphate in the sediment, as quantified on ion-exchange resin.

Table 2
Slopes of Regressions of Surface Water Parameters (mM) Against SO_4 Depletion (mg S cm^{-2})

Surface water variable (molar basis)	Regression against $(SO_4)_{Depl}$ (mg S cm^{-2})		
	Slope	R^2	p
DIC	0.235	0.89	<0.0001
DOC	0.148	0.70	<0.0001
DIC + DOC	0.383	0.84	<0.0001
DIC: DOC	0.044	0.56	<0.0001
TN	0.0121	0.56	<0.0001
TN: DIC	−0.0028	0.25	<0.01
TN: DOC	0.0004	0.01	NS
TN: DIC + DOC	−0.0006	0.08	NS
TP	6.26E−05	0.29	<0.002
TP: DIC	−7.00E−06	0.03	NS
TP: DOC	7.00E−06	0.02	NS
TP: DIC + DOC	−1.00E−07	0.00	NS
THg	2.26E−09	0.63	<0.0001
THg: DIC	9.00E−06	0.46	<0.0001
THg: DOC	6.00E−06	0.23	<0.01
THg: DIC + DOC	2.00E−05	0.42	<0.0001

Note. When a sediment constituent's ratio to DIC or DOC has a significant slope against sulfate depletion, it indicates that the constituent was mobilized to the surface water at a significantly different rate than the DIC or DOC.

In addition to increases of TP in the surface water, the sediment pore water in the highest SO_4 treatment contained 7.5-fold greater available phosphate than the controls, as quantified with ion-exchange resin (Table 1 and Figure 2h). In comparison, the increase in surface water TP was only 1.9-fold (Table 1 and Figure 2g). The difference between phosphorus response in the resin and the surface water may be partly due to (a) loss of TP from the surface water after mobilization or (b) irreversible trapping of mobilized P on the resin. If phosphorus is released from sediment en masse in response to an S-induced shift from iron oxides to iron sulfides, the sediment pore water would experience this release first, while release to surface waters would take longer due to diffusion-limited transport and potentially an iron-oxide barrier at the sediment-water (anoxic-oxic) interface.

DIC in surface water is not conservative, being subject to exchange across the air-water interface, carbonate mineral precipitation, and photosynthetic uptake. Surface water pCO_2 in all mesocosms was above saturation with respect to atmospheric equilibrium by a factor of 1.4–15.5 (based on the DIC speciation calculations discussed earlier; data not shown), so the mesocosms were losing, not gaining, C through gas exchange with the atmosphere. The pCO_2 values in the mesocosms are similar to those reported from epilimnia of small, organic-rich, temperate lakes of low to moderate salinity (Cole et al., 1994; Myrbo & Shapley, 2006). With respect to mineral precipitation, based on geochemical equilibrium calculations, surface waters were undersaturated with respect to all carbonate minerals. Thus, although DIC in surface water is subject to several transport and transformation processes, the sustained presence of CO_2 at quantities significantly above saturation with respect to the atmosphere and the observation of increasing DIC and DOC with increasing $(SO_4)_{Depl}$ (Table 1) provide strong evidence of sulfate-induced increases in net carbon mineralization in the mesocosms.

In addition to the carbon originally present in the sediment, organic carbon was also photosynthetically fixed by wild rice and algae in the mesocosms and subsequently subjected to respiration and some decomposition, adding to the DIC and DOC in surface waters. DOC may also have been released into sediment pore water as an exudate from the wild rice roots (Rothenberg et al., 2014; Windham-Myers et al., 2009). Exudate DOC, however, does not account for the observed increase in DOC, since a negative relationship between the number of wild rice plants and DOC was observed (Spearman's rho = −0.63, $p < 0.001$, Table S2).

4.2. Effects of SO_4 Reduction on Mercury and Methylmercury in Surface Water

We interpret Hg mobilization to the surface water in an analogous manner to C, N, and P, as Hg tends to associate strongly with organic matter in sediment (Feyte et al., 2010). In the mesocosm surface waters,

Table 3
Elemental Ratios in Sediment and Surface Water Across the Range of SO_4 Depletion

Molar ratio in sediment[a]		Molar ratio in surface water[b]			Efficiency of mobilization of sediment N, P, or Hg to surface water, relative to carbon		
		DIC	DOC	DOC + DIC	DIC	DOC	DOC + DIC
C: N	12[a]	19	12	32	63%	100%	38%
C: P	463[a]	3,752	2,366	6,118	12%	20%	8%
C: Hg	1.90E + 07	1.04E + 08	6.5E + 07	1.69E + 08	18%	29%	11%

Note. Together, the ratios are used to calculate the efficiency of mobilization of the constituents of particulate organic matter into the surface water.
[a]Sediment data from Hildebrandt, Pastor, and Dewey (2012), a mesocosm study that obtained sediment from the same natural wild rice stand. [b]Regression slopes of C versus N, P, and Hg in mesocosm surface waters; calculations are made based on surface water DIC alone, surface water DOC alone, and the sum of surface water DOC + DIC.

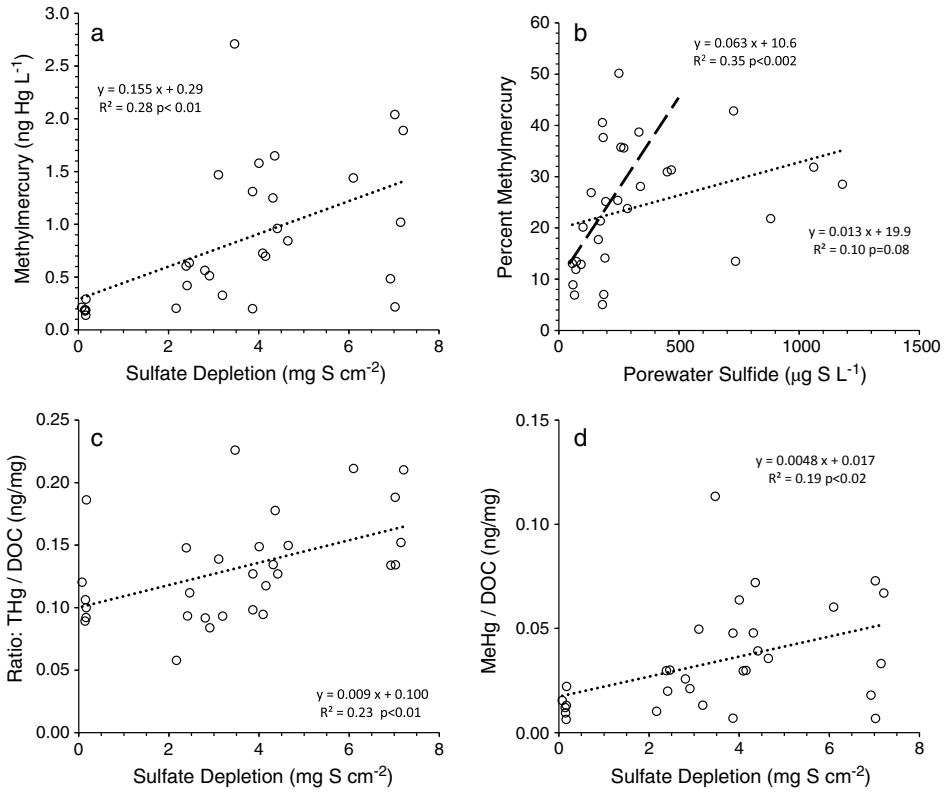


Figure 3. The response of surface water Hg variables to SO_4 depletion and the production of pore water sulfide, showing linear regressions. (a) MeHg as a function of SO_4 depletion; (b) percent MeHg as a function of pore water sulfide, showing regressions for all data (dotted line) and for the subset of data extending only to a pore water sulfide concentration of 468 µg S L^{-1} (dashed line); (c) ratio of THg to DOC as a function of SO_4 depletion; (d) ratio of MeHg to DOC as a function of SO_4 depletion.

THg, inorganic Hg (iHg), and MeHg all increased significantly with increased $(SO_4)_{Depl}$ (Table 1 and Figures 2b and 3a, $p < 0.0001$) and were greater in the highest sulfate amendment by factors of 2.6, 2.2, and 5.9, respectively (Table 1). The relative increase in THg (2.6-fold) is greater than that for DIC, DOC, TN, and TP, which range from 1.7 to 2.3-fold (Table 1). DOC enhances the solubility of both iHg and MeHg and can facilitate the movement of Hg from sediment into surface water (Ravichandran, 2004). The 5.9-fold increase in MeHg indicates that MeHg flux to surface waters was enhanced by sulfate loading disproportionately more than sedimentary release of THg (2.6-fold) and the increase in surface water DOC (1.7-fold).

The genes required to methylate Hg have been found in a wide variety of anaerobic bacteria, including SO_4-reducing bacteria, iron-reducing bacteria, and methanogens (Podar et al., 2015). Though some pure culture and experimental evidence exist for mercury methylation by other bacteria, extensive pure culture, experimental, and landscape-scale observations suggest SO_4-reducing bacteria dominate Hg methylation in many freshwater and marine environments. The relatively large increase in surface water MeHg in response to increased $(SO_4)_{Depl}$ in this experiment supports the assumption that MSR was responsible for most of the observed production of MeHg. It is likely that increased SO_4 loading to low-SO_4 aquatic systems with organic sediment will result in increased Hg methylation even though the relative importance of Hg methylation in the environment by different groups of bacteria is still a subject of debate (Paranjape & Hall, 2017).

If movement of DOC from sediment to surface water were the sole mechanism for the Hg increase in surface water, a constant Hg:DOC ratio would be expected on the $(SO_4)_{Depl}$ gradient. However, THg:DOC, iHg:DOC, and MeHg:DOC ratios in surface water are all significantly correlated with SO_4 depletion (Table S2 and Figures 3c and 3d). Therefore, all forms of Hg (THg, iHg, and MeHg) increase in surface waters more than

does DOC, indicating that a sulfate-induced enhancement of carbon mineralization may act in combination with either enhanced methylation or an enhanced capacity of DOC to carry Hg. Changes to the binding strength of the DOC in heavily S-impacted mesocosm sediment are possible, as thiol groups on DOC are dominant binding sites for Hg (Skyllberg, 2008). The dual role of organic carbon and sulfur in driving both the production of MeHg and the transport of MeHg could be responsible for the substantially larger maximum increase in MeHg:DOC ratio relative to the increase in the THg:DOC ratio (an average 206% increase relative to a 63% increase, Figures 3c and 3d), as postulated by Bailey et al. (2017).

Regnell and Hammar (2004) identified three MSR-driven processes that might cause mobilization of Hg from sediment in a wetland, (1) mineralization of organic matter; (2) extraction of iHg by reduced S compounds, which could be associated with mobilized DOC; and (3) enhanced production of MeHg, which is more mobile than iHg. They argued that enhanced production of MeHg explained THg mobilization in the minerotrophic peat bog that they studied. However, in this study, increases in surface water MeHg concentrations (Figure 3a) are not sufficient to explain the linear increase in THg observed in this experiment (Figure 2b) because most (67%) of the increase is iHg (Table 1). Some of the increase in surface water iHg could be the result of increased production of MeHg that moved to surface water and was subsequently demethylated. Regardless of the underlying mechanism, our observations clearly show increases in surface water Hg that were greater than the increases in C, N, and P (Table 3); this corroborates other studies (Bouchet et al., 2013; Merritt & Amirbahman, 2007; Regnell & Hammar, 2004) that suggest sediment Hg may be synergistically mobilized to surface waters through mineralization, methylation, and enhanced mobility with DOC.

Recent research has shown that in many ecosystems, higher concentrations of pore water sulfide may inhibit MeHg production through either thermodynamically or kinetically controlled reactions with inorganic Hg (Benoit et al., 2003; Hsu-Kim et al., 2013). We plotted %MeHg, rather than the MeHg concentration, against pore water sulfide because we are interested in identifying the pore water sulfide zone of greatest efficiency for the methylation and mobilization of mercury. In this experiment the MSR-driven mineralization of OM released THg to surface water in addition to producing pore water sulfide. Accordingly, because THg is not constant, plotting %MeHg is the most accurate way to identify peak methylation efficiency. In principle, the restricted bioavailability of Hg to methylating bacteria results in a maximum in MeHg production at intermediate concentrations of pore water sulfide. Consistent with previous research in sulfate-impacted freshwater ecosystems (Gilmour et al., 1998; Gilmour, Krabbenhoft, et al., 2007, Gilmour, Orem, et al., 2007; Bailey et al., 2017), MeHg production was most efficient at intermediate sulfide concentrations. In the control, where average sulfide was 69 $\mu g\ S\ L^{-1}$, MeHg averaged only 11% of THg in surface waters. In the intermediate SO_4 treatments, which had average sulfide concentrations of 224 and 393 $\mu g\ S\ L^{-1}$, MeHg production efficiency peaked significantly higher, at averages of 30% and 32%, respectively (Table 1). %MeHg declined to an average of 23% in the highest SO4 treatment, which had an average sulfide concentration of 728 $\mu g\ S\ L^{-1}$. Given the relatively great scatter in the relationship between %MeHg and sulfide (Figure 3b), it would be most defensible to conclude that the decrease in %MeHg began to occur somewhere between 300 and 700 $\mu g\ S\ L^{-1}$. There is a strong positive relationship ($p < 0.001$) between sulfide and %MeHg if the five sulfide concentrations greater than 727 $\mu g\ S\ L^{-1}$ are excluded from the regression (which leaves only sulfide concentrations less than 468 $\mu g\ S\ L^{-1}$, since there is a gap in sulfide concentrations; Figure 3b). Other studies have identified sulfide zones of peak methylation roughly comparable to that found here. In South Florida, Orem et al. (2011) found that sulfide ranging from 5 to 150 $\mu g\ S\ L^{-1}$ did not inhibit methylation but that sulfide concentrations greater than 1,000 $\mu g\ S\ L^{-1}$ did. In a subboreal Minnesota wetland enriched in SO_4 from mining discharge, Bailey et al. (2017) found that sulfide concentrations above ~650 $\mu g\ S\ L^{-1}$ inhibited methylation.

The relationship between surface water SO_4 and Hg methylation can be strongly affected by site-specific conditions. Because of the variable conversion of SO_4 in surface water to sulfide in pore water—primarily due to differences in OM and Fe availability (Pollman et al., 2017)—researchers have found a broad range in the SO_4 concentration associated with maximum efficiency of Hg methylation. For example, Orem et al. (2014) observed that two different areas in the Everglades Protection Area had peak surface water MeHg concentrations at SO_4 concentrations of 2 and 10–15 $mg\ L^{-1}$. In the mesocosms presented here peak surface water %MeHg was observed in the two sulfate treatments that averaged 59 and 93 $mg\ L^{-1}$ (Table 1).

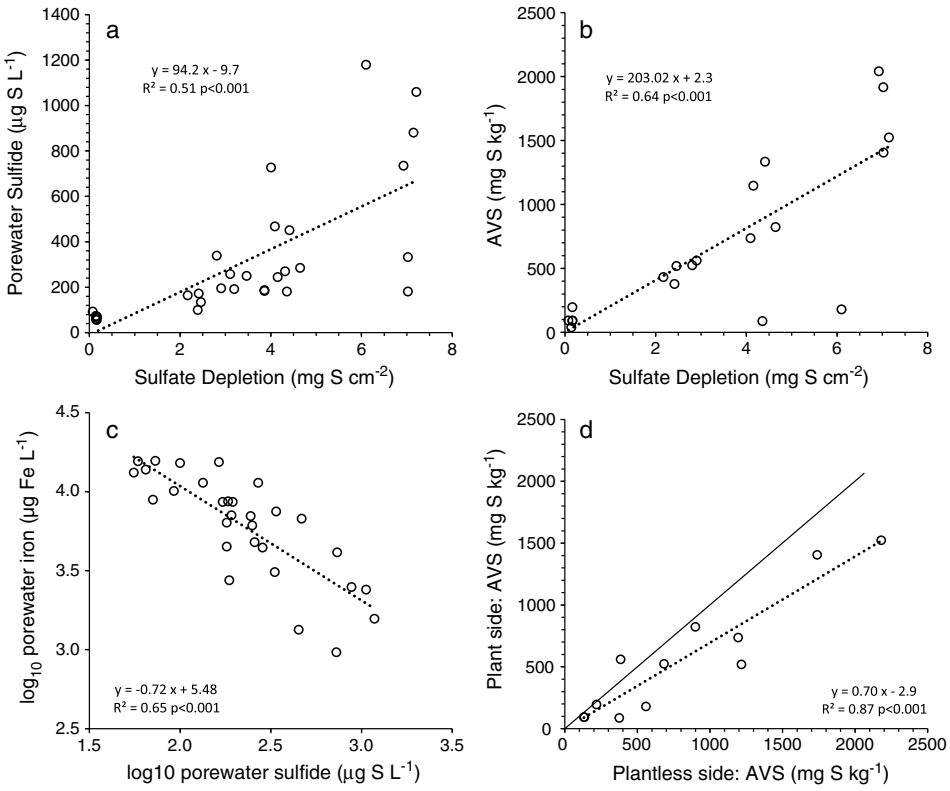


Figure 4. AVS and pore water sulfide, as related to SO_4 depletion, pore water iron, and presence of rooted plants. (a) Pore water sulfide as a function of SO_4 depletion; (b) AVS from the vegetated side of the mesocosms as a function of SO_4 depletion; (c) pore water iron as a function of pore water sulfide; (d) AVS compared between the vegetated side and nonvegetated side. The solid 1:1 line shows that in almost all mesocosms more AVS is found in the side without plants.

4.3. Effects of SO_4 Reduction on Pore Water and Sediment Sulfide

Pore water sulfide increased at higher $(SO_4)_{Depl}$, although with greater variance at higher $(SO_4)_{Depl}$ (Figure 4a), possibly as a result of variable oxidation of sulfide that may depend on the proximity of the Rhizon sampler to plant roots (Schmidt et al., 2011) or of variable bioturbation by invertebrates (Lawrence et al., 1982). When SO_4 is reduced through MSR, the sulfide produced has a number of nonexclusive potential fates: the sulfide could (1) be oxidized within the sediment; (2) remain in the sediment pore water as free sulfide; (3) diffuse into oxygenated surface water, to be oxidized; (4) react with metals in the sediment, forming insoluble precipitates (dominated by iron-sulfide compounds); or (5) be lost to the atmosphere as H_2S gas or as volatile organic sulfur compounds. Because precipitation reactions are fast relative to redox reactions and diffusion, most of the sulfide probably forms metal precipitates if metals are available. When precipitation dominates the fate of sulfide produced from MSR, the continuous reduction of SO_4 and precipitation of iron sulfides form quasi-steady states between surface water SO_4 and pore water sulfide (Figure S2b) and between pore water sulfide and pore water iron (Figures 3 and 4c). The overall mass of sulfide in the mesocosm sediment, quantified through analysis of AVS (from sediment in the vegetated area), is closely correlated with SO_4 depletion (Figure 4b) even though AVS may not include all the reduced sulfide in sediments. It is likely that most of the AVS in these sediments is present as an FeS precipitate because other metals are at low concentrations in these sediments, which came from a relatively pristine (unpolluted) lake (Fond du Lac Band, 2016; Pastor et al., 2017). Note that there are two mesocosms with especially low AVS concentrations (Figure 4b). It is possible that the AVS in the specific location in these mesocosms where sediment core samples were collected was influenced by

a spatially heterogeneous oxidization process (e.g., root oxygen or benthic invertebrates) that limited the accumulation of sulfide.

AVS was 30% lower in the vegetated side of the mesocosms, suggesting that wild rice released oxygen into the sediment, inhibiting the production of sulfide and/or decreasing sulfide concentrations through oxidation (Figure 4d; Wilcoxon paired test, $p = 0.007$). It is notable that this 30% difference developed in just one growing season, despite the previous 2 years of sulfate treatment. Pore water sulfide showed no statistically significant difference between the two sides owing to high variability within treatments. Numerous investigations have found that rooted aquatic plants release oxygen from their roots, a phenomenon that is usually interpreted as an adaptation to limit the toxicity of reduced chemical species in the pore water, especially sulfide (Lamers et al., 2013). Although oxygen release has been observed in white rice, *Oryza sativa* (Colmer, 2002), it has never been documented in wild rice, which is in the same tribe (Oryzeae) of grasses as white rice, and also develops aerenchyma (Jorgenson et al., 2013), plant structures that provide a low-resistance internal pathway for movement of oxygen to the roots. Since the growth and reproduction of rooted plants can be inhibited by sulfide (Pastor et al., 2017), there may be a tipping point of exposure to sulfide above which oxygen release is insufficient to mitigate phytotoxic effects, and the plant population declines over time, possibly to extirpation. In this experiment, in the third treatment year, the increase in pore water sulfide was the apparent cause of a decrease in the average number of wild rice stems from 17 in the control mesocosms to 3 in the highest-sulfate treatment mesocosms (Pastor et al., 2017).

4.4. Mesocosms as Models for Ecosystem-Scale Effects of SO_4 Reduction

Although mesocosms, as contained ecosystems, are useful because they mimic ecological and biogeochemical processes that occur in the field, extrapolating findings to nature is challenging when plastic walls have prevented exchange of water and materials (Petersen et al., 2009). These wall-based challenges are manifest in three phenomena in this experiment, (1) relatively long surface water residence times due to the lack of a constant throughflow; (2) the presence of the wall itself, which provides a surface for periphyton; and (3) lack of either overland or groundwater loading of external materials:

1. Relatively long surface water residence times: the increased loading of N, P, C, Hg, and MeHg to the surface water of the mesocosms was readily detected because the lack of hydraulic loading from a watershed minimized dilution and loss through the outflow. The impact of an increase in SO_4 loading on surface water concentrations of N, P, C, Hg, DIC, and DOC would be lower in waters with shorter residence times. For instance, Baker and Brezonik (1988), in modeling increases in alkalinity from atmospheric SO_4 loading, noted that net increases in alkalinity would be most important in waters with long residence times ($>$5 years) and that there would be little increase in alkalinity in waters with much shorter residence times ($<$1 year). However, the measured concentrations may not represent the maximum impact of MSR-driven mineralization because the mesocosm wall may enhance removal from the surface water (point number 2, below).
2. Presence of the mesocosm wall: the mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, enhancing the removal of surface water nutrients and Hg to periphyton or inorganic sinks such as iron oxyhydroxides. Natural aquatic systems have less proportional loss to surfaces. The quantitative estimates of internal loading of N, P, and Hg in response to MSR-induced carbon mineralization may have been underestimated by the measured surface water concentrations, given that significant loss of these constituents to periphyton may have occurred. In addition, THg was filtered prior to analysis, which would have removed any Hg associated with phytoplankton or other suspended particles.
3. Lack of either overland or groundwater loading of particulate and dissolved material, specifically iron: the availability of iron in sediment is a primary controller of the fate of MSR-produced sulfide (Pollman et al., 2017). In natural aquatic systems, iron would be supplied at a relatively constant rate from the system's watershed over the long term, although varying in magnitude from watershed to watershed (Maranger et al., 2006; Winter, 2001). This experiment was not an accurate long-term mimic of pore water sulfide concentrations because the external supply of iron was cut off at the inception of the experiment. With no loading of iron, but continued loading of SO_4, the continued production of sulfide would be expected to eventually consume all available Fe, allowing pore water sulfide levels to exceed those expected in a natural system at equivalent surface water SO_4 concentrations. This mesocosm experiment provides

evidence for just such a result. The experiment continued for 2 years after the 2013 sampling presented here. In the fifth year (August 2015) pore water sulfide was much greater than had been observed in 2013, and disproportionately so in the highest SO_4 treatment, which was most likely to consume available Fe. Between the 2013 and 2015, pore water sulfide increased in the control SO_4 treatment (about 7 mg SO_4 L^{-1}) from an average value of 69 μg L^{-1} in 2013 to 116 μg L^{-1} in 2015, a 68% increase. Pore water sulfide in the highest treatment (nominally 300 mg SO_4 L^{-1}, Table 1) increased from an average value of 728 μg L^{-1}in 2013 to 9,350 μg L^{-1} in 2015, a 1,184% increase (Pastor et al., 2017). In a survey of 108 Minnesota waterbodies with a wide range of surface water sulfate, only two exceeded a pore water sulfide level of 3,200 μg L^{-1} (Myrbo et al., 2017).

5. Conclusions

This study demonstrates that increased SO_4 loading to inland waters with organic-rich sediments can significantly increase the decomposition of sedimentary organic matter, which increases internal loading to surface water of the chemical constituents of organic matter, including DIC, DOC, P, N, and Hg. Associated changes include increased production of sulfide and methylmercury and increased alkalinity and pH. Any one of these changes could alone cause significant secondary changes in the structure of an aquatic ecosystem but, taken together, could cause a cascade of primary and secondary environmental changes: increased availability of nutrients (N and P), which can alter dominant plant species, organic carbon production, oxygen consumption, and redox; increased pore water sulfide, which can be toxic to benthic animals and plants; increased MeHg production, which can affect fish and other consumers in the aquatic food web; increased DOC, which can alter light transmission, thermal stratification, and aquatic chemistry; and increased DIC production, which increases alkalinity and pH, affecting aquatic chemistry and biota. Each of these changes resulting from higher surface water SO_4 and consequent increases in MSR has been documented in the literature, but the entire suite of associated changes in aquatic chemistry has not heretofore been demonstrated in an integrated fashion. The degree to which an increase in SO_4 loading affects the ecological structure of the receiving water will depend on the relative increases in N, P, DIC, DOC, Hg, MeHg, pH, and sulfide, which will be a function of background geochemistry and hydrology of the specific system. In this experiment, the changes in these parameters were linearly proportional to SO_4 reduction, which, in turn, was linearly proportional to the time-weighted average SO_4 concentration. The linear responses of the parameters to SO_4 additions suggest that ecologically significant changes may occur even when SO_4 concentrations are elevated only modestly and that dramatic changes may occur with higher sulfate loading.

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Article

Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments

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Abstract

Under oxygenated conditions, sulfate is relatively non-toxic to aquatic plants. However, in water-saturated soils, which are usually anoxic, sulfate can be reduced to toxic sulfide. Although the direct effects of sulfate and sulfide on the physiology of a few plant species have been studied in some detail, their cumulative effects on a plant's life cycle through inhibition of seed germination, seedling survival, growth, and seed production have been less well studied. We investigated the effect of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris* L.) in hydroponic solutions and in outdoor mesocosms with sediment from a wild rice lake. In hydroponic solutions, sulfate had no effect on seed germination or

juvenile seedling growth and development, but sulfide greatly reduced juvenile seedling growth and development at concentrations greater than 320 µg/L. In outdoor mesocosms, sulfate additions to overlying water increased sulfide production in sediments. Wild rice seedling emergence, seedling survival, biomass growth, viable seed production, and seed mass all declined with sulfate additions and hence sulfide concentrations in sediment. These declines grew steeper during the course of the 5 yr of the mesocosm experiment and wild rice populations became extinct in most tanks with concentrations of 250 mg SO_4/L or greater in the overlying water. Iron sulfide precipitated on the roots of wild rice plants, especially at high sulfate application rates. These precipitates, or the encroachment of reducing conditions that they indicate, may impede nutrient uptake and be partly responsible for the reduced seed production and viability.

Introduction

Under oxygenated conditions, sulfate, the most abundant form of dissolved sulfur in aquatic systems, is relatively non-reactive, and is therefore relatively non-toxic. However, where oxygen is absent and organic matter is present, sulfate can serve as an electron acceptor for heterotrophic microbial metabolism, producing reactive reduced sulfur species. When sulfate concentrations limit the activity of sulfur-reducing microbes, an increase in sulfate can enhance the decomposition of organic matter and initiate a cascade of interrelated biogeochemical reactions (Garrels and Christ **1965**) that alter the bioavailability of phosphorus and other nutrients (Lamers et al. **2002**), and generate alkalinity (Giblin et al. **1990**). One of the most reactive products of sulfate reduction is hydrogen sulfide, which we here term “sulfide.” If dissolved sulfide persists in the rooting zone of aquatic plants, it can inhibit root growth and metabolism (Mendelssohn and McKee 1988, Koch and Mendelssohn **1989**, Koch et al. **1990**, Lamers et al. **2002**, **2013**, Gao et al. **2003**, Armstrong and Armstrong **2005**, Geurts et al. **2009**, Martin and Maricle **2015**) and photosynthesis (Pezeshki **2001**). If root biomass and metabolism are reduced by elevated sulfide concentrations, then the plant's ability to take up limiting nutrients may be impaired (DeLaune et al. **1983**, Koch et al. **1990**, Gao et al. 2002, **2003**, Armstrong and Armstrong **2005**, Lamers et al. **2013**).

Although the direct effects of sulfide on the physiology of individual plants of a few species have been studied in some detail, the cumulative effects of sulfide on a plant's life cycle through possible inhibition of seed germination, seedling survival, and seed production have been less well studied. Sulfide could affect any or all of these stages of a plant's life cycle, either directly by toxicity to seeds and seedlings or indirectly by decreasing nutrient uptake through roots during seed formation. If so, then populations may become sparser and less viable over several life cycles. Population effects could be realized rapidly in non-clonal annual aquatic emergent plant species that rely exclusively on seed production, germination, and seedling survival to produce the next generation of emergent shoots. A seed bank in the sediment would facilitate recovery of a population after one or two catastrophic growing seasons, but would become depleted if chronic sulfide toxicity does not allow occasional successful growth and reproduction to restock the seed bank.

Northern wild rice (*Zizania palustris* L., hereafter wild rice) is an annual graminoid (Family Poaceae, Tribe Oryzeae), which is most abundant in the rivers and lakes in the Lake Superior region. Because of its widespread distribution and tendency to form large monotypic stands, wild rice is an important component of the food supply for the aquatic and avian herbivores and seed consumers, such as muskrats and waterfowl. Reduction of these wild rice populations could, therefore, have cascading effects on diverse aquatic food webs. In addition, the native Ojibwe people of the Lake Superior and Lake Michigan region teach that they

were led to this region to find “the food that grows upon the water,” which is wild rice. The Ojibwe identify their origins with wild rice and consider themselves “people of the rice” (Vennum **1998**). The resource is also important to Menominee and Dakota peoples of the region. Efforts to enhance the productivity, perpetuation, and restoration of natural wild rice populations are of great importance to state and tribal natural resource agencies for both ecological and cultural reasons.

The wild rice life cycle begins when seeds from the previous year or years germinate in mid to late May. Juvenile seedlings grow through the water column in early to mid-June. Upon reaching the surface, the seedling generates a floating leaf that fixes carbon into carbohydrates for root production and nutrient uptake. By the end of June, nitrogen and other nutrients are translocated out of the floating leaf into an aerial shoot emerging from the leaf axil, and the floating leaf dies. The early stages of the vegetative growth of the aerial shoot happen during the next two weeks and vegetative growth continues until the emergence of flowering heads in late July. Seed production and ripening begins in early to mid-August with seed production completed by early- to mid-September. The productivity of wild rice is primarily limited by nitrogen and secondarily by phosphorus; increased nitrogen supply accelerates development of the life cycle and reduces allocation to roots (Sims et al. **2012a**) and increases the number of inflorescences, seeds per inflorescence, and mean seed mass, resulting in more seedlings produced the following year, and hence greater fitness (Sims et al. **2012b**).

Historic observations suggested that wild rice usually occurs in waters where sulfate concentrations were near or below 10 mg/L and populations are uncommon where sulfate concentrations exceeded 50 mg/L (Moyle **1944**, **1945**). Based on Moyle's (**1944**, **1945**) research, the State of Minnesota sulfate standard for waterbodies supporting wild rice is 10 mg/L; Wisconsin, Michigan, and Ontario currently do not have sulfate standards for wild rice waters. For comparison, the EPA non-enforceable, aesthetic (taste) secondary water quality sulfate standard for human consumption is 250 mg/L (*available online*).[7]

This research is part of a larger study coordinated by the Minnesota Pollution Control Agency on the effect of sulfate on wild rice, which included an extensive survey of potential wild rice waters across Minnesota containing surface water sulfate ranging from <2 mg/L to >600 mg/L. This study was carried out because of recent interest in the nature of the relationship between sulfate and wild rice, especially with respect to potential anthropogenic sulfate enhancements to wild rice ecosystems such as sewage treatment plants, agricultural runoff, and mining of ores containing metallic sulfides. The mechanisms responsible for the decreased wild rice density with increased sulfate concentrations observed by Moyle (**1944**, **1945**) have not been investigated until this study.

Although we have a fairly extensive understanding of the general aspects of the life cycle of wild rice in natural stands in relation to nutrient availability and sediment chemistry (Keenan and Lee **1988**, Day and Lee **1990**, Meeker **1996**, Lee **2002**, Pastor and Walker **2006**, Walker et al. **2010**, Hildebrandt et al. **2012**, Sims et al. **2012a** , **b**), the way in which sulfate in surface water can affect the life cycle of wild rice, and hence its population dynamics, is much less well understood. The objectives of our research are to (1) determine the relative effects of sulfate and sulfide on seed germination, seedling viability, vegetative growth, and seed production; (2) determine the response of wild rice populations and population viability to sulfate in the overlying water and the production of sulfide in sediment porewaters.

Methods

The effects of sulfate and sulfide on wild rice were tested in two different ways: (1) a laboratory hydroponic culture system and (2) an outdoor mesocosm system that better mimicked natural wild rice waters, but does not control the chemical exposures as precisely as the hydroponic experiments did. Short-term (10 or 11 days) hydroponic exposures of seeds and seedlings to sulfate and sulfide were conducted to examine effects on seed germination, seedling growth, and survival. Full life cycle tests were conducted in mesocosms where wild rice grew in sediment taken from a natural wild rice lake. These multi-year outdoor tests examined the effects of elevated surface water sulfate and the associated increased sedimentary sulfide concentrations on germination, survival, growth, and reproduction.

Hydroponic experiments

Li et al. (**2009**) published one of the few dose-response studies of aquatic macrophytes (*Typha* and *Cladium*) to sulfide, which requires the maintenance of anaerobic conditions. Malvick and Percich (**1993**) developed a simple hydroponic system to investigate effects of nutrients on germination and early growth of wild rice, but their system could only be implemented under aerobic conditions. We used these two studies as starting points for the development of our methods.

Wild rice seeds used for all hydroponic experiments were collected on 30 August 2012 from Little Round Lake (Minnesota Lake ID 03-0302, 46.97° N, 95.74° W; average surface water sulfate <0.5 mg/L and porewater sulfide = 77 µg/L, $n = 5$). The seeds were stored at 4°C in polyethylene bottles in a darkened room until needed for experiments. Immediately before each experiment, a subsample of these seeds was selected that were intact, filled, not green (unripe), and not moldy. To obtain seedlings for juvenile seedling response to sulfate or sulfide, the selected seeds were allowed to germinate in aerobic deionized water until a 1–2 cm long mesocotyl shoot appeared, which usually occurred 5–7 days after germination. The mesocotyl is the embryonic stem that will develop into the mature stem.

Once the seeds or seedlings were selected, they were picked up with forceps and transferred to the appropriate test in appropriate containers. The hydroponic solution was one-fifth strength Hoagland's solution in 5 mmol/L PIPES buffer to maintain a pH of 6.8 ± 0.03 (mean ± SD) in the solution, similar to that observed in the porewater of mesocosm experiments. Nitrogen was supplied only as ammonium (0.16 mmol/L NH_4Cl) to mimic natural concentrations of inorganic nitrogen in wild rice waters (Walker et al. **2010**). The Hoagland's solution contained sulfate only in trace amounts as $ZnSO_4$ (0.5 µmol/L) and $CuSO_4$ (0.15 µmol/L). This nutrient solution was then augmented with appropriate amounts of anhydrous Na_2SO_4 or $Na_2S{\cdot}9H_2O$ to achieve desired sulfate or sulfide treatment concentrations. The one-fifth Hoagland's solution and PIPES buffer were chosen based on previous trials to determine proper strengths and buffers that would support seedling growth without adverse effects (see Appendix S1 for composition of our modification of Hoagland's Solution).

Germination of wild rice seeds under aerobic conditions subject to various concentrations of sulfate

The selected seeds were placed into each of six numbered plastic cups to total 50 seeds each, then randomly assigned and transferred to each of six 1-pint Mason jars (1 pint = 473 mL) containing six sulfate treatment concentrations of 0 (trace), 10, 50, 100, 400, or 1600 mg SO_4/L. These sulfate treatments (trace to 1600 mg/L) bracket the large range encountered across Minnesota's geologically diverse landscape (10th and 90th percentiles of 0.2 and 285 mg/L, respectively; MPCA **2016**), plus some mine pits over 1000 mg/L

that may overflow into wild rice waters. This seed counting and random transfer was repeated twice more to result in six treatment levels with three replicate jars per treatment. The jars were covered with plastic covers fitted with rubber stoppers to facilitate solution exchanges. Two holes in the plastic lids were left open to facilitate air exchange and to prevent the solutions from becoming anaerobic. The experiment proceeded in a growth chamber at 20°C in the dark to simulate conditions measured in sediments during the growing season, which we have measured in our mesocosms (see Results). The solutions were exchanged with fresh solution of the appropriate treatment concentration every three days. Dissolved oxygen in the solutions across all treatments was initially 8.280 ± 0.218 mg/L (mean ± SD) and dropped to 2.85 ± 0.60 mg/L by the end of three days, still well above anoxic levels required for production of sulfide. Solution pH and sulfate were measured on each initial batch of sulfate treatment and on the exchanged solution from each jar. The germinated seedlings were harvested after 11 days. The number of successfully germinated seeds, determined as those that produced a mesocotyl at least 1 cm in length, were counted. The length of the mesocotyl was measured for each seed. The germinated seeds were then dried at 65°C for 3 d. The mesocotyl was then carefully separated from the seed hull and weighed.

Germination of wild rice seeds under anoxic conditions subject to various concentrations of sulfide

The techniques used here were the same as for the germination trials under various sulfate concentrations, except that extra care was necessary to ensure anaerobic conditions. Fifty seeds were chosen as above and then placed in 700 mL borosilicate glass bottles capped using phenolic screw caps with chlorobutyl septa 5 mm thick. The one-fifth Hoagland's nutrient solution was deoxygenated with oxygen-scrubbed nitrogen before being added to the bottles. PIPES buffer was added to the test solution to maintain consistent pH levels of 6.8 ± 0.03 throughout an experiment. Bottles were filled completely with the deoxygenated nutrient solution and without introducing any air bubbles and then capped with the septa. Stock sulfide solutions (20–30 mmol/L) were prepared as needed by adding $Na_2S{\cdot}9\ H_2O$ (sodium sulfide nonahydrate) to deionized and deoxygenated water. The concentration of the stock sulfide solution was checked periodically against a stock solution that had been standardized using an iodimetric titration. An appropriate amount of the stock solutions was added to each bottle with a Hamilton gas-tight glass syringe through the septa while simultaneously withdrawing an equivalent volume of the Hoagland's solution by means of a second syringe through the septum. All of the syringes used in this and other experiments were purged three times with oxygen-scrubbed ultra-pure nitrogen from a tilled PVDF gas sampling bag (Saint-Gobain No. D1075016-10), which had also been purged three times before filling. Added stock sulfide solution volumes range between 0.2 and 3.0 mL depending on target exposure concentrations and the nominal concentration of stock sulfide solution. The target sulfide concentrations were 0 (trace), 96, 320, 960, and 2880 µg/L. These sulfide treatments (trace to 2880 µg/L) bracket the range encountered across shallow aquatic systems in Minnesota that potentially could host wild rice (5th and 95th percentiles of 26 and 1631 µg/L, $n = 108$; A. Myrbo, *unpublished data*).

The bottles were placed in a growth chamber in continuous darkness at 20° ± 1°C. Solutions were exchanged every two days if during the week or three days if over a weekend. The solution in each jar was sampled for sulfide analysis at the beginning and end of each two- or three-day cycle. The pH of the solution in each jar was measured at the end of each two- or three-day cycle. To obtain the initial pH of the solution, one additional replicate jar for each treatment but without seeds was filled with one-fifth Hoagland's solution, then the sulfide treatment was added using syringes as above and the jar was opened and pH was measured

immediately. Total dissolved sulfide ($H_2S + HS^-$) was measured on a Hach DR5000 spectrophotometer using a colorimetric methylene blue method (4500 S2-D; Eaton et al. **2005**) as implemented with Hach method 8131. The method was adapted for a lower detection limit (~15 µg/L) using a photo cell with a 5 cm path length. All measurements of dissolved sulfide in both hydroponics and mesocosm experiments refer to the sum of all dissolved inorganic reduced sulfur ($H_2S + HS^-$). The samples of hydroponic water were added directly from the gas tight syringe to the sulfuric acid reagent, followed immediately by the potassium dichromate reagent. After 11 days, the germinated seeds were harvested and measured as described for the experiments on effects of sulfate on germination.

Growth of juvenile wild rice seedlings under aerobic conditions subject to various concentrations of sulfate

We examined growth of juvenile seedlings at concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L. Twenty replicated 70-mL unsealed glass Kimax tubes (Cole-Parmer, Vernon Hills, IL, USA) were used for each test concentration. One seedling germinated and selected as described was placed with forceps into each Kimax tube, which was then filled with one-fifth Hoagland's solution and an appropriate amount of sulfate. The filled tubes (solution and seed) were placed into every other opening in Nalgene Resmer (ThermoFisher Scientific, Waltham, MA, USA) test tube holding racks so that light could penetrate to all sides of each tube. A total of six 40-tube racks, each containing 20 tubes, were used to hold the test tubes. Screw caps were placed loosely on the tubes to allow for oxygen exchange across the solution surface and thereby prevent the development of anaerobic conditions. The tubes were placed in a Percival environmental growth chamber where we measured 288 ± 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation immediately above the plants using a Decagon PAR – 80 Ceptometer (Decagon Devices, Pullman, WA, USA). Tests were performed under a 16 h : 8 h light : dark schedule. All racks were placed in the growth chamber so that the spaces between the racks were the same as the spaces within the racks and the tops of the tubes are within 30 cm of the bottom of the lights. The location of each rack in the growth chamber remained the same for the test duration. Test solutions in the tubes were renewed every two days. Temperature was maintained at 21°C during lighted periods and 19°C during dark periods and the humidity was maintained at 85%. Plants were harvested after 10 days and the seed hull was carefully removed. Stem and leaf length was measured to the nearest millimeter by placing the stem with leaf stretched out on a flat surface next to ruler with the zero mark aligned with the point of stem-root transition. Total root lengths were measured in duplicate scans of the entire root system using the program WinRhizo (Regent Instruments, Quebec, Canada). Seedlings were weighed after drying at 100°C for 48 h. Control juvenile seedlings did not have any visible phytotoxic or developmental symptoms at any time and the controls had additional stem growth of at least 5.0 cm during the 10-d test.

Growth of juvenile wild rice seedlings under anaerobic conditions subject to various concentrations of sulfide

Germinated seedlings were chosen using the same techniques described for aerobic conditions. Seven seedlings 1–2 cm in length that fit the criteria as described, were placed with a forceps in 125-mL borosilicate glass jars capped using phenolic screw caps with 5 mm thick chlorobutyl septa. Each sulfide concentration was replicated in this way in three separate jars. Deoxygenated Hoagland's nutrient solution was added as described above. Seedlings were grown in the same environmental growth chamber under the same

temperature and light conditions as for the sulfate experiments but with solution sulfide concentrations of 0, 96, 320, 960, and 2880 µg/L. Solutions were exchanged every two days if during the week or three days if over a weekend. Sulfide concentrations were measured at the beginning and end of each two–three day solution exchange period. Because the plants were photosynthesizing and producing oxygen, the sulfide concentration declined during these two–three day periods. This was especially so for the lowest sulfide concentrations (less than ~300 µg/L) in which less than 10% remained after two days, but 70–90% of sulfide remained after two days for sulfide concentrations greater than 650 µg/L. We therefore used the time-weighted average sulfide concentration over the 10 days period to characterize the sulfide concentrations the plants were exposed to. Seedlings were harvested after 10 days, the seed hull was carefully removed, and the stem and leaf lengths and total plant mass were determined. Because many of the plants, especially at high sulfide concentrations, did not grow at all (see *Results* below) the roots and shoots were very fragile and no attempt was made to dissect the plants into subcomponents as with the experiment on the effects of sulfate on seedling growth.

Statistical analyses of hydroponic experiments

The general procedure for each set of sulfate and sulfide exposure experiments was first to examine seed germination or seedling growth response across a wide range of concentrations spanning three orders of magnitude of either sulfate or sulfide as noted. The main effect of sulfate or sulfide concentrations on the variable of interest was then tested with an analysis of variance using SigmaPlot (SYSTAT Software, San Jose, CA), USA. When the residuals were not normally distributed or the data did not have equal variance between treatments, then the data were transformed by taking the natural logarithms, which then passed normality and equal variance tests. If there were no effects across this wide range of concentrations in this experiment, then it was repeated to test whether the results were a false negative. If there were significant main effects, then Tukey's pairwise comparisons were performed to determine in which part of the range of concentrations significant effects occurred. Further experiments were then conducted twice using this narrower range of concentrations centered on the region of significant change to more precisely refine the range of response of seedling germination or growth to sulfate or sulfide concentrations.

If there was a significant effect of sulfide on seedling growth, then the biomass growth of seedlings (mg) over the 10-d period was regressed against the time-weighted total dissolved sulfide concentrations (µg/L) with a four-parameter sigmoidal function using SigmaPlot nonlinear regression

$$\text{Plant growth} = y_{\min} + \frac{y_{\max}}{1 + \exp\{-[(S^{2-} - x0)/b]\}} \quad (1)$$

where y_{min} is the right-side (minimum) horizontal asymptote (minimum growth response) y_{max} is the height of the left-side horizontal asymptote (maximum growth response) above y_{min}, S^{2-} is total dissolved inorganic sulfide ($H_2S + HS^-$), $x0$ is the sulfide concentration at the inflection point of the curve, and b is a parameter that scales µg/L of sulfide concentration to mg of biomass growth. The 50% effects concentration (EC50, the concentration of sulfide that caused a 50% reduction in change in plant mass compared to controls) was calculated from this regression.

The sulfate experiment had to be conducted under aerobic conditions while the sulfide experiment had to be conducted under anaerobic conditions. Therefore, redox statuses of the solutions were necessarily confounded with sulfur speciation. To test the effect of redox status on seedling growth, we compared the

growth of plants from both the lowest concentrations of the sulfate (aerobic) and sulfide (anaerobic) experiment using a single-factor analysis of variance.

Mesocosm experiments

Experimental design

We constructed mesocosms using the same procedures and designs previously reported by Walker et al. (**2010**) for a 5-yr experiment on the interaction of the nitrogen cycle and wild rice population dynamics.

In late spring of 2011, polyethylene stock tanks (400 L, 132 × 78 × 61 cm; High Country Plastics, Caldwell, ID, USA) were fitted with overflow drain pipes and buried to ground level. The drain pipes are connected to 20-L polyethylene overflow buckets buried adjacent to each tank. Water tables were set by the inflow to the drain pipe at 23 cm above the sediment surface. The tanks were leveled and then partly filled with 10 cm of clean sand washed with the same well water later added to the tanks (see next paragraph). The sand layer was then covered with 12 cm of surface sediment collected from a natural wild rice bed in Rice Portage Lake (Minnesota Lake ID 09-0037, 46.70° N, 92.70° W) on the Fond du Lac Band of Lake Superior Chippewa Reservation, Minnesota. Rice Portage Lake is approximately 337 ha, of which approximately 50 ha are wild rice beds (Minnesota Department of Natural Resources **2008**). Ten to 20 cm of sediment over sand is sufficient to support the rooting depths we have observed in natural wild rice lakes. The sediments were kept saturated and then thoroughly homogenized in a large stock tank prior to distribution into the tanks. Analyses of five volumetric samples of the mixed sediment indicate a homogenous material (C = 14.8% ± 1.7%, N = 1.12% ± 0.13%, S [acid volatile sulfur] = 0.005% ± 0.003%). Sediment bulk density was 0.27 ± 0.01 g/cm^3 (Walker et al. **2010**). These nutrient and bulk density values are similar to those of other wild rice beds (Keenan and Lee **1988**, Day and Lee **1990**). No new sediment has been added to the stock tanks since the mesocosms were established in 2011.

The tanks were immediately filled with water obtained from a nearby well after sediment additions to prevent the sediment from drying. Water was added cautiously from a garden hose to prevent redistribution and suspension of sediment. During the growing season, water levels were maintained at 23 cm above the sediment surface by weekly additions of water to the drain pipe heights or by allowing water to drain through the pipe into the overflow buckets. Rainfall N concentrations as NO_3-N and NH_4-N ranged from 0.2 to 1.99 mg/L while the NO_3-N and NH_4-N concentrations in the well water are always <0.2 mg/L (Walker et al. **2010**). Sulfate concentrations in well water averaged 10.73 ± 0.75 mg/L (n = 36) and in rainwater averaged 2.13 ± 1.02 mg/L (n = 16). The sediments comprise a natural inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter remain submerged in the mesocosms year round with water levels set at approximately 20 cm in late fall.

Wild rice was planted once in late spring 2011 from seeds obtained from Swamp Lake (Minnesota Lake ID 16-0256, 47.85° N, 90.58° W), a 37-ha lake on the Grand Portage Band of Lake Superior Chippewa Reservation, Minnesota. Seeds from each year's crop were allowed to fall unimpeded into the tanks to provide the seed source for the next year's population; no further seeding from external seed sources occurred.

End-of-season plant density in Minnesota wild rice lakes monitored by the 1854 Treaty Authority averages 40 plants/m^2 (Vogt **2010**). Accordingly, the seedlings were thinned to this density (30 plants per tank) in late spring or early summer each year before the floating leaf stage was achieved. The seedlings removed from

each tank during thinning in 2012–2015 were counted to estimate seed germination and early seedling success.

Immediately after installation and seeding, beginning in late June 2011, the tanks were treated with different amounts of sulfate to achieve several target sulfate concentrations in the overlying water. There were five overlying water sulfate concentrations and six replicate tanks per sulfate concentration, for a total of 30 tanks. Nominal water column sulfate concentrations of 50, 100, 150, and 300 mg SO_4/L were maintained in sulfate-amended tanks. Aside from incidental sulfate in the make-up water from a well and rainwater, control tanks did not receive any sulfate amendments and overlying water concentrations ranged from 2 to 10 mg/L (average of 7 mg/L) depending on rainfall, evapotranspiration, and loss via sulfate reduction in the sediment. The overlying water sulfate concentrations in the mesocosm experiments bracket both the existing 10 mg/L Minnesota statutory standard for wild rice waters and the EPA drinking water standard of 250 mg/L. Samples of the water column were taken weekly and analyzed for sulfate concentration using a Lachat QuikChem 8000 Autoanalyzer (Method 10-116-10-1-A, Hach Co., Loveland, CO, USA). When necessary (approximately every two weeks), the sulfate concentration was adjusted to near the desired nominal concentrations with appropriate amounts of 10 g/L sodium sulfate (Na_2SO_4; Fisher Chemical S421, Thermo Fisher Scientific, Waltham, MA, USA) stock solution and well water. The sodium sulfate stock solution was first mixed in 1–2 L of water from the tank, then added back to the tank's overlying water with mild mixing.

Plant, sediment, and water sampling and analyses

In each year from 2011 to 2015, five plants in each tank were randomly chosen in early summer for detailed measurements throughout the growing season and to be destructively sampled at the end of the growing season. In late August to September, ripe seeds from these plants were collected every two or three days by gently removing them, leaving unripe seeds behind for the next collection date. The seeds from each individual plant were placed in a paper envelope and marked with the tank identification number. The plants were then harvested for determination of biomass, root : shoot mass ratios and total seed production by counting seed peduncles along the flowering stem.

Seeds from each of the five sampled plants were separated into filled (viable) seeds and empty (nonviable) seeds, counted, and weighed. A subsample of seeds collected in all years except 2013 were dried at 60°C for determination of moisture content to convert wet mass to dry mass. The five sample plants were separated into root and shoot (stem + leaves), and then weighed. Root : shoot ratios and seed masses and numbers from the five sampled plants were applied to total aboveground population masses and total plant numbers to determine total root and seed biomass and number and total biomass in each tank.

While harvesting the plants for growth and biomass measurements, we noticed that plants in the tanks amended with sulfate had blackened roots while plants grown in the control tanks had white or light tan or orange roots. To investigate this further, a sample of roots from a plant from one control tank and a plant from one 300 mg/L amended tank were collected and placed immediately in water in which dissolved oxygen had been purged by bubbling with oxygen-free N_2. These samples were analyzed for Fe and S concentrations by energy-dispersive X-ray spectroscopy (EDS) using a Hitachi TM-1000 scanning electron microscope (Hitachi High Technologies, Schaumburg, IL, USA) fitted with a Quantax EDS unit (Bruker Corporation, Billerica, MA, USA). The nominal spot size was 0.2 μm and the analysis volume was ~5 μm^3. The sample of blackened roots was analyzed at seven points and the sample of tan/orange control roots was analyzed at five points.

All aboveground plant material was collected from each tank at the end of the growing season and weighed

to determine total aboveground biomass. A subsample was taken to determine wet : dry ratios for moisture correction after drying at 60°C. All aboveground plant material except for the five sample plants were returned to each tank. All stems in each stank were counted at the time of harvesting the aboveground plant biomass to determine end of growing season plant density.

In 2013, significant seedling mortality occurred in all tanks after thinning but before the floating leaf stage. We believe this early season mortality was due to a record cold and late spring in northern Minnesota in April and May of 2013; ice stayed on lakes an average of 3 weeks later than the median ice-out date (data *available online*).[8] The reduced overall emergence of plants in the spring of 2013 precluded the destructive sampling of five sample plants in each tank at the end of the 2013 growing season because this harvesting would have greatly decreased the number of viable seeds returned to the sediment for the following growing season. Instead, during 2013 all seeds were harvested from each and every plant in the tanks, sorted as described above on each collection day, and returned to the tanks within 24 h of collection without drying in order to maintain their viability for future populations. To determine wet-dry conversion ratios for these seeds, additional seeds were collected at the same collection times from an adjacent experiment on wild rice (Walker et al. **2010**) for moisture determination after drying them at 60°C.

Polycarbonate porewater equilibrators (peepers) with sampling ports spaced 1.5 cm intervals were used to make in situ measurements of geochemical profiles of sulfur and iron species at discrete depths in the sediment porewater of a subset of tanks in August of 2013. Care was taken that the installation and extraction of the peepers did not disturb any plants. The method for collecting samples for sulfate, sulfide, and ferrous iron with peepers was modified from Koretsky et al. (**2007**). Sulfide and iron were quantified in samples immediately with minimal oxygen exposure using a colorimetric methylene blue method (4500 S2-D; Eaton et al. **2005**) as implemented with Hach method 8131 for sulfide and a colorimetric phenanthroline method for iron (3500-Fe-B; Eaton et al. **2005**). Sulfate was quantified with ion chromatography on a Dionex ICS 1100 system (Thermo Fisher Scientific, Waltham, MA, USA) after acidifying samples to pH < 3 using hydrochloric acid and purging gently with oxygen-free nitrogen gas.

In August 2013 and 2015, we also used 10-cm long Rhizon samplers (Rhizosphere Research Products B.V., Wageningen, The Netherlands) to obtain porewater for sulfide analysis. The sampler was inserted vertically into the sediment and connected to an evacuated 125-mL serum bottle. Sulfide samples were prepared without removing the butyl rubber stopper for inline distillation by automated flow injection colorimetric analysis (4500 S2-E; Eaton et al. **2005**).

On 6 October 2015, a 10-cm long sediment core was taken from each mesocosm and homogenized. Extractable iron was quantified following a 30-min exposure to 0.5 mol/L HCl, following Balogh et al. (**2009**), at the Minnesota Department of Health Environmental Laboratory. Total organic carbon was determined using the method of oxidative combustion-infrared analysis (U.S. EPA **2004**), after pre-treatment with acid to remove inorganic carbon, at Pace Analytical Services in Virginia, Minnesota, USA.

Statistical analyses of mesocosm experiments

The effects of sulfate concentrations on plant attributes were tested by repeated measures analysis of variance followed by pairwise comparisons between attributes of plants in the control tanks and each higher sulfate concentration. We also regressed each plant attribute against average annual sulfate concentration for each year. Correlations were assessed using Pearson's correlation test. This combination of both analysis of variance and regression was used as recommended by Cottingham et al. (**2005**). We used target sulfate

concentrations as categorical variables in analyses of variance and growing season actual sulfate concentrations in regression analyses.

Results

Hydroponic experiments

Effect of sulfate on seed germination

Between 71% and 76% of the seeds pre-selected as filled and mold-free germinated at each sulfate concentration. Sulfate exposure concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L did not affect germination success, mesocotyl lengths, or the masses of the stem plus leaf (if any) and roots ($P > 0.10$ for each test). The experiment was repeated with the same results.

Effect of sulfide on seed germination

Sulfide concentrations of 0, 96, 320, 960, and 2880 µg/L did not affect germination success of seeds, mesocotyl masses, or mesocotyl lengths ($P > 0.10$ for each test). The experiment was repeated with the same results.

Effect of aerobic and anaerobic conditions on seed germination

There were no differences in germination rates under anaerobic compared with aerobic conditions when concentrations of sulfur were at trace (<1 µmol/L) amounts of $CuSO_4$ and $ZnSO_4$ in the Hoagland's solution. Mean mesocotyl lengths in the anaerobic solutions (7.8 cm) were significantly reduced ($P < 0.05$) by 38% compared with mean mesocotyl lengths in the aerobic solutions (12.5 cm).

Effect of sulfate on seedling growth

Sulfate concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L did not affect the growth of juvenile seedling stem length, juvenile stem mass, juvenile root mass, or total juvenile seedling mass ($P > 0.10$ for each test). Sulfate decreased juvenile root length slightly ($P < 0.02$) but only at 1600 mg SO_4/L compared with 50 mg SO_4/L. The experiment was repeated with the same results.

Effect of sulfide on seedling growth

To examine the effects of sulfide on early seedling growth, we began by growing juvenile seedlings under a wide range of nominal sulfide exposure concentrations of 0, 96, 320, 960, and 2880 µg/L in anoxic solutions in a first trial. Both roots and stems of control plants (no added sulfide) increased significantly ($P < 0.05$) over the exposure, approximately doubling in size compared with initial lengths and masses. In seedlings exposed to sulfide concentrations 320 µg/L or more, stem and leaf masses ($P < 0.01$) and total plant masses ($P < 0.001$) were significantly depressed by an average of 60% and 75%, respectively, relative to controls. Root lengths were only weakly depressed with increasing sulfide concentration ($P < 0.10$).

To narrow the range of toxicity, we then conducted two additional trials focusing on the effects of sulfide on juvenile seedling growth at concentrations less than 1600 µg/L sulfide. The second trial examined growth at

exposure concentrations of 0, 200, 400, 800, 1600 µg/L sulfide and the third trial examined growth at exposure concentrations of 0, 160, 320, 640, and 1280 µg/L sulfide. Consistent with the first trial, the biomass of all control plants increased significantly ($P < 0.05$) during the 10 d of exposure, approximately doubling in size compared with initial lengths and masses, and exposure to sulfide across these narrower ranges of concentration again significantly depressed stem plus leaf lengths and total masses of juvenile seedlings.

Because all three trials produced similar effects, we performed a pooled analysis of variance using data from all three. Exposures of seedlings to sulfide concentrations of 320 µg/L or greater significantly reduced growth rates ($P < 0.01$) of wild rice seedlings compared to the control by 88% or greater; Fig. 1). Seedlings exposed to sulfide concentrations at 320 µg/L or greater hardly grew at all and in some cases their mass decreased during the 10-d course of the exposure (Fig. 1). But exposures at sulfide concentrations less than 320 µg/L did not significantly reduce growth rates ($P > 0.10$) compared with the controls (Fig. 1). There was a sigmoidal response of seedling growth to elevated sulfide concentrations, with an inflection point at approximately 245 µg/L (Fig. 1; see figure caption for parameter values and r^2 for Eq. (1)). The EC50 calculated from this regression was 227 µg sulfide/L.

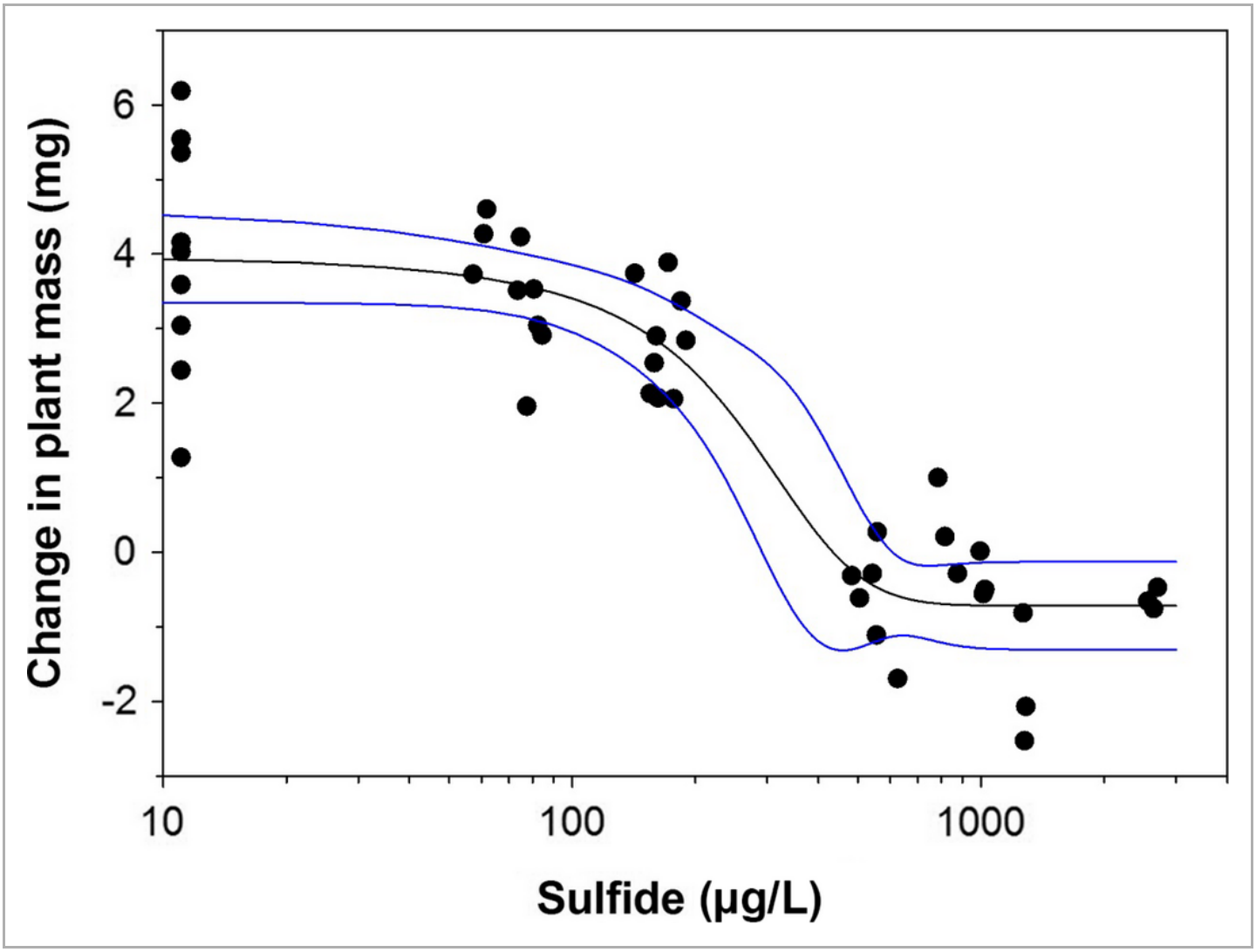


Figure 1.

Open in figure viewer

Growth of wild rice seedlings declines with increasing sulfide concentrations in hydroponic solutions. Individual data points are from three separate experimental runs (see *Methods* and *Results* sections). Fitted sigmoidal response curve (Eq. (1)) is shown in black, 95% confidence intervals in blue; $r^2 = 0.80$, $y_{min} = -0.7172$, $y_{max} = 5.1353$, $x0 = 245.9051$, $b = -103.8853$.μ (Color figure can be viewed at wileyonlinelibrary.com.)

Effect of aerobic and anaerobic conditions on seedling growth

Under micromolar concentrations of sulfur from trace amounts of $CuSO_4$ and $ZnSO_4$ in the Hoaglands solution, stem lengths were 10% longer ($P < 0.02$), root lengths were 73% shorter ($P < 0.001$), and total plant masses were 16% less ($P < 0.01$) under anaerobic conditions compared to aerobic conditions.

Mesocosm experiment

Sulfate concentrations in overlying water

The average monthly measured sulfate concentrations in amended tanks were consistently within 80–100% of nominal target concentrations of 50, 100, 150, and 300 mg/L (Table 1). The sulfate concentrations sometimes decreased after large rainfall events.

Table 1. Target and measured sulfate concentrations in overlying water in the mesocosm experiment

Target sulfate concentration	Measured growing season mean sulfate concentrations (mg/L)					
	12 Jul–30 Aug 2011	6 Jun–28 Aug 2012	5 Jun–27 Aug 2013	27 May–26 Aug 2014	5 May–4 Sep 2015	Average over all years
0	8.05 (0.34)	8.0 (0.31)	7.05 (0.18)	5.8 (0.16)	6.16 (0.25)	7.01 (0.45)
50	50.0 (1.58)	34.0 (1.26)	37.2 (1.02)	43.3 (0.8)	41.7 (1.26)	41.2 (2.73)
100	97.7 (4.33)	77.1 (1.76)	79.7 (1.41)	87.2 (1.29)	85.3 (2.03)	85.4 (3.58)
150	135.0 (3.73)	126.0 (2.08)	127.0 (1.55)	131.0 (1.68)	132.0 (2.56)	130.0 (1.57)
300	254.0 (7.35)	263.0 (3.32)	268.0 (2.37)	273.0 (2.52)	272.0 (4.08)	266.0 (3.50)

Note

Values in parentheses are SE.

Porewater sulfide concentrations with sulfate additions

Profiles of sulfate, sulfide, and iron in the mesocosm porewaters showed patterns consistent with sulfate diffusion from the overlying water into the surficial 5 cm of sediment with subsequent reduction to sulfide (Fig. 2). Concentrations of sulfide were typically highest in upper 3–5 cm, which is the rooting zone of seedlings. Sediment in tanks contained on average 8.3 ± 0.8 mg/g extractable iron; extractable iron did not vary with average surface water sulfate concentration (linear regression r^2 = 0.02). Sediment in control tanks contained less than 0.15 mg/g acid volatile sulfides (1 mol/L hydrochloric acid, Allen et al. **1991**) while sediment in 300 mg/L sulfate tanks contained over 1.75 mg/g in 2013.

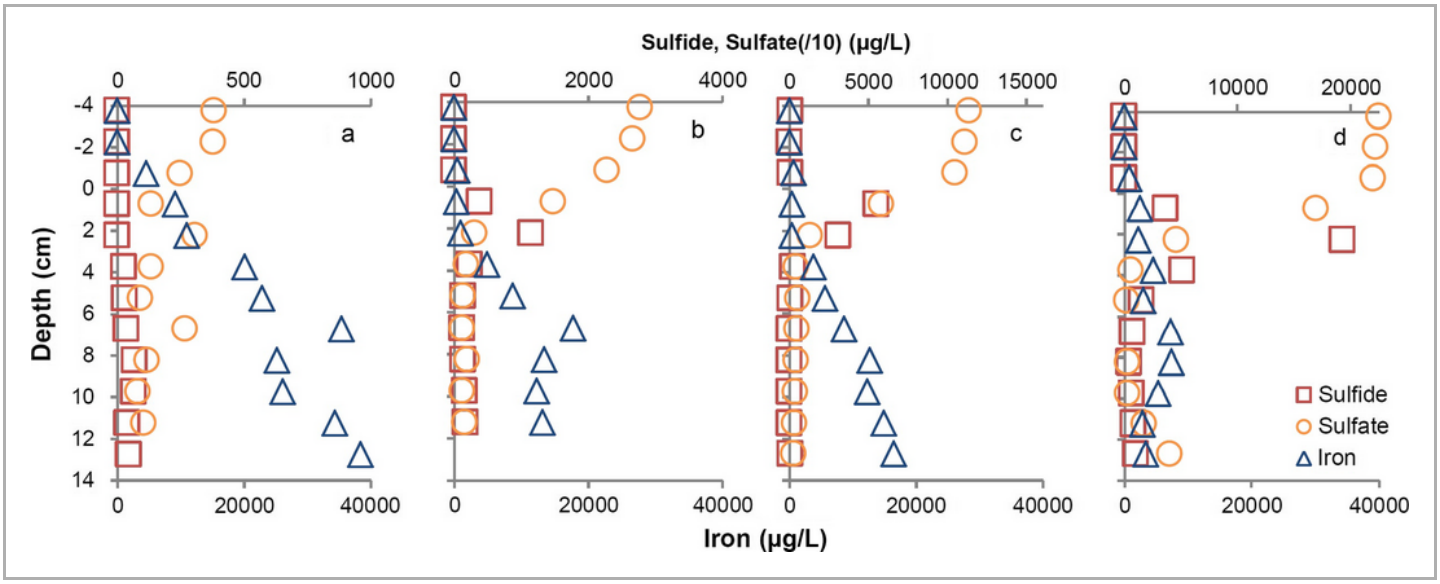


Figure 2.

Open in figure viewer

Vertical profiles of sulfate, sulfide, and iron in mesocosms with different measured sulfate concentrations in the overlying water measured during August 2013. Average annual overlying water sulfate concentrations were (a) 7.05 mg/L, (b) 37.2 mg/L, (c) 127 mg/L, and (d) 268 mg/L. Note different scales for sulfate and sulfide in panels b, c, and d. (Color figure can be viewed at wileyonlinelibrary.com.)

Porewater sulfide concentrations obtained from the upper 10 cm of sediment with Rhizon samplers were highly correlated with sulfate concentrations in the overlying water in both 2013 and 2015 (Fig. 3a). Concentrations were higher in 2015, and disproportionately higher in the higher sulfate treatments (Fig. 3b), which could be a consequence of progressively less precipitation with iron, which was a limited quantity.

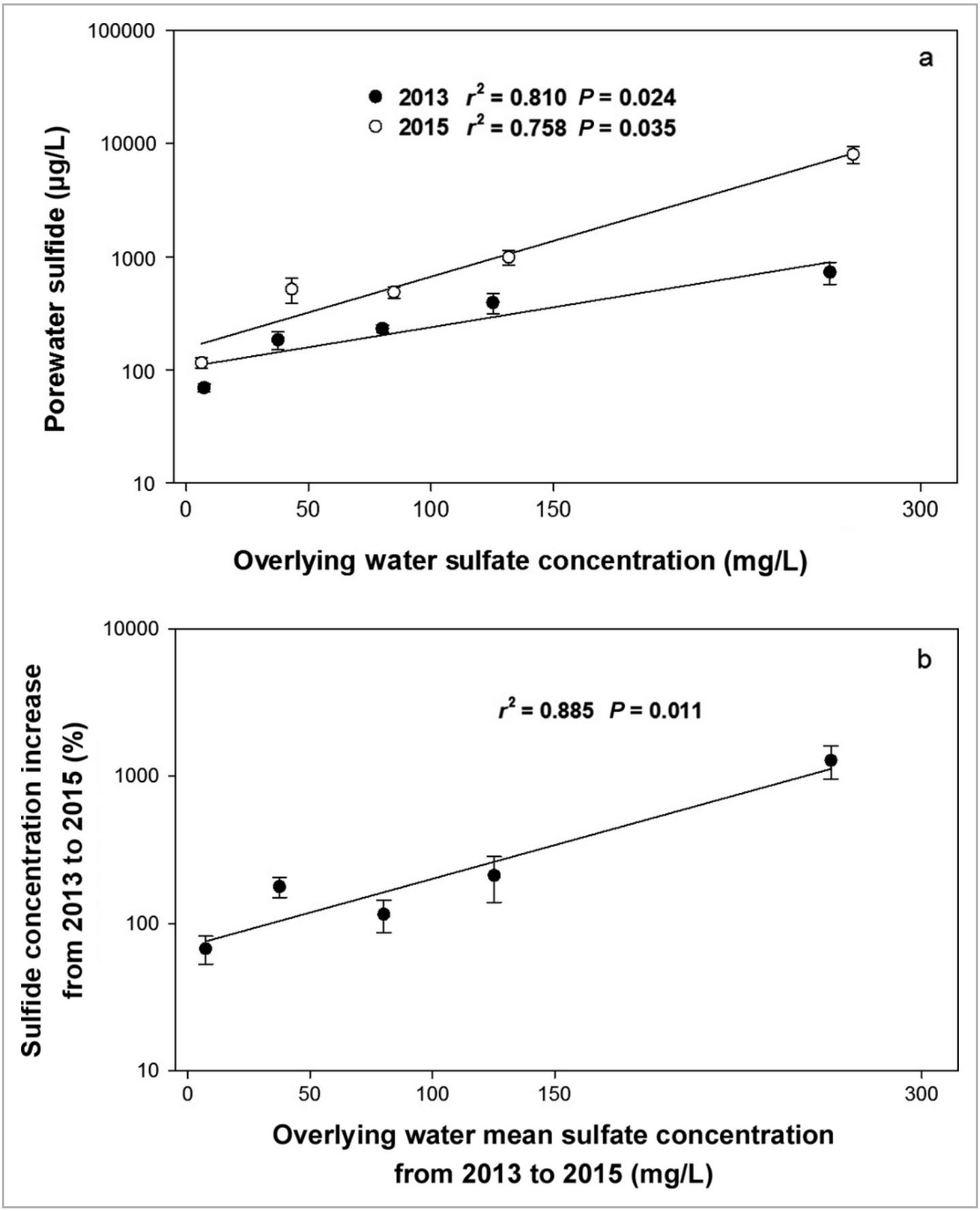


Figure 3.

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(a) Porewater sulfide concentrations are strongly correlated with measured concentrations of sulfate in overlying water in the mesocosms and (b) the sulfide concentrations increased from 2013 to 2015 in proportion to sulfate concentrations. Symbols are means and standard errors.

Effects of sulfate and sulfide on seedling emergence rate and seedling survival

In each spring after the initial planting in 2011, the number of seedlings that emerged from the sediment (Fig. 4a) declined significantly with increased sulfate concentrations ($P < 0.001$). Emergence rates differed from year to year ($P < 0.001$) but the rate of decline in seedling emergence with amended sulfate concentrations (slopes of regressions in Fig. 4a) did not change significantly from year to year (sulfate × year interaction $P = 0.598$).

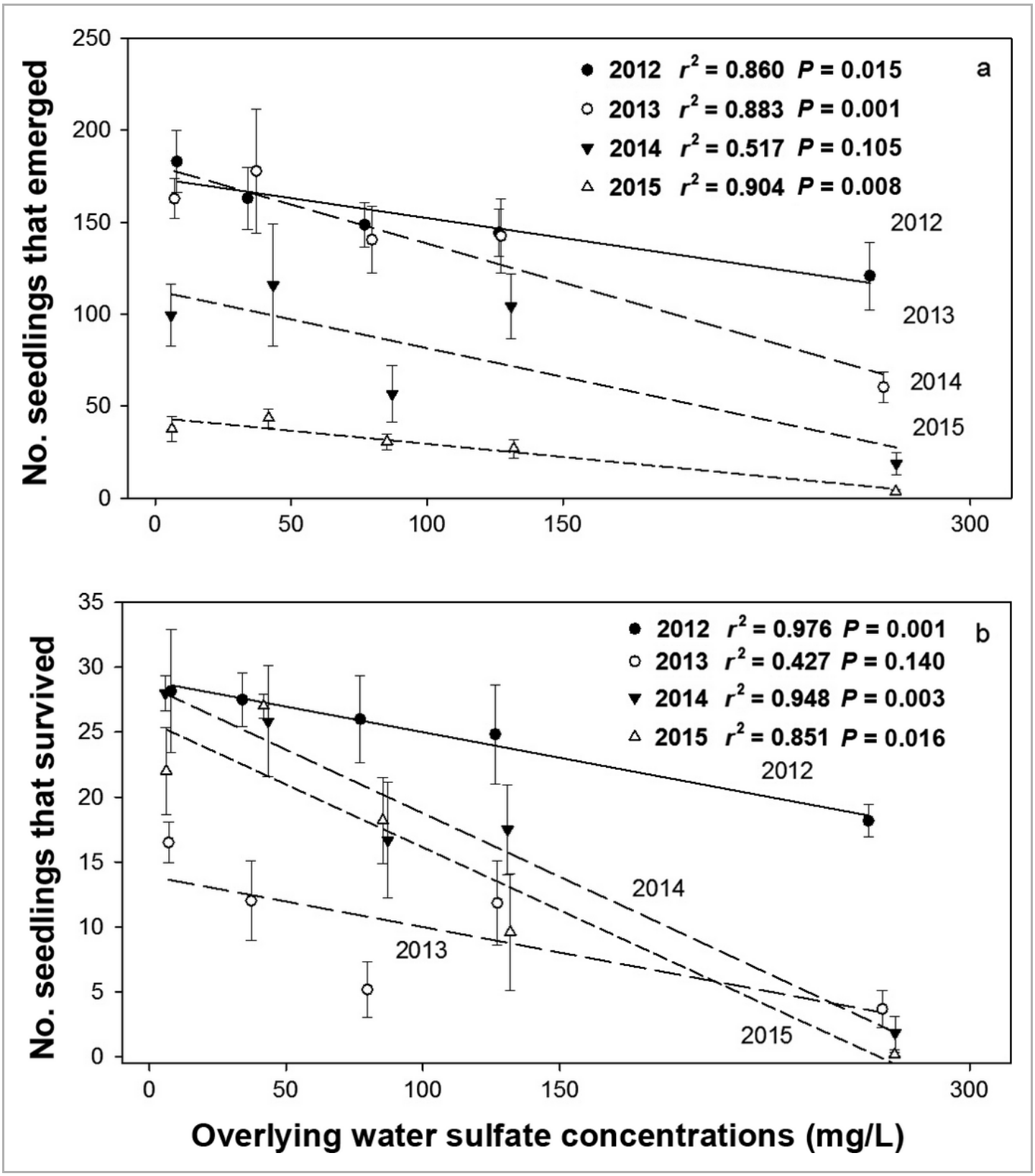


Figure 4.

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Emergence (a) and survival (b) of seedlings in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

The subsequent survival of those seedlings remaining after thinning (Fig. 4b) also declined significantly with increased sulfate concentrations ($P < 0.001$) and year ($P < 0.001$). The rate of decline in seedling survival with amended sulfate was twice as high in 2014 and 2015 than in 2012 and 2013. The number of surviving seedlings was not correlated with the number of seedlings that had been removed by thinning in any given year ($P > 0.10$), so the magnitude of thinning itself had no effect on seedling survival in the same year. The number of surviving seedlings was also not correlated ($P > 0.10$) with the production of straw litter from the previous year, so the decline in seedling survival was not an artifact of inhibition by thatch accumulation or nitrogen immobilization into fresh litter (Walker et al. **2010**).

In each year, there were no differences between control tanks and tanks amended to 50 mg/L SO_4, but seedling emergence and survival were significantly lower ($P < 0.05$) in tanks amended to 100 mg/L SO_4 or greater compared to control tanks.

Effects of sulfate and sulfide on vegetative growth

Elevated sulfate and presumably sulfide concentrations decreased plant biomass ($P < 0.001$) and the rate of decline increased significantly during the course of the experiment, but most especially in 2015 (sulfate × year interaction statistically significant at $P < 0.001$; see Fig. 5 and the figure legend for r^2 and P levels). By 2015, wild rice was extinct in all but one replicate in the 300 mg/L treatment, which supported only two plants. Root and shoot masses of individual plants were highly correlated ($r = 0.998$, $P < 0.001$) and root : shoot ratios were nearly constant between 0.210 and 0.224. Therefore, while the amounts of root and shoot productions were significantly affected by elevated sulfate concentrations, the proportional allocation of production between roots and shoots was not.

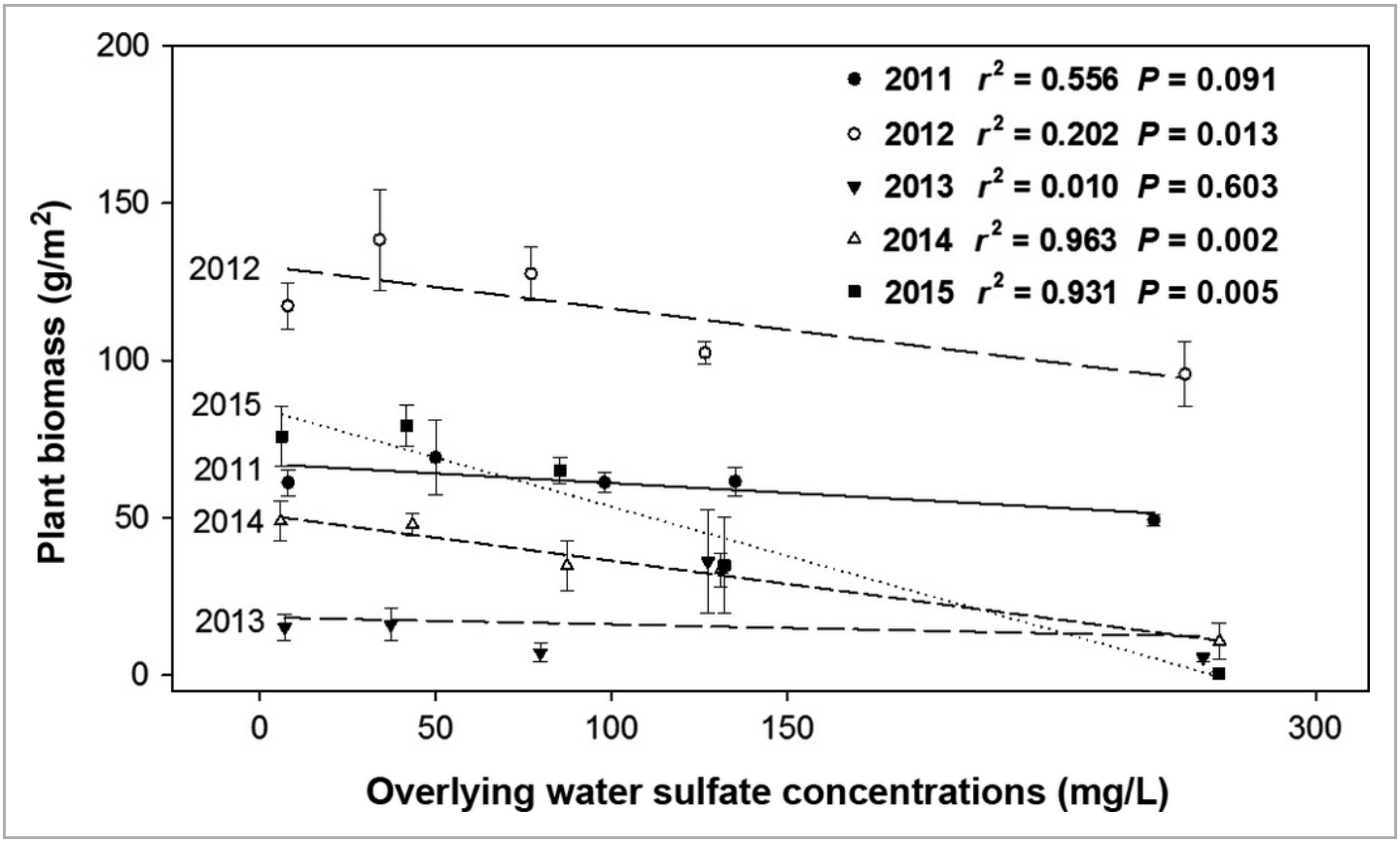


Figure 5.

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Vegetative biomass in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

Effects of sulfate and sulfide on seed production

The number of seeds produced per plant (both filled and empty, as determined from peduncle counts) did not change significantly across all sulfate concentrations (not displayed), but the proportion of seeds produced that were filled declined significantly with increasing sulfate concentrations (Fig. 6a, $P < 0.001$). Although 55–80% of seeds from control plants were filled during all four years, the slopes of the regressions of the proportions of filled seeds against sulfate concentration declined more steeply with each successive year (sulfate × year interaction significant at $P < 0.001$). By 2015, the proportions of filled seeds were as low as 25% in the tanks with the highest sulfate concentrations.

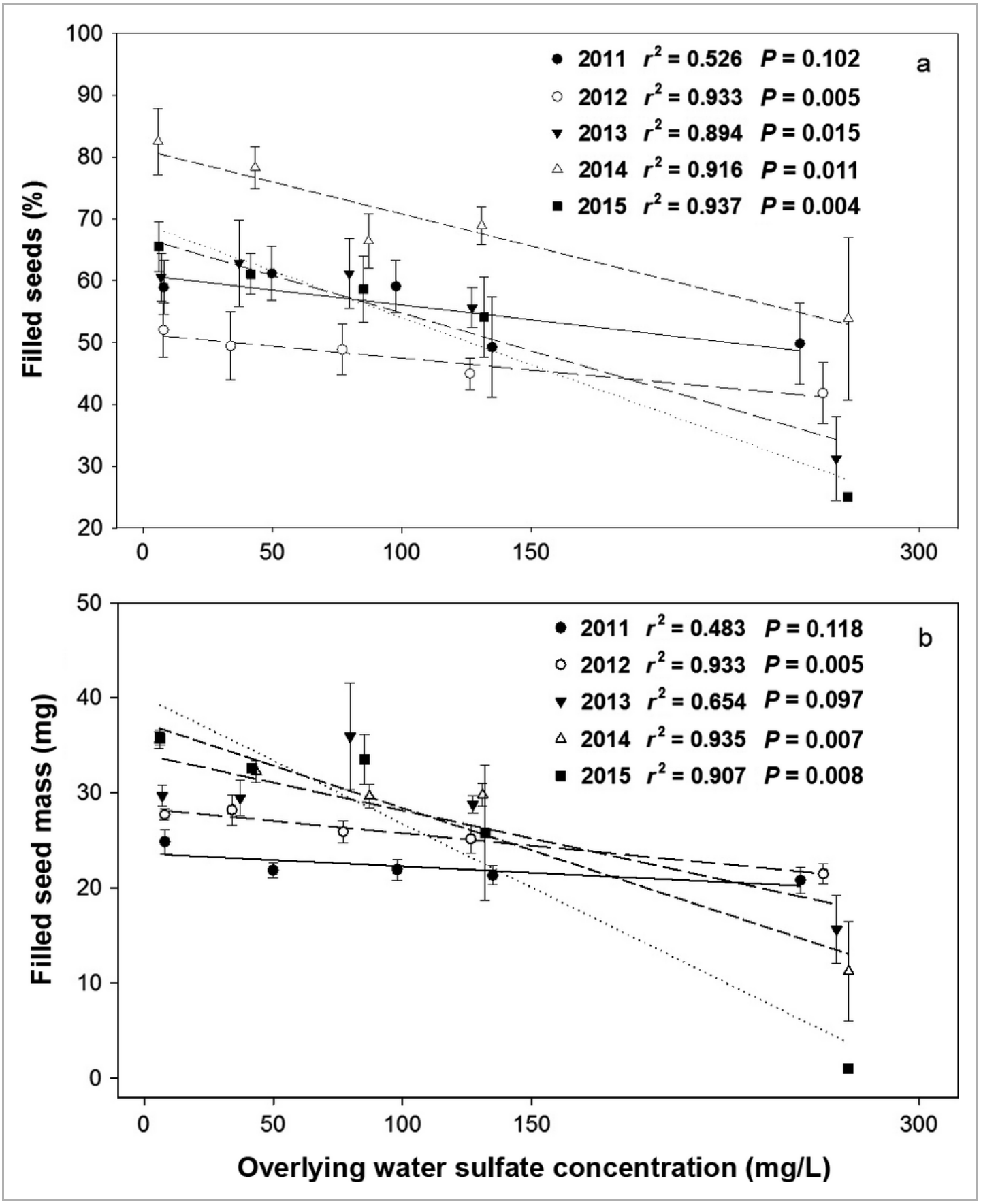


Figure 6.

Open in figure viewer

(a) The proportion of seeds that were filled and (b) the mean seed mass in mesocosms both declined with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

Individual seed masses declined with increased sulfate concentrations (Fig. 6b, $P < 0.001$). The seed masses declined more steeply with increasing sulfate concentrations with each successive year (sulfate × year interaction significant at $P < 0.001$).

In each year, seed production did not differ between control tanks and tanks amended to 50 mg/L SO_4, but seed mass and the proportion of viable seeds were significantly lower ($P < 0.05$) in tanks amended to 100 mg/L SO_4 or greater compared to control tanks.

Blackened roots associated with elevated sulfate

Beginning in 2012 and continuing for each subsequent year, plants in the tanks amended with sulfate had blackened roots while plants grown in the control tanks had white or light tan or orange roots when we harvested them at senescence. Visual estimates of the proportion of blackened roots increased progressively from approximately 50% in the tanks with sulfate concentrations approximately 50 mg/L to 100% in tanks with sulfate concentrations approximately 300 mg/L. These roots were pliable and white in cross sections cut with a knife, so they appeared to be still alive. In these cross sections, the blackening appeared to be crusted plaques on the root surfaces. The blackened roots from the 300 mg/L amended tank averaged 28.3% ± 9.8% Fe and 13.4% ± 4.6% S by mass, both much greater than tan/orange roots from the control tanks, which averaged 5.0% ± 3.9% Fe and 0.34% ± 0.29% S. We are investigating the chemistry of these plaques further, but our analyses thus far suggest that the blackening was caused by precipitation of some form of iron sulfide.

Discussion

Table 2 summarizes the major effects of sulfate and sulfide in these experiments. In the mesocosms, the correlation between sulfate concentrations in overlying water and sulfide concentrations in porewater (Fig. 3a) is so strong within a given year that we can reasonably use sulfate concentrations in overlying water as a surrogate for increased sulfide concentrations in sediment porewater. Porewater sulfide increased substantially between 2013 and 2015 (Fig. 3a, b). The sulfide production in these sulfate-amended mesocosms will eventually overwhelm the available iron and accumulate free sulfide in the porewater, which may be responsible for the disproportionately higher sulfide in the highest treatment in 2015 (Fig. 3b). The mesocosms did not mimic the steady state that occurs in the natural environment because sulfate in overlying water was resupplied but iron was not. Mechanistic models that include the interaction between sulfide and iron (e.g., Wang and Van Cappellen **1996**, Eldridge and Morse **2000**) include the continuous addition of iron from the overlying to the sediment, successfully modeling the steady-state relationship between sulfate, sulfide, and iron observed in the environment. The sedimentation of new iron to the sediment occurs in the natural environment, but was not included in this mesocosm experiment. Nevertheless, the experiment successfully exposed wild rice to progressively higher concentrations of porewater sulfide and documented the biological effects.

Table 2. Summary of the effects of sulfate and sulfide on the stages in the life cycle of wild rice

Wild rice life cycle stage	Effects of increased sulfate and/or sulfide	
	Hydroponic experiments	Mesocosm experiments
Germination rate	no effect of sulfate or sulfide	not assessed
Juvenile seedling growth	significant negative effect of sulfide, no effect of sulfate	not assessed
Seedling emergence from sediment	not assessed	significant negative effect of sulfate addition, probably a result of reduced seed viability rather than direct effects of sulfide
Seedling survival	not assessed	significant negative effect of sulfate addition, most likely through sulfide production
Mature plant growth	not assessed	significant negative effect of sulfate addition, most likely through sulfide production
Seed production (number of seeds per plant)	not assessed	no effect of sulfate or sulfide
Seed viability, both individual seed mass and proportion of filled seeds	not assessed	significant negative effect of sulfate addition, most likely through sulfide production

The porewater sulfide concentrations observed in natural waterbodies will vary depending on each site's surface water sulfate and sedimentary concentrations of organic matter and iron (Eldridge and Morse **2000**). The sediment organic matter and extractable iron in this experiment (8.1% and 8.3 mg/g) are within the range of 67 Minnesota wild rice waterbodies; organic matter is lower than the median of 9.1%, and the iron is higher than the median of 4.8 mg/g (5th to 95th percentiles of 0.9–31.0% and 1.6–15.3 mg/g, respectively; A. Myrbo, *unpublished data*).

Upwelling groundwater through sediment would cause a waterbody to deviate from the conceptual model presented here; upward groundwater flow would not only counter downward diffusion of sulfate, but could also supply water with chemistry completely different than the overlying water. In a survey of 46 Wisconsin lakes, Nichols and Shaw (**2002**) found that the occurrence of wild rice is associated with areas of inflowing groundwater. In some cases, upwelling groundwater may supply sulfate to the reduction zone in littoral sediments (Krabbenhoft et al. **1998**), so the effect of groundwater is unpredictable. Wild rice waters most likely to exhibit elevated porewater sulfide are those with relatively high organic matter, which allows enhanced microbial activity, and relatively low iron, which minimizes removal of porewater sulfide as a FeS precipitate (Heijs et al. **1999**, Eldridge and Morse **2000**).

Elevated sulfate concentrations were not directly toxic to wild rice seedlings in hydroponic solutions, in

agreement with results reported by Fort et al. (**2014**). But adding sulfate to overlying waters in the mesocosms with wild rice sediment increased porewater sulfide concentrations most strongly in the upper 5 cm of sediment in 2013, after three field seasons of sulfate amendments (Fig. 2). Sulfide was clearly toxic to early seedling growth in hydroponic experiments at concentrations above 320 µg/L, as indicated by slower growth or even zero or negative growth in a few cases (Fig. 1). Sulfide concentrations in excess of 320 µg/L were observed in the upper 5 cm of sediment when sulfate concentrations in the overlying water exceeded 20–50 mg/L (depending on season, Fig. 2).

The upper 2–5 cm of sediment is where seed germination and very early seedling growth most likely takes place. Wild rice seeds are shaped like torpedoes and penetrate the sediment aided by their long awns, which act as rudders and keep the seed vertical as it falls through the water column (Ferren and Good **1977**). It is likely that the seeds are buried in the upper 2–5 cm of this sediment where oxygen is low and sulfide concentrations are greatest (Fig. 2). To survive, the seedling must germinate in and grow through this zone of high sulfide concentrations. In nature, the mesocotyl may elongate up to 6 cm (Aiken **1986**), allowing a buried seed to emerge through up to "3 inches of flooded soil" (Oelke et al. **1982**). After emergence into the overlying oxygenated water, the mesocotyl differentiates into the mature stem. Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow between 50 and 100 cm before reaching the water surface, at which time floating leaves supply energy for root development (Aiken **1986**). This is consistent with the enhanced stem plus leaf growth of seedlings we observed under anaerobic conditions without elevated sulfide concentrations. Root growth, in contrast, was reduced by anaerobic conditions in our hydroponic experiments, as it has been previously observed for wild rice (Campiranon and Koukkari **1977**) and white rice (Kordan **1972**, **1974a** , **b**).

Elevated sulfide concentrations greatly reduced shoot and leaf elongation in our hydroponic experiments, particularly at concentrations greater than 320 µg/L. The toxic effect of sulfide on shoot and leaf elongation and seedling growth (Fig. 1) overrides the enhanced growth that normally happens under anaerobic conditions. Seedlings in the mesocosms with elevated sulfate (and hence sulfide) concentrations likely were inhibited from emerging successfully from the sediment and reaching aerobic conditions higher in the water column, resulting in reduced survival in the mesocosms.

It is possible that high ionic strength or salinity in the mesocosms with the higher concentrations of elevated sulfate could be the cause of reduced seedling emergence and survival. However, the hydroponic experiments demonstrated that seeds and seedlings could withstand sulfate concentrations of up to 1600 mg SO_4/L without adverse effects. This sulfate concentration is half the salinity of seawater (Schlesinger **1991**). Electrical conductivity in the mesocosms was correlated with sulfate concentrations but, in 2012, we saw only small effects of sulfate on seedling emergence and survival even though electrical conductivity was high then as it was in 2015. High ionic strength alone is therefore probably not the cause of the progressively greater declines in seedling emergence and survival in the mesocosms.

It is likely that the observed negative effects on wild rice seedling growth and survival can be directly attributed to the toxic effects of sulfide because of the coherence between the mesocosm experiments and the hydroponic experiments, which isolated the toxic effect of sulfide on seedling growth from any direct effect of sulfate. The progressive decline in seedling emergence and survival during the 5-yr course of the experiment could have resulted from increasingly greater sulfide concentrations (Fig. 3) and progressive titration of reactive forms of ferrous iron out of the system as insoluble iron sulfide. The cumulative effects of this progressive loss of reactive ferrous iron could have allowed more sulfide to remain in solution (Fig. 3) and

thereby have increasingly toxic effects on seedling emergence and survival. The possible loss of reactive ferrous iron during the 5-yr course of the experiment may have been partly responsible for the declines in population densities, even to extinction at the highest sulfate concentrations.

Elevated sulfate concentrations in the mesocosm water progressively reduced vegetative production over the five years, but to much less extent than seed production was reduced. The proportion of seeds that were filled, as well as their mean masses, decreased by over 30% and as much as 50% in the 300 mg/L mesocosm treatment by year five of the experiment. Reduced seed production and seed masses followed by reduced seedling emergence and survival the following year depressed population growth in successive years eventually driving wild rice populations to extinction at high sulfate concentrations. It is likely that this extinction was driven by reduced seed production, seedling emergence, and seedling survival that depleted the seed bank over the fine years of the experiment, and cumulative impacts on sediment chemistry from repeated sulfate additions could have exacerbated the decline.

The strong decline in measures of seed viability with increased sulfate concentrations at the end of the growing season (Fig. 6) compared with the weaker decline in vegetative growth in early to mid-growing season (Fig. 5) could not have been due to decreased N or P availability late in the growing season. Litter from the previous year has begun mineralizing N and P at this point in the growing season (Walker et al. **2010**, Hildebrandt et al. **2012**). The production of sulfide is correlated with many other chemical changes associated with the sulfate-enhanced anaerobic decay of organic matter (Lamers et al. **2002**), including increased phosphate solubility. Phosphorus availability could not be controlled independent of sulfide in sediment, and sediment porewater and overlying water phosphate concentrations were elevated in sulfate amended tanks (A. Myrbo, *unpublished data*) most likely because precipitation of sulfide with reduced iron liberates phosphate (Caraco et al. **1989**, Lamers et al. **2002**). Since N and P availability were likely not limiting late in the growing season, it is unlikely that reduced N or P availability were responsible for the decline in seed production with increased sulfate concentrations. Therefore, by deduction, it must have been uptake that was limiting.

Sixty percent of annual N uptake in wild rice plants occurs early in the growing season but there is a second burst of nitrogen and phosphorus uptake in August during seed filling and ripening (Grava and Raisanen **1978**, Sims et al. **2012a**). Even though N and P were most bioavailable in August when wild rice seeds were being developed and filled, there was coincident peak accumulation of sulfide in the sediment porewater (Fig. 2). When exposed to high sulfide concentrations, roots of white rice (*Oryza sativa*) often become suberized (Armstrong and Armstrong **2005**) with subsequent possible reduction in nutrient uptake across the thicker root membranes (DeLaune et al. **1983**, Koch et al. **1990**, Armstrong and Armstrong **2005**, Lamers et al. **2013**). Suberization of roots in response to high sulfide concentrations at this stage in wild rice's life cycle might inhibit nutrient uptake, resulting in fewer and smaller filled seeds.

Another possible mechanism for impaired nutrient uptake might be the precipitation of black iron sulfide plaques on the roots of plants that grew in mesocosms with elevated sulfate and sulfide concentrations. Our EDS analyses suggest that the tan or orange coatings on roots of plants grown under low sulfate concentrations may be iron hydroxide plaques, which are often found on healthy wild rice roots (Jorgenson et al. **2012**). The existence of tan or orange coatings, consistent with iron hydroxide plaques, strongly suggests that the immediate vicinity of the roots is oxidized when sulfate concentrations are low, most likely due to radial oxygen loss through the aerenchyma tissues within the roots (Stover **1928**, Colmer **2003**, Yang et al. **2014**). Blackened roots, however, are often observed in white rice (*Oryza sativa*) populations

subjected to elevated sulfate concentrations or organic carbon (Jacq et al. **1991**, Gao et al. **2003**, Sun et al. **2015**) and our EDS observations suggest that the blackened plaques on our roots are some form of iron sulfide. Sun et al. (**2015**) also found that these black plaques contain substantial amounts of iron sulfides. Precipitation of iron sulfide plaques on roots, whether a direct inhibitor of nutrient uptake or a harbinger of the encroachment of reducing conditions to nearer the root tissue, may be partly responsible for the reduced proportion of filled seeds as sulfate concentrations increased (Fig. 6). Further experiments using labeled ^{15}N would be useful to determine whether reduced nutrient uptake during seed filling is the cause of reduced seed production.

Suberization of roots and precipitation of iron sulfide plaques may not be independent. Enhanced suberization when the root tissue is exposed to sulfide (Armstrong and Armstrong **2005**) might cause decreased radial oxygen loss from roots of wetland plants (Joshi et al. **1975**, Gao et al. 2002, Armstrong and Armstrong **2005**). If radial oxygen loss from roots is essential to maintaining low concentrations of hydrogen sulfide in the immediate vicinity of roots (Eldridge and Morse **2000**), then sulfide concentrations in the rhizosphere could encroach nearer to the root surface when radial oxygen loss from roots is impaired. Iron (hydr)oxide present on or near the roots under these conditions could be reduced to iron sulfide and precipitated on the roots. Nutrient uptake during the stage of seed filling therefore might be impaired directly by suberization of roots followed by precipitation of iron sulfides on the roots if suberization reduces radial oxygen loss.

Conclusions

In our hydroponic experiments, elevated sulfide concentrations are directly toxic to seedlings. In our mesocosm experiments, sulfate amendments increased sulfide concentrations in the rooting zone, which then apparently decreased seedling emergence and survival. The reductions in seedling emergence and survival in the mesocosms are consistent with the toxic effects of sulfide on seedling growth in the hydroponic experiments.

The vegetative growth phase of wild rice's life cycle did not appear to be as strongly affected by sulfide as the production of viable seeds. The mechanisms behind reduced seed production and viability with increased sulfate and hence sulfide production in sediments are more difficult to discern, but may involve reduction of nutrient uptake during seed set by iron sulfide plaques on roots of mature plants (Jacq et al. **1991**) or by increased suberization with elevated sulfide concentrations later in the summer (Armstrong and Armstrong **2005**).

In natural wild rice ecosystems, the extent to which sulfate is reduced to sulfide, and to which sulfide persists in porewaters, are controlled by factors such as the sedimentary concentrations of iron and organic matter, and groundwater flow, among others, all of which may differ from the conditions in our mesocosms. But our experiments strongly suggest that the reduction of sulfate to sulfide in sediments, to the extent that it occurs in natural systems, may cause populations to decline by adversely affecting the reproductive phases of wild rice's life cycle.

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Notes

7 http://water.epa.gov/drink/contaminants/secondarystandards.cfm

8 http://climate.umn.edu/doc/journal/ice_out_recap_2013.htm

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RESEARCH ARTICLE
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Key Points:
- Factors controlling pore water sulfide, which can be toxic to wild rice, were identified with structural equation modeling
- Structural equation modeling was used to quantify the relative effects of sulfate, iron, and TOC on pore water sulfide concentrations
- The concentration of pore water sulfide is controlled nearly equally by sulfate in surface water and sediment organic carbon and iron

Supporting Information:
- Supporting Information S1
- Data Set S1
- Figure S1
- Figure S2
- Figure S3
- Figure S4
- Figure S5
- Figure S6
- Figure S7
- Figure S8

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The Evolution of Sulfide in Shallow Aquatic Ecosystem Sediments: An Analysis of the Roles of Sulfate, Organic Carbon, and Iron and Feedback Constraints Using Structural Equation Modeling

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Abstract The generation of elevated concentrations of sulfide in sediment pore waters that are toxic to rooted macrophytes is problematic in both marine and freshwaters. In marine waters, biogeochemical conditions that lead to toxic levels of sulfide generally relate to factors that affect oxygen dynamics or the sediment iron concentration. In freshwaters, increases in surface water sulfate have been implicated in decline of *Zizania palustris* (wild rice), which is important in wetlands across the Great Lakes region of North America. We developed a structural equation (SE) model to elucidate key variables that govern the evolution of sulfide in pore waters in shallow aquatic habitats that are potentially capable of supporting wild rice. The conceptual basis for the model is the hypothesis that dissimilatory sulfate reduction is limited by the availability of both sulfate and total organic carbon (TOC) in the sediment. The conceptual model also assumes that pore water sulfide concentrations are constrained by the availability of pore water iron and that sediment iron supports the supply of dissolved iron to the pore water. A key result from the SE model is that variations in three external variables (sulfate, sediment TOC, and sediment iron) contribute nearly equally to the observed variations in pore water sulfide. As a result, management efforts to mitigate against the toxic effects of pore water sulfide on macrophytes such as wild rice should approach defining a protective sulfate threshold as an exercise tailored to the geochemistry of each site that quantitatively considers the effects of ambient concentrations of sediment Fe and TOC.

Plain Language Summary Aquatic plants, such as wild rice, white rice, and waterlilies that have roots in the saturated soils of wetlands are vulnerable to the buildup of toxic levels of hydrogen sulfide (also called sulfide). Anaerobic bacteria in the soil make the sulfide from sulfate that penetrates the soil from the overlying water. When sulfate in the waterbody is low, sulfide in the soil is low. But when sulfate is high, sulfide has been hard to predict—sometimes low, sometimes high. The analysis of hundreds of wetland samples finds that sulfide can be predicted if two variables in addition to sulfate are considered: organic carbon in the soil, which is the food for the bacteria, and iron in the soil, which removes sulfide from solution. A model of the chemical reactions finds that the three variables, sulfate, organic carbon, and iron, are equally important in determining sulfide. The sensitivity of individual waterbodies to sulfate pollution effects on wild rice toxicity can thus be predicted from the analysis of the carbon and iron concentrations in the soil of a wetland.

1. Introduction

The evolution of sulfide concentrations in sediment pore waters can be toxic to rooted macrophytes in both marine and freshwater environments. Pore water sulfide is the product of anaerobic microbial respiration of sulfate in sediments. Despite the much different sulfate concentrations between the two environments —sulfate concentrations are generally orders of magnitude higher in marine systems than most continental waters—the biogeochemical principles governing the reduction of sulfate to sulfide are the same, and there should be recognition of the commonality of sulfide toxicity concerns and biogeochemical relationships. Here we describe a model of the roles of sulfate, sediment total organic carbon (TOC), and sediment iron (Fe) in determining potentially phytotoxic concentrations of sulfide in relatively sulfate-poor waters. Essentially the same question, the potential toxicity of sulfide to coastal seagrasses as a function of sulfate,

TOC, and Fe has been pursued since at least 2000 (e.g., Eldridge & Morse, 2000). The biggest difference in the approaches between the two environments is that the primary option to reduce sulfide toxicity in freshwater systems has been to control sulfate, whereas other controlling variables (e.g., TOC or water clarity) are not only more practical for control in coastal systems but are more likely to have been altered by human activity.

For example, sulfide has been implicated as the causative agent in a global decline of coastal seagrasses. In this case, the perturbations to sulfur cycling that lead to increased sulfide toxicity relate to anthropogenic activities that reduce light availability. Seagrasses are dependent on high light levels to produce sufficient oxygen to detoxify pore water sulfide and so are particularly vulnerable to any actions that reduce light availability, such as eutrophication or dredging (Orth et al., 2006). In addition, other factors that enhance pore water sulfide concentrations have been implicated in the decline of seagrasses, such as increased temperature (Koch & Erskine, 2001), increased sediment organic matter (Govers et al., 2014), iron-poor sediments (Marbà et al., 2008), and low water column oxygen (Borum et al., 2005).

Similar concerns about sulfide toxicity extend to freshwater systems (Lamers et al., 2013). For example, based on laboratory experiments demonstrating that *Cladium* is less tolerant of sulfide than *Typha*, sulfate enrichment has been implicated in the expansion of *Typha domingensis* (cattail) into areas once dominated by *Cladium jamaicense* (sawgrass) in the Florida Everglades (Li et al., 2009). One plant species of particular concern with respect to sulfide toxicity is *Zizania palustris* (wild rice), which is important in wetland environments across the Great Lakes region of North America, where it can occur in dense monocultures. This importance includes both its role as a food resource for waterfowl and humans and its economic significance to those who harvest and market it. Wild rice is also a very important cultural resource to many Minnesotans, including indigenous peoples such as the Ojibwe peoples who consider wild rice as sacred (Vennum, 1988).

Wild rice is an annual grass that grows best at water depths of 0.3 to 1.0 m in lakes, rivers, and wetlands with soft organic sediment, although it can be found rooting in a wide variety of sediment types, ranging from muck to sand (Aiken et al., 1988). Wild rice seeds are dropped into the water in the fall, lie in the sediment throughout the winter, and germinate in spring. Its occurrence and distribution are influenced by a variety of physical, hydrologic, and biogeochemical factors (Myrbo, Swain, Engstrom, et al., 2017). For example, the rate of seed germination declines greatly if the seeds dry; as a result, wild rice is usually found in permanent waterbodies. Reduced light availability, from turbidity or water color, limits the ability of wild rice to maintain a population in a waterbody (Myrbo, Swain, Engstrom, et al., 2017), because seedlings need photosynthesis to augment the seed's energy reserves in order to successfully reach the water surface (Aiken, 1986).

Sulfate has long been implicated as a variable that influences the occurrence and distribution of wild rice in Minnesota. The first such implication was by Moyle (1944) who, in an analysis of spatial variations in water chemistry in Minnesota surface waters, observed "No large stands of rice occur in waters having a SO_4 content greater than 10 ppm, and rice generally is absent from water with more than 50 ppm." This cognition that wild rice occurrence was associated with waters containing lower levels of sulfate, coupled with the importance of wild rice in Minnesota, led to the adoption of a sulfate standard to protect wild rice of 10 mg/L in 1973 by the state of Minnesota. However, the role of sulfate in limiting wild rice habitat has long been confusing, given that wild rice has been observed to grow well in elevated sulfate waters overlying silica sand substrate and in commercial paddies with sulfate concentrations of 170 mg/L (Aiken et al., 1988).

More recently, a series of studies commissioned by the Minnesota Pollution Control Agency (MPCA) indicate that sulfide in sediment pore water is a causative agent for adverse effects to wild rice and that any effect of sulfate is rather more likely indirect through any controls it exerts on the development of sulfide concentrations in pore waters. These studies include a field survey comprising one or more sampling trips to 93 lakes, 32 stream sites, and 7 wild rice paddies, (Myrbo, Swain, Engstrom, et al., 2017), 10 day hydroponic experiments, and multiyear mesocosm studies (Myrbo, Swain, Johnson, et al., 2017; Pastor et al., 2017).

The goal of the MPCA-sponsored research is to determine if it is appropriate to regulate sulfate in order to protect wild rice from sulfide toxicity. The relationship between surface water sulfate and pore water sulfide concentrations, however, is complex, and a standard that seeks to mitigate against the occurrence of sulfide concentrations harmful to the occurrence of wild rice by limiting sulfate concentrations should consider these complexities. These complexities are illustrated by considering the effects of both substrate limitation and geochemical thermodynamics. More specifically, sulfate reduction to sulfide is a microbially mediated process that involves sulfate-reducing bacteria using sulfate as a terminal electron acceptor to metabolize

organic matter in the absence of oxygen. Thus, the availability of both sulfate (Capone & Kiene, 1988; Cook et al., 1986; Perry et al., 1986; Sinke et al., 1992; Myrbo, Swain, Johnson, et al., 2017) and of organic carbon (Al-Raei et al., 2009; Glombitza et al., 2013) can limit sulfate reduction. Conditions favorable to sulfate reduction also can result in iron reduction from insoluble Fe(III) to soluble Fe(II). Fe(II) in turn can react with sulfide to form insoluble FeS and other iron-sulfide minerals (Hansel et al., 2015; Hines & Jones, 1985; van Cappellen & Wang, 1996).

Any model that relates surface water sulfate to pore water sulfide concentrations thus has to consider issues related to model specification—that is, whether the model includes the appropriate variables and whether the intervariable relationships posed in the model are truly representative of the underlying processes and satisfy the inherent assumptions of the model framework. Model specification errors thus can lead to erroneous coefficient estimates for a governing variable, including errors in both magnitude and direction. *Statistical* models that might be developed for the sulfate-sulfide problem also have to accommodate intervariable relationships that include both indirect (mediated) and nonrecursive (i.e., feedback) relationships. Traditional multivariate regression models only control for direct or apparent direct (net) effects and thus are ill suited for quantifying relationships that are either mediated or nonrecursive. As a result, similar to model estimation problems imposed by specificity errors, the misapplication of multivariate regression models can lead to misleading parameter coefficient estimates and a failure to properly quantify the components contributing to the *total* effect of a variable such as sulfate on a response variable such as sulfide, particularly if the model includes mediating variables (Grace et al., 2009; Pollman, 2014).

One approach that allows for and more properly accounts for the effects of mediating variables is structural equation modeling (SEM). Based on the hypothesized relationships that constitute the model, SEM seeks to reproduce the covariance structure of the observed data (Ullman, 2007). SEM can include models comprising measured or observed variables only (path analysis), models that include latent variables representing unmeasured processes (confirmatory factor analysis), or models that link both path and confirmatory analysis together. SEM is often referred to as *causal analysis* (Ullman, 2007) because the approach often is used to test or confirm an a priori, hypothesized model (Austin, 2007). While SEM does not actually prove causality (Bollen & Pearl, 2013; Weston & Gore, 2006), it does provide a framework for selecting or rejecting hypotheses based on the empirical data (Iriondo et al., 2003). Examples include using SEM to model macroinvertebrate distribution in riverine ecosystems (Bizzi et al., 2013) and estuaries (Malaeb et al., 2000), aquatic and estuarine macrophytes (Gustaffson & Boström, 2013; Hung et al., 2007), lacustrine phytoplankton dynamics (Arhonditsis et al., 2006; Korhonen et al., 2011; Liu et al., 2010), and methyl Hg cycling and bioaccumulation in the Florida Everglades, including mediated effects of surface water sulfate (Pollman, 2014). In addition, SEM has been used to construct models with a clear environmental management focus (La Peyre et al., 2001; Reckhow et al., 2005; Stober et al., 2001). For example, La Peyre et al. (2001) used SEM to model national wetland protection efforts as a function of five latent variables (defined as economic capital, social capital, government characteristics, environmental characteristics, and land use pressure). Their model suggested that variations in social capital had the greatest influence on wetland protection efforts and concluded that continued focus on social development was necessary to further wetland protection.

2. Objectives and Conceptual Model

The objective of this paper is to use SEM to define the functional role of surface water concentrations of sulfate vis à vis the development of pore water concentrations of sulfide, recognizing that the relationship is complex and likely involves direct, mediated, and feedback pathways. Based on the functional relationships we believe a priori to be important, we can construct a conceptual model that reflects those relationships and use SEM to test both the overall reasonableness of the model and the significance and importance of the individual components or pathways. Conceptually, our model has four components. The first component considers that the magnitude of sulfide production in the sediment pore water expectedly is related to sulfate concentrations in the water column (e.g., Cook & Schindler, 1983; Geurts et al., 2009; Siver et al., 2003; Myrbo, Swain, Johnson, et al., 2017). The second component is that the availability of sedimentary organic carbon can limit sulfate reduction (Al-Raei et al., 2009; Glombitza et al., 2013). The third component is that amorphous FeS or mackinawite formation governs the dynamics of pore water sulfide and Fe(II) concentrations:

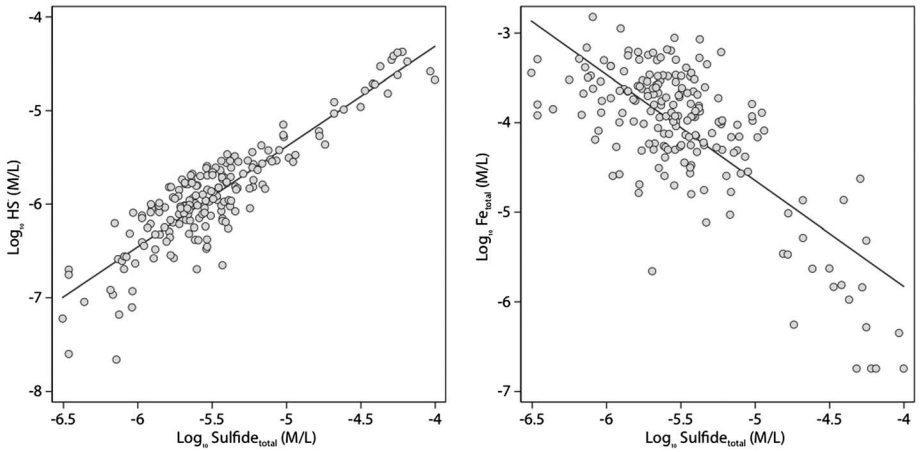


Figure 1. Key bivariate relationships for pore water sulfide relevant to assumptions in conceptual sulfide model. Left-hand panel: HS^- concentrations as a function of measured total pore water sulfide. HS^- concentrations based on acidity constants for H_2S and measured pore water pH. Right-hand panel: total dissolved pore water Fe concentrations as a function of measured total pore water sulfide. All variables are $\log_{10}$ molar concentrations. Solid lines denote line of best fit obtained from linear regression. The 70.4% of the variance in the calculated values of the ($\log_{10}$-transformed) concentration of HS^- is due to the variance in ($\log_{10}$-transformed) concentrations of total sulfide; shared variance between total sulfide and the pH-dependent speciation fraction for HS^- accounts for another 5.0%. The coefficient of determination for the pore water Fe and total sulfide relationship (both variables $\log_{10}$ transformed) is 0.547 ($p < 0.0001$).

$$Fe(II) + HS^- \leftrightarrow FeS + H^+ \quad (1)$$

Inherent in our implementation of the model is the notion that pore water concentrations of total sulfide are a reasonable proxy for HS^-. Given that assumption, pore water Fe(II) and total sulfide concentrations should be inversely related (cf. Maynard et al., 2011); both assumptions are borne out by bivariate plots of the variables of interest (Figure 1; see also Myrbo, Swain, Engstrom, et al., 2017) constructed from our field data (see section 3.1 below). The last component of the conceptual model is that sediment Fe concentrations influence the pore water Fe concentrations that can develop under reducing conditions. This is an extension of the fact that sediment Fe must be present for dissolved Fe(II) concentrations to develop in the pore water, unless an alternative source of dissolved Fe(II) such as via shallow groundwater discharge is important.

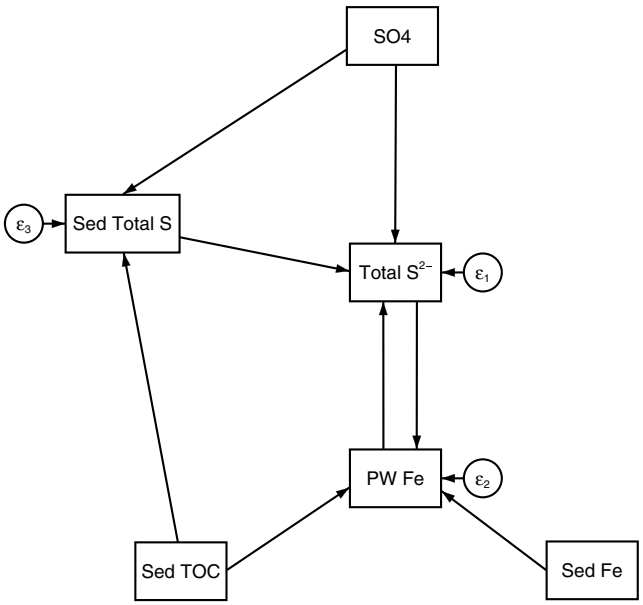


Figure 2. Conceptual model for biogeochemical controls on pore water sulfide in wetland sediments. Sed refers to sediment solid phase concentrations (mass parameter per dry mass sediment). PW Fe is pore water Fe; total S_2^- is total pore water sulfide, and SO_4 is surface water SO_4.

The resultant conceptual model is shown schematically in Figure 2 and includes pore water sulfide, pore water Fe, and sediment total sulfur (S) as dependent or endogenous variables and sulfate, sediment Fe, and sediment total organic carbon (TOC) as independent or exogenous variables. The model also includes a mediated pathway for the effect of sulfate on pore water sulfide through links between sulfate and sediment total S and between sediment total S and pore water sulfide. This mediated pathway considers that a second route for sediment sulfur accretion is by biogenic uptake of sulfate in the water column (Pérez-Castiñeira et al., 1998) followed by deposition to and incorporation within the bottom sediments. An important feature of the conceptual model is that, consistent with the thermodynamic or equilibrium chemistry defined by equation (1), the link between pore water sulfide and Fe is nonrecursive rather than recursive. Recursive models allow for causation to flow in a single direction only, while nonrecursive models allow for feedback relationships such as the thermodynamic relationship between pore water Fe(II) and HS^-. While the use of nonrecursive models to model aquatic ecosystems has precedent—for example, evaluating the reciprocal relationships between sediment substrate

characteristics and seagrass density (Folmer et al., 2012; van der Heide et al., 2011)—we believe that our use of a nonrecursive SEM to model pore water chemistry is the first such application.

3. Materials and Methods

3.1. Data

The data used to construct and evaluate the structural equation (SE) model were collected by the MPCA as part of a field survey designed to investigate the relationship between physical and chemical conditions in shallow waterbodies and the presence and absence of wild rice (Myrbo, Swain, Engstrom, et al., 2017). The field survey included pilot sampling conducted in 2011 comprising 58 samples collected from 48 different lake and stream sites, followed in 2012 and 2013 by a field survey that was broadened to include more sites with elevated sulfate concentrations and 7 different cultivated wild rice paddies. The broader survey included 121 different waterbodies, of which 40 were sampled on multiple occasions across time (2 to 6 times, except for one site sampled 10 times). The broader survey also included some sites that were also sampled during the pilot survey; thus, the total number of unique sites sampled during the entirety of the 3 year field survey included 131 different sites, with a total of 260 site visits. Field sampling included surface water, sediment, and pore water samples for a variety of physical, chemical, and habitat related variables, although some site visits were not able to collect the full suite of samples. Sampling protocols and methods for more than 65 parameters potentially measured at each site are detailed in Myrbo, Swain, Engstrom, et al., (2017).

Several considerations guided the selection of observations from the field survey data set for developing the SE model. SEM is generally regarded as data intensive, although perhaps not more so than multivariate regression (McCune & Grace, 2002). Estimates of minimum sample sizes to adequately conduct SEM vary but generally are related to the complexity of the model. Simulation studies by Jackson (2003), for example, suggest that improved model performance is achieved as the ratio of the number of observations to fitted model parameters increases. Model performance, however, appears to be more sensitive to the absolute number of samples (Jackson, 2003), with a typical sample size for SE models published in the literature approximating 200 (Kline, 2011). As a result, our overarching principle in data selection was to maximize the number of samples available to fit the model without compromising the integrity of the analysis. For example, we excluded paddy sites from the wild rice model data set because modeling natural systems is our ultimate goal, and paddies are manipulated in ways that likely cause the biogeochemistry to differ from natural sites. For instance, N and P fertilizers are used and, in a fundamental departure from natural sites, paddies are dewatered each summer, which may alter the oxidation state of the sediment. After eliminating paddies, we further screened the data set to exclude those sites that did not have a complete set of measurements for surface water sulfate, pore water sulfide, pore water Fe, sediment Fe, sediment TOC, and sediment total S. This latter set of criteria also resulted in the exclusion of a relatively small number of samples. In an effort to assess potentially redox-active Fe, sediment Fe in this data set was quantified using a 0.5 N HCl extraction (Myrbo, Swain, Engstrom, et al., 2017), rather than a stronger acid digestion, which would have included relatively inert Fe.

We conducted preliminary multiple linear regression (MLR) analyses, using pore water sulfide as the dependent variable and surface water sulfate, sediment TOC, and sediment Fe as the independent variables, to identify possible outliers and influential observations. Based on the MLR analyses, we identified two clear outliers (Hoffs Slough and one sample from Sandy Lake) based on both inspecting model residuals and plotting values for hat leverage versus Cook's D distance (Hamilton, 2013). Hoffs Slough is a ditch in an agricultural area, while the Sandy Lake observation is one of a total of 10 samples collected from Sandy Lake; as a result, both outliers were excluded from further analysis. In addition, we excluded two observations with unusually high pore water sulfide (Bean and Lady Slipper; pore water sulfide 16.0 and 14.8 mg/L, respectively; the next highest sulfide concentration was 3.19 mg/L).

The resultant screened data set, which was named for internal purposes "Class D," includes a total of 194 observations from 111 different waterbodies. This number of observations was considered sufficient to engage in SEM given the relatively simple nature of the conceptual model and is consistent with the number of observations used by many published studies (Kline, 2011). The data include 20 observations collected in 2011, 107 in 2012, and 67 in 2013. Because 43 of the Class D sites were sampled more than once, we created a subset called "Class B" with which to compare results. Class B consists of one sample from each site and excludes samples collected in 2011, which were analyzed with slightly different lab methods (see Myrbo,

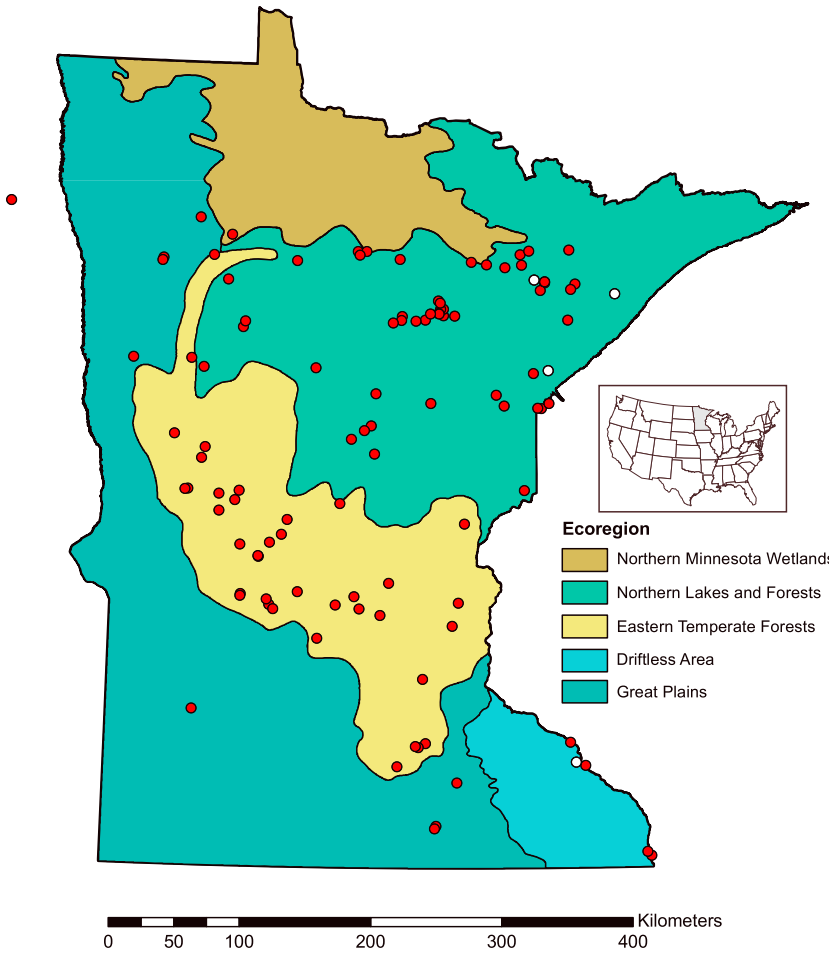


Figure 3. Locations for lakes and stream sites (Class D and Class B) sampled by Myrbo, Swain, Engstrom, et al. (2017) used to construct the SE model. Red-filled circles show sites that belong to both Class D and Class B data sets, while the open circles show sites that belong solely to Class D. The single site in North Dakota (westernmost site) lies in the Great Plains ecoregion.

Swain, Engstrom, et al., 2017). The Class B data thus include a set of 105 unique observations with a full complement of measured model parameters, including 78 lake and 27 stream sites (Figure 3).

3.2. Statistical Analysis and Modeling

3.2.1. Model Estimation

We conducted all statistical analyses, including SEM, using Stata 14 (StataCorp, 2015). Two key underlying assumptions with SEM are multivariate normality of the endogenous variables and linear relationships between the endogenous and exogenous variables (Kline, 2011; Ullman, 2007). We $\log_{10}$-transformed all variables both to improve linearity and to yield variable distributions that more closely approximated normality, although multivariate normality was not achieved.

When multivariate normality is uncertain, estimation methods that assume multivariate normality can produce misleading or incorrect estimates of the standard error of the individual model coefficients. Several approaches are potentially useful when estimating SEM models with nonnormal multivariate data distributions. These methods include *asymptotic distribution-free* (*adf*) estimation (Acock, 2013; Kline, 2011), bootstrapping (Acock, 2013; McIntosh, 2007; Tomarken & Waller, 2005), and estimation using the Satorra-Bentler scaled χ^2 test (StataCorp, 2015; Tomarken & Waller, 2005). The *adf* method makes no assumptions regarding data normality but may require in excess of 200 to 500 observations for even relatively simple models (Kline, 2011). Bootstrapping, which uses resampling with replacement of the individual observations to produce estimates of the standard errors and resulting significance of the model coefficients, also makes

no assumptions regarding underlying multivariate normality and thus produces more robust estimates of coefficient confidence. The Satorra-Bentler test seeks to correct the χ^2 statistic by scaling the estimate based on the kurtosis of the data (Kline, 2011). Initial analyses implemented all three methods. Bootstrapping was conducted using 1,000 replications. We used comparative evaluations of the fitted model coefficients (the *adf* method can yield model coefficients that differ compared to the maximum likelihood estimation used by the bootstrapping and Satorra-Bentler methods) and their significance, which indicated that estimation of the final model was relatively insensitive to the choice of estimation method coefficients. However, because *adf* methods can be misleading with sample sizes of fewer than 200, final model estimation was conducted using the Satorra-Bentler test and bootstrapping.

3.2.2. Model Robustness

A critical assumption that SEM shares with linear regression is that the underlying relationships between a given endogenous variable and the variables that influence it are indeed linear. Unlike SEM, MLR has a rich set of tools available for a variety of post hoc analyses to evaluate adherence to underlying model assumptions. Given the similarities between path analysis (of which our modeling herein is a form) and MLR (Li, 1975), we verified linearity by initially conducting MLR on pore water sulfide concentrations using the same variables used to directly model pore water sulfide in the SE model. We then evaluated the presence of nonlinearities and apparent outliers by constructing augmented component plus residual (ACPR) plots for each direct independent variable (Hamilton, 2013; Mallows, 1986; see supporting information Figure S1).

3.2.3. Assessment of Goodness of Fit

We assessed model goodness of fit using several different metrics. Because SEM seeks to reproduce the observed covariance matrix for the variables in the model, a primary metric for model performance is a chi-square (χ^2) test that assesses the magnitude and significance of the discrepancy between the fitted covariance matrix for the model in question and the observed covariance matrix. Restated, the χ^2 statistic compares the covariance matrix for the model we seek to evaluate with that of a model that has no degrees of freedom and thus fits the observed data perfectly. The null hypothesis is that the two models are not different. χ^2 p values <0.05 appear to be a commonly used threshold for rejecting the null hypothesis and thus rejecting the model (e.g., Bizzi et al., 2013; Hayduk & Glaser, 2000; Hu & Bentler, 1999), although this threshold is controversial (cf. Barrett, 2007; Lance et al., 2006; McIntosh, 2007).

The χ^2 test is the approach most used to evaluate the fit of SEM models (Grace, 2008) and was used in this study as the initial criterion for retaining a given model. Other fit indices used as alternatives or adjuncts to the χ^2 test include both absolute and incremental or approximate fit indices (Hu & Bentler, 1999; Kline, 2011). Stata provides several alternative fit indices of both types, including the root-mean-square error of approximation (RMSEA), the standardized root-mean-square residual (SRMR), the comparative fit index (CFI), and the Tucker-Lewis index (TLI). Recommended target values for the RMSEA and SRMR are 0.05 and 0.08, respectively, while values greater than 0.95 are recommended targets for both the CFI and TLI (Acock, 2013; Hu & Bentler, 1999).

3.2.4. Model Validation

We validated the SE model by conducting a "leave one out cross validation" or jackknife analysis where we refit the SEM model by withholding a single observation from model estimation and then used the refit model to predict the $\log_{10}$-transformed value for pore water sulfide of the withheld observation. This process was repeated for each of the observations comprising the Class D and Class B data sets. A major advantage of this process is that it uses all of the available observations to both fit and validate the model.

3.2.5. Alternative Model Specifications

As mentioned previously, the issue of model specification is an important consideration. As a result, we also expanded our conceptual model to consider several other model specifications. These models included

1. expanding the model to include sediment acid volatile sulfide (AVS). AVS is an operationally defined, reactive sulfide fraction that is believed to comprise dissolved hydrogen sulfides and mackinawite (Morse & Rickard, 2004) and thus arguably better represents the pool of reduced sedimentary sulfur actively exchanging with pore water sulfide. A primary feature of this model thus was to include a second reciprocal relationship involving pore water sulfide—viz., a path between pore water sulfide and sediment AVS;
2. identifying pore water dissolved organic carbon (DOC) as an exogenous variable. The rationale behind evaluating pore water DOC was twofold. First, DOC forms strong complexes with dissolved Fe^{2+} (Jansen et al., 2002) that can then reduce the concentration of free Fe^{2+} available to bind with sulfide and form amorphous FeS or mackinawite. The SE model does not consider dissolved Fe or sulfide speciation, and

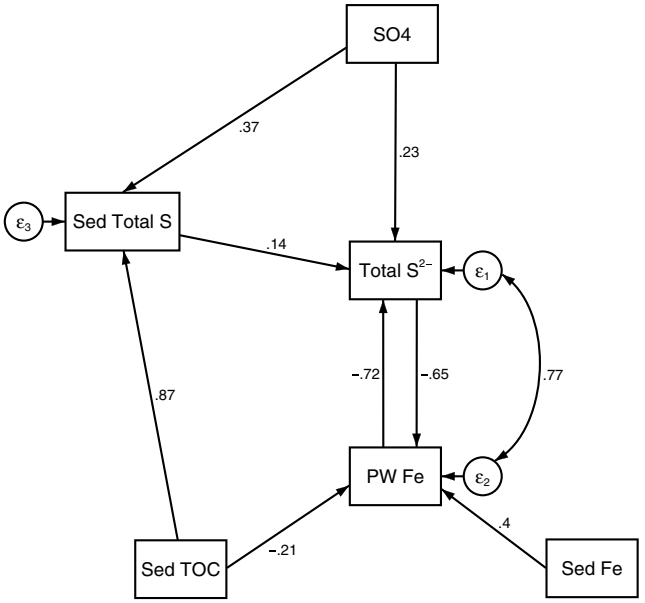


Figure 4. Final SE model for biogeochemical controls on pore water sulfide in wetland sediments. Final model extends conceptual model (Figure 1) by allowing for the correlation of errors between pore water Fe and sulfide. The diagram also shows the standardized path coefficient for each interaction pathway.

thus, inclusion of pore water DOC was considered as a first-order approximation for evaluating the potential efficacy of modeling with speciated values; and

3. including surface water total phosphorus (TP) as an exogenous variable in the model. Increasing eutrophication is expected to increase sulfide production and sedimentary AVS through both the stimulatory effects of additional phosphorus on bacterial activity and rates of organic matter decomposition and through the increased delivery of autochthonous organic matter to the sediments, which in turn serves as a substrate to drive the development of anaerobic conditions and promote dissimilatory sulfate reduction. Anaerobic conditions are a necessary prerequisite for the solubilization of solid phase Fe(III) through its reduction to Fe(II). This alternative model thus considered direct pathways between TP and both pore water Fe and sediment AVS.

4. Results and Discussion

4.1. Model Fit and Evaluation With Class D data

The initial fit of the conceptual model was conducted using maximum likelihood estimation. This resulted in a model that performed reasonably well with respect to predicting the variations in the three endogenous variables (corrected r^2 values ranging from 0.582 to 0.650) but also resulted in a χ^2 value reflecting overall model fit that was unacceptably large ($\chi^2 = 35.54$, degrees of freedom [*df*] = 4, and $p < 0.0001$). Evaluation of the effect of different structural changes to the model that would result in statistically significant reductions in the χ^2 value (modification indices) indicated several choices: (1) linking sediment Fe (negatively) to pore water sulfide, (2) linking either or both sulfate and sediment total sulfur directly (and negatively) to pore water Fe, and (3) allowing the error terms for pore water Fe and sulfide to be correlated. Correlating the error terms recognizes the possibility of either other variables missing from the model or sampling error (or both) affecting both pore water Fe and sulfide. For example, both dissolved Fe and sulfide are highly sensitive to oxidizing shifts in the redox environment of the pore water that potentially can occur during sampling or sample handling and transportation. The effect of such shifts in redox on both constituents is a reduction in concentration. Given its current framework, the other possible choices for improving the overall fit of the model are more reasonably considered only as indirect pathways and do not necessarily add to the conceptual understanding of the model or otherwise lead to an improvement in the utility of the model. For example, the conceptual model includes an indirect pathway defining the effect of sediment Fe on pore water sulfide. Allowing sediment Fe to directly interact with pore water sulfide negates the statistical significance of this pathway; removing the pathway in turn considerably weakens our ability to predict pore water sulfide.

As a result, we modified the conceptual model to allow error correlation between pore water Fe and sulfide (Figure 4). The resultant χ^2 value for the modified model was reduced to an acceptable level ($\chi^2 = 1.110$, $df = 3$, and $p = 0.775$). In addition, the values for the incremental fit indices reported by Stata were all below recommended target levels (Table 1). We further evaluated model fit by examining the matrix of fitted covariance residuals (Table 2). Kline (2011) suggests that values >0.10 indicate that the model does not fit the covariance matrix particularly well, and large residuals can provide guidance regarding possible modifications to improve model fit (StataCorp, 2015). The maximum absolute value for the covariance residuals was 0.007, which is well below this suggested threshold and provides further confidence that the fitted model is well supported by the data.

Model estimation of the modified conceptual model conducted by bootstrapping and the Satorra-Bentler method both verify that the model overall fit is good ($\chi^2 = 0.998$, $p = 0.874$, and Table 1) and that the estimates of the model coefficients are highly significant when accounting for multivariate nonnormality. With the exception of the path coefficient for the effect of sediment total sulfur on pore water sulfide ($p = 0.011$), all path coefficients had $p < 0.001$. We estimated the fit between each observed and predicted

Table 1
Comparison of Goodness of Fit Statistics Resulting From Fitting the Pore Water Sulfide SEM Model to the Class D and Class B Data Sets

Metric	Threshold or target value	Class D	Class B	Class B (no sediment TS)
χ^2—degrees of freedom		3	3	1
χ^2 (maximum likelihood)		1.110	3.019	2.268
$p > \chi^2$ (maximum likelihood)	>0.05	0.775	0.389	0.132
χ^2 (Satorra-Bentler)		0.998	3.000	2.098
$p > \chi^2$ (Satorra-Bentler)	>0.05	0.802	0.392	0.148
RMSEA[a] (maximum likelihood)	0.05	<0.001	0.008	0.110
RMSEA (Satorra-Bentler)	0.05	<0.001	<0.001	0.102
90% CI[b], lower bound	0	<0.001	<0.001	<0.001
90% CI, upper bound	0.1	0.080	0.165	0.308
p RMSEA $\leq$ 0.05		0.874	0.501	0.182
Comparative fit index (maximum likelihood)	0.95	1.000	1.000	0.993
Tucker-Lewis index (maximum likelihood)	0.95	1.014	1.001	0.954
Comparative fit index (Satorra-Bentler)	0.95	1.000	1.000	0.994
Tucker-Lewis index (Satorra-Bentler)	0.95	1.016	1.001	0.959
SRMR[c]	0.08	0.007	0.017	0.020
Residuals coefficient of determination		0.959	0.974	0.947
Stability index		0.687	0.733	0.786
Pore water total sulfide ($\log_{10}$-transformed) coefficient of determination[d]		0.622	0.633	0.623
Pore water Fe ($\log_{10}$-transformed) coefficient of determination		0.685	0.687	0.676
Sediment total S ($\log_{10}$-transformed) coefficient of determination		0.650	0.697	
N		194	105	105

Note. Pore water sulfide coefficient of determination is the Bentler-Raykov squared multiple correlation coefficient.
[a]RMSEA—root-mean-square error of approximation. [b]CI—confidence interval. [c]SRMR—standardized root-mean-square residual. [d]Bentler-Raykov squared multiple correlation coefficient.

endogenous variable using the Bentler-Raykov squared multiple correlation coefficient (StataCorp, 2015). This coefficient is a corrected version of the coefficient of determination (r^2) that should be implemented when evaluating the explained variance of variables in nonrecursive models that have reciprocal causations such as pore water Fe and sulfide. Corrected r^2 values ranged from 0.622 to 0.685 for pore water sulfide and Fe, respectively (Table 1). Figure 5 plots the actual fit between observed and predicted values for $\log_{10}$-transformed pore water sulfide. Included in the plot is a locally weighted scatterplot smoothing (LOWESS) curve that is useful for elucidating any undesired curvilinearity in the predicted relationship. The LOWESS curve shows no such evidence.

One concern with nonrecursive models relates to possible inherent instabilities owing to the fact that variables involved in feedback relationships are dependent upon themselves. The potential for such instability is evaluated through a *stability index* that estimates whether direct effects influencing variables in the model can become infinitely larger (Kline, 2011). Stata uses the method of Bentler and Freeman (1983) to calculate this index. Stability requires that the index value be less than 1; the stability index for the modified model was 0.687 (Table 1).

Results from jackknife cross-validation simulations showing the agreement between the full model predictions and the out-of-sample predictions are shown in Figure 6. Those results illustrate that the out-of-sample predictions closely match the predictions obtained from the fully calibrated model. As a result, the root-mean-square error (RMSE) for the out-of-sample predictions (0.300) agrees well with, and is only slightly

Table 2
Matrix of Fitted Covariance Residuals for the Final SE Model Fitted With Class D Data (See Figure 4 for Model Structure)

Variable	Pore water sulfide	Pore water Fe	Sediment total S	Sulfate	Sediment Fe	Sediment TOC
Pore water sulfide	0.001					
Pore water Fe	−0.001	0.002				
Sediment Total S	0.003	0.004	0.000			
Sulfate	−0.001	−0.007	0.000	0.000		
Sediment Fe	0.002	0.001	−0.003	0.000	0.000	
Sediment TOC	0.007	0.001	0.000	0.000	0.000	0.000

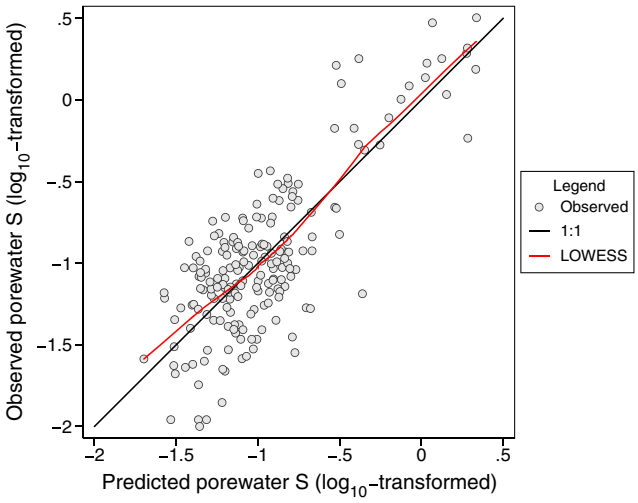


Figure 5. Comparison of observed with predicted pore water sulfide (both $\log_{10}$ transformed) for the SE model fit with Class D data. Plot includes lines showing expected 1:1 fit and the fit obtained from LOWESS smoothing (bandwidth = 0.8).

larger than, the calibration RMSE 0.294. The jackknife analysis also demonstrates no problems with unusually influential observations in the Class D data set.

As mentioned previously, the Class D data set includes a number of sites with multiple observations over time. The inclusion of sites with multiple observations raises the possibility that the resultant model errors are not truly independent from a spatial perspective, unless each observation can be regarded as an independent characterization of the biogeochemical quasi steady state existing among pore water, sediment, and surface water parameters. We tested the possibility of such spatial autocorrelation of errors post hoc using analysis of variance (ANOVA). The analysis focused on Class D sites with four or more observations (10 sites with 48 observations). Figure 7 is a box plot that shows the distribution of model residuals for each of the sites with four or more observations; the plot also includes the residuals distribution for the remaining sites with only a single observation. The ANOVA indicates no overall significant clustering of residual values related to inclusion within a given site group ($p = 0.541$) and thus supports the use of multiple observations from the same site.

4.2. Model Results With Class B Data

The conceptual model with linked covariance between the pore water Fe and sulfide error terms was fit with the Class B data to further evaluate whether excluding sites with multiple observations would result in a substantively different set of results. Initial results indicated that the path between sediment total S and pore water sulfide was no longer significant, although the overall fit was acceptable with the exception of the upper limit for the RMSEA (Table 1). Thus, we refit the model without this pathway. In addition, eliminating this pathway also rendered modeling sediment total S irrelevant; as a result, and in order to avoid biasing the overall significance of the fit statistics, we dropped sediment total S from the model.

While the overall fit of the subsequent reduced form model is still significant ($\chi^2 = 2.268$ and $p = 0.132$), the RMSEA values were further degraded, such that the RMSEA value itself, and not just the upper limit, was unacceptably high. This degradation in model performance for both forms of the model is likely more a reflection of using an inadequate number of observations to fit the model, rather than a fundamental weakness in the model structure. This evidenced by (1) the significance of all the model path coefficients (all values of $p \leq 0.002$, with exception of the sediment total S—pore water sulfide path coefficient) and (2) a comparison of the fitted model coefficients that express the total effect (direct plus indirect) of all three exogenous variables influencing pore water sulfide for the two Class B models and the Class D model (Figure 8). This comparison shows that the differences in the coefficient estimates are small and lie well within the coefficient standard errors. Model integrity was further demonstrated by MLR conducted with the Class B data using pore water sulfide as the dependent variable and each of the SE model variables with direct paths to pore water sulfide. We constructed ACPR plots post hoc to evaluate linearity for each connection. Those results, which are included in the supporting information (Figures S2 and S3) confirm the underlying linearity of these pathways.

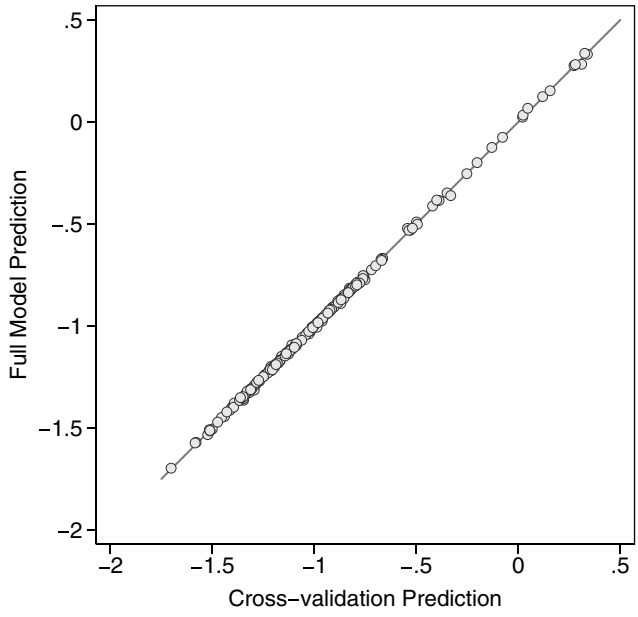


Figure 6. Comparison of predictions of pore water sulfide ($\log_{10}$ transformed) obtained for the SE model fit with Class D data and "out-of-sample" predictions obtained from jackknife cross validation.

4.3. Alternative Models

We compared the set of postulated alternative models with the original model by evaluating both the suite of exact and approximate fit statistics described previously as well as the Akaike and Bayesian

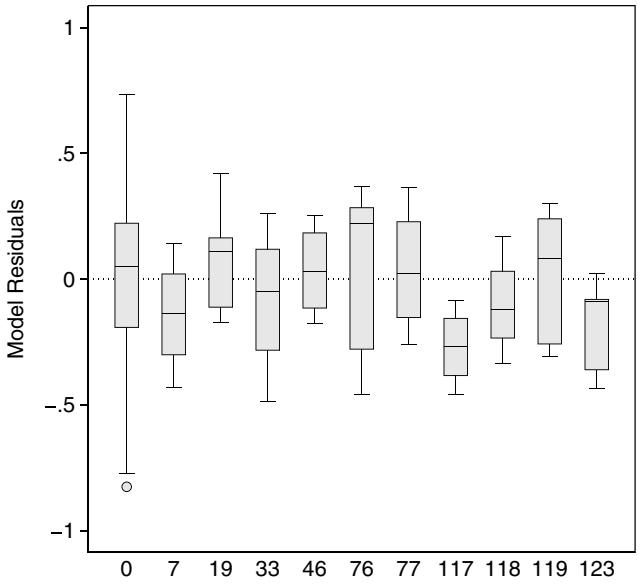


Figure 7. Box and whisker plot of the distribution of model residuals for individual sites with four or more observations. Each number on the abscissa denotes a unique site except for "0," which includes values for all sites (N = 68) without multiple observations.

information criteria (AIC and BIC) values for each model. Comparing AIC/BIC values are meaningful only if the same number of observations is used to fit each model. Because several of the alternative models included variables with a missing observation, all the models thus were fit with the same set of reduced observations (N = 193).

The path diagrams for the original conceptual model along with each of the alternative models are included in the supporting information (Figures S4–S7). The diagrams depict the models in "final form" with the fitted standardized coefficients; final form is intended to convey that no further changes in the model structure are suggested by either modification indices that are conceptually supportable or by eliminating nonsignificant ($p > 0.05$) pathways.

Based on χ^2 values, the strongest models are the original model and the conceptual model modified to include sediment AVS (AVS model, Figure S6). This latter model includes a reciprocal relationship between pore water sulfide and AVS, as well as direct pathways between sulfate and sediment AVS and pore water Fe and AVS. This structure is consistent with the hypothesis that increased sulfate leads to increased dissimilatory sulfate reduction, and thus increases in AVS (Ramamoorthy et al., 2009). While this model is conceptually more satisfying, it is not necessarily superior to the original model for several reasons. First, the value for the SRMR was somewhat higher for the AVS model (0.013 versus 0.007). Second, AIC and BIC values indicate the original, more parsimonious model is superior. Moreover, the ability to predict pore water sulfide, pore water Fe, or sediment total sulfur is not markedly improved by inclusion of sediment AVS (Table S1). Lastly, including sediment AVS as an endogenous variable does not change the overall relationship between the exogenous variables and the endogenous variables of interest. This is illustrated in Table S2, which compares the total effects of a given variable on a specific endogenous variable. Using pore water sulfide as an example, the coefficient for the total effect of sulfate for the original and AVS models is 0.539 and 0.529, respectively; for sediment Fe and TOC, the coefficients are −0.551 versus −0.557 and 0.515 versus 0.532, respectively.

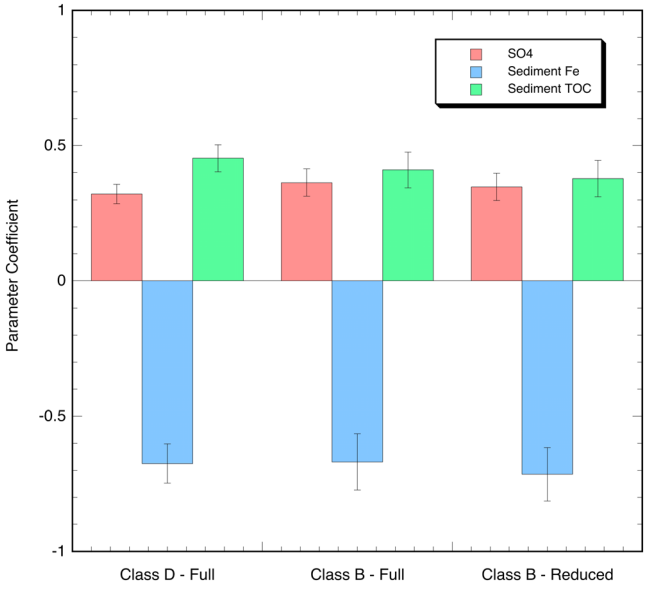


Figure 8. Comparison of the estimated coefficients depicting variable total effect on pore water sulfide for the three exogenous variables included in the SE model. The comparison includes fitting the SE model to the Class D and Class B data to the full conceptual model and fitting the Class B data to the conceptual model without including sediment total sulfur as a variable. Error bars are standard errors for the individual coefficient estimates.

The other two alternative models—inclusion of pore water DOC effects on pore water sulfide and pore water Fe (Figure S5) and inclusion of sediment AVS, pore water DOC, and surface water TP effects (Figure S7)—were somewhat inferior models, although both models were well supported based on χ^2 significance. In particular, weaker model fits were suggested based on an unacceptably high RMSEA value for the DOC model (0.058 compared to a guideline threshold of 0.05) and elevated RMSEA value for the AVS-DOC-TP model (0.028); this accordingly resulted in lower probabilities that the RMSEA $\leq$ 0.05. CFI and TLI values were somewhat lower as well. While increasing TP concentrations can directly promote higher rates of microbial activity, the lack of importance of TP in the models may indicate that the effects of TP on pore water sulfide are predominantly indirect and exerted through its effect on the accretion of sediment organic matter (which in turn stimulates sulfate reduction). Because sediment organic matter content is only weakly related to trophic state variables such as TP (cf. Brenner & Binford, 1988) and more strongly reflects the physical depositional environment, the statistical relationship between TP and sulfide dynamics expectedly would be weak as well. Nonetheless, in freshwater wetlands such as portions of the Florida Everglades where high sulfate loadings are coupled with high phosphorus loadings, extremely high sulfide concentrations can occur, although the primary

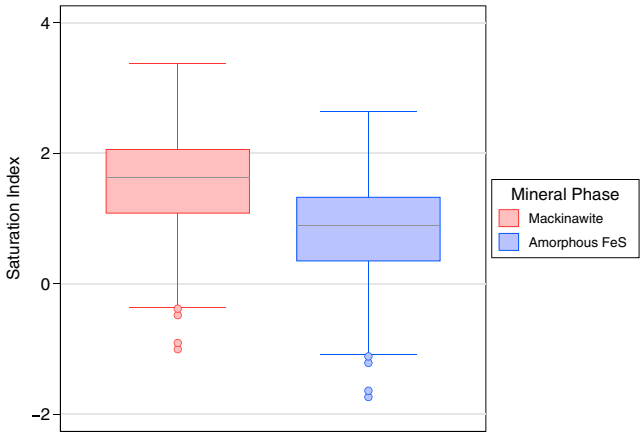


Figure 9. Distribution of amorphous FeS and mackinawite saturation index (SI) values calculated for the Class D data set. Values greater than 0 indicate supersaturation, while values less than 0 indicated undersaturation.

determinant appears to be high concentrations of sulfate more than phosphorus (Figure S8 and data from Scheidt & Kalla, 2007).

4.4. Geochemical Implications

The core of the conceptual model is the reciprocal relationship between pore water concentrations of Fe and sulfide, and which is embodied in the equilibrium dynamics of amorphous FeS or mackinawite precipitation and dissolution (equation (1)). We can test this hypothesis by calculating the ratio of the ion activity products (IAP) for equation (1) to the equilibrium solubility product constant (K_{sp}) for both solid phases ($10^{-3.915}$ and $10^{-4.648}$ for amorphous FeS and mackinawite, respectively) (Parkhurst & Appelo, 2013). Log ratios or saturation index (SI) values in excess of 0 suggest supersaturation, while values less than 0 are indicative of under or subsaturation (Appelo & Postma, 2010). Free concentrations of Fe^{2+} and HS^- were calculated based on measured pore water pH, and the hydrolysis constant for Fe^{2+} and dissociation constants for H_2S (Parkhurst & Appelo, 2013). Because they do not include corrections for ionic strength nor the possible effects of other ions such as DOC effects on Fe^{2+} speciation, the resultant IAP values are biased somewhat high. Thus, these SI calculations can provide evidence only of conditions not sufficiently adequate for amorphous FeS or mackinawite precipitation to occur, rather than confirm that either phase is controlling.

The distribution of SI values calculated for both mineral phases are shown in the box plot contained within Figure 9. Only a small fraction of Class D sites had $SI_{mackinawite} < 0$ (6.7%), with over 75% of the sites suggesting supersaturation by an order of magnitude. SI_{FeS} values were, by definition, lower but still largely supportive of supersaturation (84% of the sites with values $SI \geq 0$). Holdren and Armstrong (1986) conducted similar IAP calculations for Lake Mendota sediments and, based on those calculations, concluded that FeS precipitation may control Fe solubility in sediment pore waters as well.

Our SE modeling coupled with the SI calculations thus suggest a solid aqueous phase dynamic between Fe supply and sulfide sequestration that buffers the evolution of pore water sulfide concentrations resulting from dissimilatory sulfate reduction. This buffering system is well recognized—for example, Heijs et al. (1999) developed a quantitative measure for the ability of sediments to buffer free S^{2-} concentrations dependent on the availability of both Fe(II) and Fe(III). In part, this buffer system includes the reduction of Fe(III) minerals such as ferrihydrite by H_2S, leading to the formation of metastable FeS (Poulton et al., 2002).

It should be noted that sediment organic matter also can react with sulfides to form organic sulfur, including thiols, organic monosulfides, and organic disulfides (Zeng et al., 2013), and that this pathway can be an important removal mechanism for pore water sulfide in many lakes (e.g., Holmer & Storkholm, 2001; Rudd et al., 1986; Urban et al., 1999). Moreover, this mechanism may be particularly important as a constraint on pore water sulfide concentrations in aquatic ecosystems where low iron availability is coupled with high sediment organic content. Because the SE model does not include a sediment organic S-sulfide relationship, modifying the SE model to account for organic sulfur formation likely will be important if it is extended to such ecosystems.

A key advantage of the SE model is that it provides a framework for quantifying the relative effects of different variables on pore water sulfide concentrations. Such an analysis can include direct effects, indirect effects, and total effects. Placing the effects of different variables in context with each other is perhaps most easily understood through the use of standardized coefficients. Standardized coefficients give the corresponding response relative to a single standard deviation for a dependent variable when a given independent variable has been increased by a single standard deviation. As such, standardized coefficients provide a convenient means for directly comparing the relative importance or strength of association of different independent variables, while the unstandardized coefficients reflect the form of the relationship (Acock, 2013).

Figure 10 compares the standardized coefficients for model variable effects on pore water sulfide. These results indicate that internal dynamics between pore water Fe and sulfide exert the greatest degree of control on pore water sulfide (standardized coefficients for total effects equal to −1.37 and 0.90, respectively).

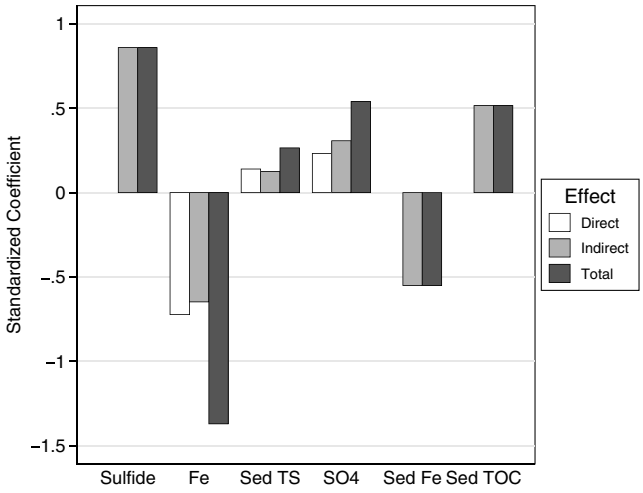


Figure 10. Standardized coefficients for direct, indirect, and total effects of each of the variables influencing pore water sulfide in the SE model. The use of standardized coefficients allows direct comparison of the relative importance of each variable relationship on variations in pore water sulfide, while the estimated coefficients (cf. Figure 8) allow one to estimate the marginal or instantaneous effects of changing a given exogenous variable on pore water sulfide while holding all other exogenous variables constant. Note that categories labeled as "sulfide" and "Fe" refer to pore water.

The effect of pore water sulfide on itself is of course indirect and exerted through its direct relationship with pore water Fe. The effect of pore water Fe in turn is largely direct, although it also exerts a substantial indirect effect through the pore water Fe-pore water sulfide feedback loop.

From a management perspective, the effects of the exogenous variables are of particular interest. Our comparison indicates the magnitudes of relative total effects for the exogenous variables are nearly identical: sulfate (0.54), sediment Fe (−0.55), and sediment TOC (0.52). These results thus suggest that given their distributional characteristics in our model data set, the ambient variations in each of the exogenous variables contribute in nearly an equivalent manner to the observed variations in pore water sulfide. In other words, while the importance of the role of sulfate as a controlling variable for pore water sulfide concentrations is validated both conceptually and statistically with respect to significance, the SE model also underscores the importance of the sediment Fe buffering system and the effect of the availability of sediment organic matter. The practical consequence of this buffering system is that the sensitivity of macrophytes such as wild rice that are adversely impacted by the occurrence of sulfide is likely exacerbated in sediments relatively low in iron. In such sediments, increases in organic carbon and sulfate will, *ceteris paribus,* result in higher concentrations of sulfide (cf. Calleja et al. 2007).

5. Practical Application of the SE Model

The ultimate goal of the MPCA-sponsored field survey upon which this modeling effort is based (Myrbo, Swain, Engstrom, et al., 2017) is to determine if it is appropriate to regulate sulfate and, if so, how to structure the regulation in order to protect wild rice from exposure to pore water sulfide concentrations above a protective threshold—the identification of which is a separate task from the modeling exercise described here. This modeling effort demonstrates that pore water sulfide concentrations can be successfully modeled with three exogenous variables—sulfate, sediment TOC, and sediment Fe. The latter two measurements are the average concentrations in the top 10 cm of sediment in a wild rice waterbody and likely reflect the natural ambient condition of the wild rice habitat. Although our modeling shows that both sediment TOC and sediment Fe are important contributors to the toxic impacts of sulfide, regulating sulfate is the only viable mechanism to protect wild rice against sulfide toxicity.

However, because each wild rice waterbody has different sediment TOC and Fe concentrations, any given fixed sulfate standard applied to multiple wild rice waters would produce a wide range of sulfide concentrations, some of which would likely be above the protective threshold. Because of the varying efficiency in converting sulfate to sulfide, if the goal is to keep wild rice waters below a particular sulfide threshold, then sulfate limits should be different for each waterbody.

To identify a protective sulfate concentration for a particular waterbody, it would be logical to employ the relationships revealed by the SE model and to work backward from an identified protective sulfide threshold to calculate a surface water sulfate standard tailored to the geochemistry of that particular waterbody. A direct way to accomplish this task would be to first arrange the SE model into a single equation that expresses pore water sulfide as a function of the exogenous variables in the model (sulfate, sediment Fe, and sediment TOC). By substituting the protective sulfide concentration in for that variable and rearranging the equation, a revised equation for the protective sulfate concentration as a function of Fe and TOC concentrations can be derived. Such an equation could be applied to any wild rice waterbody for which sediment Fe and TOC are known.

An important concern with the above approach, however, relates to both confidence intervals associated with predicted sulfide concentrations and biases in predicted values resulting from back-transforming a predicted log-transformed concentration to its original arithmetic form (Duan, 1983). Stata does not

provide confidence intervals for predicted endogenous variables obtained from SEM. With respect to the latter problem, solutions have been proposed (Duan, 1983; Manning & Mullahy, 2001), but they are complicated.

There are at least two approaches toward resolving the difficulty of practicably using the SEM results to predict sulfate concentrations that reflect a target or threshold pore water sulfide concentration. Both approaches rely on the SE model to define the driving variables of interest—viz., sediment Fe and TOC and surface water sulfate. The first approach uses generalized linear modeling with an appropriate link function (in this case a log link for the dependent variable) and specification of the residual error distribution. This approach capitalizes on the fact that path analysis and MLR are very similar (Li, 1975) and yield very similar estimated model coefficients for predicting pore water sulfide; with proper error distribution specification, it also avoids the issue of back-transformation bias. The second approach is multiple binary logistic regression (MBLR). MBLR simulates the likelihood that the response variable of interest exceeds a defined threshold given a set of independent variables. This type of binary response modeling is particularly well suited for the management question of whether, *ceteris paribus*, a given sulfate concentration is likely to result in pore water sulfide concentrations above or below a specified toxicity threshold. This approach also avoids the problem of back-transformation bias.

A MBLR model can be developed using the same field data that were used to construct, calibrate, and validate the SE model. MBLR would directly predict the probability of exceeding the protective sulfide concentration threshold as a function of sulfate, Fe, and TOC. An acceptable probability threshold can then be defined (for example, 0.5 or some other value; the choice would be a policy decision), and the model used to "inverse" predict the sulfate concentration that will produce the threshold as a function of Fe and TOC. Operationally, this equation to determine the protective sulfate threshold for any particular waterbody could then be applied in the same manner as the equation derived from the SEM, as described above.

In summary, it is desirable to use SEM to better understand and characterize the relationships between the important parameters in the system that relates sulfate and sulfide but then to use MBLR to translate the understanding gleaned from SEM into a water quality standard for sulfate. A standard that is an equation, rather than a uniform concentration that is applied to all waterbodies, would be a reflection of the biogeochemical diversity of the environment. New approaches are needed as environmental regulation progresses from protecting organisms from pollutants that are directly toxic, to pollutants whose negative effects are indirect and a function of environmental conditions that vary from site to site.

6. Conclusions

We used SEM to help elucidate and quantify the underlying structure of water quality and sediment chemistry variables governing the evolution of pore water sulfide concentrations in lakes and streams in the Upper Midwest/Great Lakes region. Three key "external" variables were initially hypothesized to be important—surface water sulfate, sediment Fe, and sediment TOC. Intermediate, mediating variables included pore water Fe and sediment total sulfur. Inherent in the model structure is a reciprocal relationship between pore water Fe and sulfide. This structure defines a sediment iron-based buffering system that is supported both empirically by other observational studies in wetlands and conceptually by the thermodynamics of amorphous FeS or mackinawite dissolution. The conceptual structure of the SE model is well supported both by model fit statistics and a suite of model diagnostic and post hoc analyses and by SI analyses that suggest that amorphous FeS or mackinawite precipitation-dissolution dynamics help control or impose constraints on the reciprocal relationship between pore water Fe and sulfide. Our use of SEM to model pore water chemistry dynamics, including the nonrecursive nature of those dynamics in particular, is believed to be the first such application in the open literature.

An important consequence of the sediment buffering system is that it serves to help limit the effects of increases in either surface water sulfate or sediment organic carbon on pore water sulfide. Based on total effects estimated from the SE model, all three external variables all essentially equivalent in importance with respect to influencing pore water sulfide. When considering the policy implications of trying to protect wild rice against the toxic effects of sulfide by limiting sulfate inputs to the ecosystem, our results thus demonstrate that the problem is multidimensional and that any such standard must consider the effects of

ambient concentrations of sediment Fe and sediment TOC to ensure the imposed sulfate standard is neither unduly or insufficiently protective. Multiple binary logistic regression would be a practical way to tailor sulfate standards to the specific geochemistry of sites so that a uniform level of protection for organisms sensitive to sulfide would be achieved.

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RESEARCH ARTICLE
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Key Points:
- Dynamic water fluxes affect sulfate reduction rates in hyporheic zones, especially where organic content is high
- Field data and reactive transport modeling suggest sulfate reduction can dominate Fe reduction in stream and wetland sediments
- High sulfate concentrations slow methanogenesis in wetlands but do not entirely suppress methane

Supporting Information:
- Supporting Information S1
- Table S1
- Figure S1
- Figure S2
- Figure S3
- Figure S4
- Figure S5
- Figure S6
- Figure S7
- Figure S8
- Figure S9
- Figure S10

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Modeling hydrologic controls on sulfur processes in sulfate-impacted wetland and stream sediments

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Abstract Recent studies show sulfur redox processes in terrestrial settings are more important than previously considered, but much remains uncertain about how these processes respond to dynamic hydrologic conditions in natural field settings. We used field observations from a sulfate-impacted wetland and stream in the mining region of Minnesota (USA) to calibrate a reactive transport model and evaluate sulfur and coupled geochemical processes under contrasting hydrogeochemical scenarios. Simulations of different hydrological conditions showed that flux and chemistry differences between surface water and deeper groundwater strongly control hyporheic zone geochemical profiles. However, model results for the stream channel versus wetlands indicate sediment organic carbon content to be the more important driver of sulfate reduction rates. A complex nonlinear relationship between sulfate reduction rates and geochemical conditions is apparent from the model's higher sensitivity to sulfate concentrations in settings with higher organic content. Across all scenarios, simulated e^- balance results unexpectedly showed that sulfate reduction dominates iron reduction, which is contrary to the traditional thermodynamic ladder but corroborates recent experimental findings by Hansel et al. (2015) that "cryptic" sulfur cycling could drive sulfate reduction in preference over iron reduction. Following the thermodynamic ladder, our models shows that high surface water sulfate slows methanogenesis in shallow sediments, but field observations suggest that sulfate reduction may not entirely suppress methane. Overall, our results show that sulfate reduction may serve as a major component making up and influencing terrestrial redox processes, with dynamic hyporheic fluxes controlling sulfate concentrations and reaction rates, especially in high organic content settings.

Plain Language Summary Unlike in oceans, sulfur reactions have not been considered to play a prominent role in the biogeochemistry of terrestrial environments because of much lower concentrations, but recent studies have been showing terrestrial sulfur reactions to be more important than previously thought. These reactions often take place in wetland, stream, and lake sediments, which can contain a mix of both surface water and underlying groundwater. Our study investigated how water fluxes through these sediments can affect sulfur reactions when the surface water and groundwater chemistry differ significantly, such as through influxes of surface water sulfur from mining activities. Using field measurements and a computer model that simulates chemical reactions and water flow, we found that water flux drives water chemistry in wetland and stream sediments. This affects how rapidly sulfur reactions occur, especially in organic-rich wetland sediments. The model showed sulfur reactions to dominate over iron reactions, which contradicts classic chemical thermodynamics. Also, when water fluxes carry high sulfur concentrations into wetland sediments, sulfur reactions out compete production of methane, a strong greenhouse gas. Our results indicate that sulfur reactions can play a prominent role in terrestrial biogeochemistry, and the degree to which it does is affected by hydrological fluxes.

1. Introduction

Because sulfur concentrations in (nonmarine) terrestrial settings such as freshwater sediments and aquifers are typically low compared to marine settings, processes such as sulfate reduction have often been overlooked there. Renewed attention on terrestrial sulfur has led to a number of recent studies demonstrating the prevalence of high sulfate reduction rates (see reviews by *Pester et al.* [2012] and *Holmer and Storkholm* [2001]),

the occurrence of "hidden" and "cryptic" sulfur cycles [*Wieder and Lang*, 1988; *Jørgensen*, 1990; *Elsgaard and Jørgensen*, 1992; *Wind and Conrad*, 1997; *Mandernack et al.*, 2000; *Heitmann et al.*, 2007; *Knorr and Blodau*, 2009; *Hansel et al.*, 2015], and previously unrecognized sulfur-reducing microbial strains [*Castro et al.*, 2002; *Loy et al.*, 2004; *Leloup et al.*, 2006; *Kondo and Butani*, 2007; *Schmalenberger et al.*, 2007; *Liu et al.*, 2009; *He et al.*, 2010; *Miletto et al.*, 2010; *Pester et al.*, 2010; *Steger et al.*, 2011; *Pester et al.*, 2012; *Hausmann et al.*, 2016]. Sulfur studies typically focus on sediment beds underlying lakes, estuaries, and wetlands — settings with sufficient organic carbon to drive sulfate reduction. Sulfur processes there have also been found to be coupled with iron (Fe) and carbon (C) cycling [*Holmer and Storkholm*, 2001; *Baldwin and Mitchell*, 2012; *Li et al.*, 2012]. Resolving terrestrial sulfate processes is thus important for understanding overall biogeochemical cycling, particularly where they may be impacted by perturbations such as atmospheric SO_2 emissions (mostly in developing countries [*Klimont et al.*, 2013]) and surface water contamination.

Sulfate (SO_4^{2-}) reduction rates are known to depend on microbial type [*Bradley et al.*, 2016; *Pallud and Van Cappellen*, 2006], organic carbon properties [*Detmers et al.*, 2001; *LaRowe and Van Cappellen*, 2011], and geochemical conditions [*Pallud et al.*, 2007; *Tarpgaard et al.*, 2011; *Laverman et al.*, 2012], but rates remain difficult to predict, with laboratory and field measurements spanning orders of magnitude (see literature compilation of rates in supporting information Table S1) [*Holmer and Storkholm*, 2001; *Holmer et al.*, 1998; *Steenbergen et al.*, 1993; *Pallud et al.*, 2007; *Dale et al.*, 2008; *Robador et al.*, 2015; *Laverman et al.*, 2012; *Roychoudhury*, 2004; *Boudreau and Westrich*, 1984; *Smith and Klug*, 1981]. Further complicating predictions of sulfur cycling, SO_4^{2-} reduction often occurs in the hyporheic zone. This zone in shallow sediments of lakes, estuaries, and wetlands is characteristically dynamic due to the mixed influence of groundwater and surface water [*Bencala*, 1993; *Findlay*, 1995; *Boulton et al.*, 1998]. Spatiotemporally complex flow fields result from bathymetry [*Harvey and Bencala*, 1993; *Tonina and Buffington*, 2007], variable surface water levels [*Boano et al.*, 2014; *Cardenas et al.*, 2004], and heterogeneous hydraulic conductivity at diverse scales [*Menichino and Hester*, 2015; *Sawyer and Cardenas*, 2009]. Differences in groundwater and surface water chemistry generate sharp chemical gradients that shift with changing hydrologic fluxes [*Triska et al.*, 1993; *Boulton et al.*, 1998]. Because of this variability, the hyporheic zone is a known hot spot for oxygen-sensitive geochemical processes such as denitrification [*Mulholland et al.*, 2008; *Krause et al.*, 2009; *Rahimi et al.*, 2015; *Zarnetske et al.*, 2015]. Hyporheic flux can thus be expected to perturb conditions controlling SO_4^{2-} reduction. Some recent field studies have evaluated the effect of intermittent streambed drying and wetting on sulfur processes [*Coleman Wasik et al.*, 2015; *Eckley et al.*, 2015; *Karimian et al.*, 2017; *Liu et al.*, 2017], yet little effort has been made toward a detailed examination of the role of surface water and deeper groundwater exchange in driving sulfur processes. In one hyporheic zone study to assess SO_4^{2-} reduction, *Rinehart et al.* [2015] performed push-pull tests in a stream riparian zone and showed that higher flow rates drove higher rates of denitrification and SO_4^{2-} reduction. Our study aims to shed light on sulfur processes in hyporheic settings, by examining how naturally variable surface water and groundwater fluxes perturb redox conditions, and how this influences SO_4^{2-} reduction and its interactions with other geochemical processes.

Our study combines reactive transport modeling with field observations at a sulfate-impacted riparian wetland. Reactive transport models have shown utility in evaluating hydrologically and geochemically dynamic and variable conditions affecting hyporheic zone settings [e.g., *Runkel and Kimball*, 2002; *Lautz and Siegel*, 2006; *Bardini et al.*, 2012; *Zarnetske et al.*, 2012; *Boano et al.*, 2014; *Engelhardt et al.*, 2014]. Here our goal with reactive transport modeling is not to exactly simulate observed conditions at our particular field site but to probe the combined physical and geochemical processes that affect sulfate reduction but are difficult to infer from field data alone. With the model, we quantify how sulfur processes in the hyporheic zone are affected by regional and local groundwater flux and chemistry, dynamic changes over a season, and hydraulic and geochemical variability across a riparian wetland setting. We also evaluate how sulfur processes interact with other geochemical processes under these varying conditions.

2. Study Site Description

Our study was conducted at Second Creek, a small stream catchment in northeastern Minnesota (USA) (Figure 1). Second Creek is underlain by glacial outwash and till. Aquifer sediment in the western upslope area is primarily composed of coarse sand and silt with smaller fractions of clay and gravel. On the east side, aquifer sediment is dominated by very coarse sand and gravel with only traces of silt and clay. The main channel of the stream is relatively narrow (2–3 m) and shallow (1–2 m) and is flanked by 20–30 m of wetlands (Figure 1a).

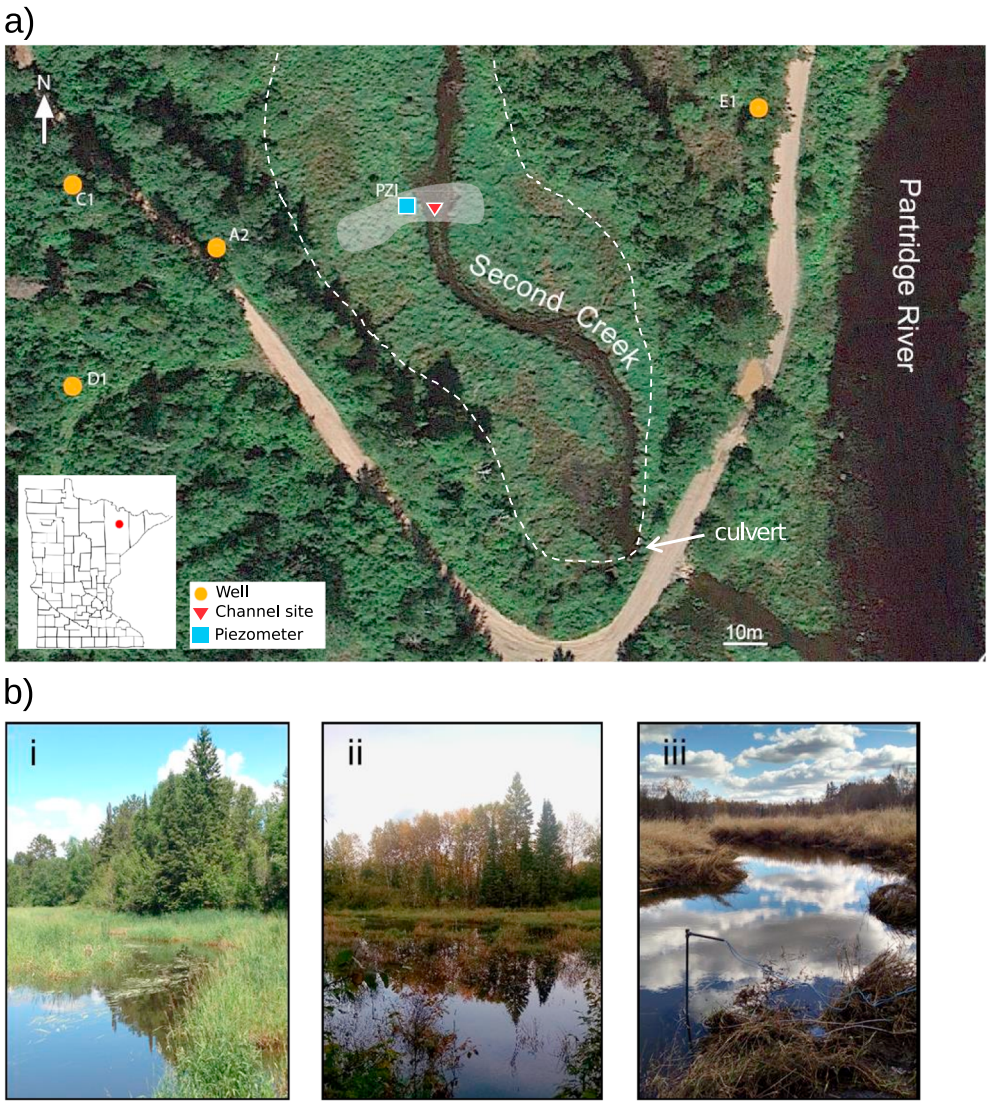


Figure 1. (a) Study area with location of groundwater wells (yellow circles), the wetland piezometer (blue square), and the channel sampling site (red triangle). Shading indicates the area of focus of the temporary installations (temperature probes, seepage meters) and geochemical sampling locations. Clearly visible in this image is the main stream channel (labeled "Second Creek"), which is flanked on both sides by heavily vegetated wetlands (outlined with dashed lines). (b) Second Creek during the nonflooded period in (i) late June, (ii) the flooded period in mid-September, and (iii) the nonflooded period in mid-October (iii).

In the summer, wild rice and submerged macrophytes grow in the main channel of the stream, and the densely vegetated adjacent wetlands contain grasses, sedges, and shrubs.

Surface water sources to Second Creek include pumping of mine waste rock pits to the north and northeast. Surface water SO_4^{2-} in many streams in the region represents a mix of point-source continuous high SO_4^{2-} discharges and lower SO_4^{2-} runoff from precipitation. Often, a lack of dilution later in the summer increases SO_4^{2-} concentrations in streams and surface waters [*Berndt and Bavin*, 2012], but transient changes in mine-water releases and localized water level fluctuations also cause deviations from this pattern [*Johnson et al.*, 2016]. The site was previously included in a field survey of Minnesota lakes and streams [*Myrbo*, 2013] that addressed a concern that elevated concentrations of SO_4^{2-} may negatively impact wild rice (*Zizania palustris*), a culturally and economically important species in the state. Despite highly elevated SO_4^{2-} levels up to 10 mM in the main channel surface water, Second Creek contains plentiful wild rice, prompting questions of whether upward flux of groundwater could be transporting water with lower SO_4^{2-} concentrations than surface water to the hyporheic zone.

3. Field Methods

We collected physical and geochemical data from Second Creek from May through October in 2015. In early June, water table groundwater wells with depths of about 4–7 m were drilled (Figure 1a). A shallow piezometer was hand installed to a depth of approximately 1.5 m in the west wetland adjacent to the main channel, and one stream gauge was hand installed in the channel. All groundwater wells, the hand-installed piezometer, and the stream gauge were equipped with Schlumberger Diver or Baro pressure transducers. Seepage meters with cross-sectional areas of 2510 cm^2 were constructed following the general method of *Rosenberry and LaBaugh* [2008]. These were installed three times over the summer, each time for 24–48 h, to directly measure fluxes in the main channel and west wetland. The seepage meters were pushed in about 7 cm into the sediment wherever possible, although hummocks often prevented uniform seating.

Approximately monthly sampling was carried out for sediment geochemistry and for aqueous chemistry of surface water, pore water in the hyporheic zone, and groundwater from the upslope wells. Pore water samples were taken from the shaded area in Figure 1 from the "channel" (red triangle in the main channel), "west wetland" (west of the channel site), and "east wetland" (east of the channel site). Groundwater wells were sampled after three well volumes were pumped out of the well. Following methods established by *Myrbo* [2013], sediment cores were collected from the main channel and the wetland using an HTH sediment gravity corer (Pylonex, Sweden) with 7 cm diameter polycarbonate tubing, and pore water samples were drawn from the cores using 10 cm long Rhizon^{TM} filters (pore size 0.12–0.18 μm) (Rhizosphere.com, Netherlands) [*Shotbolt*, 2010]. In late August 2015, passive pore water equilibrators ("peepers") were also used to sample pore water at 1.56 cm intervals using a method similar to that of *Koretsky et al.* [2006]. The gravity coring method provides instantaneous 10 cm depth-integrated pore water chemistry, while peepers provide high vertical resolution measurements integrated over the 2–3 week period over which they equilibrate. All aqueous chemistry samples were analyzed for major cations, major anions, alkalinity, and pH. Surface water and groundwater were also analyzed for dissolved oxygen (DO). All pore water samples were analyzed for sulfide, and peeper pore water was also analyzed for dissolved methane (CH_4).

Cation samples (Al^{3+}, Ba^{2+}, Ca^{2+}, $Fe^{2+/3+}$, K^+, Li^+, Mg^{2+}, Mn^{2+}, Na^+, P^{5+}, Si^{4+}, and Sr^{2+}) of about 12 mL volume were acidified in the field with one drop of 6N HCl and analyzed using a Thermo Scientific iCAP 6500 dual view ICP-OES (inductively coupled plasma-optical emission spectrometer). Anion samples (F^-, acetate, formate, Cl^-, NO_3^-, Br^-, and SO_4^{2-}) were kept cool and out of sunlight in opaque bottles and analyzed using Thermo Dionex ICS 5000+ ion chromatography system. Samples of about 25–50 mL volume were also collected from cores for pore water sulfide; these were kept anoxic, preserved with 0.2 mL of 2.0N ZnAc and 0.5 mL of 15M NaOH, and analyzed colorimetrically using a Lachat Autoanalyzer following in-line acid distillation and NaOH trapping. All alkalinity samples were collected in bottles with no headspace and were analyzed within 48 h using colorimetric acid titration. Other parameters, such as pH and dissolved oxygen (DO), were measured in the field using a Thermo Scientific Orion STAR A329. Peeper sulfide concentrations were quantified in the field directly after sampling using the methylene blue method [*Eaton*, 2005] with Hach sulfide reagents [*Hach*, 2010]. Sulfide quantified with this method includes all reduced and dissolved inorganic sulfide. Peeper CH_4 samples were collected in N_2-flushed serum bottles preloaded with 10 mg trisodium phosphate following the general method of *Baedecker and Cozzarelli* [1992]. Analysis took place after a headspace extraction on an Agilent 5973N gas chromatographer using flame ionization with hold times of approximately 4.15 min.

Sediment samples were collected using the HTH sediment gravity corer from the main channel and wetland. Samples from the top 10 cm and 10–20 cm intervals of the core were homogenized and analyzed for total carbon (TC) and acid volatile sulfide (AVS). AVS samples were collected under a nitrogen atmosphere and frozen immediately upon collection. The samples were then analyzed following a weak acid extraction (0.5N HCl) with an ion selective electrode [*Brouwer and Murphy*, 1994]. TC samples were freeze dried, and a portion was removed and tested for reactivity with 6N HCl. Of the 17 samples tested, only two samples (both from the main channel in October 2015) reacted with the acid, indicating the presence of carbonate minerals (inorganic carbon). The samples were then analyzed on the Costech 4010 elemental analyzer at the University of Minnesota Stable Isotope Lab. Because the majority of the samples did not react with HCl, TC is used as an approximation for total organic carbon.

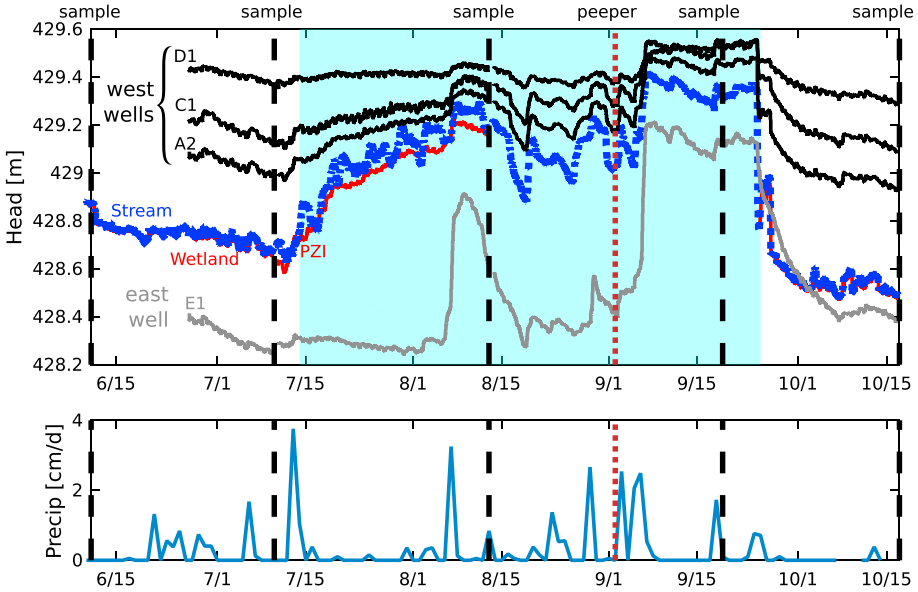


Figure 2. (top) Summer 2015 head data (in meters above mean sea level) from the stream (blue), west groundwater wells (black), east groundwater well (gray), and the wetland piezometer (red). (bottom) Precipitation data. Surface water, groundwater, and pore water core sampling dates are indicated with vertical black dashed lines. The peeper sampling date is indicated with the brown dotted line. The flood period is highlighted in light blue.

4. Field Results and Delineation of Hydrogeochemical Scenarios

4.1. Physical Hydrology

The physical hydrology at Second Creek from May through October of 2015 was characterized by a period of significant flooding from mid-July to late September. Heavy rains on 13 July 2015 washed out an upstream beaver dam, and the debris blocked the culvert directly downstream of the study area (Figure 1a), causing the stream level to rise by over 70 cm and flood the wetlands flanking the main channel. A backhoe was used to clear the culvert on 24 September 2015, allowing the stream to return to lower water levels. The midsummer flood effectively divided the season into three distinct hydrologic periods: (1) nonflood water levels from May to mid-July, (2) flooded water levels from mid-July to late September, and (3) return to nonflood water levels from late September to mid-October (Figure 1b, i–iii). The unexpected flood event provided a unique opportunity to examine stream pore water geochemical response to changes in surface water hydrology that could occur naturally, such as through meteorological forcing, or through human-controlled activities, such as active pumping of mine waters into streams. Additionally, flooding of the wetlands made it possible to evaluate the geochemical response to hyporheic fluxes in organic-rich sediments, such as may occur in lakes and wetlands with standing water. In our study, we distinguish between the main stream channel, where surface water flows all the time, and the adjacent wetland, which is mostly exposed during nonflood conditions but is continuously inundated during the flood period (Figure 1).

Hydraulic head data over the summer (Figure 2) show Second Creek to be regionally a flow-through stream, with groundwater flowing from the west wells toward the stream and then the east well (well locations shown in Figure 1a). Following the large rain events on 7 August 2015 and 6 September 2015 (Figure 2), the flooded stream overspilled the east bank and recharged the east well. At the clearing of the culvert, the head decline in the east well lagged the rapid stream head drop, resulting in a temporary reversal of hydrologic flow between the stream and east upland area. During most of the nonflood periods, the water table in the adjacent wetland areas remained very close to the stream level, as shown by the negligible head difference between the stream gauge data and head data from "PZI."

Vertical hyporheic flux below the stream was inferred using wetland head data from PZI and seepage meters installed in both the channel and wetland. In the wetland areas, hyporheic exchange of surface water and groundwater occurred only during the flood period, when the stream level rose and inundated the typically exposed wetland areas. Stream head exceeded head in the wetlands during most of the time of data availability, although brief head gradient reversals occasionally occurred between rain events (Figure 2). Wetland head

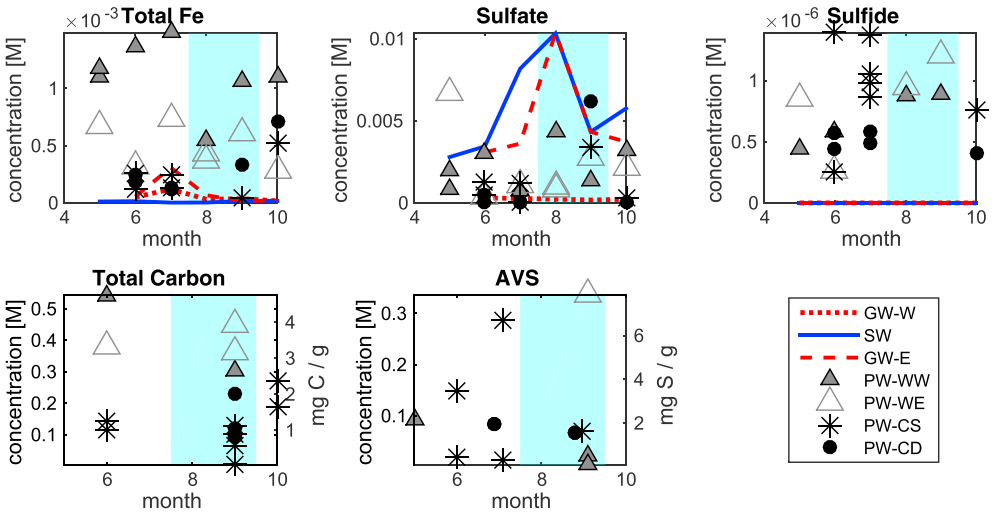


Figure 3. (top row) Water chemistry measured from monthly samples in the west groundwater (GW-W), surface water (SW), west wetland pore water (PW-WW), east wetland pore water (PW-WE), and shallow and deep channel pore water (PW-CS and PW-CSD, respectively). Samples were taken from the shaded area indicated in Figure 1. See supporting information Figure S1 for other aqueous chemistry observations. (bottom row) Sediment extraction results for wetland and channel sediments; these were converted to moles per liter [M] using average measured porosity and bulk density values to facilitate comparison with model results in those units. Only the two October samples reacted with the addition of HCl (indicating some inorganic carbon content), supporting an assumption that the other total carbon measurements consist primarily of organic carbon.

data were discarded starting 13 August 2015 until the end of the flood period on 24 August 2015, because surface water rose above the top of the top of the hand-installed piezometer (at 429.1 m), rendering the data unreliable. Corroborating the available head data, multiple seepage meters deployed in the flooded west wetland measured losing surface stream water fluxes (−0.2 to −0.5 mm/d). Replicate pairs of seepage meters installed in the main channel measured gaining stream fluxes before the flood (0.2 and 0.7 mm/d) and losing stream conditions during the flood (−0.7 and −1.4 mm/d). The range in measurements for each setting and period reflects spatial heterogeneity as well as uncertainties due to the presence of gas bubbles in the seepage meter tubing and difficulties establishing good seals in the hummocky wetland and streambed.

4.2. Geochemistry

Observations in Figure 3 show distinct geochemistry in the surface water, groundwater, and pore water throughout changing hydrological conditions over the summer of 2015 (see supporting information Figure S1 for other aqueous chemistry observations). Surface water almost always contains the highest concentrations of SO_4^{2-} among the three domains. Under nonflooded conditions, the west groundwater is the up-gradient source in the flow-through stream system and consistently shows the lowest SO_4^{2-} concentration regardless of streamflow. Pore water SO_4^{2-} concentrations in the channel and wetland pore water are consistently close to the up-gradient west groundwater concentrations in the early season but increase during the flood (August and September), indicating influence from surface water with elevated SO_4^{2-} concentrations. These SO_4^{2-} data support the physical hyporheic zone measurements showing upwelling during the nonflood periods, which would push up lower SO_4^{2-} water from the up-gradient west groundwater, and downwelling during the flood period, which would bring in high SO_4^{2-} surface water. SO_4^{2-} in the down-gradient (east) groundwater generally follows surface water concentrations closely. In August and September, east groundwater concentrations are nearly identical with surface water concentrations not because of hyporheic exchange, but because surface water overspilled the east bank and recharged the east side groundwater through oxidized conditions in the unsaturated zone. This pathway could explain how high surface water sulfate bypasses channel and wetland pore waters to reach the eastside well. Preceding the flood, east groundwater SO_4^{2-} concentrations are lower than surface water levels but still notably higher than pore water concentrations. These could be remnant concentrations from earlier spillovers, or oxygenated recharge water could be reoxidizing sulfide (dissolved or as $FeS_{(s)}$ compounds) in the sediments along its flow path from below the stream to the east well.

Sulfide concentrations are low but present in nearly all pore water samples, suggesting that SO_4^{2-} reduction occurs over the entire summer at all locations. No sulfide was detected in surface waters; negligible concentrations were assumed for groundwater based on the observation of 50–100% DO saturation levels. Higher sulfide concentrations in shallow versus deeper channel sediments indicate that most of the sulfate reduction is occurring near the streambed interface, where SO_4^{2-} concentrations are highest. Lower wetland sulfide concentrations in the early summer compared to the flood period likely result from the exposure of the wetland during this time to atmospheric oxygen. High flood waters prohibited sampling in the stream channel, making it difficult to infer temporal trends or to compare with wetland concentrations during that time. From the available data, flooded wetland and channel sulfide concentration ranges appear similar.

Total dissolved Fe is negligible in the surface water. This is consistent with the 50–100% DO saturation levels measured in the surface water, assuming that any soluble Fe would be mostly in the form of Fe^{2+}, and complexed Fe^{3+} does not significantly contribute to dissolved concentrations. Up-gradient west groundwater contains some dissolved Fe, but concentrations are highest in the pore water. This indicates that unlike SO_4^{2-}, most of the total dissolved Fe is released from in situ sediments rather than transported from surface water or groundwater sources. Of the different locations, pore water samples from the wetlands, particularly the west wetland, contain the highest total dissolved Fe concentrations.

High vertical resolution aqueous geochemistry samples, collected with peepers in August, help fill some data gaps during the flood, especially in the channel. In the wetland, they showed higher concentrations of sulfide and total dissolved Fe and lower concentration SO_4^{2-} compared to the channel. Furthermore, high concentrations of dissolved CH_4 (up to 0.65M) were found in the west wetlands, while only very low concentrations (on the order of μM) were detected in the channel. Further details about the peeper results are provided in section 6 alongside the high spatial resolution model simulations.

Figure 3 shows sediment carbon and acid volatile sulfide (AVS) content in both the measured mass units, as well as in units of moles per liter water to facilitate later comparison with model results. The units conversion was carried out using an average porosity of 0.51 and bulk density of 700 g/L previously measured at Second Creek [*Myrbo*, 2013]. Sediment total carbon content is assumed to represent organic carbon, the e^- donor driving redox reactions in the pore water. TC content is consistently much higher in the wetlands compared to the main channel (Figure 3). No notable differences could be attributed to sample depth, sample month, or wetland location. The measured TC could include some amount of inorganic carbon [*Schumacher*, 2002], and the extracted organic component likely includes forms that are not readily labile over the study period [*Smith et al.*, 2013]. Thus, actual concentrations of degradable organic carbon may be lower than these data, which also means that smaller magnitude variations within degradable organic carbon concentrations may be difficult to detect with this method.

Sediment AVS (Figure 3) indicates the presence of sulfide precipitation at all locations and throughout the summer. Previous work by *Myrbo* [2013] found low aqueous concentrations of metals other than Fe in the region, indicating that AVS measurements can be used to represent $FeS_{(s)}$. Also supporting this assumption, observed pore water chemistries represent (over)saturated $FeS_{(s)}$ conditions based on typical solubilities of fresh $FeS_{(s)}$ precipitates [*Berner*, 1967; *Helgeson*, 1978; *Morse et al.*, 1987; *Davison*, 1991; *Chen and Liu*, 2005]. Main channel and wetland samples cover a significant spread in AVS results, with no notable trends with depth or month. The large range in AVS is potentially a result of small-scale variability in topography or transient changes to redox conditions. The small number of samples makes it difficult to directly compare wetland AVS to observations in the channel.

4.3. Hydrogeochemical Scenarios

Collectively, physical and geochemical measurements indicate that over the summer of 2015, Second Creek provides a useful test bed for examining the influence of variable hyporheic flux on sulfate reduction and interacting S-Fe-C reactions over different hydraulic and geochemical conditions represented by the stream channel and wetlands. While groundwater wells clearly show that Second Creek is a flow-through stream with regional flow from west to east, our physical observations within the hyporheic zone, supported by geochemical data, suggest upwelling of groundwater in the stream channel in the early and late summer, and downwelling of surface water in the stream channel and submerged wetland during the midsummer flood (Figure 4). Consistent with earlier studies examining sulfate-impacted waters in Minnesota [*Myrbo*, 2013], our geochemical data further indicate that in all time periods, pore water is influenced by the high SO_4^{2-} concentration surface water, some of which is reduced to sulfide due to the reservoirs of organic carbon in the

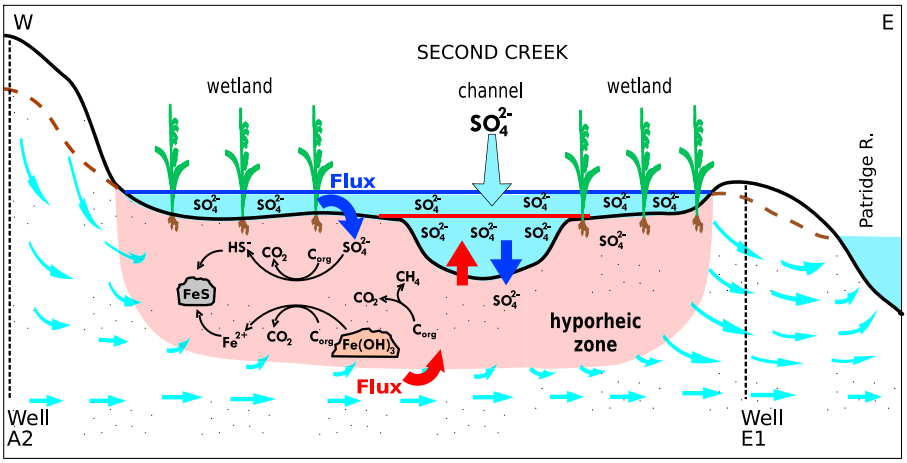


Figure 4. Conceptual model for the hydrological regimes and geochemical processes inferred from field data. Regionally a flow-through stream system, Second Creek experiences dynamic local flux conditions in its hyporheic zone (shaded in pink). Surface water with elevated SO_4^{2-} concentrations is discharged to Second Creek from upstream mining sources. At our study site on Second Creek, local upwelling of groundwater (red arrows) occurs in the early and late summer in the main channel when surface water levels are low, and locally downwelling of surface water (bright blue arrows) occurs during the midsummer flood in the main channel and adjacent wetlands. Local flow dictates conditions within the shallow streambed hyporheic zone, while regional flow establishes lower boundary conditions. These fluxes control concentrations of sulfate, which together with organic carbon content in sediments, drive geochemical reactions in the hyporheic zone.

sediments; measurements of AVS and high pore water total Fe concentrations also show that much of the sulfide precipitates as $FeS_{(s)}$ compounds. With low total Fe concentrations found in both surface water and underlying groundwater, we demonstrated that pore water total Fe originates from the sediments, likely by reduction of solid ferric oxyhydroxides. Additionally, high concentrations of CH_4 detected with peepers in the wetland demonstrate that mixed and/or competing redox zonation may occur below sulfate-impacted waters.

Because of highly dynamic physical and geochemical conditions, identifying and quantifying the impact of hydrologic flux on sulfate reduction and coupled geochemical reactions in the hyporheic zone is difficult using field measurements alone. In the context of our field observations, we implemented a reactive transport model to further interpret and quantify the impact of hydrologic flux on sulfur cycling processes in stream and wetland sediments. Prompted by our field data, we evaluate five different hyporheic zone scenarios with characteristic hydrologic and geochemical conditions. Three scenarios are modeled for the stream channel, including the preflood, flooded, and postflood periods. The other two scenarios simulate the submerged wetland during the flood, including the west wetland and the east wetland. Dividing the summer into only three time periods oversimplifies the complexity of hyporheic processes in natural stream settings, but it delineates distinct hydrologic regimes for which to examine groundwater and stream water influence on pore water geochemistry in SO_4^{2-}-impacted streams and wetlands. Modeling these scenarios allows us to quantify sulfate reduction rates under varying hydrologic fluxes and geochemical settings, with the specific goals of evaluating the relative roles of surface water and groundwater, sediment organic content, and interactions with Fe and CH_4 (through mineral reactions and redox competition).

5. Model Description

We use the reactive transport model PHT3D [*Prommer et al.*, 2003] to examine the geochemical response to hydrologic fluxes over the summer of 2015 at Second Creek for each of the five scenarios outlined above. PHT3D couples the numerically robust transport algorithms from MT3DMS [*Zheng and Wang*, 1999] with the flexible geochemical model PHREEQC-2 [*Parkhurst and Appelo*, 1999]. The transport component uses groundwater flow fields produced with MODFLOW [*Harbaugh*, 2005].

5.1. Domain and Flow Model

To evaluate the most geochemically dynamic portion of the hyporheic zone, we simulated 1-D vertical profiles focusing on the dynamic top 50 cm of streambed sediment that also includes the root zone of aquatic plants.

The model domain was further extended to 3 m depth to represent deeper groundwater conditions, with the pore water-deeper groundwater interface set at 2.5 m depth based on preliminary model tests to match observed water chemistry in the upper profile. The model domain was discretized at 1.5 cm thick layers. The geochemical model domain is divided into three zones based on observed variations in the field data: the top layer represents surface water chemistry, middle layers are sediment pore water in the streambed, and the bottom layers below 2.5 m are deeper groundwater.

To simplify the model based on our approximately monthly sampling intervals, we divide the summer into 30 day periods of steady state flux conditions for both the wetland and stream channel. We associate each 30 day period to either the preflood, flood, and postflood period, and we impose constant groundwater upwelling or downwelling accordingly. Flux magnitudes, which were difficult to measure in the field, were further constrained based on observed pore water geochemistry.

5.2. Geochemical Model

5.2.1. Background and Partial Equilibrium Approach

To simulate processes controlling S and interactions with Fe and C, the geochemical model must represent different terminal electron accepting processes (TEAPs) and relevant solid phase reactions. Kinetic reduction of terminal electron acceptors (TEAs) in groundwater and sediment pore water is often modeled using a Monod-based approach to represent preferential reduction of TEAs based on reduction potential [*Van Cappellen and Gaillard*, 1996; *Hunter et al.*, 1998; *Schäfer et al.*, 1998; *Gwo et al.*, 2001; *Mayer et al.*, 2001]. However, redox zonation does not always occur in the exclusive order of reduction potential [*Jakobsen and Postma*, 1996; *Ludvigsen et al.*, 1998; *McGuire et al.*, 2002; *Laverman et al.*, 2012; *Hansel et al.*, 2015], and the irreversible nature of the reactions in the Monod approach can cause thermodynamically infeasible reactions and unrealistic fluctuations in pH [*Curtis*, 2003]. Representing TEA reduction kinetics in models is further complicated by their dependency on a complex range of biogeochemical conditions.

We adopt a partial equilibrium approach to simulate overall observed geochemical trends. The partial equilibrium approach [*Jakobsen and Postma*, 1999] avoids the strict redox zonation and pH problems of Monod-based model by representing only the oxidation half of the redox reaction (organic carbon degradation) as kinetic, while allowing the reduction half of the reaction (e.g., aerobic respiration, SO_4^{2-} reduction, Fe(III) reduction, or methanogenesis) to occur at equilibrium. The high concentrations of surface water SO_4^{2-} (3.15–8.72 mM) indicate that organic carbon availability is likely the limiting factor in SO_4^{2-} reduction [*Holmer and Storkholm*, 2001]. Previously measured total sediment Fe extractions exceed AVS content at Second Creek (not shown here), suggesting that abundant Fe reservoirs may similarly be available for Fe(III) reduction. We generically represent the e^- donor as labile organic carbon with stoichiometry CH_2O. This leads to the following coupled anoxic redox reactions:

$$2(CH_2O) + SO_4^{2-} \rightarrow 2(HCO_3^-) + H_2S \quad (1)$$

$$4Fe(OH)_{3(s)} + CH_2O + 7H^+ \rightarrow 4Fe^{2+} + HCO_3^- + 10H_2O \quad (2)$$

$$2CH_2O \rightarrow CH_4 + CO_2. \quad (3)$$

To implement the partial equilibrium approach in the model, we use the first-order decay rate equation for organic carbon with parameter k':

$$\frac{d[CH_2O]}{dt} = -k'[CH_2O], \quad (4)$$

where $[CH_2O]$ is the concentration of labile organic carbon. We implement the initial concentration of readily degradable organic carbon based on observed total carbon concentrations and then use the existing physical and geochemical surface water, pore water, and groundwater data at Second Creek to calibrate the decay parameter k'. Other kinetic rate equations have been proposed [*Li et al.*, 2010; *Curtis*, 2003; *Jin and Bethke*, 2002, 2005], which aim to represent additional factors affecting redox processes, such as microbial activity [*Bradley et al.*, 2016; *Pallud and Van Cappellen*, 2006; *Tarpgaard et al.*, 2011; *Okabe et al.*, 1992], organic carbon forms [*Holmer and Storkholm*, 2001; *Detmers et al.*, 2001; *Larowe and Van Cappellen*, 2011], mineral phases [*Hering and Stumm*, 1990; *Bonneville et al.*, 2009] sediment surface properties [*Roden*, 2006], and microenvironment conditions [*Groffman and Crossey*, 1999; *Sobolev and Roden*, 2002; *Roden*, 2012; *Armstrong*, 1964; *Grayston et al.*, 1997; *Bais et al.*, 2004; *Bruijn*, 2013]. Instead of attempting to incorporate this multitude of effects, we calibrate

a first-order decay model based on readily accessible measurements and minimize the complexities that can introduce difficult-to-constrain uncertainties in the model.

5.2.2. Geochemical Model Implementation

Aqueous components in the model include nearly all the measured species (inorganic C, dissolved CH_4, DO, Al, Ba, Ca, Cl, Fe, S, K, Mg, Mn, Na, P, S, Si, and pH). Some components are found in small concentrations and play an insignificant geochemical role in S processes, but they are also included to facilitate calibration of hydrologic fluxes. We assume simulated Fe^{2+} to represent the dominant form of total dissolved Fe, although Fe^{3+} can be functionally dissolved in water when chelated to organic compounds [*Mortensen*, 1963; *Jones*, 1998]. The standard PHT3D/PHREEQC-2 geochemical database was used for equilibrium constants for aqueous species reactions.

Sediment phase components in the model include both minerals and cation exchange. With ferrihydrite as the dominant rhizosphere Fe(III) oxide form [*Emerson et al.*, 1999; *Hansel et al.*, 2001], we implement amorphous $Fe(OH)_{3(s)}$ as the main sediment source for pore water Fe. Sustained elevated concentrations of total dissolved Fe at Second Creek suggest Fe supply is not limiting, and so an arbitrarily high initial concentration of $Fe(OH)_{3(s)}$ is set in the model. $FeS_{(s)}$ precipitation is allowed in the model, based on observed pore water Fe, sulfide, and AVS content. Both $Fe(OH)_{3(s)}$ and $FeS_{(s)}$ are represented as equilibrium phase minerals in the partial equilibrium model:

$$Fe(OH)_{3(s)} + 3H^+ \leftrightarrow Fe^{3+} + 3H_2O \tag{5}$$

$$FeS_{(s)} + H^+ \leftrightarrow Fe^{2+} + HS^-. \tag{6}$$

Similar to the organic carbon decay parameter k', we calibrate thermodynamic equilibrium parameters (logK*) for equations (5) and (6) to incorporate complex processes related to SO_4^{2-} and Fe dynamics. For example, specific sediment mineralogy is unknown at Second Creek but could include a variety of Fe(III) oxides, oxyhydroxides, and mixed Fe(III)/Fe(II) compounds, all of which is effectively folded into a single parameter for $Fe(OH)_{3(s)}$. $FeS_{(s)}$ forms vary in their logK constants (−2.9 to −4.648 [*Berner*, 1967; *Helgeson*, 1978; *Morse et al.*, 1987; *Davison*, 1991; *Chen and Liu*, 2005]). A higher logK* value corresponding to less stable phases would likely be most appropriate for fresh precipitates. The model also includes cation exchange for H^+, Fe^{2+}, Mn^{2+}, Ca^{2+}, and Mg^{2+}, which are implemented with an equilibrium constant of −4.03 to model exchange for all divalent cations:

$$D^{2+} + 2X^- \leftrightarrow DX_2 \tag{7}$$

(with generic divalent cation "*D*" and exchange site *X*), following a glacial outwash aquifer model for northern Minnesota [*Ng et al.*, 2015]. We include this cation exchange model in order to represent the possible reservoir of sorbed Fe^{2+} [*Liger et al.*, 1999; *Zachara et al.*, 2004; *Ng et al.*, 2015], as well as interactions with pH and other major cations such as Mg^{2+}. Like SO_4^{2-}, Mg^{2+} is released at high concentrations due to mining activity in northeastern Minnesota [*Berndt and Bavin*, 2009]. To simplify the cation exchange model, we did not include monovalent ions, which occur at lower concentrations than Mg^{2+} at Second Creek (up to 0.5 mM K^+ and 2 mM Na^+ compared to up to 12 mM Mg^{2+}, see supporting information Figure S1). The cation exchange capacity (CEC) is calibrated.

Sediment-bound organic carbon was included for all pore water model layers. Surface water DO was set to a suboxic level (0.1875 mM) based on reducing conditions observed in very shallow sediments. While k' sets the overall organic carbon degradation rate, the calibrated solid phase parameters ($Fe(OH)_{3(s)}$ logK*, $FeS_{(s)}$ logK*, and CEC) ultimately determine the redox distribution among Fe(III) reduction, SO_4^{2-} reduction, and methanogenesis in the partial equilibrium model.

Initial solution chemistry for each zone (surface water, pore water, and groundwater) is based on observed monthly water chemistry data and further charge-balanced and equilibrated with sediment phase components using PHREEQC-2. Observed surface water chemistry at the start of the 30 day simulation period was used as constant concentration top boundary conditions. The average observed west groundwater concentration was used as constant concentration bottom boundary conditions for the west wetland and channel, and the average observed east groundwater concentration was used for the east wetland. Pore water

Table 1. Calibrated Model Parameters, Compared to Observations or Original Model Database Values Where Applicable

Water Flux (Positive up) (mm d^{-1})	Calibrated	Seepage Meter
Channel Jun–Jul	1.00	0.2 to 0.7
Channel Jul–Aug	−0.72	−0.7 to −1.4
Channel Sep–Oct	8.64	Not available
West wetland Jul–Aug	−0.27	−0.2 to −0.5
East wetland Aug–Sep	−0.01	Not available
Sediment organic carbon (mol L_w^{-1})[a]	Calibrated	Measured TC
Channel (all)	0.08	0.003 to 0.27
Wetland (all)	0.41[b]	0.30 to 0.54
Geochemical model parameters	Calibrated	Original database
Aerobic k' (s^{-1}) (equation (4)	1.00E−03	NA
Anaerobic k' (s^{-1}) (equation (4)	2.61E−09	NA
$Fe(OH)_{3(s)}$ logK* (equation (5)	2.119	4.891
$FeS_{(s)}$ logK* (equation (6)	−2.349	−3.92
Cation exchange capacity (mol L_w^{-1})[a] (equation (7)	0.005	NA

[a] L_w refers to liters of water.
[b] Wetland organic carbon set to average measured total carbon (TC).

chemistry observations at the following monthly sampling time were used as calibration targets at the end of the 30 day simulation period for calibrating model parameters.

Parameter calibration of flux, organic carbon concentration and decay, and mineral properties (see Table 1) was carried out using the parameter estimation and uncertainty analysis program PEST version 14.02 [*Doherty*, 2016], PEST optimizes parameter estimates by minimizing an objective function consisting of the sum of weighted squared residuals (misfits between simulations and observations). In our implementation, weights for the target pore water observations consist of the observed minimum to maximum range observed for a specific aqueous species at that location (channel, west wetland, or east wetland); we additionally weighted by a factor of 5 the residuals for pore water SO_4^{2-}, sulfide, Fe^{2+}, and CH_4, which are the main focuses of this study. In-field measurements of sulfide from peepers were also removed from the optimization because of high in-field analytical uncertainties for low dissolved sulfide concentrations (<3 μM). Pore water CH_4 above 20 cm depth in the west wetland was similarly omitted, because preliminary tests showed the partial equilibrium implementation to be unable to with simulate simultaneous methanogenesis alongside Fe(III) and SO_4^{2-} reduction, which is observed there. Other details of the PEST implementation are described in the supporting information.

6. Model Results and Discussion

6.1. Calibration Results

Data for the five scenarios described above were used to calibrate the model parameter values presented in Table 1. Full details on the PEST results and uncertainty analysis are provided in the supporting information, including calibrated parameter standard deviations, correlations, eigenvector analyses, and composite sensitivities. Observations from all scenarios together served as strong constraints on $Fe(OH)_{3(s)}$ logK*, $FeS_{(s)}$ logK*, and degradation parameters. Degradation parameters were particularly sensitive to high CH_4 concentrations observed in the west wetland. Negligible CH_4 measured in the channel in August led to a calibrated organic carbon concentration for the channel that falls within the observed range and is much lower than wetland concentrations. Transient solute fronts measured in the channel and west wetland in August allowed confident calibration of downward flux magnitudes for the corresponding scenarios. In other scenarios, observations indicate that strong downward flux was unlikely, but flux magnitude estimates there were far less certain, because simulated profiles are only weakly sensitive to differences in zero to upward flux magnitudes. A correlation of 0.64 between the calibrated $Fe(OH)_{3(s)}$ logK* parameter and anaerobic decay parameter k' indicate some nonuniqueness in their estimates.

The calibrated $Fe(OH)_{3(s)}$ logK* of 2.119 is significantly lower than the original database value of 4.891 for amorphous $Fe(OH)_{3(s)}$ and corresponds to a lower potential for reductive Fe dissolution (equation (5)). This lower value was calibrated in order to simulate sufficient concentrations of sulfide to match observations, but it is unexpected given that the dynamic redox conditions in the hyporheic zone and rhizosphere should produce unstable Fe(III) oxide forms [*Groffman and Crossey*, 1999; *Sobolev and Roden*, 2002; *Roden*, 2012; *Begg et al.*, 1994; *Emerson et al.*, 1999; *Hansel et al.*, 2001] that correspond to higher logK*. The implications of this unexpected parameter result on redox zonation is discussed further below. Observed supersaturation of $FeS_{(s)}$ relative to the original database solubility prompted a notably higher calibrated $FeS_{(s)}$ logK* (−2.349) than the original database value (−3.92). *Chen and Liu* [2005] also found the PHREEQC database value to be high compared to a compilation of field measurements and suggested a revision of logK = −3.00, which is closer to our calibrated value. Our high calibrated value for $FeS_{(s)}$ logK* results in less precipitation (equation (6)) and could indicate actual kinetic effects slowing the precipitation of $FeS_{(s)}$ [*Rickard and Luther*, 2007]. Although $Fe(OH)_{3(s)}$ provides the major sediment source of dissolved Fe^{2+} in the model, cation exchange acts as another possible reservoir; however, calibrated cation exchange capacity (CEC) of 0.005 mol/L is lower than the 0.02 mol/L used for a glacial outwash aquifer in Minnesota by *Ng et al.* [2015].

6.2. Hydrogeochemical Scenario Simulations

6.2.1. Stream Channel Scenarios

Simulated stream channel profiles for $Fe(OH)_{3(s)}$ and SO_4^{2-} and their reduced forms (dissolved sulfide, dissolved Fe^{2+}, and $FeS_{(s)}$) in Figure 5 show marked differences over the three hydrologic periods, establishing hydrologic flux as an important driver of hyporheic zone geochemistry at the study site. See supporting information (Figures S3a–S3c) for model results of additional geochemical components.

Preflood (mid-June to mid-July) (Figure 5a). At the start and end of the preflood period, replicate pore water samples taken at different channel locations reflect spatial heterogeneity but are sufficiently consistent with each other to indicate overall upward hydrologic flux that helps prevent high surface water SO_4^{2-} from penetrating further than about 5–10 cm into the profile. Although dissolved Fe^{2+} and sulfide profiles remain mostly constant over the 30 day simulation, changes in $Fe(OH)_{3(s)}$ and $FeS_{(s)}$ over the simulation clearly indicate active Fe(III) and SO_4^{2-} reduction. Note that 30 day $FeS_{(s)}$ accumulation in most of the model (0.24 mM) is much less than the measured AVS range at Second Creek (3.5 to 7847 mM, see Figure 3), suggesting that a large preexisting reservoir could make it difficult to observe seasonal SO_4^{2-} reduction based on AVS. $FeS_{(s)}$ decreases in the upper few centimeters of the model domain results from oxidation by surface water DO. The DO is quickly depleted within these top few centimeters, and anaerobic TEAPs dominate through the rest of the profile. Simulations of other aqueous components (see supporting information Figures S3a–S3b) mostly fall within the range of observation replicates, with most discrepancies likely attributable to spatial variability across the channel or temporal changes within a month in surface water concentrations not represented in the model.

Flood (mid-July to mid-September) (Figure 5b). During the flood, gravity core sampling of the channel was not possible in August due to high surface water levels, and instead, the simulation results are compared against peeper pore water measurements in July and sediment core pore water measurements in September. Peeper measurements correspond to about 15 days following the mid-July sampling, but 30 day model results are shown to standardize simulation duration across scenarios. High-resolution peeper data show the SO_4^{2-} solute front reaching a depth of about 20 cm by August, which is well represented by the model with downward hydrologic flux. Even though peeper sulfide measurements were omitted from the calibration due to high in-field analysis uncertainty, they are shown in Figure 5b for visual comparison. It can be seen that simulated sulfide concentrations peak where the highest sulfide concentration is observed, corresponding to where SO_4^{2-} penetrates. Simulated Fe^{2+} matches the peeper profile closely. Unlike the approximately constant Fe^{2+} profile in the preflood period, simulations during the flood show an increase in pore water Fe^{2+} over time that forms a bulge moving downward. In the model, advection of high surface water Mg^{2+} into the sediment desorbs H^+ to help promote further Fe(III) reduction, which intensively consumes H^+ (equation (2) (see supporting information Figure S3). The lower model domain shows similar $Fe(OH)_{3(s)}$ decrease and $FeS_{(s)}$ accumulation as in the preflood scenario, but less net $Fe(OH)_{3(s)}$ depletion occurs at about 5–12 cm, coincident with where high sulfate concentrations enter the profile, sulfide concentration peaks, and slightly higher $FeS_{(s)}$ concentrations accumulate. It thus appears that Fe^{2+} concentrations in the upper profile decrease over time because SO_4^{2-} reduction there out competes Fe(III) reduction and then triggers a sink of $FeS_{(s)}$. This result is at odds with the traditional thermodynamic ordering of TEAPs but is consistent with recent findings by *Hansel et al.* [2015] (discussed further below).

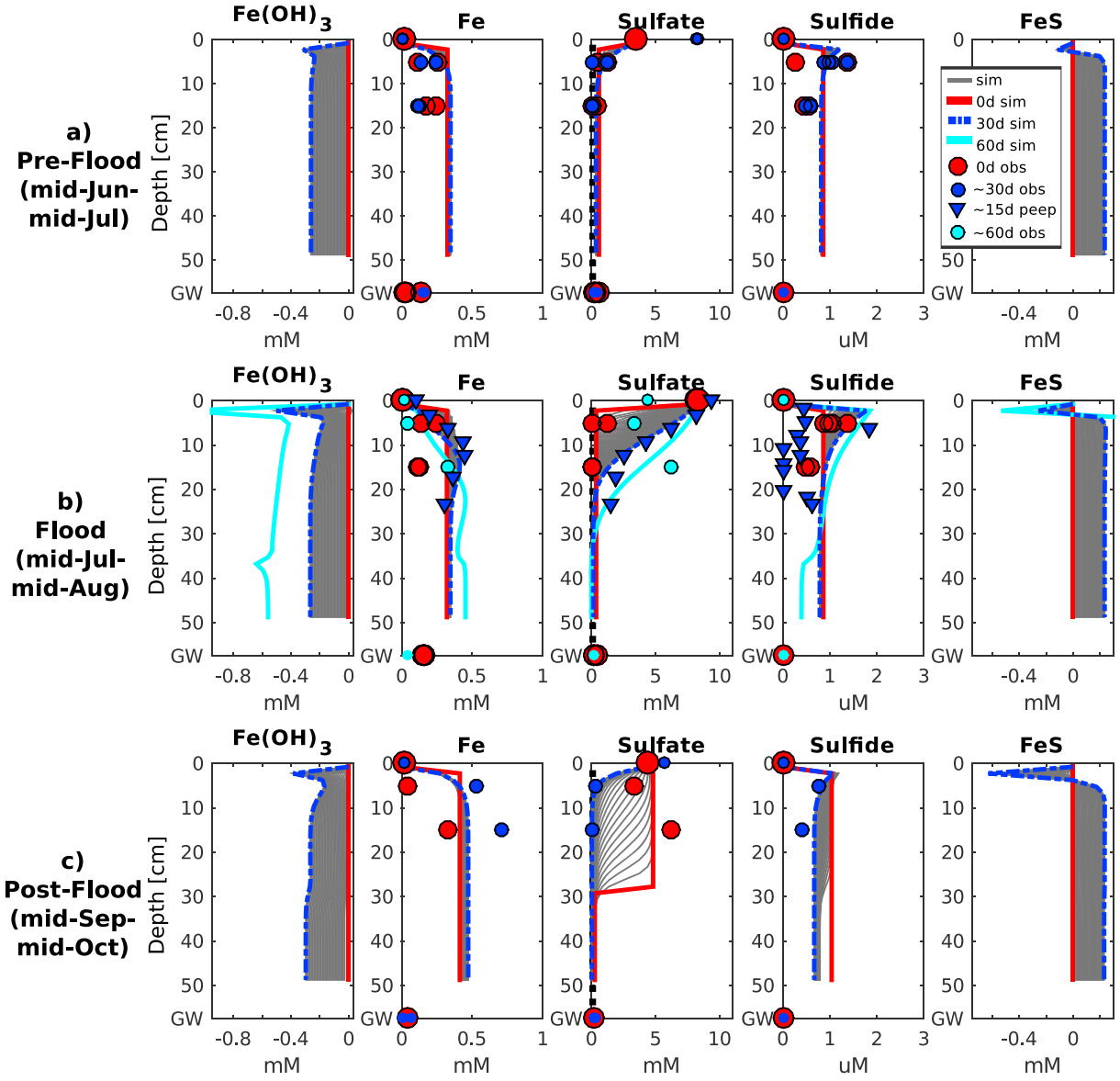


Figure 5. Main species for channel scenarios. Simulations (lines) over 30 days (and at 60 days for flood scenario) are compared against observations (symbols). For the sediment phases $Fe(OH)_{3(s)}$ and $FeS_{(s)}$, simulations are shown for the net change relative to arbitrary initial conditions. For the other aqueous species, initial, top boundary, and bottom boundary conditions in the model are set to initial (day 0) surface water, groundwater, and sediment core pore water observations. Note that the domain bottom boundary is set at 3 m depth, below the shown profile section. The model is calibrated to match pore water data ("obs" for sediment cores and "peep" for peepers). Assuming Fe^{2+} to be the dominant soluble valence state at the observed circumneutral pH conditions (see supporting information Figures S3a and S4a), we compare simulated Fe^{2+} against observed total dissolved Fe.

Postflood (mid-September to mid-October) (Figure 5c). In the preflood and flood scenarios, a constant initial pore water profile based on shallow pore water samples was likely a reasonable approximation because pore water and groundwater chemistry were relatively similar to each other but very different than surface water chemistry. Peeper data and flood period simulations show this not to be the case for the postflood period, which begins with some solute profiles—particularly SO_4^{2-}—reflecting transient, downward advecting conditions. Thus, we initialized the flood period in a similar manner as the other scenarios (using pore water data), but we then assigned groundwater SO_4^{2-} concentrations to the lower part of the profile, corresponding to the simulated 60 day solute front depth for the flood scenario (Figure 5b). Pore water sulfide was not measured during the flood due to difficulties coring the channel under high water levels, and so a concentration between July and October observations was selected as an initial condition.

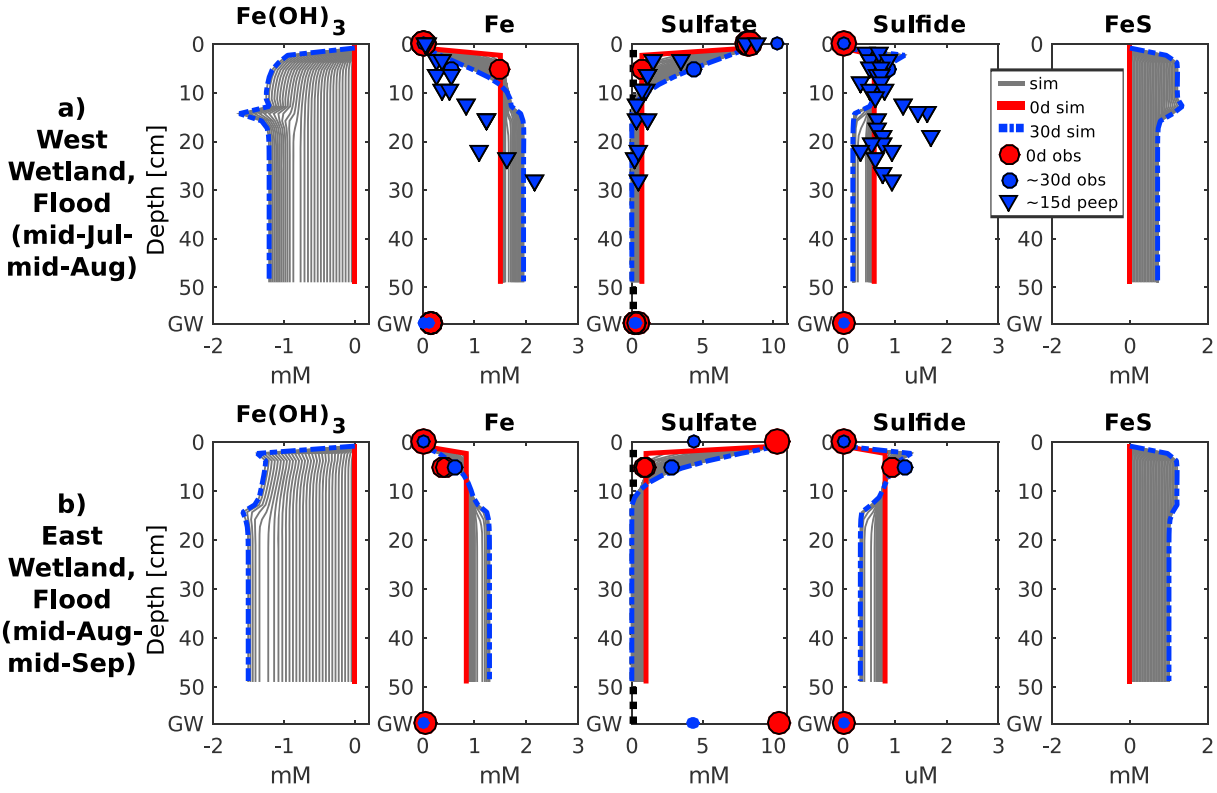


Figure 6. Main species for wetland scenarios. Simulations (lines) over 30 days are compared against observations (symbols). For the sediment phases $Fe(OH)_{3(s)}$ and $FeS_{(s)}$, simulations are shown for the net change relative to arbitrary initial conditions. For aqueous species, initial, top boundary, and bottom boundary conditions in the model are set to initial (0 day) surface water, groundwater, and sediment core pore water observations. Note that the domain bottom boundary is set at 3 m depth, below the shown profile section. The model is calibrated to match pore water data ("obs" for sediment cores and "peep" for peepers).

Pore water measurements at the end of the postflood simulation period show that high SO_4^{2-} pore water concentrations that appeared during the flood quickly returned to lower preflood levels (Figure 5c). The model simulates this using a much higher calibrated upward flux compared to the preflood flux. Seepage meters were not deployed in September, but lower stream head and greater head difference between up-gradient wells and the stream (Figure 2) support the possibility of greater upward flux in the channel during that time. Pore water Fe^{2+} simulations do not match observations as closely as SO_4^{2-} in Figure 5c, possibly due to difficulties in accurately simulating pH buffering by sorption and other mineral phases, but concentrations fall within the range of channel pore water measurements over that period. Corresponding to the decrease in SO_4^{2-} over time, simulated sulfide concentrations slightly decrease toward October peeper concentrations, which indicates some slowing of SO_4^{2-} reduction. However, accumulated concentration of $FeS_{(s)}$ throughout most of the profile remains very similar to the other two channel scenarios. It should be noted that although upward flux brings in groundwater with lower SO_4^{2-} concentrations than surface water, these groundwater concentrations (average 0.3 mM) are still considered high for terrestrial settings. This means that although higher sulfate concentrations from surface water increase sulfate reduction in the simulations, this increase is relatively small given the pervasively high SO_4^{2-} concentrations throughout all domains of the Second Creek study site.

6.2.2. Flooded Wetland Scenarios

Hyporheic exchange of surface water and deeper pore water also occurs in the wetlands adjacent to the channel when they were submerged during the flood period (July through September). Scenarios for the flooded west and east wetlands were both examined (Figures 6 and 7), because of distinct geochemical and hydrological conditions observed on the up- and down-gradient sides of this flow-through stream system (Figure 4). Additional model components are shown in the supporting information (Figures S4a–S4c).

West wetland (July–August) (Figures 6a and 7a). In the flooded west wetland, a high SO_4^{2-} concentration front is not observed to penetrate as deep in the sediment profile as in the flooded channel (about 10 cm versus 20 cm) (compare Figures 5b and 6a). The observed downward transport of other less geochemically active ions

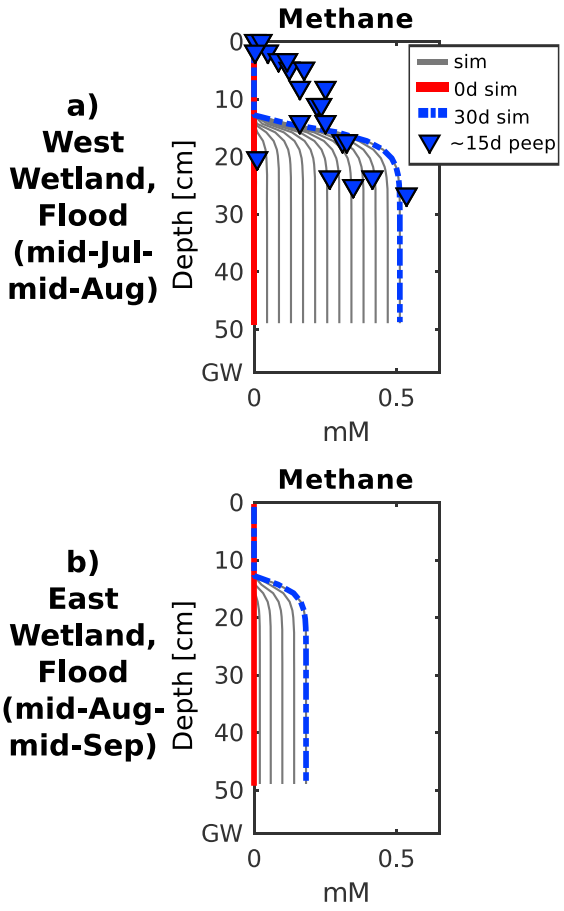


Figure 7. Dissolved CH_4 for wetland scenarios. Simulations (lines) over 30 days are compared against observations (symbols). The model is calibrated to match pore water data from peepers.

such as Mg^{2+}, K^+, and Na^+ helps constrain a downward flux in the west wetland that is less than half that in the flooded channel. The lower wetland flux magnitude is consistent with lower hydraulic conductivity, which can be expected for the finer sediments [*Yourd*, 2017] and higher organic carbon content found in the wetlands compared to the channel. Five times higher organic carbon concentration in the wetland drives correspondingly higher redox reaction rates, resulting in much greater $Fe(OH)_{3(s)}$ depletion and $FeS_{(s)}$ accumulation in the wetland model compared to the channel. Because of the highly reducing conditions, net $FeS_{(s)}$ depletion due to oxidation does not occurs. Similar to in the wetland, the wetland peepers show a bulge in the Fe^{2+} profile with lower concentration near the top where SO_4^{2-} concentrations are highest. However, the model simulates higher dissolved Fe than observed in the upper profile; as discussed next, this is related to interactions with CH_4 in the model.

The calibrated organic carbon decay parameter was constrained to match observed high concentrations of CH_4 in the west wetland below 20 cm depth, which the model does quite well (Figure 7b). As mentioned above, observed lower CH_4 concentrations in the upper 20 cm were omitted from the calibration procedure because of difficulties representing simultaneous methanogenesis alongside Fe(III) and SO_4^{2-} reduction with the partial equilibrium approach. Simulated CH_4 concentrations drop to zero in the upper profile above about 12 cm. This attenuation occurs in the model because high SO_4^{2-} concentrations there allow SO_4^{2-} reduction to thermodynamically out compete methanogenesis, and because any upward dispersion of CH_4 is oxidized coupled to Fe(III) and SO_4^{2-} reduction. The coupling of CH4 with Fe(III) and SO_4^{2-} reduction is evidenced by the sharp depletion of $Fe(OH)_{3(s)}$ and accumulation of $FeS_{(s)}$ above 12 cm (Figure 6a). In freshwater and groundwater systems, anaerobic methane oxidation (AMO) coupled to Fe(III) and SO_4^{2-} reduction has been proposed [*Gupta et al.*, 2013; *Segarra et al.*, 2015; *Weber et al.*, 2016], but this process may be oversimulated in our model, considering the observed nonzero CH_4 concentrations above 12 cm. Oversimulation of AMO coupled to Fe(III) reduction may help explain the oversimulation of dissolved Fe in the upper profile.

East wetland (August–September) (Figures 6b and 7b). In the east wetland, the similarly low pore water SO_4^{2-} concentrations measured in July (just before the flood) as in August (midflood) (Figure 3) are difficult to reconcile with generally downwelling conditions attributed to the flood period, which should introduce higher surface water concentrations over time. The low pore water SO_4^{2-} concentrations measured on 13 August could have resulted from a temporary reversal of flux to upwelling conditions, while the eastside aquifer head decreased back toward original levels following the east bank spillover on 7–8 August (Figure 2). To maintain consistency with the general hydrologic regime delineation, we implement the flooded east wetland scenario using August–September data, which show an increase in pore water SO_4^{2-} (Figure 3) that is more consistent with downwelling. The calibrated downward flux is nonetheless close to zero (Table 1), which could be due to heterogeneity in hydraulic conductivity and/or to smaller head gradients caused by sustained high east groundwater head levels following the second east bank spillover on 6 September (Figure 2). Like the other

flood period scenarios, though, this simulation shows lower Fe^{2+} in the upper profile where SO_4^{2-} concentrations and reduction rates are highest (Figure 6b). Similar to the flooded west wetland scenario, CH_4 appears below the SO_4^{2-} front in the model (Figure 7b). One distinction from the west wetland model is the slightly greater depletion of $Fe(OH)_{3(s)}$ and accumulation of $FeS_{(s)}$ in the east wetland (Figure 6b), which corresponds to greater Fe and SO_4^{2-} reduction rates throughout the profile. This apparently occurs because lower initial pore water Fe^{2+} (0.8 mM in the east wetland versus 1.5 mM in the west wetland) and slightly higher pore water SO_4^{2-} (1 mM in the east wetland versus 0.7 mM in the west wetland) promote greater Fe and SO_4^{2-} reduction, respectively, in the partial equilibrium implementation.

6.3. Sulfate Reduction Rates and Interactions With Fe and CH_4

As described in the section 5, SO_4^{2-} reduction and other TEA redox rates are not explicitly specified in the partial equilibrium model, but instead, the bulk organic carbon degradation rate is set according to a calibrated first-order decay. How the organic carbon degradation is then distributed among different TEAPs depends on the calibrated equilibrium solubility parameters for $Fe(OH)_{3(s)}$ and $FeS_{(s)}$ as well as other geochemical conditions. The TEA-specific redox rates for each scenario were determined from the total mass balance reaction changes calculated in the model for the corresponding species over the 30 day simulation; the mass changes were then normalized by the volume of the pore water model domain (the only portion of the model with organic carbon) to produce the overall mass transformation rates. We simulated SO_4^{2-} reduction rates of 3 to 10 nmol cm^{-3} d^{-1}, which falls within the wide range of 0.5 to 2233 nmol cm^{-3} d^{-1} (found for a SO_4^{2-} concentration range of 0.08 to 98.1 mM) compiled from both marine and terrestrial studies (see literature compilation table in supporting information Table S1). Many of the highest literature rates were measured in flow-through reactors [*Laverman et al.*, 2012; *Pallud et al.*, 2007], which may not reflect natural environmental conditions; other studies [*Robador et al.*, 2015; *Smith and Klug*, 1981] used sediment incubation at optimal reduction temperatures (e.g., 30°C), which are higher than our study site pore water temperatures (15–22°C) and would increase microbial activity. Our simulated SO_4^{2-} reduction rates closely resemble AVS-based field measurements of 5–25 nmol cm^{-3} d^{-1} sulfate reduction in an oligotrophic lake in Denmark [*Holmer and Storkholm*, 2001; *Holmer et al.*, 1998].

Figure 8 shows SO_4^{2-} reduction rates in terms of e^- equivalence for each of the five scenarios. It can be seen that the main driver of differences in sulfate reduction rates across the scenarios is sediment organic carbon, while hydrologic flux plays a far secondary role. In fact, the channel, which had a only a fifth of the organic carbon concentration of the wetlands, produced very a similar SO_4^{2-} reduction rate for the downwelling flood scenario as for the two nonflood periods. Channel profile results discussed above did reveal redox sensitivities to the high SO_4^{2-} concentration solute front (e.g., less Fe(III) reduction corresponding to higher SO_4^{2-} and sulfide concentrations during the flood), but these effects are difficult to detect in the bulk-domain mass balance results, because most of the 2.5 m deep pore water profile in unaffected by the very high surface water SO_4^{2-}. As noted earlier, the deeper channel pore water and westside groundwater SO_4^{2-} concentrations (0.3 to 0.6 mM) may be low compared to surface water concentrations at Second Creek (2.3 to 6 mM), but they are still considered high for terrestrial settings (see supporting information Table S1) and are sufficient to support SO_4^{2-} reduction throughout the pore water profile. This means that although hydrologic flux notably impacts the overall geochemical profiles in the upper pore water domain (as can be seen in Figure 5), the difference between downward flux of extremely elevated SO_4^{2-} concentrations and upward flux of moderately elevated SO_4^{2-} concentrations may not change bulk SO_4^{2-} reduction rates very notably in the channel.

Compared to the channel, the wetland scenarios have higher simulated SO_4^{2-} reduction rates and exhibit greater variability in rates (Figure 8). Sensitivity tests with the model indicate that the flux magnitude difference between the two scenarios plays a minor role in their respective SO_4^{2-} reduction rates. Instead, the higher total domain SO_4^{2-} reduction rate in the east wetland can be attributed to its higher pore water SO_4^{2-} concentrations. Interestingly, in the high degradation setting represented by the wetlands, SO_4^{2-} reduction rates are far more sensitive to SO_4^{2-} concentrations compared to the low degradation setting represented by the channel. This points to a nonlinear and complex relationship between SO_4^{2-} reduction rates and SO_4^{2-} concentrations (Figure 8) that also depends on multiple geochemical factors. This is consistent with the diverse rate relationships reported in previous studies: *Pallud et al.* [2007] found that SO_4^{2-} availability affects rates below SO_4^{2-} concentrations of 1 mM, while *Tarpgaard et al.* [2011] found high reduction rates at SO_4^{2-} concentrations

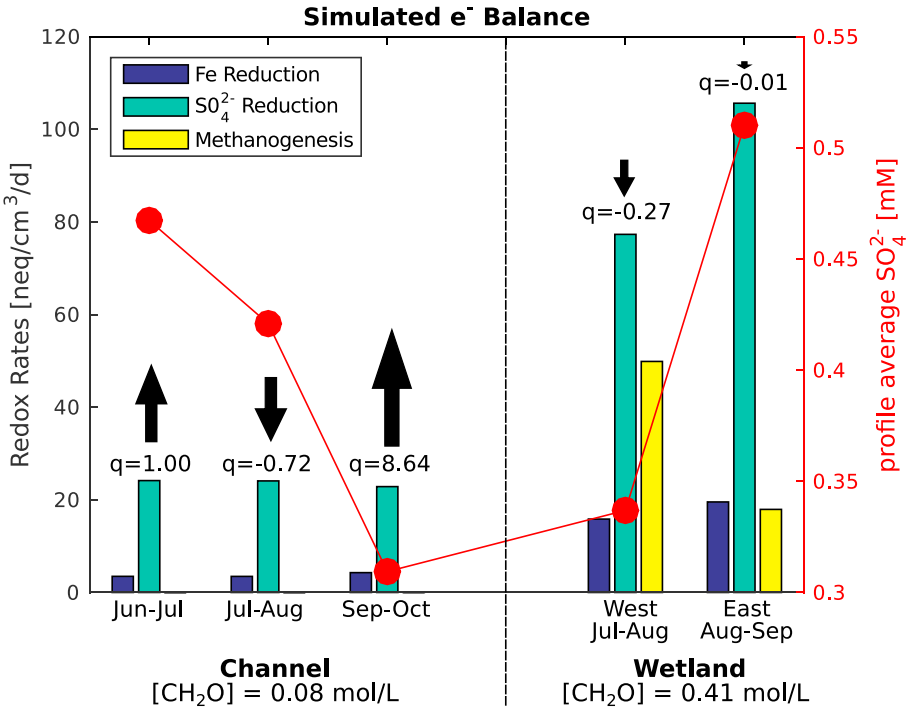


Figure 8. Bar graph of simulated e^- redox rates (left axis) for the five different scenarios. Symbols show average simulated concentrations (right axis) over the 2.5 m soil profile and over the 30 day simulation period. Model flux rates (q) and organic carbon concentrations ($[CH_2O]$) are also indicated.

of 1–100 μM. Also noting the effect of organic content, *Holmer and Storkholm* [2001] measured higher SO_4^{2-} reduction rates in eutrophic lakes compared to oligotrophic lakes.

The most obvious coupling of sulfur and Fe in the model is through $FeS_{(s)}$ precipitation, which the model predicts to be the overwhelming sink of reduced S. More subtle is the interaction through redox zonation. Converting SO_4^{2-} reduction and other TEA-specific redox rates to e^- equivalence in Figure 8 makes it possible to compare the relative roles of different TEAPs. The dominance of SO_4^{2-} reduction over Fe(III) reduction was detected earlier in the scenario profile results; here e^- balance calculations clearly demonstrate that SO_4^{2-}-reduction is the most important TEAP in all scenarios. These field data-calibrated model results corroborate recent flow-through column experiments using freshwater sediments by *Hansel et al.* [2015], in which SO_4^{2-} reduction was observed to precede Fe(III) reduction, even when sediments were amended with highly soluble ferrihydrite. Such findings represent a reversal of the classically held redox sequence, and this reversal had been earlier attributed to only very stable crystalline Fe forms [*Postma and Jakobsen*, 1996; *Jakobsen and Postma*, 1999; *Bethke et al.*, 2011]. Dynamic hyporheic zone and rhizosphere processes at Second Creek likely cycle DO to produce poorly crystalline Fe(III) oxide minerals, yet calibration of a low solubility parameter was necessary for simulating sufficient sulfide concentrations to match observations. *Hansel et al.* [2015] suggest sulfide toxicity, sulfidization, and other metabolic constraints [*Burdige*, 1993; *Koretsky et al.*, 2003] as possible mechanisms for inhibiting Fe(III) reduction, while they found SO_4^{2-} reduction to be facilitated by "cryptic" S cycling involving sulfur intermediates and abiotic sulfide oxidation coupled to Fe processes. Other recent studies have also detected concurrent SO_4^{2-} and Fe(III) reduction [*Kirk et al.*, 2004; *Komlos et al.*, 2008; *Akob and other*, 2008; *Choi et al.*, 2016; *Karimian et al.*, 2017], but to our knowledge, our model results are the first to provide evidence from a field setting for the dominance of SO_4^{2-} reduction over Fe(III) reduction.

The e^- balance results in the wetlands also shows that greater SO_4^{2-} reduction rate in the east wetland compared to the west wetland can notably inhibit methanogenesis. Our model simulations support studies suggesting that high terrestrial SO_4^{2-} concentrations can inhibit CH_4 release from wetlands [*Gauci et al.*, 2004], while our field data are also consistent with the recent field study by *Dalcin Martins et al.* [2017] that detected CH_4 in the active SO_4^{2-} reduction zone.

7. Summary and Conclusions

We calibrated a reactive transport model to field observations at a sulfate-impacted riparian wetland to examine sulfate reduction and its interactions with Fe and CH_4 under different hydrogeochemical scenarios. Surface water, pore water, and deeper groundwater geochemical data provided effective constraints on hyporheic zone flux, organic carbon degradation properties, and sediment parameters related to Fe. Our study area was found to be regionally a flow-through stream, which established the up-gradient groundwater source; however, local hyporheic flux was controlled more directly by stream levels. One-dimensional vertical streambed simulations showed that changes in hyporheic flux direction and magnitude strongly affect solute profiles through relative influxes of deeper groundwater and surface water. At our study site, very high concentrations of SO_4^{2-} from mining-derived surface water (2.8 to 10.3 mM) penetrates deeper into sediments under downwelling flood conditions, while lower concentrations in the up-gradient groundwater buffer against the influx of surface water during upwelling conditions.

Although hyporheic flux drives solute fronts, we found that SO_4^{2-} reduction rates respond most strongly to organic carbon concentration, because of its control on overall redox rates in the implemented partial equilibrium model. A factor of five greater organic carbon content in the wetland scenarios compared to the stream channel scenarios led to about 3 to 4 times greater SO_4^{2-} reduction rates there. Very similar total SO_4^{2-} reduction rates were simulated across the different channel scenarios, because even though deeper pore water and groundwater SO_4^{2-} concentrations are up to two orders of magnitude lower than surface water concentrations, they are still sufficiently high (0.3–1.0 mM) to be driving SO_4^{2-} reduction throughout the bulk of the channel profiles. Wetland simulations showed consistently higher SO_4^{2-} reduction rates than the channel and also exhibited greater variability between wetland scenarios. Wetland rates showed greater sensitivity to differences in SO_4^{2-} concentrations in the profile.

Together, the five scenarios covering different flux conditions and sediment settings indicate that hydrologic flux strongly controls geochemical profiles in streambeds, but how much this influences SO_4^{2-} reduction rates depends on organic carbon content and on surface water and groundwater chemistry. Unsurprisingly, the impact is less if both groundwater and surface water SO_4^{2-} concentrations are sufficiently high to support SO_4^{2-} reduction. Less obvious was the model result that orders of magnitude lower SO_4^{2-} concentrations in the groundwater compared to surface water could produce similar rates in the channel. Furthermore, simulations revealed a complex and nonlinear effect in which SO_4^{2-} reduction rates show much greater sensitivity to SO_4^{2-} concentrations in high organic content (high degradation) settings. A range of about 0.3 to 0.5 mM average profile SO_4^{2-} negligibly affected in SO_4^{2-} reduction rates in the channel scenarios, while a similar range contributed to 37% change in rates in the wetlands. These results indicate that perturbations in SO_4^{2-} concentrations brought by dynamic hyporheic fluxes could have a stronger impact on SO_4^{2-} reduction rates in settings with higher organic content. Also, regional groundwater chemistry becomes important for constraining conditions in the deeper pore water profile in such settings.

Across all hydrogeochemical scenarios, e^- balance model calculations showed SO_4^{2-} reduction to dominate over Fe(III) reduction, despite unlimited reservoirs of Fe(III) oxide specified in the model; SO_4^{2-} reduction accounted for 5 to 7 times greater redox activity (in terms of e^- equivalence). In the partial equilibrium approach, distribution of TEAPs is mainly controlled by the solubility parameter for $Fe(OH)_{3(s)}$. In order to simulate sufficient sulfide to match observations, we calibrated far less soluble properties than typical for amorphous forms, even though dynamic hyporheic zone and rhizosphere processes at our study site likely generate unstable Fe(III) minerals such as ferrihydrite. Detailed profile simulations show that where high SO_4^{2-} concentrations enter the streambed, Fe(III) reduction slows and produces lower concentrations of pore water Fe^{2+}. This reversal of the classic thermodynamic ladder in the model corroborates recent experimental findings by *Hansel et al.* [2015] and, to our knowledge, provides the first evidence from a field setting for its occurrence. Our results invite questions about "cryptic" sulfur cycling that could be facilitating the high sulfate reduction rates, as well as about processes that may be retarding the reductive dissolution of unstable Fe forms. The possible dominance of SO_4^{2-} reduction brings to the forefront the importance of S in biogeochemical cycling in terrestrial settings.

Simulations of redox zonation also reveal that SO_4^{2-} reduction precedes methanogenesis as expected, with influxes of high SO_4^{2-} concentrations from surface water strongly attenuating CH_4 concentrations in wetlands. The model further suggests the possibility of Fe(III) and SO_4^{2-} reduction coupling to anaerobic methane oxidation at the boundary of the methananogenic zone; however, such processes may be oversimulated,

because low concentrations of CH_4 are observed in the SO_4^{2-} and Fe(III) reducing areas. Our results suggest that hyporheic flux may affect CH_4 emissions through its influence on streambed SO_4^{2-} concentrations, because methane-producing environments consist of the highly reducing conditions under which SO_4^{2-} reduction rates are most sensitive to SO_4^{2-} concentrations and can compete with methanogenesis. Also, hyporheic fluxes drive the spatial distribution of the redox zones within the streambed profile, which likely influences gas release to the atmosphere.

Our findings stem from a relatively simplistic reactive transport modeling approach, in which the many complexities related to organic carbon structure, Fe mineral diversity, microbial details, and microenvironment processes are folded into calibrated effective parameters for a partial equilibrium model implementation. Furthermore, high spatiotemporal variabilities in hyporheic flux are reduced to five constant flux scenarios based on gross hydrological conditions and sediment setting. While some important factors are thus not taken explicitly into account, our approach enables clear conceptual models that illuminate overarching processes while avoiding uncertainties from poorly constrained processes. Future work motivated by the results of this study will include microbial and detailed solid phase analyses to assess processes that allow SO_4^{2-} reduction to dominate in the field. Tighter constraints on solid phase Fe will especially be crucial, given their role in both the redox source ($Fe(OH)_{3(s)}$) and sink ($FeS_{(s)}$) in the model. We will also monitor hydrologic conditions in greater detail to evaluate how microbial and geochemical processes respond to hyporheic flux on varying spatiotemporal scales.

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NMW Exh. 8

Final Technical Support Document: Refinements to Minnesota's Sulfate Water Quality Standard to Protect Wild Rice



CLEAN WATER LAND & LEGACY AMENDMENT

MINNESOTA POLLUTION CONTROL AGENCY

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Availability of manuscripts based on MPCA-sponsored research that are cited in this document

From 2011-2013 MPCA sponsored extensive research into the effect of elevated sulfate and sulfide on wild rice and potential wild rice habitat. Much of the research was carried out by researchers at the University of Minnesota (at both the Duluth campus and Twin Cities campus) and the St. Croix Watershed Research Station of the Science Museum of Minnesota. Four manuscripts were prepared for publication in peer-reviewed scientific journals. A paper by Pastor et al. (see below) was published in January 2017. A group of three papers that refer to each other were submitted to the Journal of Geophysical Research. Because they refer to each other, they cannot be published until all three are accepted for publication. As of August, 2017, two of the three papers have been accepted, and one is still in review.

The MPCA is not posting the manuscripts on the MPCA website, because it is unethical to publish a scientific paper more than once, and most journals now regard the posting of a paper on a website as a publication. However, sharing a copy of a manuscript is not considered to be a publication. Therefore, copies of the four manuscripts are available from the MPCA, and may be requested but must not be posted on a website.

Published:

(Pastor et al., 2017) Pastor, J., B. Dewey, N. W. Johnson, E. B. Swain, P. Monson, E. B. Peters, and A. Myrbo. 2017. Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments, *Ecological Applications*, 27, 321-336. This paper is available from the MPCA.

Accepted for publication:

(Myrbo et al., in press-1) Myrbo, A., E.B. Swain, D.R. Engstrom, J. Coleman Wasik, J. Brenner, M. Dykhuizen Shore, E.B. Peters, and G. Blaha. In press-1. Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. This manuscript is available from the MPCA.

(Pollman et al., in press) Pollman, C.D., E.B. Swain, D. Bael, A. Myrbo, P. Monson, and M. Dykhuizen Shore. In press. The evolution of sulfide in shallow aquatic ecosystem sediments – an analysis of the roles of sulfate, organic carbon, iron, and feedback constraints using structural equation modeling. This manuscript is available from the MPCA.

Submitted, but not yet accepted:

(Myrbo et al., submitted-2) Myrbo, A., E.B. Swain, N.W. Johnson, D.R. Engstrom, J. Pastor, B. Dewey, P. Monson, J. Brenner, M. Dykhuizen Shore, and E.B. Peters. Submitted-2. Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. This manuscript is available from the MPCA.

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Introduction

The federal Clean Water Act requires states to designate beneficial uses for all water bodies (i.e. "waters") and develop water quality standards to protect each use. Water quality standards include several components:

- Beneficial uses — identification of how people, aquatic communities, and wildlife use waters.
- Numeric standards — typically the allowable concentrations of specific chemicals in a waterbody, established to protect beneficial uses. Can also include measures of biological health.
- Narrative standards — statements of unacceptable conditions in and on the water.
- Antidegradation protections — extra protection for high-quality or unique waters and existing uses.

Minnesota Rules Chapter 7050 assigns a series of beneficial use classifications to all waters of the state. These use classifications set out the beneficial uses that apply to Minnesota waters. Water use classifications, and their accompanying narrative and numeric standards and antidegradation provisions, make up the state's set of water quality standards. Aquatic life and recreation, industrial uses, agriculture and wildlife, and domestic consumption are some of the beneficial uses that these standards protect. Although there is a lot of commonality among the beneficial uses established by states – for example, every state designates and protects drinking water as a beneficial use – states may also set beneficial uses that reflect the unique nature of their waters and aquatic resources.

Minnesota's Class 4 water quality standards protect the waters of the state so that they are suitable for "the agriculture and wildlife designated uses." One subclass of Class 4 is Class 4A waters (Minn. R. 7050.0224, Subp 2), which must be clean enough "to permit their use for irrigation without significant damage or adverse effects upon any crops or vegetation usually grown in the waters or area." In 1973, Minnesota established a unique beneficial use by establishing a subset of the Class 4A use class called "water used for production of wild rice" and setting a numeric standard to protect the production of the wild rice grain. Wild rice is an important plant species in Minnesota. It provides food for waterfowl, is economically important to those who harvest and market it, and is also an important cultural resource to many Minnesotans.

The specific pollutant from which the "water used for production of wild rice" is protected is sulfate. Sulfate is a natural chemical commonly found in surface and groundwater. It can also be found at varying concentrations in discharges from permitted facilities such as mining operations, municipal wastewater treatment plants, and industrial facilities. The observed relationship between the presence of wild rice in waters with lower sulfate levels, and its absence in waters with elevated sulfate, led to the adoption of the wild rice sulfate standard in 1973.

Minnesota's wild rice sulfate standard came under scrutiny in the 2000s. Of particular interest was better understanding the effects of sulfate on wild rice in order to understand the appropriateness of the standard and its implementation. The need to clarify which waters support the wild rice beneficial use was also identified.

In 2011 the Minnesota Legislature provided $1.5 million in funding for the MPCA to conduct a Wild Rice Sulfate Standard Study to gather additional information about the effects of sulfate and other substances on the growth of wild rice. The legislation also required the MPCA to undertake rulemaking to identify wild rice waters and to make any other needed changes to the sulfate standard following completion of the study.

Following the completion of the Wild Rice Sulfate Standard Study in December 2013, MPCA reviewed the results and developed a preliminary analysis of the research, which it then shared with stakeholders

in March 2014 (MPCA, 2014). MPCA staff met with many partners and stakeholders, and continued to refine the analysis of the research based on comments received, review of additional literature and additional statistical analyses. The result of this effort was completion of the Analysis of the Wild Rice Sulfate Standard Study — Draft for Scientific Peer Review in June 2014 (MPCA, 2014).

MPCA then contracted with Eastern Research Group, Inc. (ERG) to convene and facilitate a scientific peer review of the study and analysis. Full details, background documents and the final report from ERG on the scientific peer review process can be found on the MPCA's webpage about the wild rice sulfate standard study.

MPCA refined its analysis based on the peer review and tribal and Advisory Committee feedback, and in March 2015 MPCA released a Draft Proposal for Protecting Wild Rice from Excess Sulfate (MPCA, 2015).

The Draft Proposal included:

- A proposed draft approach to the wild rice water quality standard;
- A draft list of waters where the standard would apply; and
- Draft criteria for adding waters to the list over time as new or additional information becomes available.

The Draft Proposal was shared broadly, including through a Request for Comments (RFC) asking the public for comments and information about wild rice sulfate standard rulemaking.

As a result of comments and questions received following release of the Draft Proposal, MPCA has re-analyzed data from the studies using different statistical approaches. This reanalysis included review of the following:

- Field survey data used to relate wild rice presence to sulfide in the sediment;
- Field survey data that relate sulfate to sulfide;
- Basic assumptions relating sulfate to wild rice;
- Choice of which data set of sites from 2011-2013 field work would be most appropriate to use in analyses; and
- Variables controlling conversion of sulfate to sulfide.

The MPCA then published a draft Technical Support Document (draft TSD; MPCA, 2016) in 2016 as the next step in the ongoing effort to better understand the effects of sulfate on wild rice to inform an evaluation and, as needed, a revision to the standard.

The Draft TSD was shared broadly and the MPCA received partner and stakeholder input. This Final TSD provides the main scientific support for the MPCA's proposed changes to the wild rice sulfate standard. It revises and updates the draft TSD, providing additional analyses and explanations. Some information provided in the Draft TSD has been moved into the Statement of Need and Reasonableness (SONAR, MPCA 2017). Together, the SONAR and this TSD, along with their exhibits and reference materials, support the MPCA's proposed changes to the wild rice sulfate standard.

Chapter 1. Numeric wild rice sulfate standard

This chapter of the TSD focuses on the mechanism by which sulfate impacts wild rice, and the numeric standard for protecting wild rice from excess sulfate. This chapter is organized as follows:

Part A introduces the primary hypothesis that guided MPCA's technical investigation (namely: if sulfate is harmful to wild rice, sulfate acts by being converted to hydrogen sulfide (sulfide) in the sediment in which wild rice grows), and presents evidence that sulfide exerts significant control over the presence and absence of wild rice in Minnesota's shallow aquatic systems.

Part B refutes the argument that it is not necessary to protect wild rice from elevated sulfide, given that there are multiple other environmental stressors affecting the occurrence of wild rice in water bodies aside from sulfide, such as changes in water levels, impacts of watershed development, and the presence of invasive or competitive species.

Part C presents the evidence used to identify 120 micrograms per liter (µg/L) in sediment porewater as the sulfide concentration that is protective of wild rice populations in natural water bodies. Identification of a protective sulfide concentration is a necessary step in the development of a sulfate standard. The next step is to develop a quantitative relationship between sulfate and sulfide.

Part D shows that the relationship between surface water sulfate and porewater sulfide differs among water bodies, and is dependent on sediment concentrations of organic matter and iron.

Part E presents an equation that allows the calculation of a numeric sulfate standard for each wild rice water, as an alternative to maintaining the existing 10 mg/L standard or establishing a different fixed number as the sulfate standard for all wild rice waters.

Part F shows that the equation-based sulfate standard is more accurate than any fixed standard at protecting wild rice from elevated sulfide. Any fixed standard has a higher error rate (being too high or too low than necessary to be protective) than the equation.

A. Confirmation that porewater sulfide is a primary controller of wild rice occurrence

The MPCA began its investigation of the effect of sulfate on wild rice in 2010 by reviewing the scientific literature. After this initial evaluation, MPCA determined that additional studies were needed to better understand the effects of sulfate on the growth of wild rice. In early 2011, MPCA staff scientists prepared a draft research protocol that was designed to further investigate the effects of sulfate on wild rice. On May 9, 2011, MPCA sponsored a discussion of the draft research protocol that included 36 scientists with pertinent expertise (13 from the University of Minnesota, seven from Federal agencies, six from Minnesota tribes, five from the MDNR, and five scientists with other affiliations). The scientists discussed the draft research protocol, which hypothesized that if sulfate is important in controlling the occurrence of wild rice, the active agent would be a result of bacterial conversion of the sulfate to hydrogen sulfide (H_2S) in the sediment where wild rice seeds germinate and grow. In water-saturated sediment, which are usually anoxic, some bacteria that degrade organic matter in the sediment "respire" sulfate, rather than oxygen, producing hydrogen sulfide. The chemical form of hydrogen sulfide varies with pH; below pH 7 H_2S dominates, and above pH 7 the bisulfide ion (HS^-) dominates. For simplicity in this document the sum of the two chemical species is referred to as sulfide.

The 2011 Legislature provide funding to research the effects of sulfate and other substances on wild rice. The research protocol was revised in response to the expert discussion, and finalized on November 8, 2011 (MPCA, 2011). Following a preliminary data collection effort in 2011, in 2012 the MPCA issued a Request for Proposals and ultimately contracted with groups of scientists at the University of Minnesota Duluth and Twin Cities campuses to undertake a study to better understand the effects of sulfate and other substances on wild rice. The MPCA study focused on collecting data on the relationship between sulfate, sulfide, and wild rice through three major parallel study components.

The components each had a specific purpose and associated strengths and limitations (Table 1-1). The study was designed so that the individual components together provided a better understanding of the effects of sulfate on wild rice. The three major study components were:

- Field surveys of wild rice habitats to investigate physical and chemical conditions correlated with the presence or absence of wild rice, including sulfate in surface water and sulfide in the sediment porewater of the rooting zone.
- Controlled laboratory hydroponic experiments to determine the effect of elevated sulfate and sulfide on early stages of wild rice growth and development.
- Outdoor container (mesocosm) experiments using natural sediments to determine the multi-year response of wild rice and other variables to a range of sulfate concentrations in the surface water.

Scientists also conducted additional research via a laboratory experiment to examine the effect of temperature on the movement of sulfate between water and sediment.

Table 1-1. Purpose, strengths, and limitations of the components of the MPCA-sponsored wild rice research.

	Field survey	Laboratory hydroponic experiments		Outdoor container experiment	Sediment incubation laboratory experiment
		Sulfate	**Sulfide**		
Main purpose	Expand understanding of environmental conditions correlated with the presence & absence of wild rice	Evaluate effects of sulfate on wild rice seed germination and growth of seedlings	Evaluate effects of sulfide on wild rice seed germination and growth of seedlings	Evaluate effects of sulfate loading on sulfide and wild rice life cycle, over multiple years	Evaluate effect of temperature on movement of sulfate into and out of underlying sediment
Endpoints	Concentrations of chemicals in surface water, porewater, & sediment (e.g., sulfate & sulfide) vs. wild rice occurrence	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds	Growth of wild rice sprouts (biomass, root and shoot elongation); germination rate of seeds.	Growth of wild rice (biomass, plus number and weight of seeds); sulfide concentrations in rooting zone	Sulfate concentrations in overlying water over time; sulfate, iron, sulfide, and anion tracers in sediment porewater; simple model
Key strengths	Most reflective of actual environmental conditions; multiple wild rice stands and breadth of characteristics sampled	Controlled dose-response experiment; controlled exposure to known concentrations of SO_4	Controlled dose-response experiment; controlled exposure to known concentrations of sulfide	Controlled dose-response experiment. Includes natural sediment matrix as rooting environment; involves entire growth cycle, multiple years	Controlled experiment with natural sediment and water
Key limitations	Least controlled; annual visit for most sites, 3x/year for a subset; not definitive on cause and effect	Only evaluates early growth stages; leading hypothesis is that sulfate is converted to sulfide, which is directly toxic	Only evaluates early growth stages; unable to simultaneously keep roots anaerobic and shoots aerobic	Eventual steady states with various sulfate loads may not mimic the environment, since there is no loading of other key constituents, such as iron, from groundwater or the watershed.	Provides preliminary assessment of sediment from two sites that may inform, but is not fully transferrable to other sites; no groundwater movement; no wild rice plants grown
Reference	Myrbo et al. (in press-1.)	Pastor et al. (2017)		Pastor et al. (2017); Myrbo et al. (submitted-2)	DeRocher & Johnson (2013) Report to the MPCA.

Impact of porewater sulfide on plants and animals

In 2010, when the MPCA began its investigation on the impact of elevated sulfate and sulfide on wild rice, MPCA could find no scientific information specific to sulfide impacts on wild rice. However, elevated sulfide is a well-documented concern for other aquatic plants that root in sediment (reviewed by Lamers et al., 2013).

EPA has provided guidance on surface water criteria for sulfide, setting a level of 2 micrograms per liter (µg/L) as the maximum that can be present in a surface water before sulfide is likely to harm aquatic life. Though EPA produced this value 30 years ago (EPA, 1986), remarkably little attention has been given since then to the potential toxicity of sulfide to benthic animals and aquatic plants that root in sediment. In a major review, Bagarinao (1992) concluded that sulfide had been "…largely overlooked as an environmental factor for aquatic organisms." In a discussion of sediment toxicity testing, Wang and Chapman (1999) also observed that the biological implications of sulfide in sediments are poorly understood and "all too often ignored."

Little information has been developed about how sulfide controls the occurrence of plants and animals in water-saturated sediments and soils. A recent review (Kinsman et al., 2015) concluded that the potential toxicity of porewater sulfide is likely shaping the plant and animal communities of aquatic ecosystems, yet little data has been collected. In an exception to the paucity of data, Simkin et al. (2013) showed that porewater sulfide controlled the distribution of wetland plants more than nutrients. In their introduction, they wrote "…it is puzzling that there has not been more work to investigate the possible role of sulfide as a master variable controlling plant community composition within inland wetland ecosystems." Lamers et al. (2013), in a review of sulfide toxicity to aquatic plants, pointed out that traditional toxicity testing generally neglects the chemistry of the rooting zone.

Aquatic plants that root in marine sediment have evolved in a high-sulfate, high-sulfide environment. Ocean water averages 2,800 mg/L sulfate, so anoxic bacteria in marine sediment can potentially produce high concentrations of sulfide as bacteria degrade sedimentary organic matter. Recently, elevated sulfide has been implicated as the causative agent in a worrying global decline of marine seagrasses, which in some ways are a marine analog to wild rice. Seagrasses, which are perennial, and wild rice, which is an annual plant, are distantly related monocots that both inhabit shallow waters, although seagrasses are often totally submerged. Seagrasses colonize shallow coastal areas worldwide, providing habitat for animals and numerous ecological services. Seagrasses successfully live in the presence of high sediment sulfide by producing high amounts of oxygen through photosynthesis and transporting that oxygen to the roots, which detoxifies the sulfide by converting it back to sulfate. As a result, seagrasses require some of the highest light levels of any plant group (Orth et al., 2006). The primary hypothesis to explain the global decline of seagrasses is that sulfide toxicity is occurring more often as a result of a variety of human activities, including those that reduce underwater light or oxygen levels. In freshwater systems, elevated sulfide could be a result of sulfate pollution, so it makes sense to regulate sulfate in freshwaters. But because sulfate is uniformly high in ocean water, factors other than sulfate have been implicated in increased sulfide production or toxicity. Seagrasses are particularly vulnerable to any processes that reduce light availability, such as eutrophication or dredging of sediment (Orth et al., 2006). In addition, factors that enhance porewater sulfide concentrations have been implicated in the decline of seagrasses, including increased temperature (Koch and Erskine, 2001), increased sediment organic matter (Govers et al., 2014), and iron-poor sediment (Marbà et al., 2008). Sulfide concentrations are greater in iron-poor sediment because iron can remove sulfide from solution. It is thought that only dissolved sulfide is potentially toxic to plants and animals.

Evidence that elevated sulfide can exclude wild rice from otherwise suitable habitat

The MPCA-sponsored field survey of potential wild rice habitat, conducted 2012-2013 (Myrbo et al., in press-1), involved sampling 108 different sites and quantifying 65 field variables (Table 1-2). Some waterbodies were sampled on more than one date. For statistical purposes, a sub-set of the data (called "Class B") was identified where each waterbody is represented by the sample closest to August 11 (the median sample date), in an effort to control for any seasonal variability. Myrbo et al. used the Class B dataset to evaluate the relationship between wild rice presence (or absence) and environmental variables through binary logistic regression (Table 1-3). In addition, Myrbo et al. correlated wild rice density at a site to the environmental variables through nonparametric Spearman tests (Table 1-3).

Field data used for statistical tests of variables that may control wild rice

The Class B data set was used for statistical tests that correlate the presence or density of wild rice against environmental variables that may control wild rice. Class B was used because this data set is the best available approximation of a random sample of potential wild rice waters that includes porewater sulfide and other variables pertinent to wild rice hypotheses. The primary hypothesis prior to conducting the MPCA field survey was that sulfide, rather than sulfate, controlled wild rice presence and absence (MPCA, 2011). It was essential to sample sites that did not have wild rice, in addition to sites with wild rice, so that variables that control the absence of wild rice could be assessed (see analysis below for further discussion of this point). Therefore, sampling only known wild rice sites would not have allowed the assessment of potentially important variables such as sulfate and sulfide. Furthermore, there was no existing data on porewater sulfide in wild rice habitat, and incomplete data on sulfate in surface waters, although the general trend of sulfate concentrations across Minnesota was known. Since it was likely that porewater sulfide was a function of sulfate in surface water, the field survey was conducted to sample a range of sulfate in waterbodies that could potentially host wild rice. Since it had been noted by naturalists and scientists that both white and yellow waterlilies co-occur with wild rice (Pillsbury and McGuire, 2007), the presence of waterlilies was used to identify potential wild rice habitat when wild rice plants could not be found in a waterbody (for further discussion, see below). Therefore, in an effort to determine the effect of elevated sulfate on wild rice, known waters with wild rice (which tended to be low in sulfate) were sampled, plus potential wild rice habitat where elevated sulfate was suspected based on geography or local land use, resulting in the Class B data set. The representativeness of the Class B data can be assessed by comparing quantile sulfate concentrations against other data sets (Table 1-2). Class B includes data from both lakes and streams. The distribution of sulfate concentrations in the Class B data set is appreciably higher than wild rice lakes, but closely approximates the randomized survey of Minnesota lakes conducted by the EPA in 2012 (Table 1-2).

In general, Minnesota streams and rivers have higher concentrations of sulfate than lakes; randomized EPA surveys found that the median, or typical, stream sulfate concentration is 17.0 mg/L, compared to 3.0 mg/L in lakes. The median concentration in Class B streams of 10.0 mg/L is appreciably higher than the median of Class B lakes, 4.1 mg/L, but not as high as the EPA's random survey. It is unclear why the Class B stream data did not better follow the distribution of sulfate across Minnesota, but it may be because a smaller number of streams were sampled than lakes (27 compared to 81) and because the field crews were mainly sent to sites known to host wild rice (80% of stream sites had wild rice, compared to 55% of lakes).

Overall, the Class B data set is the best available approximation of a random sample of potential wild rice waters. Extrapolation of the data is mainly performed later in this TSD to assess the accuracy of models that relate sulfate in surface water to sulfide in porewater.

Table 1-2. Summary statistics for sulfate concentrations in various datasets.

Data Set	Quantile Sulfate Concentration (mg/L)			Number of sites
	25%	50%	75%	
Lakes with reported wild rice (Available sulfate data for lakes listed in MDNR, 2008)	1.0	1.8	3.6	520
All Minnesota Lakes (2012 EPA National Lakes Assessment)	0.3	3.0	13.1	50
Class B Lakes	1.0	4.1	14.6	81
Class B Streams	1.6	10.0	16.6	27
All Minnesota Streams and Rivers (2008 EPA National Rivers and Streams Assessment)	2.0	17.0	47.3	52
All Class B waterbodies (Lakes and Streams)	1.2	5.9	15.6	108

When the field crews could not find wild rice in a waterbody, they sampled the water and sediment at a location where wild rice would most likely be growing if it were to grow in that waterbody. These "non-wild rice" sampling locations were usually identified by the presence of either white or yellow waterlilies. The presence of waterlilies is taken to indicate that the habitat is similar to the habitat required by wild rice, because waterlilies and wild rice frequently co-occur (Pillsbury and McGuire, 2009). In addition, in an analysis of 1,753 MDNR aquatic plant surveys from shallow Minnesota lakes, the odds of finding wild rice where there are water lilies are 27 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20-36 times. This high odds ratio is strong evidence that wild rice and waterlilies share many habitat requirements, although it appears that waterlilies may have a higher tolerance to elevated sulfide concentrations.

Statistical evaluation of variables that may control wild rice

Binary logistic regression (BLR) is the classic method for scientists to identify environmental variables that control the suitability of habitat for a particular species of interest (Hosmer and Lemeshow, 1989; Peeters and Gardeniers, 1998; van der Heide et al., 2009). BLR is "binary" in the sense that it classifies field sites as having, or not having, the species of interest—in this approach, the density of the species is irrelevant to the classification. Conservation biologists use binary information (presence/absence) in the analysis of habitat suitability; density is rarely used because representative density data are difficult to obtain and density can be a function of factors unrelated to the long-term suitability of the habitat. For example, even in excellent wild rice habitat the density of wild rice in a given year can be reduced by a hydrologic event or by animals that use wild rice for food or for nesting material. The entire wild rice plant provides food during the summer for herbivores such as Canada geese, trumpeter swans, muskrats, beaver, white-tailed deer, and moose, and the stems are harvested for nesting material by loons, red-necked grebes, and muskrats (MDNR, 2008, p. 9).

The field survey quantified the wild rice density (stems/m^2) in four 1-m diameter circles around the boat where the sediment samples were collected; this does not represent the entire waterbody. The field crew did not attempt to sample a site that was "typical" of the wild rice bed, which would have been

difficult to determine. Rather, it was considered adequate to take the canoe or small boat anywhere into the wild rice bed for sampling of the water and sediment. There was no reason to expect that the wild rice density at the sampling site would be well correlated with any single environmental parameter, because wild rice density fluctuates significantly from year to year for many reasons, such as temporary nitrogen immobilization in plant litter, hydrologic fluctuation, herbivory, and other disturbances (Tables 1-6 and 1-7).

Using BLR, Myrbo et al. (in press-1) identified 12 variables that may be important in controlling the presence or absence of wild rice, as they were all significant at the 0.05 probability level or better (Tables 1-2 and 1-3). To examine the relationships among all the field variables, a Spearman correlation matrix was calculated (Table 1-4), which revealed that many of the 12 variables are correlated with each other. Because Spearman correlations are not designed for binary data, the correlation matrix included the wild rice density (stems/m^2), despite the fact that the density variable just represents wild rice density around the boat at the sediment sampling site and did not characterize the wild rice bed as a whole. Somewhat surprisingly, the Spearman correlations between wild rice stem density and environmental variables generally agreed the BLR results, although sometimes at lower significance levels (Table 1-4).

Identification of three causative variables in wild rice presence/absence

Myrbo et al. (in press-1) concluded that, out of the 12 identified potentially causative variables, there are just three factors that have direct effects on wild rice — and that the other potentially causative variables are actually correlated with the truly causative factors. The three causative factors are porewater sulfide, surface water transparency, and surface water temperature. These can be understood as having effects on wild rice that, although independent of each other, also interact, especially in reinforcing correlations with other variables such as a negative correlation with total nitrogen in the surface water (see Table 1-5). Surface water nitrogen is an example of a field variable that does not act directly on wild rice, but nevertheless is significantly correlated with the absence of wild rice because it has mechanistic relationships to more than one of the three directly causative variables (Table 1-5).

The available evidence, coupled with the established scientific understanding of the biogeochemical processes of shallow aquatic ecosystems, suggest that the three causative variables act as follows: Elevated porewater sulfide reduces the growth of wild rice, either by direct toxicity or indirectly by impairing nutrient uptake (Pastor et al., 2017); water transparency below 30 cm essentially excludes wild rice from a waterbody due to light limitation (Myrbo et al., in press-1); and elevated temperatures limit the geographic range of this species of wild rice, *Zizania palustris* (Myrbo et al., in press-1).

Regarding the temperature effect, although the measured temperature variable was summer surface water temperature, the actual mechanism is more likely that the sites with higher summer temperatures also are the sites where winters fail to be sufficiently cold. The seeds of *Zizania palustris* are said to need at least three months of submersion in near-freezing water in order to break dormancy (Cardwell et al., 1978), but the needed winter and spring temperatures to break dormancy are incompletely understood (Atkins et al., 1987; Kovach and Bradford, 1992). Atkins et al. (1987) performed an experiment that incubated wild rice seed at 5 C for 5, 6, and 7.5 months, and found the highest germination after 7.5 months, but did not investigate other incubation temperatures. They also found that germination rates were greater in diurnally fluctuating temperatures rather than in constant temperatures.

Cultivated varieties of Minnesota's wild rice are grown successfully in the Central Valley of California, which has a warmer summer than Minnesota (Fig. 1-1), indicating that high summer temperatures are likely not the limitation of wild rice range. But the winters of the Central Valley of California are too warm to break the seed dormancy, so Central Valley wild rice farmers store the seed for the next growing season over the winter under water in refrigerated facilities (Marcum, 2007). Therefore, a reasonable hypothesis generated by the observed negative correlation of wild rice occurrence with temperature is that the southern border of the wild rice range may be limited by too-warm winter temperatures, in addition to a progressively greater loss in potential shallow-water habitat due to geographic patterns in both geology and land use. MDNR (2008, p. 30) suggested that climate change-caused increases in winter temperatures could threaten the occurrence of wild rice at the southern portion of its range, due to inadequate seed exposure to cold temperatures. The strongest temperature effect of climate change in Minnesota is warming winters (Seeley, 2006, p. 84).

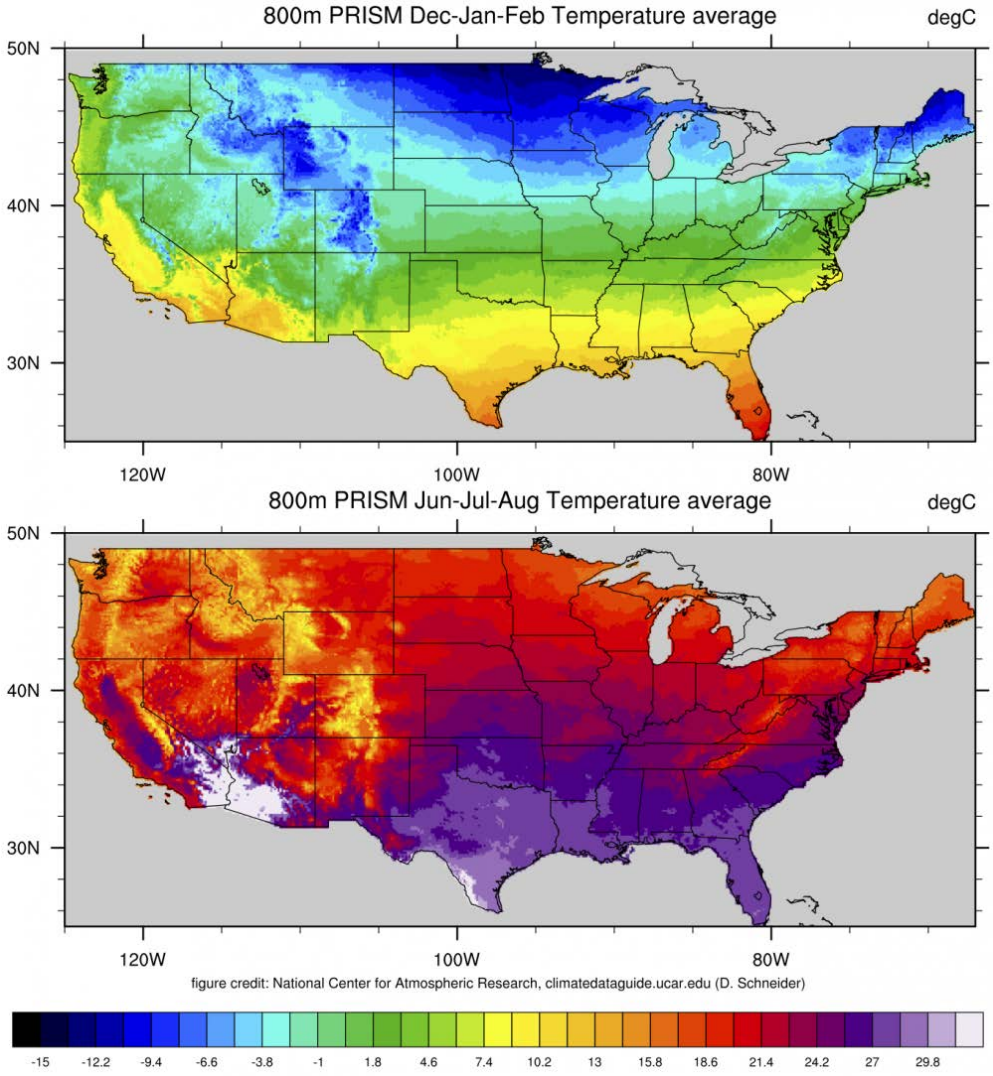


Figure 1-1. Average temperatures across the United States in winter (top) and in summer (bottom). From https://climatedataguide.ucar.edu.

Interaction of the three causative variables with other environmental variables

Even though the three causative variables may act independently of each other, there are likely interactions among the three variables, plus secondary effects that are correlated with wild rice presence/absence but are not causative (Myrbo et al., in press-1). For instance, additions of sulfate to waterbodies increases sulfide production, which interacts with iron to release phosphate that had been associated with iron. Furthermore, sulfate-stimulated microbes decompose sedimentary organic matter that would have not otherwise decomposed, which releases the nutrients phosphorus and nitrogen to the surface water, allowing increased phytoplankton growth. Therefore, sulfide production is correlated with increased phytoplankton, which reduces water transparency, inhibiting wild rice growth. Elevated phosphorus and nitrogen in surface water are significantly correlated with the absence of wild rice in waterbodies. These correlations most likely occur because a) the nutrients were released as a result of sulfate-enhanced decomposition of organic matter, producing toxic levels of sulfide (Myrbo et al., submitted-2), and b) their increased concentrations in surface water produced lower water transparency, which limits wild rice growth (Myrbo et al., in press-1; Table 1-5).

The production of sulfide, while negative for wild rice growth at higher porewater concentrations, also affects other variables, causing other observed correlations with wild rice (Tables 1-2, 1-3, and 1-4). These sulfide-related correlations with wild rice can be either negative, such as between wild rice and porewater potassium (K), or positive, such as the positive correlation of wild rice with porewater iron. The latter is the easiest to understand, because dissolved sulfide and dissolved iron react with each other to form a solid precipitate of iron sulfide. When porewater iron is high, sulfide is low, resulting in a positive correlation between porewater iron and wild rice, which is weaker ($p < 0.01$) than the negative correlation between porewater sulfide and wild rice ($p < 0.001$).

By performing multiple binary logistic regression (MBLR) of variables against the presence/ absence of wild rice, it is possible to determine if a variable acts independently of the three causative variables, or is simply correlated with one or more of the causative variables. In MBLR, porewater iron does not improve a model with just porewater sulfide as the predictor, and therefore the positive correlation of porewater iron with wild rice is likely just caused by the effect of sulfide on wild rice. Similarly, the significant correlation of wild rice with the concentration of total sulfur in the sediment ($p=0.048$) is probably a consequence of the strong correlation between total sulfur and porewater sulfide ($p<0.001$). The addition of the variable total sulfur to a regression does not improve the explanatory power of a regression just based on porewater sulfide, indicating that total sulfur is negatively correlated with wild rice because it is correlated with porewater sulfide, the actual causative factor (Myrbo et al., in press-1).

The negative correlations of porewater potassium (K) and surface water total nitrogen (TN) with wild rice are likely the result of their positive correlations with porewater sulfide (Table 1-5). These correlations are most easily understood in terms of the role that sulfide production plays in shallow aquatic systems: when sulfate-respiring bacteria dominate microbial activity, sulfide production is proportional to the decomposition of sedimentary organic matter, which releases the nutrients contained in the decaying plants, including potassium and nitrogen (Myrbo et al., in press-1; Myrbo et al., submitted-2; Lamers et al., 1998). In the field survey data, porewater sulfide is also positively correlated with surface water potassium and porewater concentrations of total nitrogen, ammonia, and silica (Table 1-4), all of which are released as plants decompose. The controlled sulfate-addition outdoor mesocosm experiment provides evidence that sulfide production also mobilizes phosphorus from the sediment into the overlying water, an effect supported by a positive correlation between sediment acid-volatile sulfide (AVS) and surface water phosphorus in the field data (Table 1-4). (Note that when sulfide is produced, it can either stay in the porewater or precipitate with iron; AVS is the sum of the two forms).

The strong correlation between porewater potassium with the absence of wild rice (Tables 1-4, 1-5) is remarkable, as potassium is an essential plant nutrient and therefore it is unlikely that the association is based on toxicity to wild rice. Rather, it is likely that the association is a result of the simultaneous mobilization of potassium with the production of sulfide as plant matter is decomposed simultaneously with the conversion of sulfate converted to sulfide by bacteria. Potassium does not bond covalently with organic compounds and is readily leached out of dead organic matter (Troeh and Thompson, 2005). Silica phytoliths dissolve as plant matter decomposes, allowing additional potassium that had been trapped within the phytoliths to be released into sediment porewater (Nguyen et al., 2015). Wild rice and other wetland macrophytes develop abundant phytoliths that release dissolved silica upon the decomposition of the plant tissue (Struyf and Conley, 2009). Porewater silica, potassium, and sulfide are all significantly correlated with each other (Table 1-4). The negative correlation of porewater potassium with wild rice may be magnified by its additional positive correlation with elevated water temperature (Table 1-5), which plausibly not only accelerates decomposition , but also the dissolution of silica phytoliths in the decomposing organic matter (Kamatani, 1982; Gudasz et al., 2010).

The release of potassium as sulfide is produced during the decomposition of plants is the likely explanation for an observed negative correlation between elevated potassium in surface water and wild rice growth and abundance metrics (Walker and Tuominen, 2014). Walker and Tuominen surveyed wild rice density and sampled surface water lakes and streams in northeastern Minnesota, but did not sample porewater sulfide.

Even though nutrients that limit plant growth (phosphorus and nitrogen) are not toxic to wild rice, the release of plant nutrients associated with the conversion of sulfate to sulfide can increase phytoplankton growth, reducing the light available to wild rice. Water transparency is negatively correlated both with the occurrence of wild rice and wild rice density (Table 1-3). In the field survey data, water transparency is negatively correlated with surface water phosphorus and nitrogen, but not with porewater sulfide. The lack of correlation with porewater sulfide implies that lowered transparency is negative for wild rice regardless of the porewater sulfide concentration, a conclusion confirmed in MBLR analysis (Myrbo et al., in press-1). Aside from phytoplankton abundance, transparency is also controlled by water color, with which it is highly correlated (Spearman's rho= -0.68). Water color is also negatively correlated with the stem density of wild rice, but is not correlated with porewater sulfide (Table 1-4).

The temperature of the surface water measured when each site was sampled is negatively correlated with the presence of wild rice, but temperature is not correlated with porewater sulfide or transparency (Tables 1-2, 1-3, and 1-4), suggesting that temperature limits the range of wild rice independently of porewater sulfide and transparency. In MBLR, water temperature improves a model based on porewater sulfide, suggesting that temperature acts independently of sulfide. Higher temperatures may increase sulfide production by enhancing the activity of the microbial community, but that effect is already accounted for in the concentration of sulfide, so temperature must have an independent negative effect on wild rice (Myrbo et al., in press-1). Consistent with the overall climatic patterns across Minnesota, the surface water temperature variable is negatively correlated with both latitude and longitude (Table 1-4). Although wild rice occurrence is correlated with the measured surface water temperature during the summer field surveys, as discussed above the actual mechanism acting on wild rice habitat may be winters that fail to be sufficiently cold to break seed dormancy (sites with warmer summer temperatures would be the same sites with warmer winter temperatures).

Minnesota varieties of wild rice grow well in the warmer summer temperatures of the California Central Valley, but the Central Valley winter is too warm to break the dormancy of wild rice seeds. Therefore,

the correlation in the field survey between higher summer temperatures and lower probability of wild rice occurrence is more likely due to overly warm winter temperatures than overly warm summers. In the field survey data, temperature is not correlated with other variables that are known to control wild rice occurrence (sulfide and water transparency), and therefore elevated temperatures seem to have an independent negative effect on wild rice occurrence.

How access to oxygen may allow wild rice to detoxify sulfide

Wetland plants, including wild rice, have adaptations to survive long-term rooting in anoxic sediment, a condition that is fatal to virtually all terrestrial plants. Wetland ecologists recognize the production of sulfide in anoxic sediment as one of the major challenges facing plants that root in water-saturated soils (Ponnamperuma, 1972; Kirk, 2004; van der Valk, 2012). To deal with elevated sulfide, wetland plants have adaptations that allow them to decrease the toxicity of sulfide, increasing their chances of successful growth and reproduction. Wetland plants can detoxify sulfide by two broad routes involving oxygen: 1) releasing oxygen from their roots to oxidize porewater sulfide, thereby decreasing sulfide concentrations and associated toxicity, and/or 2) transporting oxygen to their roots to allow internal detoxification of sulfide that has penetrated the root tissue.

Each of these detoxification mechanisms require oxygen to be transported to the roots of the plants. Oxygen is moved to the roots of wetland plants through specialized tissue, aerenchyma, that forms a conduit from the leaves to the roots. Plants adapted to growth in water-saturated soil (or sediment), such as wild rice, transport oxygen to tissues under water and to the roots because there is no oxygen in the sediment. On a volumetric basis, the maximum oxygen content of water is at least 27 times lower than in the atmosphere (Caraco et al., 2006).

Wild rice could obtain oxygen to send to the roots from either the atmosphere (if it has grown enough to reach the water surface) or photosynthetically-produced oxygen, or both. Experiments with another grass genus, *Phragmites,* have shown that release of oxygen from roots is much greater if the plant has access to the atmosphere, rather than being completely submerged (Armstrong et al., 1999). There is no evidence that wild rice would not also transport more oxygen to the roots when emergent from water compared to completely submerged. In the *Phragmites* experiments, oxygen transport to the roots by submerged plants was always at least 40% less than in plants that emerged into the atmosphere. Submerged plants released more oxygen from roots when illuminated, due to photosynthesis, but the roots of emergent plants released more oxygen even in the dark night than the roots of illuminated submerged plants during the day.

Access to the atmosphere could help explain the large difference between the apparent toxicity of sulfide to wild rice as measured in a hydroponic test in which wild rice seedlings were completely submerged (Pastor et al., 2017) and a hydroponic test in which wild rice seedlings were allowed to emerge into the atmosphere (Fort et al., 2017). The lesser toxicity of sulfide in the latter experiment would be explicable if the wild rice seedlings were able to use the elevated oxygen concentrations from the atmosphere to enhance internal detoxification. However, under natural conditions, the seedlings (a maximum of 21-days old) would not have access to the atmosphere because the stems would not yet have elongated sufficiently to reach the water surface. Perhaps neither hydroponic test perfectly mimicked the natural environment, given that it is not definitively known to what sediment depth wild rice seedlings begin development. If seeds germinate at depth in the anoxic sediment, the elongating stem has the potential to be exposed to elevated sulfide; it should not be assumed that only the roots are exposed elevated sulfide. When seeds ripen in the fall, plants drop their seed into the water. The

individual seeds sink into the water, oriented by a rudder-like awn, and work their way into soft sediment near the parent plant (Aiken et al., 1988). Viable wild rice seeds can stay buried for multiple years in the sediment. Little is known about the environmental cues that causes a seed to germinate, after which it utilizes the energy stored in its starch to elongate its stem upward out of the sediment and through the overlying water to the surface of the waterbody. Oelke et al., (1982) observed that seeds may germinate and emerge successfully from sediment while buried in up to "3 inches in flooded soil" (7.6 cm). Meeker (2000) performed experiments where he examined the ability of seeds to successfully germinate and emerge after burial at sediment depths of 0 cm (the control), 4 cm, or 8 cm. Seeds buried 4 cm emerged from the sediment at a similar rate to the control, but the 8 cm treatment emergent rate was significantly lower. Meeker was not studying exposure to sulfide, so it is unknown what the porewater sulfide concentration was in his experiments.

Pastor et al. (2017) began their experiments with 1- to 2-cm long germinated seeds and exposed the whole seedling to the sulfide treatment. Fort et al. (2017) sprouted wild rice seed at a depth of 1 cm in the hydroponic solution and provided a trellis so that the developing seedling could emerge into the atmosphere, which allowed access to the atmosphere much earlier in development than would occur in nature. In contrast, the hydroponic exposure in the Pastor et al. experiment may actually mimic the exposure of seeds that germinate while buried in up to "3 inches in flooded soil" (Oelke et al., 1982) (7.6 cm). In the three Pastor et al. hydroponic experiments, the controls (zero sulfide) grew to 11 to 14 cm (Pastor, 2013), but the seedlings in the highest sulfide treatments only grew to a maximum of 5.3 to 7.6 cm, a similar distance that germinated wild rice might elongate through anoxic sediment with elevated sulfide concentrations. Thus, the Pastor et al. experiment may mimic the sulfide exposure of seeds that germinate while buried under 8 cm of sediment.

In an outdoor mesocosm experiment, sulfide was significantly lower in sediments with wild rice, indicating that adult wild rice releases oxygen from its roots, oxidizing sulfide (Myrbo et al. mesocosm paper, submitted-2). However, wild rice plants may not need to oxidize the entire pool of elevated sulfide in the porewater to reduce sulfide toxicity if the plant can increase the supply of oxygen to roots and submerged tissues, detoxifying sulfide through the second route. Given the *Phragmites* finding, the ability of wild rice to transport oxygen to the roots and detoxify sulfide would likely be enhanced once the growing seedling reaches the water surface, where it not only can access much higher oxygen concentrations, but where it can photosynthesize at higher rates, producing more oxygen. Therefore, germination in shallow water might allow wild rice to detoxify porewater sulfide more efficiently, both internally and externally. Internal detoxification of sulfide has not been looked for in wild rice, but has been demonstrated in plant tissues from other wetland plants (Lee, 2003; Lamers et al., 2013).

Two mechanisms of internal detoxification of sulfide have recently been described in plants: (1) the conversion of sulfide to the amino acid cysteine by the enzyme OAS-TL C (Alvarez et al., 2012), and (2) the oxidation of sulfide by sulfur dioxygenase (SDO), which produces thiosulfate (Krüssel et al., 2014). The first mechanism does not depend on oxygen availability. The second mechanism, the enzyme SDO, not only requires oxygen, but the detoxification of sulfide can be quantified by measuring the consumption of oxygen (Krüssel et al., 2014; Birke et al., 2015).

In summary, it likely that wild rice is better able to detoxify sulfide after a seedling has grown long enough to reach the atmosphere at the water surface, at which time it grows a floating leaf. The floating leaf can be the source of oxygen that is sent to the roots, where SDO can detoxify sulfide by combining sulfide with oxygen. Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow between 50 and 100 cm before reaching the water

surface, at which time floating leaves supply oxygen and energy for root development (Aiken, 1986; Pastor et al., 2017).

Wild rice may be able to tolerate higher levels of porewater sulfide when the seedlings can reach the water surface faster, which would be aided by shallower water and more nitrogen availability. This may be a reason for the lack of an observed negative sulfide effect on cultivated wild rice, since cultivated wild rice is fertilized with nitrogen and water levels are managed. In addition, there is evidence that wetland plants fertilized with nitrogen can better oxidize sulfide around the roots, reducing the potential toxicity (Howes et al., 1986). Five of seven cultivated paddies sampled during the MPCA-sponsored field study exhibited dense wild rice stands where porewater sulfide exceeded 120 µg/L, the concentration identified as protective for natural stands of wild rice. The sulfate standard to protect wild rice needs to maintain porewater sulfide at levels low enough to allow growth and reproduction in natural waters of varying depths and nutrient contents.

Table 1-3. Correlations of field variables with wild rice and porewater sulfide. For sites where multiple samples were collected, the site is represented by the closest sample to August 11, in either 2012-2013 (termed the "Class B" data set; N=108). The variables are ordered by the significance of the variable's correlation with the presence or absence of wild rice, as measured by binary logistic regression (Myrbo et al., in press-1). (PW=porewater; SW=surface water; Sed=Sediment; *=p <0.05; **=p <0.01; ***=p <0.001).

	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
Field Variable	Porewater sulfide Correlation (rho)	PW sulfide Correlation significance	Wild rice density Correlation (rho)	Wild Rice Density correlation significance	Regression P value	Regression significance
PW K	0.46	***	-0.36	***	0.0008	***
PW sulfide	1.00		-0.35	***	0.0012	**
Water Depth (m)	0.11	not sig	-0.24	*	0.0028	**
Transparency (cm)	-0.07	not sig	0.24	*	0.0031	**
SW TN	0.22	*	-0.23	*	0.0054	**
Sed Se % dry	0.08	not sig	-0.22	*	0.0059	**
SW Temp	0.17	not sig	-0.17	not sig	0.0077	**
PW Fe	-0.58	***	0.21	*	0.0109	*
SW pH	0.28	**	-0.24	*	0.0200	*
SW TP	0.05	not sig	-0.11	not sig	0.0353	*
Latitude	-0.06	not sig	0.19	*	0.0376	*
Sed TS % dry	0.40	***	-0.21	*	0.0483	*
PW Na	0.33	***	-0.25	**	0.0670	not sig
PW Zn	-0.08	not sig	-0.09	not sig	0.0746	not sig
SW Cl	0.29	**	-0.18	not sig	0.0783	not sig
SW K	0.29	**	-0.08	not sig	0.0922	not sig
Sed Cu % dry	0.00	not sig	-0.14	not sig	0.0940	not sig
Sed Al % dry	-0.05	not sig	-0.11	not sig	0.1109	not sig
Sed AVS % dry	0.29	**	-0.10	not sig	0.1317	not sig
SW sulfate	0.44	***	-0.17	not sig	0.1475	not sig
Sed NAI P % dry	-0.06	not sig	-0.04	not sig	0.1958	not sig
Longitude	-0.15	not sig	-0.16	not sig	0.2141	not sig
SW Ca	-0.06	not sig	0.22	*	0.2489	not sig
PW As	-0.43	***	0.15	not sig	0.2642	not sig
Sed TP % dry	0.07	not sig	-0.10	not sig	0.2697	not sig
SW Alkalinity	0.22	*	0.24	*	0.2786	not sig

(continued)

	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
Field Variable	Porewater sulfide Correlation (rho)	PW sulfide Correlation significance	Wild rice density Correlation (rho)	Wild Rice Density correlation significance	Regression P value	Regression significance
PW TN	0.31	**	-0.23	*	0.2963	not sig
Sed % Coarse of ORG	-0.06	not sig	0.05	not sig	0.3360	not sig
Sed % Fine of ORG	0.05	not sig	-0.05	not sig	0.3575	not sig
Sed Zn % dry	-0.06	not sig	-0.03	not sig	0.3825	not sig
PW Ca	-0.01	not sig	0.22	*	0.4443	not sig
PW NH_4	0.33	***	-0.22	*	0.4505	not sig
Sed TEFe % dry	-0.35	***	-0.01	not sig	0.4795	not sig
PW DOC	-0.05	not sig	-0.01	not sig	0.4865	not sig
Sed Org P % dry	0.07	not sig	-0.08	not sig	0.5468	not sig
PW Si	0.33	***	-0.02	not sig	0.5548	not sig
PW Cu	-0.09	not sig	-0.07	not sig	0.5704	not sig
SW Na	0.26	**	-0.05	not sig	0.5859	not sig
SW Conductance	0.35	***	0.12	not sig	0.6028	not sig
SW Color	-0.11	not sig	-0.20	*	0.6122	not sig
PW TP	0.12	not sig	-0.06	not sig	0.6341	not sig
Sed TIC % dry	0.20	*	0.05	not sig	0.6519	not sig
Sed Inorg LOI	-0.16	not sig	-0.04	not sig	0.6668	not sig
Sed coarse org % dry	0.07	not sig	0.08	not sig	0.6737	not sig
Sed TN % dry	0.14	not sig	-0.08	not sig	0.6807	not sig
SW Fe	-0.33	***	0.02	not sig	0.6827	not sig
Sed fine org % dry	0.09	not sig	-0.07	not sig	0.6971	not sig
SW Mg	0.40	***	0.10	not sig	0.7151	not sig
Sed coarse inorg % dry	-0.15	not sig	0.00	not sig	0.7194	not sig
Sed fine inorg % dry	0.11	not sig	0.07	not sig	0.7267	not sig
Sed Water content	0.15	not sig	-0.07	not sig	0.7274	not sig
Sed Exchangeable P % dry	0.17	not sig	-0.03	not sig	0.7350	not sig
Sed % Coarse INORG	-0.15	not sig	-0.05	not sig	0.7489	not sig
PW Mn	-0.30	**	0.10	not sig	0.7608	not sig
Sec Ca % dry	0.21	*	0.08	not sig	0.7614	not sig

(continued)

	Spearman Correlation with Field Variable				Binary Logistic Regression for the presence/absence of wild rice	
Field Variable	**Porewater sulfide Correlation (rho)**	**PW sulfide Correlation significance**	**Wild rice density Correlation (rho)**	**Wild Rice Density correlation significance**	**Regression P value**	**Regression significance**
Sed % Fine of INORG	0.15	not sig	0.06	not sig	0.7661	not sig
Sed TOC % dry	0.10	not sig	-0.06	not sig	0.7854	not sig
Sed Mg % dry	0.23	*	0.09	not sig	0.8195	not sig
Sed Apatite P % dry	0.08	not sig	0.01	not sig	0.8495	not sig
PW pH	0.03	not sig	-0.04	not sig	0.8976	not sig
Sed org LOI	0.08	not sig	-0.06	not sig	0.9263	not sig
Sed CO_3 LOI	0.25	**	0.06	not sig	0.9677	not sig
PW Mg	0.33	***	0.11	not sig	0.9843	not sig
Sed As % dry	-0.13	not sig	0.17	not sig	0.9913	not sig
Sed Mn % dry	-0.06	not sig	0.13	not sig	0.9915	not sig

Table 1-4. Spearman correlation coefficients for selected environmental variables measured at the field sites. **For sites where multiple samples were collected, the site is represented by the closest sample to mid-August, 2012-2013 (Class B; N=108) sw=surface water; pw=porewater; sed=sediment.**

Spearman Correlation Coefficients P < 0.05 if > 0.19 P < 0.01 if>0.25 P < 0.001 if > 0.33; N = 108	Wild Rice (stems/m2)	Latitude	Longitude	Water Depth (m)	Transparency (cm)	sw pH	sw Temperature	sw Conductance	sw Ca	sw Mg	sw K	sw Na	sw Fe	sw sulfate	sw Cl	sw Alkalinity	sw TP	sw TN	sw Color	pw pH	pw sulfide	pw DOC	pw K	pw Na	pw Fe	pw Mn	pw TP	pw TN	pw NH4	pw Si	pw As	pw Cu	pw Zn	sed Water	sed TS % dry	sed AVS % dry	sed Fe % dry	sed Zn % dry	sed As % dry	sed Cu % dry	sed TP % dry	sed TN % dry	sed TOC % dry
Wild Rice (stems/m2)		0.19	-0.16	-0.24	0.24	-0.24	-0.17	0.12	0.22	0.10	-0.08	-0.05	0.02	-0.17	-0.18	0.24	-0.11	-0.23	-0.20	-0.04	-0.35	-0.01	-0.36	-0.25	0.21	0.10	-0.06	-0.23	-0.22	-0.02	0.15	-0.07	-0.09	-0.07	-0.21	-0.10	-0.01	-0.03	0.17	-0.14	-0.10	-0.08	-0.06
Latitude	0.19		0.05	-0.38	0.13	-0.21	-0.51	-0.21	-0.11	-0.14	-0.30	-0.20	0.20	0.09	-0.54	-0.15	-0.31	-0.25	0.03	0.10	-0.06	-0.06	-0.25	-0.16	0.07	-0.08	-0.26	-0.30	-0.16	-0.28	-0.08	-0.19	0.05	0.01	0.15	-0.09	0.14	0.14	0.19	0.00	-0.04	0.02	0.11
Longitude	-0.16	0.05		0.00	-0.22	-0.38	-0.19	-0.43	-0.16	-0.55	-0.38	0.03	0.55	0.16	-0.03	-0.61	0.06	0.09	0.52	-0.07	-0.15	0.08	-0.41	0.01	0.32	0.30	-0.02	-0.36	-0.27	-0.35	0.04	0.04	0.27	-0.09	-0.06	0.05	0.42	0.42	-0.01	0.40	0.03	-0.08	-0.04
Water Depth (m)	-0.24	-0.38	0.00		0.22	0.16	0.19	-0.05	-0.02	-0.07	-0.07	-0.06	-0.20	-0.03	0.09	-0.09	-0.02	-0.02	-0.20	-0.04	0.11	0.00	0.03	0.00	0.02	0.04	0.05	0.03	0.03	0.10	-0.10	-0.02	0.13	0.13	0.10	0.08	0.09	0.01	-0.05	0.08	0.01	0.17	0.09
Transparency (cm)	0.24	0.13	-0.22	0.22		-0.05	-0.08	0.08	0.09	0.07	-0.18	-0.16	-0.34	-0.07	-0.19	0.11	-0.58	-0.61	-0.68	-0.09	-0.07	-0.21	-0.10	-0.25	0.04	-0.08	-0.26	-0.20	-0.17	0.07	-0.13	0.03	-0.11	0.10	0.03	-0.13	-0.10	-0.16	0.02	-0.12	-0.14	0.06	0.10
sw pH	-0.24	-0.21	-0.38	0.16	-0.05		0.35	0.31	-0.03	0.45	0.44	0.40	-0.39	0.32	0.38	0.27	0.04	0.05	-0.26	0.32	0.28	-0.24	0.45	0.27	-0.31	-0.11	0.11	0.34	0.30	0.26	-0.03	-0.12	-0.21	-0.18	-0.04	0.08	-0.45	-0.41	-0.27	-0.33	-0.25	-0.24	-0.28
sw Temperature	-0.17	-0.51	-0.19	0.19	-0.08	0.35		0.14	0.01	0.22	0.33	0.12	-0.23	0.04	0.35	0.17	0.27	0.23	-0.06	-0.21	0.17	0.09	0.33	0.18	-0.09	0.09	0.26	0.34	0.26	0.29	0.20	0.05	-0.04	0.02	-0.08	0.11	-0.06	-0.07	0.08	0.06	0.14	-0.06	-0.06
sw Conductance	0.12	-0.21	-0.43	-0.05	0.08	0.31	0.14		0.61	0.89	0.63	0.61	-0.53	0.45	0.56	0.87	0.11	-0.17	-0.41	0.26	0.35	-0.28	0.42	0.42	-0.31	-0.05	0.20	0.18	0.16	0.32	0.00	-0.11	-0.19	-0.21	0.04	0.36	-0.35	-0.37	-0.11	-0.39	-0.20	-0.23	-0.30
sw Ca	0.22	-0.11	-0.16	-0.02	0.09	-0.03	0.01	0.61		0.37	0.13	0.22	-0.31	0.10	0.19	0.65	0.24	-0.26	-0.23	0.09	-0.06	-0.35	-0.01	0.05	-0.06	0.17	0.22	-0.04	-0.04	0.08	0.14	-0.13	-0.03	-0.05	0.04	0.20	-0.03	-0.14	0.07	-0.22	-0.04	-0.02	-0.10
sw Mg	0.10	-0.14	-0.55	-0.07	0.07	0.45	0.22	0.89	0.37		0.68	0.52	-0.55	0.46	0.50	0.85	0.05	-0.15	-0.41	0.31	0.40	-0.25	0.50	0.35	-0.35	-0.12	0.11	0.23	0.22	0.38	-0.01	-0.16	-0.24	-0.17	0.08	0.35	-0.37	-0.37	-0.08	-0.35	-0.17	-0.20	-0.26
sw K	-0.08	-0.30	-0.38	-0.07	-0.18	0.44	0.33	0.63	0.13	0.68		0.61	-0.40	0.37	0.66	0.51	0.14	0.13	-0.21	0.28	0.29	-0.09	0.61	0.49	-0.34	-0.13	0.05	0.18	0.08	0.19	-0.05	0.06	-0.16	-0.23	-0.05	0.21	-0.41	-0.34	-0.19	-0.30	-0.22	-0.25	-0.30
sw Na	-0.05	-0.20	0.03	-0.06	-0.16	0.40	0.12	0.61	0.22	0.52	0.61		-0.19	0.63	0.74	0.36	0.17	-0.07	-0.16	0.35	0.26	-0.16	0.34	0.73	-0.13	0.15	0.15	-0.03	-0.05	0.09	0.02	-0.12	0.03	-0.46	-0.12	0.31	-0.27	-0.28	-0.26	-0.26	-0.34	-0.51	-0.52
sw Fe	0.02	0.20	0.55	-0.20	-0.34	-0.39	-0.23	-0.53	-0.31	-0.55	-0.40	-0.19		-0.15	-0.24	-0.57	0.13	0.26	0.68	-0.04	-0.33	0.40	-0.41	-0.15	0.45	0.16	0.09	-0.09	-0.08	-0.36	0.24	0.03	0.40	-0.01	-0.09	-0.02	0.56	0.44	0.19	0.31	0.22	0.03	0.10
sw sulfate	-0.17	0.09	0.16	-0.03	-0.07	0.32	0.04	0.45	0.10	0.46	0.37	0.63	-0.15		0.41	0.21	0.05	-0.09	-0.09	0.32	0.44	-0.27	0.25	0.48	-0.31	0.00	0.02	-0.12	0.02	0.00	-0.06	-0.31	0.04	-0.35	0.12	0.45	-0.12	-0.15	-0.15	-0.08	-0.22	-0.41	-0.41
sw Cl	-0.18	-0.54	-0.03	0.09	-0.19	0.38	0.35	0.56	0.19	0.50	0.66	0.74	-0.24	0.41		0.32	0.24	0.09	-0.09	0.12	0.29	-0.14	0.46	0.60	-0.22	0.09	0.18	0.15	0.07	0.14	-0.09	0.04	-0.06	-0.27	-0.09	0.24	-0.32	-0.28	-0.32	-0.28	-0.21	-0.28	-0.34
sw Alkalinity	0.24	-0.15	-0.61	-0.09	0.11	0.27	0.17	0.87	0.65	0.85	0.51	0.36	-0.57	0.21	0.32		0.11	-0.23	-0.46	0.21	0.22	-0.31	0.35	0.20	-0.31	-0.08	0.16	0.20	0.18	0.37	0.06	-0.17	-0.20	-0.08	0.04	0.26	-0.33	-0.38	0.01	-0.37	-0.09	-0.10	-0.17
sw TP	-0.11	-0.31	0.06	-0.02	-0.58	0.04	0.27	0.11	0.24	0.05	0.14	0.17	0.13	0.05	0.24	0.11		0.52	0.48	0.06	0.05	0.12	0.08	0.18	-0.05	0.24	0.51	0.11	0.07	0.03	0.29	-0.06	0.22	-0.17	-0.10	0.29	0.13	0.09	0.05	0.14	0.20	-0.14	-0.17
sw TN	-0.23	-0.25	0.09	-0.02	-0.61	0.05	0.23	-0.17	-0.26	-0.15	0.13	-0.07	0.26	-0.09	0.09	-0.23	0.52		0.61	-0.05	0.22	0.26	0.15	0.12	-0.18	-0.13	0.27	0.28	0.23	0.07	0.04	0.08	-0.02	0.10	0.07	0.08	0.00	0.14	-0.04	0.15	0.24	0.10	0.05
sw Color	-0.20	0.03	0.52	-0.20	-0.68	-0.26	-0.06	-0.41	-0.23	-0.41	-0.21	-0.16	0.68	-0.09	-0.09	-0.46	0.48	0.61		-0.06	-0.11	0.39	-0.20	-0.04	0.21	0.12	0.23	0.04	0.01	-0.20	0.19	0.16	0.16	0.01	-0.04	0.04	0.37	0.38	0.09	0.28	0.24	0.05	0.07
pw pH	-0.04	0.10	-0.07	-0.04	-0.09	0.32	-0.21	0.26	0.09	0.31	0.28	0.35	-0.04	0.32	0.12	0.21	0.06	-0.05	-0.06		0.03	-0.30	0.12	0.19	-0.11	-0.04	0.03	-0.13	0.03	-0.24	0.09	-0.09	-0.04	-0.47	-0.16	0.14	-0.22	-0.35	-0.28	-0.25	-0.40	-0.46	-0.50
pw sulfide	-0.35	-0.06	-0.15	0.11	-0.07	0.28	0.17	0.35	-0.06	0.40	0.29	0.26	-0.33	0.44	0.29	0.22	0.05	0.22	-0.11	0.03		-0.05	0.46	0.33	-0.58	-0.30	0.12	0.31	0.33	0.33	-0.43	-0.09	-0.08	0.15	0.40	0.29	-0.35	-0.06	-0.13	0.00	0.07	0.14	0.10
pw DOC	-0.01	-0.06	0.08	0.00	-0.21	-0.24	0.09	-0.28	-0.35	-0.25	-0.09	-0.16	0.40	-0.27	-0.14	-0.31	0.12	0.26	0.39	-0.30	-0.05		-0.03	-0.15	0.42	0.20	0.23	0.23	0.08	0.09	0.24	0.23	0.16	0.10	-0.12	-0.10	0.31	0.33	0.17	0.20	0.22	0.11	0.19
pw K	-0.36	-0.25	-0.41	0.03	-0.10	0.45	0.33	0.42	-0.01	0.50	0.61	0.34	-0.41	0.25	0.46	0.35	0.08	0.15	-0.20	0.12	0.46	-0.03		0.58	-0.37	-0.12	0.22	0.43	0.40	0.34	-0.11	0.05	-0.17	-0.11	0.03	0.11	-0.38	-0.30	-0.20	-0.15	-0.09	-0.14	-0.18
pw Na	-0.25	-0.16	0.01	0.00	-0.25	0.27	0.18	0.42	0.05	0.35	0.49	0.73	-0.15	0.48	0.60	0.20	0.18	0.12	-0.04	0.19	0.33	-0.15	0.58		-0.25	-0.01	0.15	0.12	0.13	0.11	-0.01	-0.02	-0.03	-0.35	-0.05	0.23	-0.20	-0.25	-0.16	-0.18	-0.18	-0.35	-0.36
pw Fe	0.21	0.07	0.32	0.02	0.04	-0.31	-0.09	-0.31	-0.06	-0.35	-0.34	-0.13	0.45	-0.31	-0.22	-0.31	-0.05	-0.18	0.21	-0.11	-0.58	0.42	-0.37	-0.25		0.60	0.13	-0.18	-0.19	-0.19	0.48	0.06	0.12	-0.15	-0.31	0.00	0.63	0.34	0.24	0.22	0.08	-0.13	-0.07
pw Mn	0.10	-0.08	0.30	0.04	-0.08	-0.11	0.09	-0.05	0.17	-0.12	-0.13	0.15	0.16	0.00	0.09	-0.08	0.24	-0.13	0.12	-0.04	-0.30	0.20	-0.12	-0.01	0.60		0.27	-0.16	-0.17	-0.08	0.40	-0.13	0.22	-0.29	-0.29	0.06	0.29	0.15	-0.02	0.15	-0.02	-0.29	-0.27
pw TP	-0.06	-0.26	-0.02	0.05	-0.26	0.11	0.26	0.20	0.22	0.11	0.05	0.15	0.09	0.02	0.18	0.16	0.51	0.27	0.23	0.03	0.12	0.23	0.22	0.15	0.13	0.27		0.62	0.60	0.33	0.26	-0.02	0.00	-0.01	0.02	0.30	0.14	0.17	0.09	0.18	0.34	-0.02	-0.04
pw TN	-0.23	-0.30	-0.36	0.03	-0.20	0.34	0.34	0.18	-0.04	0.23	0.18	-0.03	-0.09	-0.12	0.15	0.20	0.11	0.28	0.04	-0.13	0.31	0.23	0.43	0.12	-0.18	-0.16	0.62		0.88	0.54	-0.08	0.10	-0.21	0.32	0.22	0.14	-0.14	0.03	0.07	0.02	0.37	0.29	0.26
pw NH4	-0.22	-0.16	-0.27	0.03	-0.17	0.30	0.26	0.16	-0.04	0.22	0.08	-0.05	-0.08	0.02	0.07	0.18	0.07	0.23	0.01	0.03	0.33	0.08	0.40	0.13	-0.19	-0.17	0.60	0.88		0.42	-0.11	0.02	-0.26	0.28	0.30	0.22	-0.07	0.06	0.11	0.11	0.37	0.23	0.20
pw Silica	-0.02	-0.28	-0.35	0.10	0.07	0.26	0.29	0.32	0.08	0.38	0.19	0.09	-0.36	0.00	0.14	0.37	0.03	0.07	-0.20	-0.24	0.33	0.09	0.34	0.11	-0.19	-0.08	0.33	0.54	0.42		-0.01	0.08	-0.20	0.23	0.16	0.18	-0.14	-0.04	0.11	0.07	0.21	0.19	0.14
pw As	0.15	-0.08	0.04	-0.10	-0.13	-0.03	0.20	0.00	0.14	-0.01	-0.05	0.02	0.24	-0.06	-0.09	0.06	0.29	0.04	0.19	0.09	-0.43	0.24	-0.11	-0.01	0.48	0.40	0.26	-0.08	-0.11	-0.01		0.06	0.10	-0.39	-0.51	-0.09	0.28	-0.04	0.17	-0.05	-0.06	-0.38	-0.36
pw Cu	-0.07	-0.19	0.04	-0.02	0.03	-0.12	0.05	-0.11	-0.13	-0.16	0.06	-0.12	0.03	-0.31	0.04	-0.17	-0.06	0.08	0.16	-0.09	-0.09	0.23	0.05	-0.02	0.06	-0.13	-0.02	0.10	0.02	0.08	0.06		-0.22	0.01	-0.20	-0.19	0.01	-0.05	0.01	-0.02	0.00	0.04	0.06
pw Zn	-0.09	0.05	0.27	0.13	-0.11	-0.21	-0.04	-0.19	-0.03	-0.24	-0.16	0.03	0.40	0.04	-0.06	-0.20	0.22	-0.02	0.16	-0.04	-0.08	0.16	-0.17	-0.03	0.12	0.22	0.00	-0.21	-0.26	-0.20	0.10	-0.22		-0.15	-0.13	0.00	0.26	0.13	0.01	0.14	-0.04	-0.16	-0.08
sed Water	-0.07	0.01	-0.09	0.13	0.10	-0.18	0.02	-0.21	-0.05	-0.17	-0.23	-0.46	-0.01	-0.35	-0.27	-0.08	-0.17	0.10	0.01	-0.47	0.15	0.10	-0.11	-0.35	-0.15	-0.29	-0.01	0.32	0.28	0.23	-0.39	0.01	-0.15		0.76	0.10	0.24	0.55	0.51	0.40	0.72	0.93	0.94
sed TS % dry	-0.21	0.15	-0.06	0.10	0.03	-0.04	-0.08	0.04	0.04	0.08	-0.05	-0.12	-0.09	0.12	-0.09	0.04	-0.10	0.07	-0.04	-0.16	0.40	-0.12	0.03	-0.05	-0.31	-0.29	0.02	0.22	0.30	0.16	-0.51	-0.20	-0.13	0.76		0.42	0.15	0.41	0.40	0.29	0.56	0.71	0.70
sed AVS % dry	-0.10	-0.09	0.05	0.08	-0.13	0.08	0.11	0.36	0.20	0.35	0.21	0.31	-0.02	0.45	0.24	0.26	0.29	0.08	0.04	0.14	0.29	-0.10	0.11	0.23	0.00	0.06	0.30	0.14	0.22	0.18	-0.09	-0.19	0.00	0.10	0.42		0.38	0.28	0.32	0.26	0.30	0.04	0.02
sed Fe % dry	-0.01	0.14	0.42	0.09	-0.10	-0.45	-0.06	-0.35	-0.03	-0.37	-0.41	-0.27	0.56	-0.12	-0.32	-0.33	0.13	0.00	0.37	-0.22	-0.35	0.31	-0.38	-0.20	0.63	0.29	0.14	-0.14	-0.07	-0.14	0.28	0.01	0.26	0.24	0.15	0.38		0.70	0.64	0.64	0.52	0.24	0.30
sed Zn % dry	-0.03	0.14	0.42	0.01	-0.16	-0.41	-0.07	-0.37	-0.14	-0.37	-0.34	-0.28	0.44	-0.15	-0.28	-0.38	0.09	0.14	0.38	-0.35	-0.06	0.33	-0.30	-0.25	0.34	0.15	0.17	0.03	0.06	-0.04	-0.04	-0.05	0.13	0.55	0.41	0.28	0.70		0.64	0.76	0.68	0.49	0.57
sed As % dry	0.17	0.19	-0.01	-0.05	0.02	-0.27	0.08	-0.11	0.07	-0.08	-0.19	-0.26	0.19	-0.15	-0.32	0.01	0.05	-0.04	0.09	-0.28	-0.13	0.17	-0.20	-0.16	0.24	-0.02	0.09	0.07	0.11	0.11	0.17	0.01	0.01	0.51	0.40	0.32	0.64	0.64		0.47	0.66	0.47	0.52
sed Cu % dry	-0.14	0.00	0.40	0.08	-0.12	-0.33	0.06	-0.39	-0.22	-0.35	-0.30	-0.26	0.31	-0.08	-0.28	-0.37	0.14	0.15	0.28	-0.25	0.00	0.20	-0.15	-0.18	0.22	0.15	0.18	0.02	0.11	0.07	-0.05	-0.02	0.14	0.40	0.29	0.26	0.64	0.76	0.47		0.59	0.32	0.37
sed TP % dry	-0.10	-0.04	0.03	0.01	-0.14	-0.25	0.14	-0.20	-0.04	-0.17	-0.22	-0.34	0.22	-0.22	-0.21	-0.09	0.20	0.24	0.24	-0.40	0.07	0.22	-0.09	-0.18	0.08	-0.02	0.34	0.37	0.37	0.21	-0.06	0.00	-0.04	0.72	0.56	0.30	0.52	0.68	0.66	0.59		0.64	0.69
sed TN % dry	-0.08	0.02	-0.08	0.17	0.06	-0.24	-0.06	-0.23	-0.02	-0.20	-0.25	-0.51	0.03	-0.41	-0.28	-0.10	-0.14	0.10	0.05	-0.46	0.14	0.11	-0.14	-0.35	-0.13	-0.29	-0.02	0.29	0.23	0.19	-0.38	0.04	-0.16	0.93	0.71	0.04	0.24	0.49	0.47	0.32	0.64		0.94
sed TOC % dry	-0.06	0.11	-0.04	0.09	0.10	-0.28	-0.06	-0.30	-0.10	-0.26	-0.30	-0.52	0.10	-0.41	-0.34	-0.17	-0.17	0.05	0.07	-0.50	0.10	0.19	-0.18	-0.36	-0.07	-0.27	-0.04	0.26	0.20	0.14	-0.36	0.06	-0.08	0.94	0.70	0.02	0.30	0.57	0.52	0.37	0.69	0.94	
sed Se % dry	-0.22	-0.07	0.38	0.05	-0.21	-0.30	0.13	-0.29	-0.06	-0.26	-0.20	-0.18	0.20	-0.05	-0.13	-0.27	0.21	0.26	0.29	-0.34	0.08	0.16	-0.11	-0.09	0.10	0.18	0.11	-0.06	0.00	0.13	-0.07	-0.10	0.17	0.44	0.35	0.27	0.57	0.73	0.50	0.81	0.64	0.34	0.38

Table 1-5. The 12 field variables that are significantly correlated with the presence/absence of wild rice, as determined through binary logistic regression (Myrbo et al., in press-1). Below the name of each field variable is the nature of the correlation with wild rice presence (positive or negative) and the average value at the sites with wild rice present and at sites with wild rice absent. The Spearman correlation coefficient is termed "rho." (*=p<0.01; **=p<0.01; ***=p<0.001)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/ absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide		Water Transparency		Water Temperature	
			rho	Significant?	rho	Significant?	rho	Significant?
Porewater sulfide (negative) 165, 795 µg/L	0.0012	Elevated porewater sulfide is correlated with the absence of wild rice most likely because elevated sulfide reduces the growth of wild rice.	1.00		-0.07	not sig	0.17	not sig
Surface water temperature (negative) 22.1, 24.4 ºC	0.0077	Elevated surface water temperature is negatively correlated with wild rice occurrence, independent of water transparency. Temperature is weakly correlated with porewater sulfide (rho= 0.17); warmer summer and winter temperatures likely have a negative effect on wild rice that is independent of sulfide.	0.17	not sig	-0.08	not sig	1.00	
Latitude (positive) 46.6, 46.1 degrees	0.0376	Minnesota has strong latitudinal gradients in many environmental factors, but latitude's strongest correlation in this data set is with water temperature (transparency, sulfate, and sulfide are not significantly correlated). It is most likely that northern latitude sites are correlated with the presence of wild rice presence because they are colder.	-0.06	not sig	0.13	not sig	-0.51	***
Water transparency (positive) 84, 66 cm	0.0031	Reduced transparency is correlated with the absence of wild rice, independent of porewater sulfide and surface water temperature. Wild rice is rarely observed at transparencies below 30 cm.	-0.07	not sig	1.00		-0.08	not sig
Porewater potassium (negative) 3.5, 6.1 mg/L	0.0008	There is no reason to expect that elevated porewater potassium (K) is harmful to wild rice, as it is a plant nutrient. Rather, it is likely that porewater K is simply correlated with porewater sulfide because sulfide production is associated with enhanced decomposition of organic matter, which releases the plant nutrients K, N, and P. The negative correlation of porewater K with wild rice is magnified by its additional correlation with temperature, which could be driving non-sulfide related organic decomposition, which would also release K to porewater.	0.46	***	-0.10	not sig	0.33	***

(continued)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/ absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide		Water Transparency		Water Temperature	
			rho	Significant?	rho	Significant?	rho	Significant?
Surface water total nitrogen (negative) 0.9, 1.2 mg/L	0.0054	There is no reason to expect that elevated nitrogen is directly harmful to wild rice, although elevated N likely encourages growth of algae or macrophytes that shade or compete with wild rice. Total nitrogen is correlated with all 3 causative factors that are negative for wild rice occurrence, in that it is not only associated with reduced transparency, but it is also correlated with sulfide and temperature, which are both associated with enhanced decomposition of sediment organic matter.	0.22	*	-0.61	***	0.23	*
Surface water total phosphorus (negative) 41, 62 µg/L	0.0353	There is no reason to expect that elevated phosphorus is directly harmful to wild rice, although elevated P likely encourages growth of algae or macrophytes that shade or compete with wild rice. Total phosphorus is not only correlated with reduced transparency, but it also correlated with water temperature, which is associated with enhanced decomposition of organic matter.	0.05	not sig	-0.58	***	0.27	**
Porewater Fe (positive) 11.0, 7.0 mg/L	0.0109	Porewater iron is negatively correlated with porewater sulfide, which is most likely the causative factor for the correlation with the occurrence of wild rice.	-0.58	***	0.04	not sig	-0.09	not sig
Sediment total sulfur (negative) 3.9, 6.9 mg/g	0.0483	Sediment total sulfur is positively correlated with porewater sulfide, which is most likely the causative factor.	0.40	***	0.03	not sig	-0.08	not sig
Sediment total selenium (negative) 1.0, 1.3 µg/g	0.0059	The slightly higher selenium at sites without wild rice is most likely caused by the co-precipitation of selenium and sulfur by sulfate-reducing bacteria, as shown by Hockin and Gadd (2003). Selenium is correlated with sediment total sulfur (rho=0.35, $p < 0.001$)).	0.08	not sig	-0.21	*	0.13	not sig

(continued)

Field Variable (positive or negative correlation with wild rice presence) Site averages: Present, Absent	Significance of binary logistic regression with wild rice presence/ absence (p value)	Likely reason for correlation with wild rice presence/absence	Spearman Correlations					
			Porewater Sulfide		Water Transparency		Water Temperature	
			rho	Significant?	rho	Significant?	rho	Significant?
Surface water pH (negative) 7.9, 8.3 pH units	0.0200	It is unlikely that elevated pH (8.3 $\pm$ 0.9 at sites without wild rice compared to 7.9 $\pm$ 0.8 at sites with wild rice) is harming wild rice. Rather, elevated surface water pH is reflecting elevated water temperature, which reduces the solubility of CO_2, raising the pH. Synergistically, pH is also correlated with porewater sulfide, which is stoichiometrically related to the generation of alkalinity, raising the pH.	0.28	**	-0.05	not sig	0.35	***
Water Depth (negative) 52 , 67 cm	0.0028	Although water depth could control wild rice presence, this metric does not characterize the waterbody, but rather where the field crews took the sample. When wild rice was not present, field crews usually sampled at water lilies, which tended to grow in slightly deeper water than wild rice. The correlation with temperature likely is driven by the tendency of warmer sites to not host wild rice. The significant positive correlation with transparency does not drive the negative correlation with wild rice occurrence, as it is the wrong sign, and therefore inexplicable.	0.11	not sig	0.22	*	0.19	*

B. That other factors affect wild rice does not negate the need to protect wild rice from excess sulfide

Multiple stressors affect wild rice in nature.

Some comments received in regards to the March 2015 Draft Proposal (MPCA, 2015) focused on regulating sulfate. Others suggested that a) it is inappropriate to regulate sulfate without also addressing the many other factors, aside from sulfide, that likely control the presence of wild rice, and b) factors other than sulfate (and sulfide) are more important in controlling the suitability of wild rice habitat. It was further suggested that it is not appropriate to use field data to identify a sulfide concentration that is protective of wild rice both because field data are inherently variable and in light of the multiple stressors that were not studied in the MPCA-sponsored research, especially a) changes in water levels from year to year, b) impacts of development, and c) presence of invasive or competitive species.

It is true that there is more "noise" in field data than in a controlled experiment. Because of this noise, or data variability, it is more challenging to detect a statistically significant impact of a particular stressor in field data; there is more statistical power in controlled laboratory experiments (Chapman, 2002). It is important to conduct controlled laboratory experiments to determine that a particular stressor (such as sulfide) has the potential to negatively affect a species, but the ecological significance of that effect is ambiguous until mesocosm or field data are collected (Chapman, 2002). If, despite environmental variability, a statistically significant relationship is demonstrated in the field that reinforces the laboratory finding, then there is little question that the chemical is important in controlling the occurrence of that species in the environment.

Despite the challenge of documenting a statistically significant relationship in field data, the binary logistic regression (BLR) analysis found a statistically significant negative correlation between the concentration of sulfide in the sediment porewater and the occurrence of wild rice (p=0.001, Table 1-5). Performing *multiple* BLR with more than one variable demonstrated that porewater sulfide is one of three primary independent variables correlated with wild rice occurrence (Myrbo et al., in press-1): porewater sulfide, water transparency, and water temperature. The statistical analysis strongly supports the conclusion that sulfide independently affects wild rice presence and absence (p=0.001; Table 1-3), which implies that limiting sulfate availability has the potential to protect wild rice from elevated sulfide. Analysis of the MPCA field data shows that porewater sulfide is simultaneously controlled by surface water sulfate and sediment concentrations of total organic carbon (TOC) and total extractable iron (TEFe) (Pollman et al., in press; discussed in Part D of this chapter). Interestingly, sulfate, TOC, and TEFe do not have any statistically significant effect on wild rice occurrence when considered individually (p= 0.15, 0.79, and 0.48, respectively; Table 1-3; Myrbo et al., in press-1). These three environmental variables only have a relationship to the occurrence of wild rice when they are considered simultaneously, given that particular combinations of the three can produce excessive concentrations of porewater sulfide (Part E of this Chapter).

Factors that act independently of porewater sulfide may also affect wild rice growth, such as hydrological changes and exotic species (Tables 1-6 and 1-7), but unless a factor has an affect on the relationship between sulfate and sulfide, consideration of such a factor is irrelevant to the mission of protecting wild rice from excess sulfide. The only factors that have been identified that have an effect on porewater sulfide are sulfate, sediment TOC, and sediment iron (Pollman et al., in press). However, one exception may be sites with upwelling groundwater; it has been reported that such sites may be favorable habitat for wild rice (Table 1-6). Consistent upward groundwater flow would break the usual relationship between sulfate in surface water and sulfide in porewater, because sulfate would be less likely to move downwards into the sediment when groundwater is moving upwards. Therefore, at some

sites the sulfate concentration of the groundwater may be more important than the surface water in controlling the production of porewater sulfide, but statistical analysis shows that at most sites porewater sulfide is a function of surface water sulfate (Pollman et al., in press). Even if this were not the case, the possibility that groundwater, rather than surface water, controls porewater sulfide in a specific wild rice bed does not negate the validity of the empirically observed, statistically significant, relationship between surface water sulfate, sediment iron, sediment TOC, and porewater sulfide as a general matter (Part D of this chapter, below; Pollman et al., in press).

Table 1-6. Reported environmental requirements for suitable wild rice habitat. Most reports comment on factors affecting the relative growth or density of wild rice rather than factors that control the presence and absence of wild rice, which is how conservation biologists identify factors that are critical to favorable habitat for a species.

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
Wild rice grows best when surface water sulfate is less than 10 mg/L	
"No large stands of rice occur in waters having a SO_4 content greater than 10 ppm, and rice generally is absent from water with more than 50 ppm." (Moyle, 1944) "Wild rice has marked preference for the quality of water in which it grows and is not found in prairie waters which have appreciable amounts of sulfate or "alkali" salts. In Minnesota the range is mostly limited to waters with concentration of sulfate or "alkali" salts lower than 10 parts per million of sulfate ion. Plantings of wild rice seed in prairie waters with higher concentrations of sulfates have generally failed. (Moyle and Krueger, 1964)	The effect of sulfate depends on the background sediment chemistry of the particular waterbody. Elevated sulfate sometimes allows excessive porewater sulfide to develop (Myrbo et al., in press-1; Pollman et al., in press).
Wild rice grows best when surface water alkalinity greater than 40 mg/L	
"Best growth is made in carbonate waters having total alkalinity greater than 40 ppm." (Moyle, 1944) "Lakes that have had wild rice for many years usually have the following characteristics: (4) they usually are fairly limy and have a total alkalinity exceeding 40 parts per million (there are stands, however, in softer water);" (Moyle and Krueger, 1964)	There is no statistically significant effect of alkalinity on wild rice presence (Table 1-3; Myrbo et al., in press-1).
High phosphorus can have adverse effects on wild rice	
"Wild rice grows within a wide range of chemical parameters (i.e. alkalinity, salinity, pH, and iron; Meeker 2000). However, productivity is highest in water with a pH of 6.0 to 8.0 and alkalinity greater than 40 ppm. While researchers have observed that natural wild rice stands are relatively nutrient rich, excess levels of some nutrients, especially phosphorus, can have significant adverse effects on productivity (Persell and Swan, 1986)." (MDNR, 2008, p. 14)	Wild rice absence is correlated with elevated surface water pH and phosphorus, which are both associated with high phytoplankton levels, reducing transparency (Table 1-3; Myrbo et al., in press-1).

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
Nitrogen and phosphorus are limiting nutrients for wild rice	
"Nitrogen and phosphorus are limiting nutrients for wild rice (Carson 2002)." (MDNR, 2008, p. 14). Five years of experimental data as well as a model of N cycling in wild rice ecosystems (Pastor and Walker 2006) suggest that delays in nutrient availability the year following deposition of large amounts of immobilizing litter cause biomass and population oscillations over a cycle potentially four years in length (Walker et al., 2010).	Rooted aquatic plants obtain their nutrients from the sediment, rather than from the surface water (Barko and Smart, 1986). There is no statistically significant relationship between sediment nitrogen or phosphorus and wild rice presence (Table 1-3; Myrbo et al., in press-1). Greater sedimentary N and P may increase growth of individual wild rice plants, but not control presence/absence of wild rice.
Wild rice grows best when carp populations are low	
Judging from lake names, it once grew farther south in central Minnesota where it has probably been exterminated by carp." (Moyle and Krueger, 1964)	No data were collected on carp presence.
"Common carp feed primarily on invertebrates in bottom soils. Their feeding action dislodges plants and suspends fine particles into the water column. The increased turbidity, caused both by disturbed sediments and by algae stimulated by the phosphorus released from disturbed sediments, shades out aquatic plants. Turbidity then increases as non-vegetated lake bottoms are disturbed by wind. The reduction in aquatic vegetation also allows for increased boat traffic and wave action that can further dislodge plants such as wild rice (Pillsbury and Bergey, 2000)." (MDNR 2008, p. 27)	Low water transparency can likely cause the absence of wild rice (Myrbo et al., in press-1).
Wild rice grows best in habitat with moving water	
"The crop grows best in lakes having some water moving through and often is lacking from stagnant lakes and pools. It is frequent along streams and at lake inlets and outlets." (Moyle, 1944)	No data were collected on water movement.
"Lakes that have had wild rice for many years usually have the following characteristics: (2) they are wide enough to have heavy wave action in spring or have a flow of water through them;" (Moyle and Krueger, 1964)	
"Natural wild rice generally requires some moving water, with rivers, flowages, and lakes with inlets and outlets being optimal areas for growth. Seasonal water depth is critical, however. Water levels that are relatively stable or decline gradually during the growing season are preferred. In particular, abrupt increases during the early growing season can uproot plants. Wild rice grows well at depths of 0.5 to 3 feet of water, although some plants may be found in deeper waters (M. McDowell, J. Persell personal communication)." (MDNR, 2008, p. 14)	

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
Groundwater inflow areas can be favorable habitat for wild rice	
Natural wild rice generally requires moving water, with rivers, flowages, and lakes or wetlands with inlets and outlets being optimal areas for growth. In some areas groundwater flows are apparently adequate to meet the need for flowing water (Norrgard, 2014). Wild rice is in a group of emergent plant species that had a mild statistical association with groundwater inflow areas of lakes (Nichols & Shaw, 2002).	No information was collected on groundwater movement at the field sites. Upward flow would break the usual relationship between surface water sulfate and sulfide, because sulfate would be less likely to move downwards into the sediment when groundwater is moving upwards.
Surface water with high transparency	
Often lacking from bog lakes with dark brown water. (Moyle, 1944) "…clear to moderately colored (stained) water is preferred, as darkly stained water can limit sunlight and may hinder early plant development." (MDNR, 2008, p. 14) "Waters in which wild rice grows are often somewhat brownish or tea-colored — but dark brown water is not favorable, for this cuts down penetration of the light that the first submerged leaves must have if the plant is to grow. In such waters, especially over mucky bottoms, the rice seedlings may be crowded by such submerged plants as coon tail, flat-stemmed pondweed and star duckweed. Waterlilies may invade wild rice stands and shade out the rice." (Moyle and Krueger, 1964)	Lower water transparency is correlated with absence of wild rice (Table 1-3; Myrbo et al., in press-1).
Wild rice occurs in shallow water between 0.5 and 3 feet depth	
"Lakes that have had wild rice for many years usually have the following characteristics: (1) they contain much water shallower than four feet; "(Moyle and Krueger, 1964) "Wild rice grows well at depths of 0.5 to 3 feet of water, although some plants may be found in deeper waters (M. McDowell, J. Persell personal communication)." (MDNR, 2008, p. 14)	Consistent with observations of Myrbo et al. (in press-1).

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
Wild rice grows best in waters with organic sediment	
"Lakes that have had wild rice for many years usually have the following characteristics: (3) they have an organic bottom a few inches to a few feet thick, overlying a hard bottom;" (Moyle and Krueger, 1964) "Although wild rice may be found growing in a variety of bottom types, the most consistently productive are lakes with soft, organic sediments (Lee, 1986). The high organic matter content with a rather low carbon/nitrogen ratio is necessary to meet the rather high nitrogen needs of wild rice (Carson, 2002). Nitrogen and phosphorus are major limiting nutrients for wild rice (Carson, 2002). Flocculent sediments with nitrogen and phosphorus concentrations less than one gram per square meter are typically incapable of supporting sustained production (Lee, 1986)." (MDNR, 2008, p. 97)	Myrbo et al. (in press-1) found no significant correlations between wild rice occurrence and sediment concentrations of organic matter, nitrogen, or phosphorus, or flocculent sediments (as measured by the water content). Note that Myrbo et al. examined what factors controlled the occurrence of wild rice, not what factors controlled the quality or density of wild rice.
The best wild rice habitat has some summers with high water to reduce the dominance of perennial plants	
"As an annual plant sprouting each year from seed, wild rice can have difficulty competing with aggressive perennial vegetation, particularly where natural hydrologic variation has been reduced. Cattail spp.), particularly hybrid cattail (*Typha* x glauca), yellow water lily (*Nuphar variegata*), and pickerelweed (*Pontederia cordata*) are examples of plants that have been cited as competing with wild rice (Norrgard, David, and Vogt, personal communication)" (MDNR, 2008, p. 91). "Lakes that have had wild rice for many years usually have the following characteristics: (6) the drainage area feeding the lakes is usually fairly large and the outlet such that there is high water in some summers (times when high water drowns out cattails and other perennial emergent plants that would otherwise crowd out the rice);" (Moyle and Krueger, 1964) "Cattails and perennial reeds and rushes will crowd out wild rice if allowed to become established. Such plants should be eradicated in paddies and in wild stands drowned out by occasional flooding. Usually there are years of high water (about one year in four) on wild rice stands that have remained as such for a long period of time." (Moyle and Krueger, 1964) Yellow water lily (*Nuphar variegate*) and *Utricularia vulgaris* occurrence patterns show that these plants prefer environmental conditions similar to optimal rice habitat, indicating that these plants are likely competitors. Resource managers often assert that wild rice competes with perennial plants such as cattails spp.), pickerelweed (*Pontederia cordata* L.), and waterlilies (*Nymphaea* and *Nuphar*) as a major factor in the disappearance of wild rice stands, but this hypothesis has rarely been tested (Pillsbury and McGuire, 2009).	No pertinent information was collected on competition with perennial vegetation or the effect of high water on the control of perennial vegetation.

(continued)

Environmental requirement for suitable wild rice habitat (source)	Relevant findings from MPCA-sponsored research
In most summers the best wild rice habitat does not have an abrupt rise in water level of more than 6 inches	
"Lakes that have had wild rice for many years usually have the following characteristics: (7) water levels which in years of normal or deficient rainfall do not rise sharply (more than 6 inches) at any time during June or July when the wild rice is in the floating-leaf stage." (Moyle and Krueger, 1964) "Water levels that are relatively stable or decline gradually during the growing season are preferred. Abrupt water level increases during the growing season can uproot plants. Wild rice is particularly sensitive to this disturbance during the floating leaf stage. However, some observers feel that water levels kept stable over the long term (multiple years) tend to favor perennial aquatic vegetation over wild rice (David and Vogt, personal communication)." (MDNR, 2008, p. 90)	No pertinent information was collected on the effect of an abrupt increase in water level on the occurrence of wild rice.
"Water levels that are relatively stable or decline gradually during the growing season are preferred. Abrupt water level increases during the growing season can uproot plants. Wild rice is particularly sensitive to this disturbance during the floating leaf stage. However, some observers feel that water levels kept stable over the long term (multiple years) tend to favor perennial aquatic vegetation over wild rice (David and Vogt, personal communication)." (MDNR, 2008, p. 90)	
Germination of wild rice seeds requires winters with at least 3 months of nearly freezing water	
"As an annual plant, natural wild rice develops each spring from seeds that fell into the water and settled into the sediment during a previous fall. Germination requires a dormancy period of three to four months of cold, nearly freezing water (35 F or colder). Seeds are unlikely to survive prolonged dry conditions." (MDNR, 2008, pp. 14-15). The seed of *Z. palustris* must experience at least 3 months in water at 1 to 3º C (Cardwell et al., 1978).	Warmer summer water temperature is correlated with absence of wild rice, which may be caused by a correlation with winters that are insufficiently cold or long to break seed dormancy (Myrbo et al., in press-1.).
Residential development in a watershed is negative for habitat; deeper habitat may be more sensitive to stress	
Wetlands that have lost most of their rice tended to have an increase in residential development within the watershed, and higher ammonium, pH, and water depth. In general, low density rice wetlands tended to be slightly deeper than other sites. Any additional stress may have caused a decline of rice in deeper wetlands while not affecting the rice in shallower wetlands (Meeker 2000, cited in field survey report by Pillsbury and McGuire, 2009)	Cause and effect not investigated, but if development increases nutrients, reduced transparency would affect deeper habitat more than shallow habitat, and pH is correlated with higher nutrients.

Table 1-7. Reported threats to existing natural stands of wild rice.

Increased external loading of sulfate or phosphorus
An increase in sulfate loading to surface water, causing an exceedance of the existing Minnesota sulfate standard of 10 mg/L (MDNR, 2008, p. 25).
An increase in sulfate loading to a surface water that is particularly efficient at converting sulfate to sulfide, increasing porewater sulfide to toxic levels (this document).
A decrease in transparency due to increased phytoplankton growth caused by a) increased external loading of phosphorus (Myrbo et al., in press-1) or b) increased internal loading of phosphorus caused by increased external sulfate loading (Myrbo et al., submitted-2.).
Shoreline and watershed development
Development may entail threats to wild rice apart from enhanced phosphorus and sulfate loading; increased shoreline development reduces aquatic plant cover (Radomski, 2006), although wild rice was not specifically studied, and mechanisms of loss are not documented. Boat traffic may dislodge wild rice (Pillsbury and Bergey, 2000).
Hydrological threats
Dams that maintain stable water levels can favor perennial vegetation over wild rice. (MDNR 2008, p. 22)
Increases in the frequency of rapid increase in water levels, particularly in early summer (MDNR, 2008, p. 21), "…although wild rice is well-adapted to annual fluctuations in water levels, while other aquatic plants may be less suited to such changes." (MDNR, 2008, p. 24) "The emergent stage begins with the development of one or two floating leaves and continues with the development of several aerial leaves two to three weeks later. The floating leaves are apparent in late May to mid-June in Minnesota, again dependent on water depth, latitude, and weather. It is at this stage of growth that wild rice is most susceptible to uprooting by rapidly changing water levels due to the natural buoyancy of the plant. Rising water levels can significantly stress the plant even if it remains rooted." (MDNR, 2008, p. 88)
Groundwater extraction that dries out wild rice habitat. (MDNR, 2008, p. 25)
Impoundments or beaver activity that raise water level in wild rice beds over 3 feet. (MDNR, 2008, p. 21)
Native and exotic species
Carp feeding action dislodges plants and suspends fine particles into the water column. The increased turbidity, caused both by disturbed sediments and by algae stimulated by the phosphorus released from disturbed sediments, shades out aquatic plants. Turbidity then increases as non-vegetated lake bottoms are disturbed by wind. The reduction in aquatic vegetation also allows for increased boat traffic and wave action that can further dislodge plants such as wild rice (Pillsbury and Bergey, 2000). (MDNR,2008, p. 27)
Grazing by Canada Geese (MDNR 2008, p. 24)
Non-native invasive species may harm wild rice. The common carp dislodges plants and reduces water clarity, both by suspending fine particles and releasing phosphorus that enhances algal growth. Hybrid cattail (*Typha x glauca*), a cross of native and non-native cattail (*Typha latifolia* L. and *Typha angustifolia* L., respectively), competes directly with natural wild rice for shallow-water habitat. These plants aggressively form thick mats of roots that can float as water levels fluctuate. A relatively new threat to natural stands of wild rice is the non-native flowering rush (*Butomus umbellatus* L.). Found in similar habitats as native bulrush (*Scirpus* L. spp.), which it resembles, flowering rush can persist in either emergent or submergent forms. Though its distribution in Minnesota is limited, its range is expanding. Another potential threat to natural wild rice in Minnesota is the non-native form of phragmites, or common reed [*Phragmites australis* (Cav.) Trin.] (MDNR, 2008, pp. 27-29).

(continued)

Climate change

Climate change may harm wild rice:

- By allowing carp to spread north (MDNR, 2008, p. 30).
- By excessive warmth, which decreases the occurrence of cold dormancy in southern portion of range that is required for high germination rates (MDNR, 2008, p. 30, Myrbo et al., in press-1).
- Spread of wild rice diseases, such as brown spot (MDNR, 2008, p. 30).
- Extreme precipitation events that increase water depth abruptly (MDNR, 2008, p. 30-31).

Genetic threat

Because wild rice pollen is airborne, some have expressed concerns about unplanned cross-pollination between cultivated stands and natural stands. At this point in time, however, traditional wild rice breeding programs are not thought to pose a threat to natural stands since the cultivated varieties reflect the selection of genes from within the naturally occurring gene pool (MDNR, 2008, p. 26).

C. Identification of 120 µg/L as the protective sulfide concentration

As part of the MPCA-sponsored wild rice research, data were collected to identify a sulfide concentration that would be protective of wild rice in natural waters. Most published information on sulfide toxicity is about effects on animals. The U.S. Environmental Protection Agency (EPA) established national criteria for sulfide in surface waters of 2.0 µg/L to protect aquatic life (EPA, 1986). It is possible for sulfide to accumulate to this concentration in surface waters that cannot be re-supplied with oxygen from the atmosphere and that have low photosynthetically-produced oxygen, such as the hypolimnion of deeper lakes (Wetzel, 2001) or ice-covered shallow lakes (Scidmore, 1957). However, because exposure to atmospheric oxygen quickly detoxifies sulfide by oxidation to sulfate, surface waters in ice-free shallow lakes that are not thermally stratified are unlikely to exceed this criterion for surface water (2.0 µg/L). The challenge faced here is to identify a porewater sulfide concentration that would be protective of wild rice.

Wild rice habitats are vulnerable to accumulation of sulfide in the sediment porewater in which the plants grow. The vulnerability is the result of the combination of the high oxygen consumption by bacteria in sediment containing decaying plant litter, and the low solubility of oxygen in water (about 10 ppm, compared to 210,000 ppm in the atmosphere). On a volumetric basis, there is 27 times as much oxygen in a liter of air than a liter of water (Caraco et al., 2006). Although the scientific literature has long identified rooted aquatic plants as vulnerable to sulfide toxicity (see review by Lamers et al., 2013), at the start of the MPCA-sponsored research effort in 2011 there was no published information specific to the effect of sulfide on wild rice. There is some information on the toxicity of sulfide to white rice (*Oryza sativa*), which is related to wild rice (*Zizania palustris*) and inhabits similar environments, and therefore faces similar environmental challenges. However, it is unclear how applicable data from white rice are to wild rice. Furthermore, many of the studies identify *toxic* levels of sulfide (e.g., 50% effect levels, or EC50), levels that would result in a significant loss of plants. In contrast, the MPCA needs to identify a *protective* level of sulfide. Lamers et al. (2013) reviewed three publications regarding the toxicity of sulfide to white rice, and reported sulfide toxicity as low as 10 micromoles per liter (320 µg/L, or 0.320 mg/L).

Protective concentrations of a chemical have often been identified by exposing organisms to a range of concentrations, and then calculating the concentration at which a minimal effect is observed, such as a 10% or 20% negative effect on growth relative to a control. Effect concentrations of 10% and 20% are termed EC10 and EC20. In an earlier analysis (MPCA, 2014), MPCA had proposed identifying a protective sulfide concentration based on a 20% negative effect (EC20). However, the independent peer review panel recommended that a more conservative protective concentration, such as EC10 or EC5, would be more appropriate for the protection of wild rice. A more conservative (lower) concentration was recommended because this effort involves identifying a protective concentration of a toxin for a single species, in contrast to an ecological community, which is assumed to have functional redundancy among species.

The MPCA has received comments that the use of EC10 is inappropriately over-protective, and that the EPA recommends that water quality standards be based on EC50 for acute exposure of a chemical, and EC20 or EC25 for chronic exposure (MCC, 2015, pp. 16-19) to protect aquatic life. The commenter is referring to EPA guidelines (EPA, 1985 and EPA, 2010), but those guidelines also anticipate that rote application of the basic procedures may not yield the most appropriate standard. Consequently, the EPA guidance provides flexibility for deviation from the normal procedures. For instance, the guidance (EPA, 1985) states:

> "Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important."

In this case, the flexibility is needed because of the difference between the MPCA's goal of protecting a singular species, wild rice, and the fact that the EPA guidance is designed to protect 95% of a community's species and to preserve the ecological functioning of the community, and not to protect an individual species. The EPA guidance is meant not only to protect multiple species in an aquatic community, but also to be applied when effect concentration data are available from at least eight different groups of aquatic organisms (EPA, 2010). The data are then graphed as a species sensitive distribution (SSD) and generally the 5th percentile of the distribution (the lowest) is accepted as a matter of policy as the concentration that would maintain the viability of most species. Preference is given to using the lower confidence limit of the 5th percentile (NRC, 2013) as the numeric criteria.

The MPCA is not applying this method to the identification of the protective sulfide concentration for wild rice because: (a) the MPCA is updating an existing standard that is specific to just wild rice, rather than the whole aquatic community; and, (b) even if the goal were to develop a standard to protect the community of organisms that inhabit the sediment of shallow aquatic ecosystems, there are not enough high-quality data on the effect of sulfide to rooted aquatic plants and sediment-dwelling animals to perform such an analysis.

EC10 has been identified as a suitable threshold when the goal is to only allow negligible exposure to a potentially toxic chemical (e.g., Merrington et al., 2014; Hommen et al., 2015). The EPA's Science Advisory Board also notes that EC10 has been used when a protective concentration is calculated for a particular species of concern (SAB, 2008).

MPCA staff calculated EC10 values from the hydroponic, mesocosm, and field data (Fig. 1-2, Table 1-8), which are updated from values presented in earlier MPCA reports, such as the March 2015 Draft Proposal (MPCA, 2015), and the Draft Technical Support Document (MPCA, 2016). The following paragraphs explain those EC10 calculations, which are further detailed in the referenced papers and Appendices 5 – 7.

Table 1-8. Estimates of protective sulfide concentrations for wild rice from hydroponic, mesocosm, and field data, based on change-point analysis, EC10 estimates, and visual identification of a decrease in a graph of the proportion of field sites with wild rice present.

	Data set	Protective Sulfide Concentration (µg/L)	
		Estimate	95% Confidence Interval
Minnesota Chamber-sponsored hydroponic experiment (data from Fort et al., 2017)			
EC10, based on hydroponic experiment (MCC, 2015)*	Hydroponic	963	not given
MPCA-sponsored hydroponic experiment (Appendix 5; data from Pastor et al., 2017)**			
EC10, based on regression of weight gain on average initial sulfide*	Hydroponic	251	<11 - 285
EC10, based on regression of weight gain on time-weighted arithmetic mean of sulfide	Hydroponic	106	<11 - 158
EC10, based on regression of weight gain on time-weighted geometric mean of sulfide*	Hydroponic	39	<11 - 66
MPCA-sponsored mesocosm experiment (Appendix 6; data from Pastor et al., 2017)			
EC10, based on regression of percent of filled seeds	Mesocosm	228	0 - 414
EC10, based on regression of number of plants that germinated	Mesocosm	163	0 - 242
MPCA-sponsored field survey (Appendix 7; data from Myrbo et al., in press-1)			
Visual identification of reduction in proportion of waterbodies with wild rice present (N=108)	All sites	120	not applicable
Change-point analysis, based on wild rice density (N=67)	All sites with wild rice	112	25 - 368
EC10, based on binary logistic regression of wild rice presence (transparent sites, N=96)	Transparency > 30 cm	93	14 - 239
EC10, based on binary logistic regression of wild rice presence (all sites, N=108)*	All sites	58	<11 - 117

*Estimates identified in the text as deserving less weight in the weighing of multiple lines of evidence.
**Data from three experiments were merged for the logistic regressions.

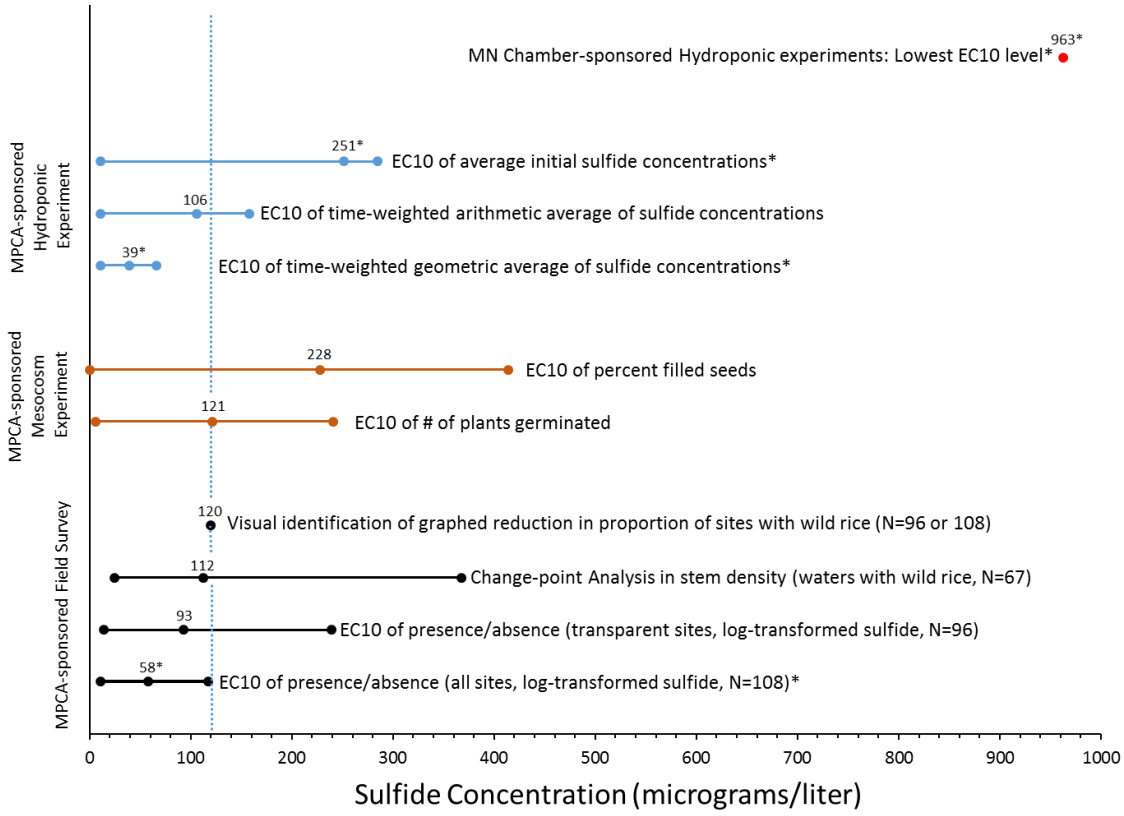


Figure 1-2. Estimates of protective sulfide concentrations for biological endpoints from hydroponic, mesocosm, and field data, based on EC10 estimates, change-point analysis, and visual examination of trends. Estimates marked with an asterisk (*) are identified in the text as deserving less weight in the weighing of multiple lines of evidence.

MPCA-sponsored hydroponic experiments: estimates of protective sulfide concentrations

Three different hydroponic EC10 values were produced by combining growth data from multiple experiments and performing logistic regressions (Ritz et al., 2015). EC10 estimates were made for three different representations of sulfide exposure (initial concentration, arithmetic average, and geometric average) yielding EC10 values of 251, 106, and 39 µg/L, respectively (Appendix 5). The peer review panel (ERG, 2014) concluded that the use of the initial concentration EC10 (251 µg/L) is not warranted, and that it would be more defensible to use either of the time-weighted EC10 values (39 or 106 µg/L). Their reasoning was based on the observation that the sulfide concentrations were measured every two to three days when the hydroponic solution was renewed, and concentrations declined significantly between hydroponic renewals, so that the plants were only exposed to the initial concentration for a short time. The photosynthesizing seedlings produced oxygen that decreased the sulfide concentrations between renewals, especially at low concentrations of sulfide.

The EC10 based on the time-weighted geometric average is lower than the arithmetic average (39 µg/L, compared to 106 µg/L) because a geometric average assumes that the rate of sulfide oxidation was faster at first, and then declined. There is no evidence for a changing oxidation rate, so an EC10 of 106 µg/L is most defensible. Furthermore, it has been argued that calculation of a geometric average is

rarely appropriate when calculating average chemical concentrations for investigations of environmental impact (Parkhurst, 1998). Parkhurst points out that geometric averages are biased low, which accounts for the unusually low EC10 sulfide concentration of 39 µg/L. Accordingly, the arithmetic average of 106 µg/L is the most defensible EC10 estimate derived from the hydroponic data. (These EC10 values supersede estimates reported earlier in draft MPCA documents of 299, 160, and 71 µg/L, which were calculated with normalized data from each of the three experiments. Further examination of the results indicated that normalizing the data skewed the results, so this updated analysis uses the raw data.)

Of the three approaches to analyzing the hydroponic data, the EC10 estimate that is based on the time-weighted arithmetic average sulfide concentrations (106 µg/L) is likely the most reliable estimate (Fig. 1-2). Note that the EC10 is determined by calculating the sulfide concentration associated with a 10% decrease in wild rice growth relative to the growth in the control treatments. The control growth rate is taken as the flat area of "no effect" observed at the lowest sulfide concentrations (for example, the flat area in the left side of the logistic curve in Fig. 1-3).

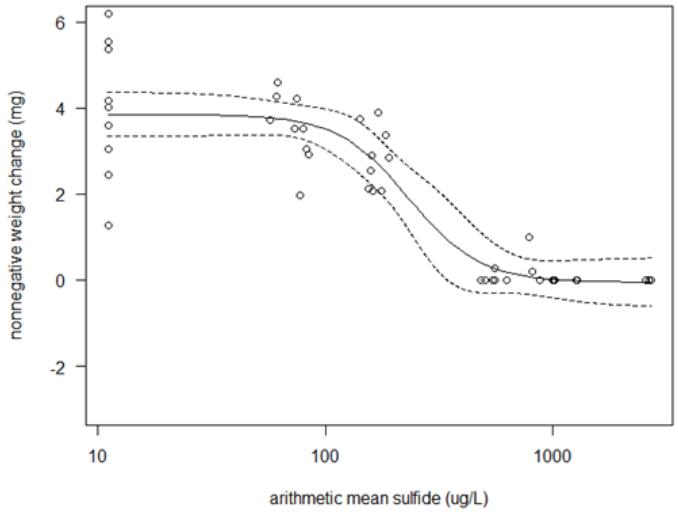


Figure 1-3. Logistic fit of wild rice seedling weight gain against time-weighted arithmetic average sulfide concentrations, using package *drc* in R. Raw data from three experiments are merged together. The EC10 was 106 µg/L, with a 95% confidence interval of 11 to 158 µg/L.

MPCA-sponsored mesocosm experiments: estimates of protective sulfide concentrations

The MPCA-sponsored mesocosm experiments (described in Pastor et al., 2017) yielded two statistically-significant effects of sulfide on wild rice, (1) percent filled, or viable, seeds and (2) number of plants that emerged in the spring. Calculation of EC10 values from linear regressions (Appendix 6) yields EC10 values of 228 and 121 µg/L, respectively, with relatively wide 95% confidence intervals (Fig. 1-2, Table 1-8).

MPCA-sponsored field survey: estimates of protective sulfide concentrations

The field survey of 108 different waterbodies offers several different ways to identify potential protective sulfide concentrations (Appendix 7): binary logistic regression (BLR), change-point analysis, and a visual examination of a graph of the proportion of sites with wild rice present—a non-statistical approach suggested by the 2014 independent peer review panel.

The EC10 derived from a binary logistic regression of wild rice presence/absence of the field data is less precise than expected because when the sulfide data are log-transformed to achieve an appropriate statistical distribution, the curve does not exhibit a flat area of "no effect" at the lowest sulfide concentrations (Fig. 1-4; see Appendix 7 for a comprehensive presentation). Because there is no flat area of no effect, the calculated EC10 value is dependent on selection of the baseline value from which to calculate a 10% effect.

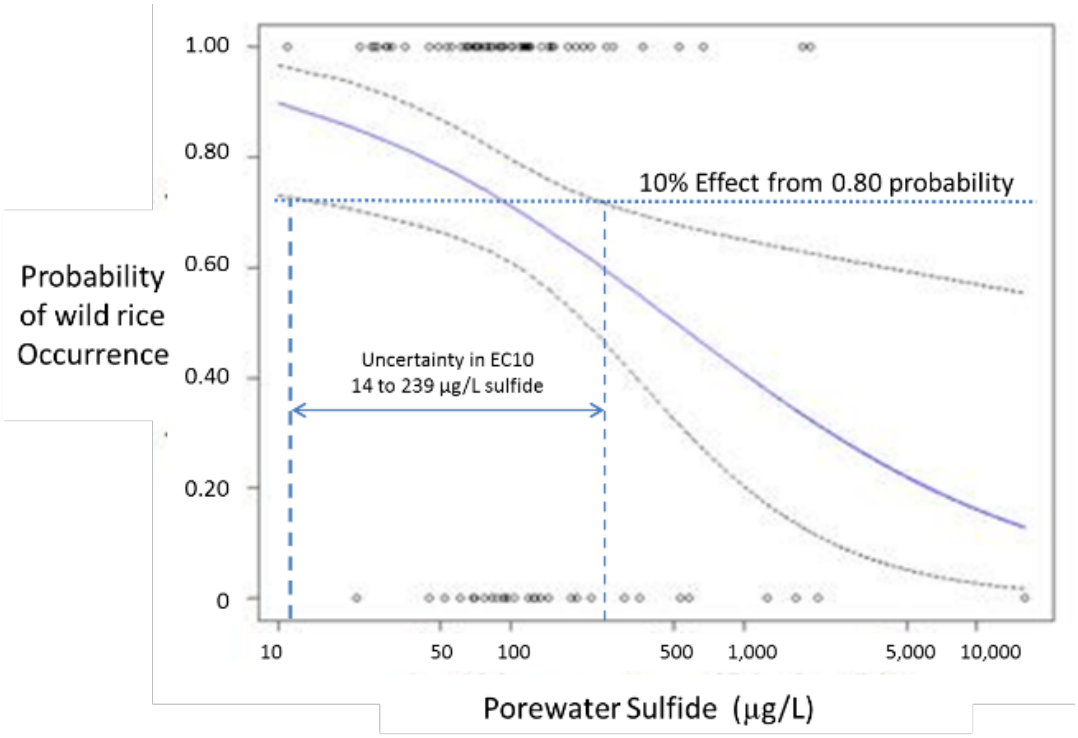


Figure 1-4. Binary logistic regression of the presence and absence of wild rice for field survey sites with water transparency greater than 30 cm (Class B data, N=96). The uncertainty of the EC10 estimate (93 µg/L) was quantified by identifying the range of sulfide concentrations that contain a given EC10 wild rice proportion in their 95% confidence interval based on the binary logistic regression

In the absence of a flat area of the curve, MPCA defined the baseline value as the proportion of sites with wild rice for the 10 sites with the lowest sulfide concentrations (0.80; Appendix 7). Given that definition, binary logistic regression can be used to identify two different protective sulfide concentrations depending on whether low-transparency (< 30 cm water transparency) sites that would not support wild rice are included (Table 1-8). These EC10 values are 58 µg/L for all sites, and 93 µg/L for the 96 sites with sufficient transparency to support wild rice. It is not reasonable to calculate a protective sulfide concentration with data from sites that would not support wild rice no matter how low the sulfide concentration is. Therefore, regression of just sites that would support wild rice, yielding an EC10 of 93 µg/L (95% confidence interval 14 – 239 µg/L), is the most defensible EC10 in this case.

A change-point analysis of wild rice density against sulfide yields an EC10 of 112 µg/L (95% confidence interval 25 – 368 µg/L), which is broadly compatible with the EC10 of 93 µg/L derived from the sites that have suitable transparency to support wild rice.

The third way in which the field data were used to identify a protective sulfide concentration was a non-statistical approach involving direct examination of the data. Visual identification of a reduction in the proportion of sites with wild rice present yields a value of 120 µg/L (Fig. 1-5), which is compatible with both the field survey EC10 based on wild rice presence/absence (93 µg/L, confidence interval of 14 - 239 µg/L) and change-point analysis based on wild rice density (112 µg/L, confidence interval of 25 – 368 µg/L; Table 1-8, Fig. 1-2).

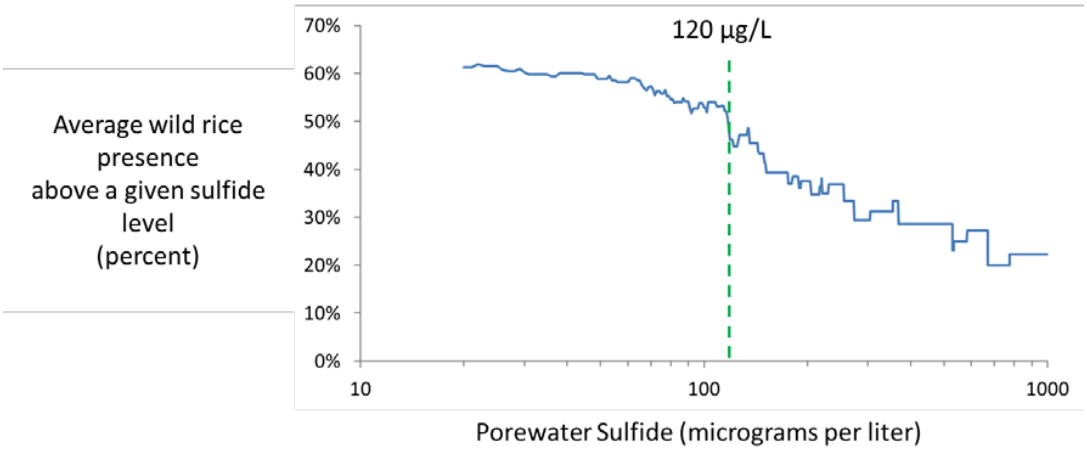


Figure 1-5. Empirical examination of the average proportion of sites with wild rice above a given porewater sulfide concentration (sites excluded with transparency < 30 cm). There is a noticeable decline in the proportion of sites with wild rice when sulfide exceeds 120 micrograms per liter (vertical dashed line).

Minnesota Chamber-sponsored hydroponic experiment: estimate of protective sulfide concentrations

A 21-day hydroponic study was sponsored by the Minnesota Chamber of Commerce (Fort Environmental Laboratory, 2015; Fort et al., 2017) in which wild rice seeds from a Minnesota lake were germinated in solution with a range of sulfide concentrations. Fort et al. (2017) did not calculate effect concentrations, but an EC10 of 963 µg/L was calculated from the Fort study data (MCC, 2015), suggesting that sulfide is less toxic to wild rice than was found in the three MPCA-sponsored studies (hydroponic, outdoor mesocosm, and field survey).

MPCA staff reviewed the design and results of the Fort hydroponic experiments to explore whether there were differences in the experimental approaches that could help account for these differing results. One potential explanation for the difference in the observed toxicity effects lies in the way that the germinated seeds were exposed to sulfide. In the Fort study, seeds were placed on a mesh that was submerged 1 cm in an aquarium open to the atmosphere that initially contained an anaerobic hydroponic solution of a given sulfide concentration; the solution was renewed and monitored daily. During the 21-day experiment, the sprouts were enabled to grow above the surface of the water, into the room air, as the mesocotyl (stem) developed and elongated. As the Fort study report states, “The mesocotyl developed in aerobic conditions under this design. Plastic wire mesh was placed inside the aquaria to provide a trellis to support vegetative growth above the hypoxic culture media." (Fort Environmental Laboratory, 2015, p. 14).

MPCA staff hypothesize that once the wild rice sprouts emerged into the room air, access to oxygen in the room air allowed the sprouts to internally detoxify sulfide by oxidizing it to non-toxic forms of sulfur (see *How access to oxygen may allow wild rice to detoxify sulfide*, in Part A of this chapter). There is

evidence in the scientific literature that aquatic plants can detoxify sulfide through two broad routes that require oxygen. Aquatic plants have special channels in the stem for transporting air, called aerenchyma, for this purpose (Colmer, 2003). Access to the atmosphere is significant because the atmosphere is 21% oxygen (210,000 parts per million, ppm), in contrast to the availability of oxygen in water (a maximum of about 10 ppm). However, as noted in Part A of this chapter, under natural conditions 21-day old wild rice plants would not have access to the atmosphere because the seeds germinate in water that is much deeper than 1 cm, and the stems would not yet have elongated sufficiently to reach the water surface.

Uncertainty surrounding potential sulfide concentrations to protect wild rice

MPCA examined the range of EC10 and change-point estimates from MPCA-sponsored research on hydroponic, mesocosm, and field data (39 to 251 µg/L), plus the EC10 of 963 µg/L estimated from the Fort et al. (2017) hydroponic study (MCC, 2015). The discussion above identifies potential protective concentrations on which there are reasons to place less weight when considering the balance of evidence: two of the three MPCA-sponsored hydroponic EC10 values, the EC10 derived from field survey data that included low-transparency sites that would not support wild rice, and the Minnesota Chamber-sponsored hydroponic experiment. Those estimates with lower weight are identified with asterisks in Fig. 1-2 and Table 1-8.

In a review of plant toxicity endpoints, Clark et al. (2004) suggested that although plant growth is an important metric, successful reproduction is the most important metric in assessing the toxicity of a substance to an annual plant, such as wild rice. In the MPCA-sponsored investigations into the effect of sulfide on wild rice, the best metrics of successful growth and reproduction are (1) the percent of filled seeds (an indicator of seed viability) in the mesocosm experiment, (2) the number of plants that germinated in the mesocosm experiment, (3) the occurrence of wild rice in the field survey, and (4) the density of wild rice in the field survey. The estimates of protective sulfide concentrations from these metrics broadly agree with each other (Fig. 1-2).

The MPCA acknowledges that there is uncertainty in all of the EC10 calculations. The EC10 estimates from the field survey are uncertain due to the lack of a flat curve at low sulfide concentrations (Fig. 1-3). The EC10 derived from the MPCA-sponsored hydroponic experiment is uncertain because (1) sulfide concentrations declined during exposures and (2) the whole seedling was exposed to sulfide, which may not occur in nature except when the plant is germinating from a seed buried several inches in the anoxic sediment. The EC10 values derived from the outdoor mesocosms do not suffer from any obvious flaw, although it should be acknowledged that the mesocosms were not perfect mimics of the environment in that porewater sulfide concentrations were probably not in steady state. Pastor et al. (2017) point out that mesocosms cannot be perfect mimics of natural wild rice waters and be in steady state with controlling variables, because the watershed sources of iron were cut off even as sulfate kept being supplied. As a result, porewater sulfide concentrations increased over time, rather than reaching a steady-state concentration.

In addition, recent publications question whether EC10 can be used as a precise estimate of “no effect,” “negligible effect” or a “protective concentration” (e.g., Hommen et al., 2015; Fox and Landis, 2016). In addition to considering the multiple EC10 values and the change-point estimate, MPCA used a more empirical approach to identify a potential protective sulfide concentration by directly examining the field data for a visual threshold that might be used to identify a protective concentration—an approach explicitly recommended by the peer review panel (ERG 2014, p. 6). The data were examined for a threshold by calculating the average proportion of sites with rice above any given sulfide concentration (Fig. 1-4; Appendix 7), and the pattern simply examined, without any statistical analysis. Such an examination shows that although the percent of sites with wild rice declines as sulfide increases, the decline is relatively slow until the sulfide concentration exceeds 120 µg/L, where there is a notable drop

in the percentage of sites with wild rice present. While a small uptick in the proportion of sites with wild rice occurs between 130-150 µg/L, the percentages never return to the 60% or greater that are observed below 120 µg/L (Fig. 1-5).

Identification of 120 µg/L as the protective sulfide concentration

Based on the analyses described above, MPCA proposes 120 µg/L as the protective concentration of sulfide. Not only is 120 µg/L at a visual break in the proportion of sites with wild rice, but it is within the range of the most defensible estimates of protective sulfide concentrations: 106 µg/L (from hydroponic experiments), 91 µg/L (the field survey EC10 based on wild rice presence), 112 µg/L (the field survey change-point based on wild rice density), 121 µg/L (EC10 based on mesocosm plant germination), and 228 µg/L (EC10 based on mesocosm seed viability) (Fig. 1-2; Table 1-8).

Of the 67 sites sampled in the field survey where wild rice was present, 73% had sulfide concentrations below 120 µg/L. The median sulfide concentration was 85 µg/L, and the average was 165 µg/L; 10% of sites had sulfide concentrations above 235 µg/L. In contrast, the median and average sulfide concentration at sites without wild rice was 126 and 795 µg/L, respectively. It is important to keep in mind that porewater sulfide is not the only environmental variable that affects the presence of wild rice, as discussed above. The analysis of the MPCA field data showed that reduced water transparency and elevated temperature also are associated with the absence of wild rice (Myrbo et al., in press-1), and other factors such as large carp populations and unfavorable hydrology have also been associated with the absence of wild rice (Table 1-6).

D. Relationship between surface water sulfate and porewater sulfide

The relationship between sulfate and sulfide is mediated by biology and chemistry, leading to a situation where superficially there seems to be little relationship between the two.

In the sediment of waterbodies, sulfate in the overlying water can diffuse into the underlying sediment and be converted by bacteria to sulfide. Diffusion of sulfate into sediment and the net retention of sulfur in sediment (as sulfide or as iron sulfide) has been shown in numerous lakes to be a function of the sulfate concentration (Urban et al., 1994). Based on that information, in a survey of many waterbodies one might expect porewater sulfide concentration to be strongly and directly correlated to sulfate concentration in the surface water. However, a plot of porewater sulfide against surface water sulfate (Fig. 1-6) shows only that when sulfate is low, sulfide is also low. But when sulfate is high, sulfide can range anywhere from low to high. This wedge-shaped distribution of data makes sense when one considers that sulfide must be produced from a sulfur-bearing chemical, and that sulfate is relatively mobile and available for the anaerobic bacteria that convert sulfate to sulfide. These bacteria necessarily can produce only limited quantities of sulfide when sulfate concentrations are low.

When sulfate concentrations are higher, there are two possible processes that may serve to limit sulfide concentrations. First, organic matter may be in short supply, limiting the bacteria's active metabolism and subsequent production of sulfide. Second, even if the bacteria are not limited by the availability of organic matter and do produce sulfide, sulfide may be removed from the porewater by precipitation with iron (Pollman et al., in press). As a result, sulfide concentrations associated with waters high in sulfate range from low to high.

Sulfide can also be produced in sediment by the putrefaction of sedimentary sulfur-bearing protein, which has been demonstrated by Dunnette (1989), who studied two eutrophic lakes. Dunnette found that putrefaction accounted for 5% and 57% of the sedimentary sulfide production in the two lakes. However, the overwhelming majority of sulfur retention in oligotrophic lake sediments can be accounted for by the conversion of sulfate to sulfide (Urban et al., 1994). Natural wild rice waters are normally low in nutrients, in contrast to the lakes studied by Dunnette, and so the findings of Dunnette may not be pertinent. Regardless, the following sections demonstrate that a satisfactory model can be developed that predicts porewater sulfide from just sulfate in surface water, sediment organic matter, and sediment iron.

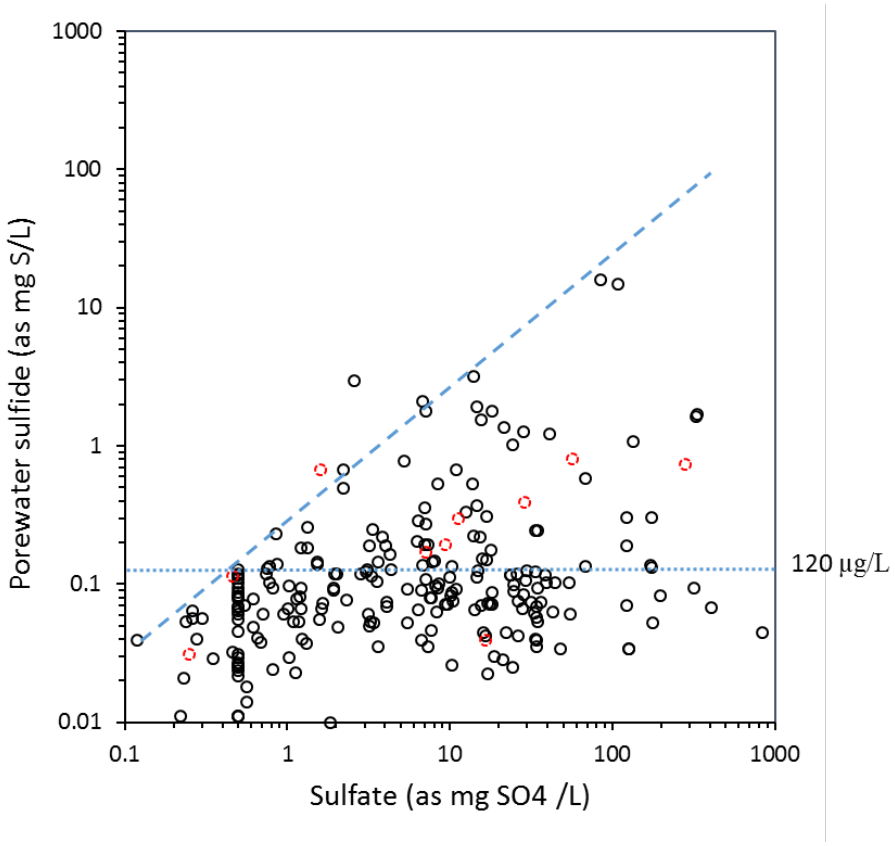


Figure 1-6. Relationship between sulfate in surface water and sulfide in porewater. The dashed line describes the empirically-observed highest net efficiency for the conversion of sulfate to sulfide (Class G data, plus cultivated paddies; N=233). Black symbols = natural waterbodies; Red dashed symbols = cultivated wild rice paddies. The protective sulfide concentration of 120 µg/L is shown.

Use of field data to model the effect of increasing sulfate in surface water

In the development of the research protocol (MPCA, 2011), potentially important environmental variables were identified based on a conceptual model of the processes relating sulfate and sulfide (graphically presented in Fig. 1-7). It has long been known that sulfate, organic matter and iron control porewater sulfide (e.g., Canfield, 1989; Giordani et al., 1996; Eldridge and Morse, 2000). In the interest of also collecting data to evaluate alternative hypotheses, in addition to measuring surface water sulfate, porewater sulfide, dissolved organic carbon (DOC), sediment organic carbon, and dissolved and sediment iron, over 60 other field variables were measured at each field site (Table 1-3).

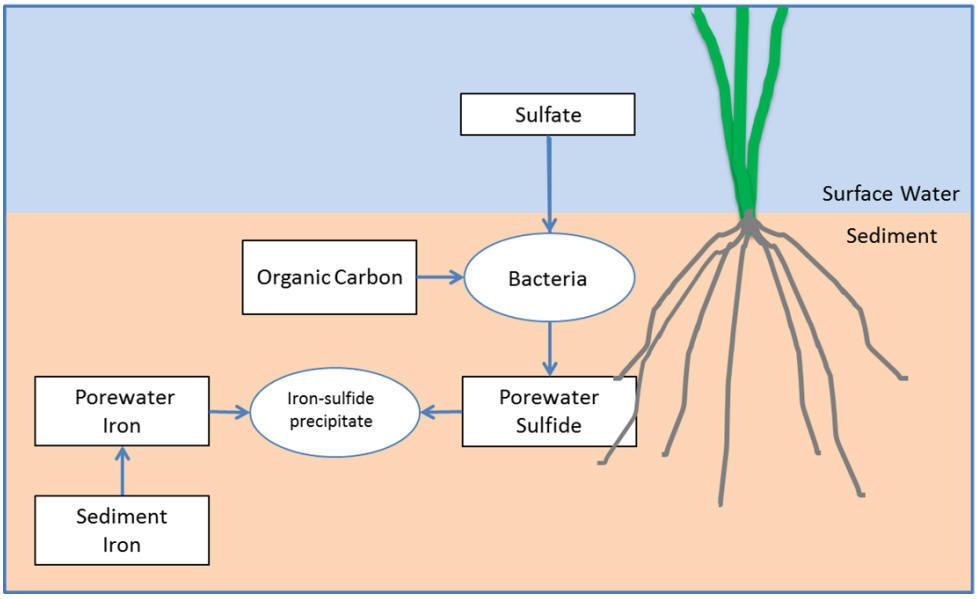


Figure 1-7. Conceptual model of the primary variables affecting the relationship between surface water sulfate and porewater sulfide. As bacteria utilize the energy in organic carbon, they respire sulfate, releasing sulfide. If iron is available, iron-sulfide precipitates form, which detoxifies the sulfide.

MPCA considered two different approaches to modeling the relationship between sulfate in surface water and sulfide in the sediment porewater underlying that surface water: mechanistic and statistical. A mechanistic model uses first principles of chemistry, physics, and biology to quantitatively describe the relationship between variables; to accomplish such a mechanistic model, a relatively complete understanding of the pertinent processes is required. In contrast, a statistical model is developed by fitting field data to mathematical relationships hypothesized by *a priori* understandings of likely chemical, physical, and biological processes operating in the environment (EPA, 2009a).

Mechanistic models, despite the goal of being based on first principles, often are empirically modified with field data to produce more accurate predictions, a process called calibration or parameter estimation (EPA, 2009a). In contrast, a statistical model is fundamentally based on the empirical relationships observed in the field between the variables of interest.

MPCA relied on a model of the statistical relationships of empirically observed data because a statistical model was judged likely to be more reliable than a mechanistic model given the greater data requirements for calibrating a mechanistic model and incomplete knowledge of the processes affecting the net conversion of sulfate to porewater sulfide. The most pertinent attempt to mechanistically model the water-sediment system concerned the potential toxicity of porewater sulfide to seagrass (Eldridge and Morse, 2000), a marine aquatic plant that is analogous to wild rice. A mechanistic model of the chemistry, physics, and biology of a marine system is exactly the same as modeling a freshwater system, except the concentration of sulfate is much higher (about 2,800 mg/L compared to Minnesota's wild rice waters, which range from below 1 to above 500 mg/L). Eldridge and Morse adapted a general model of the decomposition of organic matter by an array of bacteria that respired oxygen, nitrate, iron, and sulfate. The model required input of the concentrations of 13 different chemical species (e.g., organic matter, sulfate, porewater iron, oxygen, alkalinity), and predicted porewater sulfide as one of the seven calculated chemical species, from the simultaneous simulation of 24 different reactions. The model was run to a steady state and the results were compared to environmental data. The modeling was a success in the sense that comparing model results to empirical data reveals how well the processes are

understood. For example, the model fit the measured sulfide better when the model included oxygen release from the roots of the seagrass.

It might have been possible to adapt a mechanistic model like that of Eldridge and Morse (2000) to wild rice, run it to steady state, and compare to the environment. But the model is very complicated and requires a great deal of data to run. The point of developing such a model is to assess how well the processes are understood, and to determine which processes need to be included in the model in order to come close to the observed data. MPCA has a different goal, which is to find a practical model that can relate sulfate to sulfide, to protect wild rice from elevated sulfate concentrations that result in in porewater sulfide concentrations that harm wild rice. Therefore, MPCA did not pursue a mechanistic model approach.

Assumption that sulfate, TOC, iron, and sulfide are in a steady state at field sites

Wild rice waters are dynamic ecological systems, with continuous external loading of sulfate and iron, coupled with variable amounts of annual production of wild rice plants, followed by variable decomposition. Yet it is likely that for decades most wild rice waters have experienced relatively constant processes, such as watershed loading of nutrients and sulfate, and soil erosion that carries organic matter and iron. Sulfate concentrations do fluctuate seasonally, but the field data from the MPCA study showed that sulfide concentrations do not fluctuate to a statistically significant degree. It is likely that porewater sulfide is a function of the long-term (e.g., year or more) average sulfate concentration.

It is common, when modeling relatively undisturbed ecosystems, to assume there is not much net change over seasons or years in the concentrations of important variables (e.g., Eldridge and Morse, 2000). In the case of the MPCA field survey, it is reasonable to assume that most of the 108 different sites have experienced no significant recent change in average surface water sulfate or sediment concentrations of TOC, iron, or sulfide. Monthly sampling at 15 different wild rice waters showed no significant change in TOC, iron or porewater sulfide from June through September (Myrbo et al., in press-1). When there is no significant change in concentrations over time, environmental modelers term a dynamic system to be in a “steady state” (Schnoor, 1996, pp. 4-5).

Both mechanistic and statistical modelers often rely on the assumption that a system is in steady state. For instance, in their mechanistic model Eldridge and Morse (2000) assumed that sulfate, organic matter, iron, and sulfide, among other variables, were in steady state. MPCA made the same steady-state assumption to pursue a statistical model that empirically relates the variables that are known to control porewater sulfide (sulfate, sediment organic carbon, and sediment iron). Although the model is based on concentrations of the variables, each concentration reflects the balance between continual input and loss. For instance, the concentration of sediment iron in the model (average concentration in the top 10 cm of the sediment) reflects the balance between new iron arriving to the sediment (inputs), and burial that pushes the layer of recently deposited material successively deeper in the sediment until it is deeper than 10 cm (losses).

Development of a statistical model using structural equation modeling (SEM)

At the suggestion of the peer review panel (ERG, 2014, p. 6), the MPCA employed structural equation modeling (SEM) to test the hypothesized conceptual model (Fig. 1-7). SEM is often referred to as “causal analysis” because it provides a framework for testing hypotheses with empirical data collected in field surveys (Iriondo et al., 2003). The structural equation (SE) model that was developed supports the hypothesis that there is a dynamic relationship between production of sulfide from sulfate and precipitation as iron-sulfide solids. A key result from the model is that variations in three external variables (sulfate, sediment TOC, and sediment iron) contribute nearly equally to the observed variations in porewater sulfide (Pollman et al., in press). The model provides strong evidence that

development of a sulfate standard to protect wild rice from elevated sulfide should quantitatively consider the effects of ambient concentrations of sediment iron and organic carbon, in addition to surface water sulfate.

The SE model was validated by conducting a jackknife analysis where the model was refit by withholding a single observation from model estimation, and then using the refit model to predict the log10-transformed value for porewater sulfide. The out-of-sample predictions closely matched the predictions obtained from the fully calibrated model, and also found no problems with unusually influential single observations. In addition, models based on alternative hypotheses and involving additional variables (phosphorus, acid volatile sulfide, dissolved organic carbon) were evaluated and found to offer no advantage over the original hypothesis in the prediction of porewater sulfide (Pollman et al., in press).

In summary, structural equation modeling found that porewater sulfide is controlled equally by the concentrations of surface water sulfate, sediment iron, and sediment organic carbon. Thus, all three variables need to be considered when developing a strategy to ensure sulfide remains at or below a protective level in the sediment of wild rice beds.

E. Development of an equation to calculate a numeric sulfate standard for each wild rice water

The consideration of multiple variables in calculating a numeric concentration for a water quality standard is becoming more common as the scientific understanding of the environment improves. For instance, over time EPA's guidance for developing standards for ammonia has progressed from initially considering a fixed concentration to the present recommendation of adjusting ammonia concentrations for both the pH and temperature of the ambient water (EPA, 2013). Similarly, EPA's 2007 aquatic life freshwater criterion for copper is based on a model termed the Biotic Ligand Model (EPA, 2007 revision). This metal bioavailability model uses receiving waterbody characteristics and monitoring data to develop a numeric copper standard. Input data include temperature, pH, dissolved organic carbon, major cations (Ca, Mg, Na, and K), major anions (SO_4 and Cl), alkalinity, and sulfide.

When the consideration of multiple variables allows the calculation of a significantly more accurate numeric water quality standard, it can make sense to utilize that scientific understanding. The structural equation modeling effort demonstrates that porewater sulfide concentrations can be successfully modeled with three variables: surface water sulfate, sediment TOC, and sediment iron measured as total extractable iron (TEFe). If this scientific understanding can be incorporated into an equation, then the equation can be adopted as the water quality standard, similar to EPA's approach to ammonia and copper standards. A standard that is an equation, rather than a uniform concentration that is applied to all waterbodies, is a reflection of the biogeochemical diversity of the environment. New approaches such as this are needed as environmental regulation progresses from protecting organisms from pollutants that are directly toxic, to protecting them from pollutants whose negative effects are both indirect and a function of environmental conditions that vary from site to site. In the case of sulfate, an equation-based standard would reflect the evidence (discussed below) that an equation will be more accurate than a fixed standard

To identify a protective sulfate concentration for a particular water, it would be logical to employ the relationships revealed by the structural equation model and to work backwards from the protective sulfide concentration (120 micrograms/liter, µg/L) to identify the particular concentration of sulfate that would protect wild rice in that waterbody. A direct way to accomplish this task would be to first arrange the structural equation model into a single equation that expresses porewater sulfide as a function of the variables in the model (sulfate, sediment iron, and sediment TOC). Substituting the protective sulfide concentration for that variable and rearranging the equation would then derive an equation for the protective sulfate concentration as a function of iron and TOC concentrations. Such an equation could be applied to any wild rice water for which TEFe and TOC are known. However, rearranging the equation in that way results in re-transformation bias, reducing the accuracy of the equation (MPCA, 2015; Pollman et al., in press). Therefore, the MPCA has taken a different approach.

The re-transformation bias seen using the structural equation model can be avoided by predicting the protective sulfate concentration using multiple binary logistic regression (MBLR) with the variables identified from the structural equation model (Pollman et al., in press). By first relying on the structural equation model development to identify the appropriate variables, an MBLR model can be developed using the same field data. MBLR directly predicts the probability of exceeding the protective sulfide concentration threshold as a function of sulfate, TEFe, and TOC.

MBLR is a predictive analysis; in this case the regression predicts the probability that sulfide is greater than 120 µg/L. The inputs to the regression are the field survey data from 108 different sites for the observed sediment iron, sediment TOC, surface water sulfate and porewater sulfide (the Class B data). All of the Class B is used—including low-transparency waters, waters with wild rice, and waters without wild rice—because the resulting equation is a model of chemical relationships, and does not, and should not, include information on the presence or absence of wild rice. To include only samples where wild

rice was present would certainly bias the data toward lower sulfide concentrations. The model could not be expected to predict higher sulfide concentrations as accurately if a high proportion of high-sulfide sites are excluded (which would happen if only sites with wild rice were included in the regression).

When all 108 samples are used, the MBLR regression is:

logit(sulfide>120 µg/L) = 9.3176 + 1.8962*log10sulfate - 3.6443*log10 iron + 2.2698*log10TOC (equation 1)

An equation to predict a protective concentration of sulfate can be derived if a probability is inserted into the equation. A probability of 0.5 (that sulfide is greater than 120 µg/L) produces sulfate values that most accurately predict the sulfide concentrations that were observed during the field survey (see Appendix 8 for a discussion of why 0.5 is the most accurate probability). At a probability of 0.5, the likelihood that sulfide is greater than 120 µg/L is equal to the likelihood that sulfide is less than 120 µg/L—in other words, using a probability of 0.5 to develop the equation produces the sulfate concentration that best matches a porewater sulfide concentration of 120 µg/L. Probabilities greater than 0.5 would be under-protective, and probabilities less than 0.5 would be over-protective.

Using a simplified version of the formula, the equation becomes:

Logit(Sulfide >120) = constant + a $\log_{10}$ Sulfate + b $\log_{10}$ Iron + c $\log_{10}$ TOC (equation 2)

$$\text{Log}\left(\frac{\text{Probability Sulfide>120 µg/L}}{1-\text{Probability Sulfide>120 µg/L}}\right) = \text{constant} + a \log_{10}\text{Sulfate} + b \log_{10}\text{Iron} + c \log_{10}\text{TOC} \quad \text{(equation 3)}$$

When a probability is chosen, a constant is produced for the left side of the equation (constant2):

constant2 = constant1 + a $\log_{10}$ Sulfate + b $\log_{10}$ Iron + c $\log_{10}$ TOC (equation 4)

If Sulfate is moved to one side and everything else to the other side, the equation becomes:

constant2 - constant1 - b $\log_{10}$ Iron - c $\log_{10}$ TOC = a $\log_{10}$ Sulfate (equation 5)

$$\log_{10}\text{Sulfate} = \frac{\text{constant2} - \text{constant1}}{a} - \frac{b}{a}\log_{10}\text{Iron} - \frac{c}{a}\log_{10}\text{TOC} \quad \text{(equation 6)}$$

$\log_{10}$ Sulfate= new constant- new b $\log_{10}$ Iron - new c $\log_{10}$ TOC (equation 7)

Sulfate = $10^{\text{new constant}}$ x Iron $^{\text{new b}}$ x $\text{TOC}^{\text{new c}}$ (equation 8)

Use of a probability of 0.5 produces a logit(sulfide >120)=0, which creates the proposed equation:

$$Calculated\ Sulfate\ Standard\ = 0.0000121 \times \frac{Iron^{1.923}}{organic\ carbon^{1.197}} \quad \text{(equation 9)}$$

Or, alternatively,

MBLR120 Sulfate = 0.0000121 x $\text{TOC}^{-1.197}$ x $\text{TEFe}^{1.923}$ (equation 10)

where sulfate is expressed as mg/L, TOC as percent dry weight, and TEFe as mg/kg.

Operationally, this equation to determine the protective sulfate threshold can be applied to any waterbody for which sediment TOC and sediment TEFe data are available. Of course, the data need to be produced using methods that are consistent with the procedures used to produce the field data on which the MBLR-based equation was derived.

The effect of different sediments in different wild rice waterbodies is illustrated by calculating the protective sulfate concentrations for three wild rice lakes with contrasting sediment quality. All three lakes had low ambient sulfate concentrations—less than 0.5 mg/L—but because of differences in sediment chemistry, the three have widely different MBLR-calculated protective sulfate concentrations, ranging from 1.2 to 186 mg/L (Table 1-9).

Table 1-9. Calculated protective surface water sulfate concentrations for three wild rice lakes. Note that these examples are for illustrative purposes only.

Study Site	State ID	Sediment Total Organic Carbon (%)	Sediment Iron (mg/ kg)	MBLR120-Calculated Protective Sulfate Concentration (mg/L)
Little Round Lake	03-0302	27.5	3,069	1.2
Elk Lake	15-0010	10.2	8,480	27
Rice Lake	18-0053	35.6	50,389	186

In summary, SEM was used to understand and characterize the relationships between the important parameters in the system that relates sulfate and sulfide, and then MBLR was used to translate the understanding gleaned from SEM into a relationship that can be used to calculate a numeric sulfate standard for each wild rice water.

F. Comparison of an equation-based standard to fixed standards: error rates and concerns

In the development of numeric water quality standards, even when a statistically valid stressor-response relationship is developed there will be a proportion of the waterbodies for which any given standard will be either under-protective or over-protective (EPA, 2009b; McLaughlin, 2012a,b; Vermont DEC, 2014; Smeltzer et al., 2016). To explore this using the MPCA-sponsored field survey data, each waterbody can be compared against a potential sulfate standard to see if the standard would be consistent with its measured porewater sulfide concentration. In other words, does any given sulfate standard that is predicted to be protective of wild rice also result in a porewater sulfide concentration that is protective of wild rice? Conversely, does a sulfate level that is predicted to not be protective of wild rice also result in sulfide levels that are not protective of wild rice? When the sulfate standard is consistent with the measured sulfide, the standard has a correct, or true, classification. A true negative occurs when ambient sulfate is less than the standard and sulfide is less than the protective concentration of 120 µg/L. A true positive occurs when sulfate is greater than the standard and sulfide is greater than the protective concentration of 120 µg/L.

The MPCA field survey data can be examined to determine the proportion of misclassifications (false positive and false negative prediction errors). A false positive error (called a Type 1 error in statistics) occurs when the ambient sulfate concentration exceeds the standard, but porewater sulfide is actually below the protective concentration of 120 µg/L; in such a case, the standard is overly stringent. False positives are a concern because they potentially could cause unneeded investment in sulfate control to keep sulfide at non-toxic levels. A false negative prediction error (a Type 2 error in statistics) occurs when the ambient sulfate concentration is less than the standard, but the porewater sulfide is actually above the protective concentration; in such a case, the standard is insufficiently stringent and adverse impacts may occur where they were not expected. In the development of a water quality standard, while the primary goal is to protect beneficial uses, it is also desirable to minimize the sum of these errors, which is the overall proportion of misclassifications. The total misclassification rate can be calculated for each proposed water quality standard. It has been proposed that it may be optimal to choose a water quality standard that balances misclassifications between false positives and false negatives, so that when an error does occur it is equally likely to be over-protective as under-protective (Vermont DEC, 2014).

The misclassification rate can be calculated for all possible fixed standards using the Class B dataset. At low potential sulfate standards, below 5 mg/L, the misclassification rate is high. For instance at a potential sulfate standard of 1.0 mg/L the misclassification rate is 46% because of a high proportion of false positive errors, indicating that a standard set at 1.0 mg/L would be overly stringent (44% of the sites false positive; 2% false negative; Fig. 1-8). The misclassification rate declines to 32% as the potential sulfate standard rises to 5 mg/L, and then varies between 32% and 37% up to a standard of 40 mg/L. The analysis presented here is limited to potential fixed sulfate standards up to 40 mg/L, above which the proportion of false negatives rises (indicating that fixed standards set above this level would not be reasonable). The lowest misclassification rate, 32% is produced at three potential fixed sulfate standards, 5, 10, and 26 mg/L. At 5 mg/L 24% of the sites would be false positive, and 8% false negative. At 26 mg/L, 4% of the sites would be false positive, and 28% false negative. A fixed sulfate standard of 10 mg/L would be the most balanced between false positives and false negatives, since at 10 mg/L the proportions of the two types of error are equal, at 16%, summing to a total of 32%.

In March 2015 the MPCA proposed to use a SEM-based equation that incorporates sulfate, iron, and organic carbon to calculate sulfate standards for wild rice waters (MPCA, 2015). This approach would result in a misclassification rate of 26% (Fig. 1-8). The refined approach presented in section E of this TSD

of calculating the sulfate standard using multiple binary logistic regression (MBLR) and the same three environmental variables produces a misclassification rate of 16% (9% false positive and 7% false negative). The MBLR equation was validated by applying it to an independent data set (dataset Class V, N=47), which produced a slightly higher misclassification rate of 19% (11% false positive and 8% false negative). The proportion of false negative errors is therefore at least twice as high for a fixed standard of 10 mg/L than for the MBLR-calculated equation-based standard (16%, compared to 8% from Class V, or 7% from Class B) (Fig. 1-8). A major question is whether or not the lower overall error rate of the MBLR equation when compared to a fixed standard (16-19%, compared to 32%) justifies the additional investment in collecting iron and organic carbon data at each wild rice water.

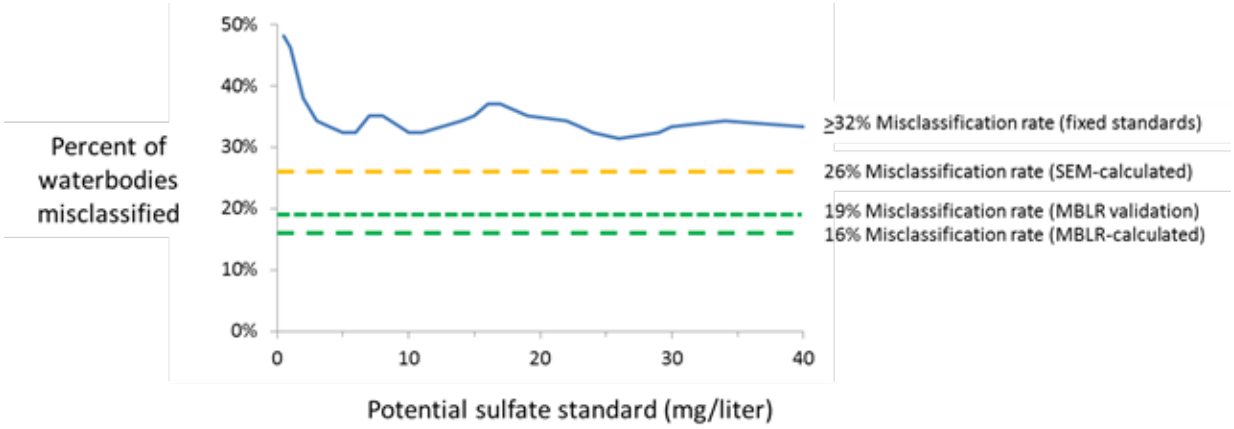


Figure 1-8. The misclassification rate compared across a range of potential sulfate standards. The misclassification rate is the sum of the percentage of false positive and false negative errors at a given potential sulfate standard.

Interplay between protective sulfide concentration and prediction errors

The analysis presented above shows that the MBLR-based equation produces sulfate standards that have fewer false positives and false negatives than any fixed sulfate standard. The equation produces about half the total misclassifications compared to the best fixed sulfate standards. Such comparisons are based on how well matched potential sulfate standards are to sulfide concentrations of 120 µg/L.

But what would the equation-based misclassification rate be if protective sulfide concentrations other than 120 µg/L were chosen? If errors were fewer for a different protective sulfide concentration, would it make sense to base the equation on that sulfide concentration? The interplay between protective sulfide concentrations and prediction errors can be confusing, because it may be tempting to recommend a sulfide concentration as being protective simply because it has relatively few prediction errors. However, it would not be reasonable to promote such a sulfide level unless it were in a range of sulfide concentrations that have a high probability of protecting the beneficial use.

It therefore would be a mistake to designate a sulfide concentration with a low misclassification rate as "protective" without evaluating how well it protects wild rice from that level of sulfide. A critical point to keep in mind is that misclassifications are not correlated with the degree of protection offered by a particular sulfide concentration. If a proposed protective sulfide concentration produces few errors, but the sulfide concentration is not actually protective of wild rice, then the enticement of low errors should be ignored.

Accuracy of calculated sulfate standards across potential protective sulfide concentrations

The potential consequences of designating sulfide concentrations other than 120 µg/L as protective can be investigated through analysis of the Class B dataset. First, the false positives and false negatives can be presented as a percentage of all 108 equation-based predictions across the spectrum of potential protective sulfide concentrations (Fig. 1-9). The sum of false positives and false negatives (the total misclassification rate) dips to 16% between 120 and 130 µg/L, and exceeds 16% at higher sulfide levels until a continuous decline to 9% that begins at 180 µg/L (Fig. 1-9).

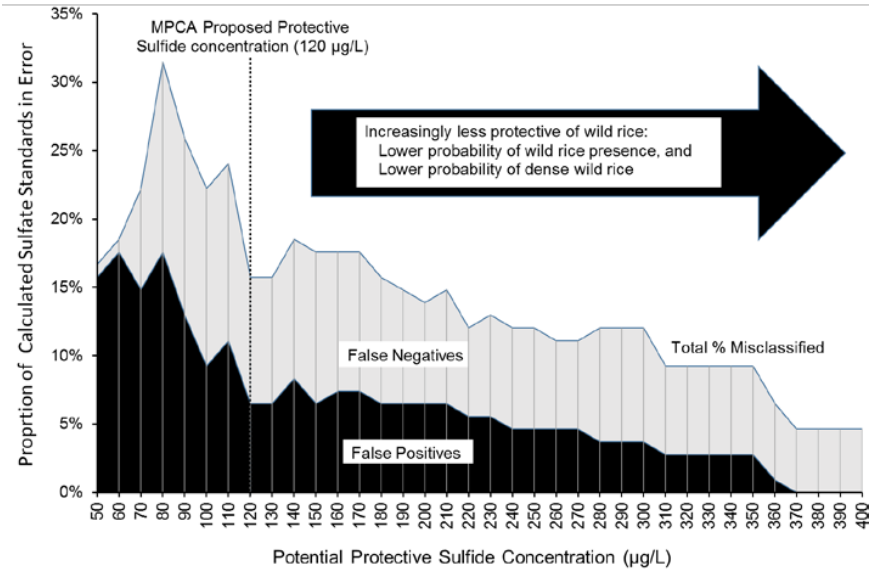


Figure 1-9. The proportion of false positive and false negative prediction errors calculated for potential protective sulfide concentrations ranging from 50 to 400 µg/L (in increments of 10 µg/L). The sum of false negatives and false positives is the total misclassification rate.

Although prediction errors decline when potential protective sulfide concentrations exceed 180 µg/L, such sulfide concentrations would not be as protective of wild rice as 120 µg/L. For instance, the probability that wild rice will be present declines as sulfide concentrations increase (Fig. 1-10), which was one of the lines of evidence included in the identification of 120 µg/L as an appropriate protective sulfide concentration (see Chapter 1, part C, above). In addition, the density of wild rice declines as sulfide concentrations increase; change-point analysis found a statistically significant decrease in density at 112 µg/L, which was another line of evidence examined. The probability that a wild rice water will exhibit dense wild rice (e.g., greater than 25 stem/m^2 or 40 stems/m^2) also declines as sulfide concentrations increase (Fig. 1-11).

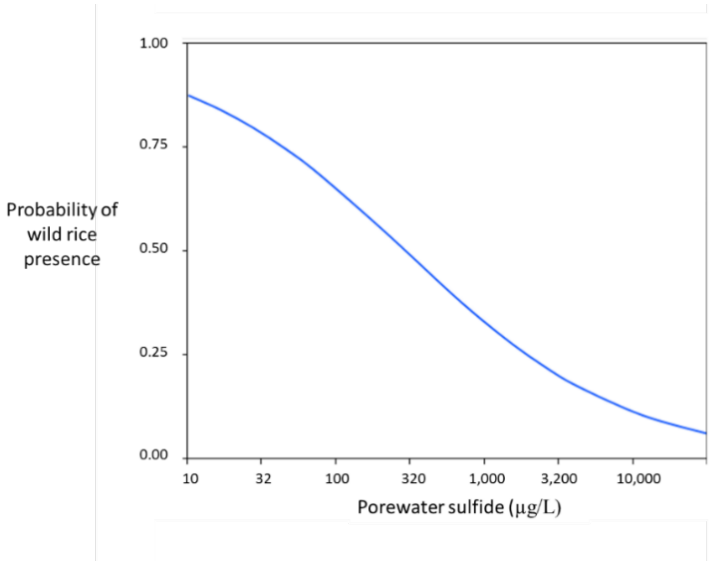


Figure 1-10. Probability of wild rice presence as a function of porewater sulfide (binary logistic regression; p=0.001; N=108).

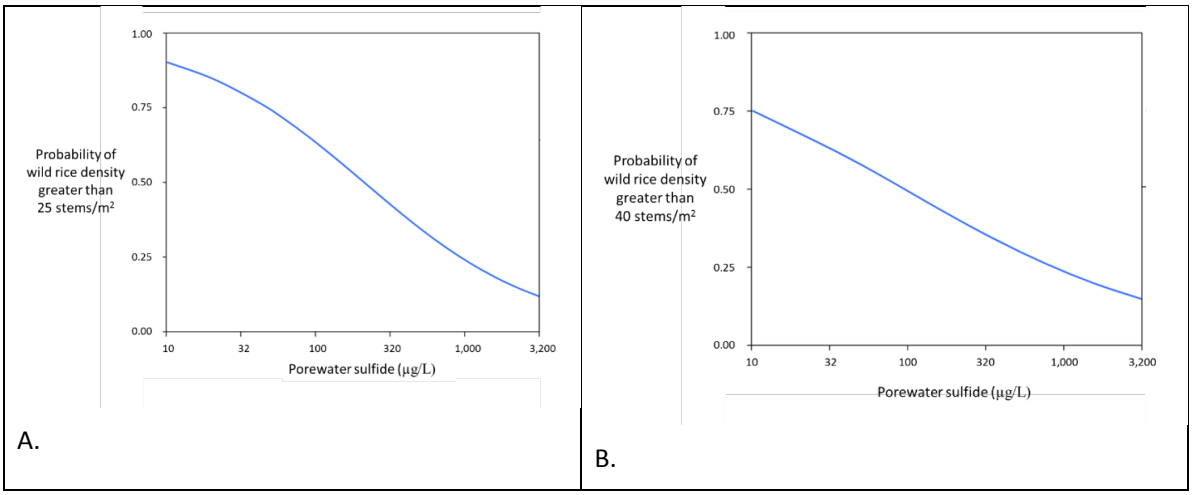


Figure 1-11. Probability of wild rice present at densities (A) greater than 25 stems/m^2 (binary logistic regression; p=0.013) and (B) 40 stems/m^2 (binary logistic regression; p=0.076). (Based on sites with wild rice in the Class B dataset, N=67.)

In contrast, the probability that a wild rice water will exhibit sparse wild rice (e.g., less than 10 stems/m^2) increases significantly as sulfide concentrations increase (Fig. 1-12). In other words, as sulfide increases and the probability of wild rice even being present declines (Fig. 1-10), it becomes more probable that the wild rice that is present has a low density (Fig. 1-13). For instance, at 120 µg/L, there is a 24% chance that wild rice density is less than 10 stems/m^2, but at 300 µg/L the probability more than doubles, to 52%. Simultaneously, the probability that wild rice density is greater than 25 stems/m^2 declines from 60% at a protective level of 120 µg/L to 44% at 300 µg/L.

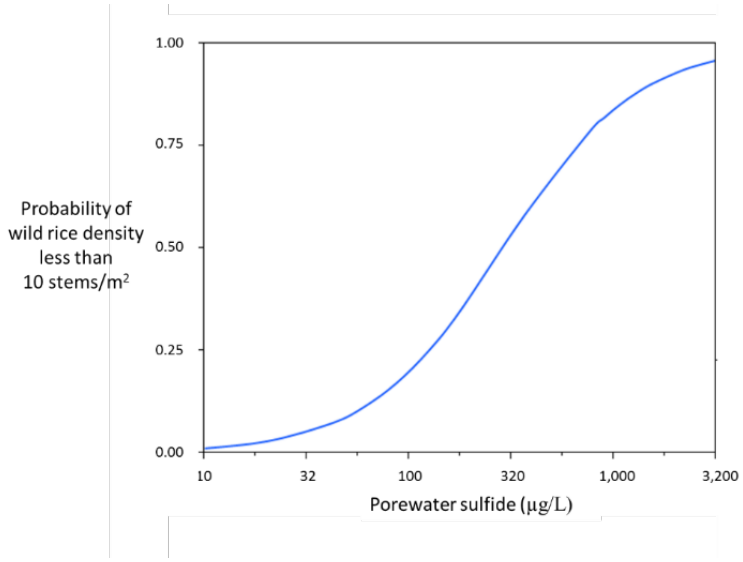


Figure 1-12. Probability of wild rice being present at a density of less than 10 stems/m^2 (binary logistic regression; p=0.0003). (Based on sites with wild rice in the Class B dataset, N=67.)

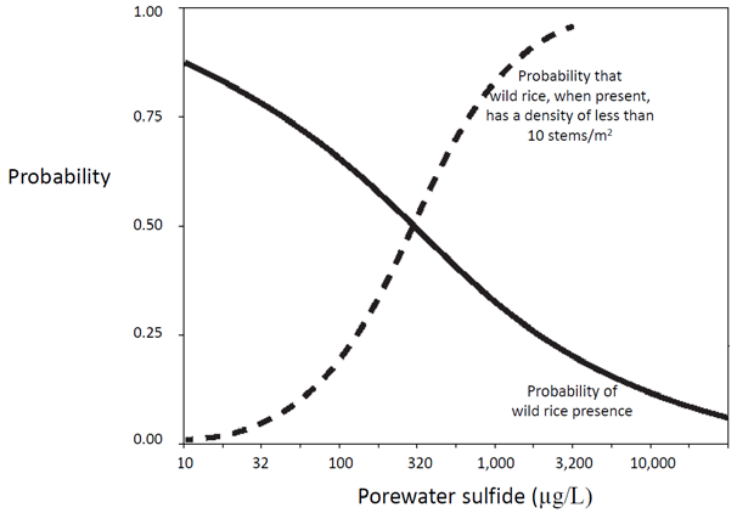


Figure 1-13. Probability of wild rice presence and the probability that the wild rice has a density of less than 10 stems/m^2, plotted against porewater sulfide concentrations.

Why not make the protective sulfide concentration zero?

It is evident that the highest probability of wild rice presence and a dense population occurs when porewater sulfide is lowest. However, it is unrealistic to have a goal of zero sulfide in the sediment porewater of wild rice waters. Sulfide is a natural chemical that is produced in the environment by naturally occurring bacteria from sulfate, which itself is a common natural chemical. Sulfate concentrations vary naturally across the landscape (Myrbo et al., in press-1), as do the other variables that control the production of sulfide, iron and total organic carbon (Pollman et al., in press). As a result, even under natural conditions there are waterbodies that are not hospitable to sulfide-sensitive species such as wild rice. In addition, there are likely areas within some wild rice waters that have lower iron or higher total organic carbon that naturally produce porewater sulfide that is incompatible with wild rice. For instance, an isolated bay of a wild rice water could plausibly have low sediment iron concentrations because the local watershed is poor in iron or there is no emergent groundwater rich in iron (Maranger et al., 2006). Similarly, a bay of a wild rice water could plausibly have higher sediment organic carbon because it lacks water movement to carry away decaying macrophyte plant material; it has been observed that natural wild rice generally grows best in waters that have some movement (Moyle, 1944; DNR, 2008; Table 1-6).

It is good that sulfate is present at least at minimal concentrations, since sulfate is an essential nutrient for plant growth. Zero sulfide and zero sulfate are not reasonable goals, so the question is, what is a reasonable goal for the protection of wild rice?

The field survey sponsored by the MPCA yields information about the range of sulfide in the porewater of wild rice waters. Of the 108 waterbodies in the Class B dataset, only two were less than the analytical lab's reporting limit of 11 µg/L. Sulfide was therefore likely present in the porewater of sediment as wild rice evolved over the millennia. It is also likely that wild rice, like other wetland plants, has adaptations that allow it to grow and reproduce in the presence of some concentration of sulfide in porewater. The multiple lines of evidence indicate that wild rice populations can thrive if porewater sulfide is less than 120 µg/L.

If it is true that wild rice populations can thrive at sulfide concentrations up to 120 µg/L, why do the graphs of presence and density (Figs. 1-10 and 1-11) imply that wild rice would benefit from every incremental decrease of sulfide below 120 µg/L? The graphs extend down to 10 µg/L, where the probability of wild rice presence is predicted to be 88%, and 75% of wild rice stands are predicted to have density greater than 40 stems/m^2. If lower sulfide is apparently associated with better wild rice growth, why not make the protective sulfide concentration as low as possible, such as the analytical limit of 11 µg/L?

There are multiple reasons why making the protective sulfide concentration as low as possible is not reasonable, or necessary to protect wild rice:

Firstly, cause and effect is likely backwards at very low concentrations of sulfide. The graphs relating sulfide to wild rice presence and density (Figs. 1-10 and 1-11), support the overall conclusion that wild rice presence and density can be controlled by exposure to sulfide, (Myrbo et al., in press-1; Fort et al., 2017; Pastor et al., 2017). However, when sulfide is low enough for wild rice to grow to dense levels, it is reasonable to assume that oxygen release by the wild rice would decrease sulfide to even lower concentrations, producing the continuous slope observed in the graphs. It is well established that wetland plants can release oxygen from their roots, which is thought to be an adaptation that decreases the toxicity of sulfide by converting it to sulfate (Lamers et al., 2013). Myrbo et al. (submitted-2) found that sulfide concentrations are significantly lower when wild rice plants are present, an observation that provides support for the idea that high densities of wild rice can drive porewater sulfide to very low concentrations. Myrbo et al. (submitted-2) go on to suggest that there may be a tipping point in the exposure of wild rice to sulfide, above which oxygen release is insufficient to detoxify the sulfide and a

wild rice population cannot persist over the long term. In other words, the capacity of wild rice to detoxify sulfide is limited. The shape of the graphs in Figures 1-10 and 1-11 are explicable if sulfide concentrations above 120 µg/L control wild rice presence and density, but that at some concentration below 120 µg/L wild rice starts controlling the sulfide concentration.

Secondly, the accuracy of the equation that would calculate sulfate standards deteriorates for protective sulfide concentrations below 120 µg/L (Fig. 1-9, Table 1-10). The sum of false positives and false negatives is 16% at 120 and 130 µg/L, but from 70 to 110 µg/L ranges between 22% and 32%. Both false positives and false negatives are serious errors. False negative errors could result in ongoing use impairment. False positive errors could lead to inappropriate management interventions using resources that would be better directed elsewhere (Smeltzer et al., 2016). Below 70 µg/L the errors are essentially all false positives (Table 1-10)—where ambient sulfate is greater than the calculated standards, but porewater sulfide is less than the assumed protective sulfide concentration of 50 or 60 µg/L (MPCA did not calculate the misclassification rates below 50 µg/L).

Wild rice exhibits atypical dose-response curves

It was noted earlier (in "MPCA-sponsored field survey: estimates of protective sulfide concentrations," above) that the logistic curve relating wild rice presence to sulfide does not exhibit a flat area of "no effect" at low sulfide concentrations (Figs. 1-4 and 1-10; Appendix 7). Similarly, the probability of observing high wild rice density does not exhibit a flat area of no effect at low sulfide levels (Fig. 1-11). MPCA staff were initially surprised at the shape of these dose-response curves, since environmental toxicologists typically observe, and expect, a sigmoid-shaped relationship between the growth of an organism and the dose, or concentration, of a chemical (e.g., Landis and Yu, 2003, p. 32). The expectation of a sigmoid-shaped curve is based on the assumption that a chemical has no effect at very low concentrations, but increasingly greater effect as concentrations of the chemical increase. However, environmental toxicologists generally have no expectation that organisms have the ability to decrease the environmental concentration of the toxic chemical, an ability that dense populations of wild rice appear to have when growing in low concentrations of sulfide (through oxidation as described above). The expectation that chemicals affect organisms, and not the other way around, can be explained by the focus of environmental toxicologists on synthetic chemicals that are not natural in the environment, and the assumption that sensitive species do not possess adaptations to reduce the environmental concentration of a toxic chemical. In fact, in their book *Introduction to Environmental Toxicology*, Landis and Yu characterize the sigmoid shape as the typical response of organisms to a "xenobiotic"—xenobiotic being their term for a toxic chemical. The EPA defines a xenobiotic as a chemical "…that does not occur naturally in the environment. Xenobiotics occur as a result of anthropogenic activities such as the application of pesticides and the discharge of industrial chemicals to air, land, or water." (EPA, 1992, p. 38). Environmental toxicologists have generally not evaluated the effect of a natural toxin such as sulfide on organisms, although there is increasing interest in the effect of sulfide on plants (Lamers et al., 2013). Because high densities of wild rice can further decrease low concentrations of porewater sulfide, as seen by Myrbo et al. (submitted-2), atypical dose-response curves are produced.

Alternative metrics for evaluation of false positives and false negatives

There are multiple metrics of the accuracy of predictions that can be calculated when false positives (FP), false negatives (FN), true positives (TP), and true negatives (TN) are known (Fielding and Bell, 1997). In the discussion above, the false predictions are calculated as the proportion of the total

predictions, which Fielding and Bell call the misclassification rate (misclassification rate = (FP +FN)/ (FP+FN+TP+TN). The overall misclassification rate for potential fixed sulfate standards is presented in Fig. 1-8, and the components (false positives and false negatives) are presented in Fig. 1-14a.

A different way to examine the accuracy of predictions is to treat predictions as though they were medical diagnoses, and then to calculate the rate that the diagnosis is incorrect. For instance, a false negative is actually a positive; the "false negative rate" (FNR, as defined by Fielding and Bell) is the proportion of all positives that are false negatives: FNR=FN/(FN+TP). From a medical point of view, the FNR is the rate at which people with a disease are incorrectly diagnosed as not having the disease. Similarly, the false positive rate (FPR) is the rate at which healthy people are incorrectly diagnosed as having a disease. In the world of sulfate water quality standards, the false positive rate is the rate at which the ambient sulfate concentrations in waterbodies exceed the standard, but porewater sulfide is actually below the protective concentration of 120 µg/L.

The State of Vermont identified phosphorus standards to protect against eutrophication by finding the phosphorus concentration where FPR and FNR are equal, so that the standard was equally likely to be over-protective and under-protective (Smeltzer et al., 2016). It is possible to perform this type of analysis for fixed sulfate standards (Fig. 1-14b), but not for equation-based standards, where the error rates are not functions of potential fixed numeric sulfate standards. (Although it is possible to calculate the misclassification rate for the identified protective sulfide concentration of 120 µg/L.)

For potential fixed sulfate standards, the two accuracy metrics (misclassification rate and error rate) produce similarly shaped curves characterizing false positives and false negatives (Fig. 1-14 a,b). In addition, the curves cross at similar sulfate concentrations (10 mg/L and 7 mg/L, respectively), the concentrations where over-protection and under-protection would be balanced.

Early in this section (F) of the TSD it is noted that the overall misclassification rate for the best fixed sulfate standard (32%) is much greater than the misclassification rate for the proposed equation (16% for the dataset it was developed on, and 19% for an independent dataset).

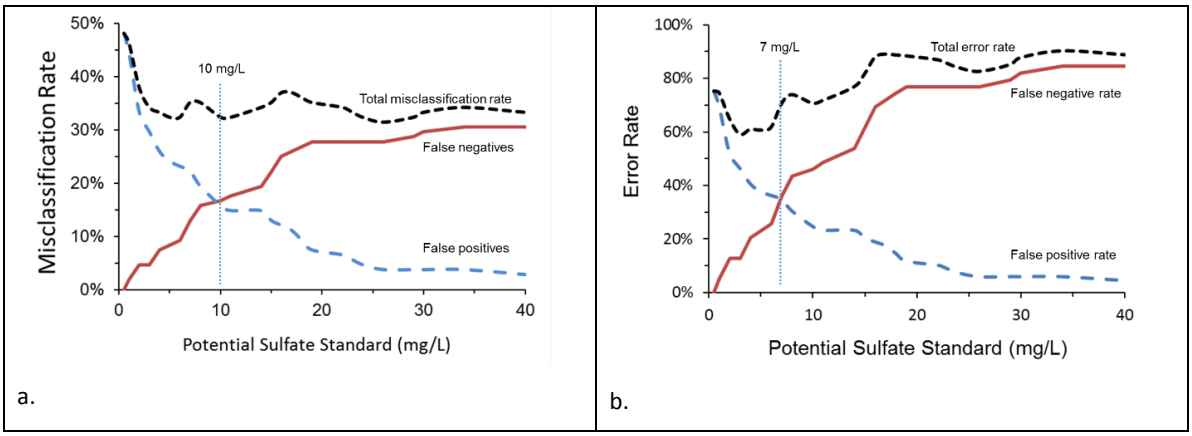


Figure 1-14. Two different ways (as described by Fielding and Bell, 1997) of interpreting the false negatives and false positives associated with a spectrum of potential sulfate criteria (potential standards) and their classification accuracy associated with a protective sulfide concentration of 120 µg/L: a) The total misclassification rate, showing that false negatives and false positives percentages are equal at 10 mg/L, summing to 32%. The total also sums to 32% at 5 mg/L and 26 mg/L, but the former is dominated by false positives (24%) and the latter is dominated by false negatives (28%). b) The total error rate, showing that the false negative rate and false positive rate are equal at 7 mg/L.

Table 1-10. Calculated false classification percentages for potential protective sulfide concentrations from 50 to 400 µg/L (Class B dataset, which approximates a probabilistic sample of waterbodies that could potentially host wild rice; N=108).

Potential Protective Sulfide (µg/L)	True positives	False positives	False negatives	True negatives	False Predictions		
					Percent false positive	Percent false negative	Total percent misclassified
50	89	17	1	1	15.7%	0.9%	16.7%
60	85	19	1	3	17.6%	0.9%	18.5%
70	68	16	8	16	14.8%	7.4%	22.2%
80	51	19	15	23	17.6%	13.9%	31.5%
90	47	14	14	33	13.0%	13.0%	25.9%
100	40	10	14	44	9.3%	13.0%	22.2%
110	35	12	14	47	11.1%	13.0%	24.1%
120	29	7	10	62	6.5%	9.3%	15.7%
130	26	7	10	65	6.5%	9.3%	15.7%
140	22	9	11	66	8.3%	10.2%	18.5%
150	17	7	12	72	6.5%	11.1%	17.6%
160	17	8	11	72	7.4%	10.2%	17.6%
170	17	8	11	72	7.4%	10.2%	17.6%
180	17	7	10	74	6.5%	9.3%	15.7%
190	16	7	9	76	6.5%	8.3%	14.8%
200	16	7	8	77	6.5%	7.4%	13.9%
210	14	7	9	78	6.5%	8.3%	14.8%
220	14	6	7	81	5.6%	6.5%	12.0%
230	12	6	8	82	5.6%	7.4%	13.0%
240	11	5	8	84	4.6%	7.4%	12.0%
250	11	5	8	84	4.6%	7.4%	12.0%
260	11	5	7	85	4.6%	6.5%	11.1%
270	11	5	7	85	4.6%	6.5%	11.1%
280	8	4	9	87	3.7%	8.3%	12.0%
290	8	4	9	87	3.7%	8.3%	12.0%
300	8	4	9	87	3.7%	8.3%	12.0%
310	9	3	7	89	2.8%	6.5%	9.3%
320	9	3	7	89	2.8%	6.5%	9.3%
330	9	3	7	89	2.8%	6.5%	9.3%
340	9	3	7	89	2.8%	6.5%	9.3%
350	9	3	7	89	2.8%	6.5%	9.3%
360	9	1	6	92	0.9%	5.6%	6.5%
370	9	0	5	94	0.0%	4.6%	4.6%
380	9	0	5	94	0.0%	4.6%	4.6%
390	9	0	5	94	0.0%	4.6%	4.6%
400	9	0	5	94	0.0%	4.6%	4.6%

Table 1-11. Calculated false classification percentages and diagnosis error rates for potential sulfate standard concentrations from 0.5 to 40 mg/L (Class B dataset; N=108).

					False predictions			Diagnosis Error Rates	
Potential Sulfate Standard (mg/L)	True positives	False positives	False negatives	True negatives	Percent false positive	Percent false negative	Misclass-ification rate	False Positive Rate	False Negative Rate
0.5	39	52	0	17	48%	0%	48.1%	75%	0%
1	37	48	2	21	44%	2%	46.3%	70%	5%
2	34	36	5	33	33%	5%	38.0%	52%	13%
3	34	32	5	37	30%	5%	34.3%	46%	13%
4	31	28	8	41	26%	7%	33.3%	41%	21%
5	30	26	9	43	24%	8%	32.4%	38%	23%
6	29	25	10	44	23%	9%	32.4%	36%	26%
7	25	24	14	45	22%	13%	35.2%	35%	36%
8	22	21	17	48	19%	16%	35.2%	30%	44%
10	21	17	18	52	16%	17%	32.4%	25%	46%
11	20	16	19	53	15%	18%	32.4%	23%	49%
14	18	16	21	53	15%	19%	34.3%	23%	54%
15	15	14	24	55	13%	22%	35.2%	20%	62%
16	12	13	27	56	12%	25%	37.0%	19%	69%
17	11	12	28	57	11%	26%	37.0%	17%	72%
18	10	10	29	59	9%	27%	36.1%	14%	74%
19	9	8	30	61	7%	28%	35.2%	12%	77%
22	9	7	30	62	6%	28%	34.3%	10%	77%
23	9	6	30	63	6%	28%	33.3%	9%	77%
24	9	5	30	64	5%	28%	32.4%	7%	77%
26	9	4	30	65	4%	28%	31.5%	6%	77%
29	8	4	31	65	4%	29%	32.4%	6%	79%
30	7	4	32	65	4%	30%	33.3%	6%	82%
34	6	4	33	65	4%	31%	34.3%	6%	85%
40	6	3	33	66	3%	31%	33.3%	4%	85%

False negative sites under the current 10 mg/L standard (but correctly classified by the MBLR-based equation)

Some sites have sedimentary concentrations of iron and organic carbon that allow relatively efficient conversion of sulfate to porewater sulfide. If surface water carried sulfate into the sediment, 100% conversion of only 0.4 mg/L would exceed a sulfide concentration of 120 µg/L, an indication of why regulation of sulfate concentrations less than 10 mg/L would be appropriate when iron availability is low. Highly efficient conversion to sulfide was observed in some sites in the MPCA field survey data set. Among the sites in the MPCA field survey, there are nine sites where a fixed standard of 10 mg/L would not be protective, but the MBLR equation would be protective (Table 1-12). The ambient sulfate concentrations at these sites range from 1.3 to 7.8 mg/L, and observed porewater sulfide concentrations range from 145 to 2,525 µg/L, above the protective concentration of 120 µg/L sulfide. At these sites, wild rice density is generally low (zero to 10.4 stems/m^2) with an exception of 69.7 stems/m^2 observed in the single visit to Bowstring River.

Table 1-12. Examples of false negatives relative to a fixed numeric standard of 10 mg/L: sites with sulfate less than 10 mg/L, but with greater than the calculated protective sulfate concentration, as calculated by the multiple binary logistic regression (MBLR120). As predicted by the MBLR equation, sulfide concentrations are greater than 120 µg/L, the protective sulfide concentration. (Values are average when the site was sampled more than once.)

Site	State ID	Ambient sulfate (mg/L)	MBLR120 (mg/L)	Wild Rice density stems/m^2	Porewater Sulfide (µg/L)	Trans-parency (cm)	Number of Field Samples
Anka Lake	21-0353-00-201	4.3	0.7	10.4	565	89	3
Big Sucker Lake	31-0124-00-203	7.8	2.1	3.8	145	101	1
Bowstring River	S007-219	1.3	0.6	69.7	256	101	1
Gilchrist Lake	86-0064-00-201	7.0	1.7	0.0	355	101	1
Rice Lake	02-0008-00-206	3.6	2.6	0.0	145	31	1
Rice Lake	66-0048-00-203	5.2	2.4	0.0	777	20	1
Rice Lake	73-0196-00-216	4.7	0.9	0.0	2,525	101	2
Rice Lake	74-0001-00-201	3.8	3.2	0.0	217	3	1
Westport Lake	61-0029-00-204	6.7	3.1	3.3	998	86	2

It is useful to examine the implications of the data from the Bowstring River in some detail, because the calculated protective sulfate concentration was extremely low. The Bowstring River was sampled at only one location during the field survey, so it was uncertain how representative the single analyses of TOC and TEFe were. The following discussion should therefore not be taken as a thorough description of the waterbody, but rather as an opportunity to discuss the effect of sulfide on the probability of wild rice occurrence in a waterbody. This site on the Bowstring River was later sampled in detail as part of an implementation pilot project, described in Chapter 3, *Implementation of the Wild Rice Standard.*

Based on the single sample from the field survey, Bowstring River would have an extremely low calculated sulfate standard, 0.6 mg/L sulfate, based on the MBLR equation (TOC is high, and TEFe is low). The ambient sulfate concentration was just 1.3 mg/L (1.0 mg/L when sampled in the implementation pilot project 6/23/2015), but produced a porewater sulfide concentration of 256 µg/L, appreciably higher than the protection goal of 120 µg/L sulfide. Despite exceeding the protective porewater sulfide level of 120 µg/L, Bowstring River supported a dense population of wild rice, which is a reminder that 120 µg/L is not a stark threshold below which wild rice can exist and above which wild rice dies. Rather, above 120 µg/L the *probability* of observing wild rice declines progressively as sulfide concentrations increase. According to the logistic regression, the probability of observing wild rice is 69% at 120 µg/L, and declines to a probability of 59% at 256 µg/L (Fig. 1-4). The probability curve suggests that the wild rice population on the Bowstring River is at risk if even slight increases in sulfate occur above the measured ambient concentration of 1.3 mg/L, given that the MBLR-calculated protective sulfate concentration was 0.7 mg/L. If ambient sulfate were allowed to approach 10 mg/L, it is likely that porewater sulfide would become much higher and that the wild rice population would decline in this waterbody. If sulfide reached 500 µg/L, the probability of wild rice occurrence would decline to 50%. As the ambient sulfate concentration increases above the calculated protective concentration for that waterbody, the likelihood of elevated porewater sulfide increases dramatically (Fig. 1-15 a).

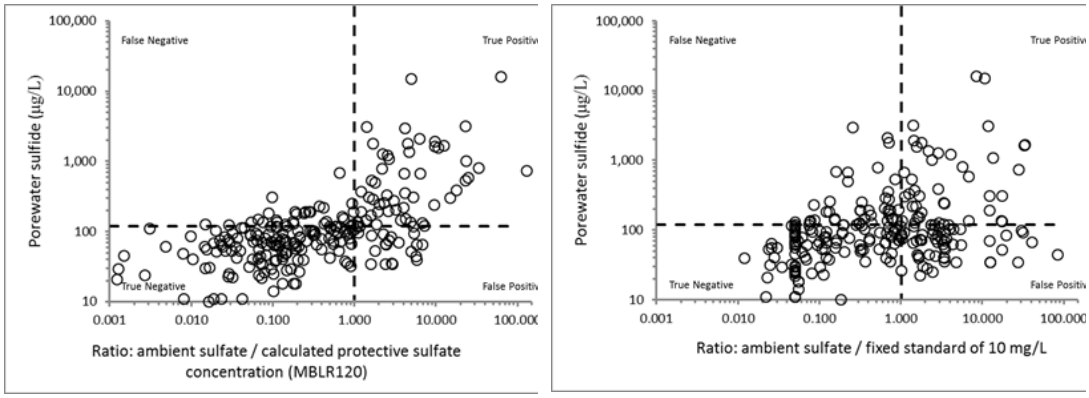


Figure 1-15. Observed accuracy observed when the MPCA survey data are assessed against (A) standards calculated with the proposed equation, and (B) a fixed sulfate standard of 10 mg/L. Sites to the right of the vertical dashed line exceed the potential sulfate standard, the goal of which is to keep porewater sulfide below the protective concentration of 120 µg/L. Sites above the horizontal dashed line actually had porewater sulfide greater than 120 µg/L. The sites in the upper right quadrants (above and to the right of the dashed lines) are correctly classified as exceeding the standard (true positives), whereas the sites in the lower right quadrant are false positives. Sites in the upper left quadrant are false negatives—sites where the ambient sulfate concentration was falsely identified as protective of wild rice, but the porewater sulfide was greater than 120 µg/L. (All survey data are graphed, so that some waterbodies are represented by multiple points; N=222.)

Note that four of the nine sensitive sites noted above that are negatively affected by ambient concentrations of sulfate below 10 mg/L are called “Rice Lake” (Table 1-12), which some assume means that the early settlers had observed obvious wild rice populations. No wild rice plants were observed in these lakes during the field survey. These sites are not notably enriched with sulfate – ambient sulfate ranges from 3.6 to 5.2 mg/L – yet porewater sulfide is elevated above the protective sulfide concentration of 120 µg/L.

If wild rice used to grow in these four lakes, porewater sulfide may have been below the protective sulfide concentration, which implies that these lakes experienced an increase in sulfate or organic matter, or, less likely, the loading rate of iron has decreased. These four lakes lie in areas where there is significant anthropogenic land use, including agriculture. Agricultural activity could increase sulfate loading, either through the use of groundwater for irrigation (since groundwater often has higher sulfate concentrations than surface water) or by the application of sulfur-containing fertilizers. Tile drainage in a watershed may also increase the export of sulfate to receiving waters, because of increased aerobic oxidation of sulfide minerals in the soil (Lamers et al., 2013).

In addition, anthropogenic activity almost always increases loading of phosphorus to surface waters, which likely would decrease transparency. As discussed in section A of this TSD, reduced transparency reduces the probability of wild occurrence independent of the porewater sulfide concentration in that waterbody. Wild rice can be excluded from a site if the water transparency is less than 30 cm (Part B of Chapter 1, above). Three of the four Rice Lakes had low observed transparency (3, 20, and 31 cm), in addition to exceeding the protective sulfide concentration.

False positive sites under the current 10 mg/L standard (but correctly classified by the MBLR-based equation)

Some sites have sediment concentrations of iron and organic carbon that do not result in relatively efficient conversion of sulfate to porewater sulfide. Among the wild rice sites in the MPCA field survey there are six sites where the observed sulfate is greater than 10 mg/L but sulfide concentrations are less than 120 µg/L—sites that under a 10 mg/L fixed sulfate standard would falsely be classified as areas where wild rice is not sufficiently protected. The MBLR equation correctly predicted that these sites would have sulfide below 120 µg/L (Table 1-13), thereby protecting wild rice.

At these six sites, average wild rice densities range from 31 to 141 stems/m^2. Ambient sulfate concentrations range from 18 to 32 mg/L, well below the calculated protective sulfate concentrations, which range from 93 to 546 mg/L. Observed sulfide concentrations range from 53 to 112 µg/L.

Table 1-13. Examples of false positives relative to a numeric sulfate standard of 10 mg/L: sites with sulfate greater than 10 mg/L, but with sulfate concentrations lower than the calculated protective sulfate concentration. As predicted by the MBLR equation, sulfide concentrations do not exceed 120 µg/L, the protective sulfide concentration. (Values are average when the site was sampled more than once.)

Site	State ID	Ambient sulfate (mg/L)	MBLR120 (mg/L)	Wild Rice density stems/m^2	Porewater Sulfide (µg/L	Trans-parency (cm	Number of Field Samples
Hay Lake	31-0037-00-202	26.9	218	141	59	86	2
Mississippi Pool 4 / Robinson Lake	79-0005-02-201	29.6	262.6	46.5	67	90	3
Mississippi Pool 5 / Spring Lake	S007-660	32.5	546.1	39	53	88	5
Mississippi Pool 8 at Genoa	S007-222	31.2	93	31.2	112	86	4
Mississippi Pool 8 at Reno Bottoms	S007-556	18.1	187.6	72.3	71	57	1
Partridge River	S007-443	24.1	302	42.5	80	79	6

Practical implications of false positive and false negative prediction errors

If the ambient sulfate concentration is greater than the standard, the state of Minnesota is required to take action, pursuing either a) completion of a Total Maximum Daily Load (TMDL) study to determine how to reduce sulfate concentrations below the standard, or b) an EPA-approved site-specific standard for the site. The latter would be pursued if there is evidence that the exceedance of the sulfate standard is a *false positive error*. The false positive rate is greater for fixed standards, so a fixed standard would require the pursuit of more TMDLs or site-specific standards when, in fact, neither are needed to protect wild rice.

Because any fixed standard would be less accurate than an equation-based standard, under a fixed standard there would be more cases in which a wild rice water has a rice population judged to be healthy, but where the ambient sulfate concentration exceeds the standard. In such situations, it would be necessary to develop a site-specific standard to protect the beneficial use, which involves significant staff time and resources. This effort would occur less often under the implementation of an equation-based sulfate standard.

When a false positive is calculated for a sulfate discharge, the determination may result in unneeded investment in water treatment—which is why the false positive rate should be minimized.

False negative errors—where it is thought that sulfide will not accumulate to levels that harm the wild rice population when, in fact, it does—will not be recognized for a number of years, because it takes multiple years for sulfide to accumulate in the sediment and harm the rice (Pastor et al., 2017). False negative errors, therefore, potentially cause environmental harm—harm that may be difficult to

reverse, because there is little experience for intentionally oxidizing and detoxifying sulfide once it accumulates in sediment (Ning et al., 2011).

The prediction error rate, the need for site-specific standards, and the occurrence of harm due to false-negative classification can all be reduced by adopting the MBLR equation as the sulfate standard to protect wild rice.

The Mississippi River below the Twin Cities is a good example of the ramifications of retaining a fixed sulfate standard. The MPCA field survey sampled four pools of the Mississippi River below the Minneapolis-Saint Paul Metropolitan Area that have had large populations of wild rice for many years. Ambient sulfate concentrations (18 to 32 mg/L) were well above 10 mg/L. The observed sulfate concentrations are well below the protective sulfate values calculated from the MBLR equation (93 to 546 mg/L). If the wild rice sections of the Mississippi River listed in Table 1-13 were evaluated against a fixed sulfate standard of anything less than 18 mg/L, either a site-specific standard would need to be developed or, under section 303(d) of the federal Clean Water Act, Minnesota would be required to develop a TMDL plan to reduce sulfate concentrations to below the standard. A TMDL plan would entail the calculation of the maximum amount of sulfate that could be discharged to the Mississippi and its tributaries, which would lead to the allocation of the necessary reductions to achieve compliance with the sulfate standard. Development and implementation of such a TMDL would be costly in terms of staffing resources and the potential for additional treatment requirements, and would not appreciably benefit the wild rice populations in these pools of the Mississippi since porewater sulfide concentrations are already less than the protective sulfide concentration of 120 µg/L. However, if the wild rice sulfate water quality standard were based on calculated values generated using the MBLR model, then a TMDL would not have to be developed, Minnesota would not have to determine how the sulfate concentration in the Mississippi could be reduced by as much as 70%, and wild rice would remain sufficiently protected from sulfate impacts.

Comparison of error rates to Vermont's phosphorus standards

The state of Vermont recently adopted, and EPA approved, fixed phosphorus standards to protect aesthetic use in lakes and aquatic biology in streams. Numeric standards were derived in a way to minimize false positive and false negative rates (Vermont DEC, 2014; Smeltzer et al., 2016). Eleven different phosphorus standards were developed, depending on the applicable tiered water use objective. The misclassification rates varied from 17 to 40% (Table 1-14), with a median of 35% - about the same as the best misclassification rate for possible fixed sulfate standards for wild rice (32%).

Table 1-14. Phosphorus standards developed by the state of Vermont (Vermont DEC, 2014) and associated misclassification rates.

Use Objective	Tier*	Phosphorus Criterion (µg/L)	Misclassification rate
Lake Aesthetics	Excellent aesthetic value	17	24%
	Good aesthetic value	18	17%
Aquatic Life	1	10	39%
(Small, high-gradient streams)	2	10	35%
	3	12	36%
Aquatic Life	1	9	40%
(Medium high-gradient streams)	2	9	39%
	3	15	22%
Aquatic Life	1	18	32%
(Warm-water, moderate gradient	2	21	31%
streams)	3	27	39%

*Tier Level of Aquatic Life Use Support

Analysis of Suggested Alternatives to the Protective Sulfide Level and Equation

During the process of developing the proposed revisions, the MPCA received a great deal of comment and advice from stakeholders and interested parties, many of which contained suggested alternate proposals for the sulfate standard. The MPCA considered a number of possible alternatives, including possible fixed standards and that a higher protective sulfide level (and related changes to the equation) might be appropriate.

With the release of the Draft TSD in July 2016 and in discussions of subsequent analyses, primarily with the Wild Rice Advisory Committee, MPCA staff frequently discussed the error rates of the equation. Commenters suggested that there were alternatives to the MPCA's identified protective sulfide level and equation that would result in a lower error rate (4% compared to 16%) but a similar level of wild rice protection.

Accordingly, MPCA staff have carefully reviewed the suggested changes in the derivation of an equation to calculate protective levels of sulfate. Ramboll (2017) suggested developing an equation with two changes to the work undertaken by the MPCA. The first change is adjusting the dataset used to perform the multiple binary logistic regression (MBLR), using only the 67 waterbodies where wild rice was observed. The second change is to use a protective porewater sulfide concentration of 300 µg/L, and developing the equation to relate sulfide to sulfate from there. Both of these changes would affect the resulting equation to calculate a numeric sulfate standard for a wild rice water.

Dataset used to perform the MBLR

Ramboll (2017) asserted that the chemical relationship between sulfate (in surface water) and sulfide (in sediment porewater) should be developed using regression analysis on a dataset that includes only waterbodies that have wild rice, because such a dataset "is most relevant to the receptor of concern

which is a longstanding approach used by EPA in determining criteria." In support of this position, Ramboll (2017) asserted:

> This is similar to the longstanding EPA policy and practice of data use in development of criteria (USEPA 1994; Stephan et al., 1985; USEPA 2010). For example, when developing aquatic life criteria, EPA uses toxicity data from freshwater species to derive freshwater criteria and saltwater species are used to derive saltwater criteria. Likewise, criteria for warmwater fisheries are derived without toxicity data for coldwater species. [p. 3-1]

and

> In keeping with EPA policy and practice, only sites on the proposed wild rice list and with wild rice present should be included. [p. 4-1]

MPCA staff inspected the EPA documents cited by Ramboll (2017) and found no guidance directly pertinent to the question of what datasets should be analyzed when establishing a protective sulfide level and translating a protective sulfide level to a numeric standard to protect wild rice. The primary EPA guidance for the development of a water quality standard is that a state needs to demonstrate that its development procedure is fully protective of designated uses—and that EPA will review proposed standards by looking for a sound scientific rationale (EPA, 1994. p. 3-21, Water Quality Standards Handbook).

As described throughout this TSD, MPCA staff used the field survey data for two purposes: (1) to identify a porewater sulfide concentration that would be protective of wild rice growth and reproduction, and (2) to develop an equation that calculates a protective sulfate concentration that corresponds to the protective sulfide concentration identified in (1). Different subsets of the field data were used in support of each of the two purposes:

(1) Identification of a protective sulfide concentration: As MPCA staff reviewed the multiple lines of evidence for the identification a sulfide concentration to protect wild rice, different subsets of the field dataset were used, depending on the question being asked. For instance, when asking how porewater sulfide affects the probability that wild rice (of any density) will be observed in a waterbody, it was necessary to include waterbodies where no wild rice was observed. When addressing the probability of wild rice being present, two different datasets were analyzed, all 108 waterbodies (yielding an EC10 of 58 μg/L), and a subset that consisted only of sites with sufficient transparency to support wild rice (yielding an EC10 of 91 μg/L). MPCA staff regard the latter estimate as more defensible, since elevated sulfide is not responsible for the lack of wild rice when transparency is inadequate to support wild rice. In contrast, when the question was how sulfide affects the density of wild rice, only waterbodies with wild rice were included in the change-point analysis, which revealed a statistically significant decline in wild rice density at 112 μg/L. (Including waterbodies with no wild rice in this analysis would not have clearly addressed the question of how sulfide affects wild rice density.)

(2) Development of a protective sulfate concentration: MPCA staff developed a mathematical relationship that characterizes the chemical relationship between sulfate and the protective level of sulfide (120 μg/L) by including all 108 waterbodies. MPCA used all available data because the goal was to statistically describe a chemical relationship in the environment, not the effect of sulfide on wild rice.

Using only the 67 wild rice waterbodies has the effect of calculating higher sulfate levels than if all 108 waterbodies are used. This is perhaps because excluding waterbodies without wild rice also excludes many waterbodies with high sulfide, skewing the data used to calibrate the equation.

One way to see the effect of calibrating the equation with different waterbody datasets (and protective sulfide concentrations) is to compare the median potential sulfate standard that each equation produces when applied to the 67 waterbodies within the Class B dataset that had wild rice. The MPCA proposal (protective sulfide of 120 μg/L, equation developed with all Class B data) yields a median

sulfate concentration 14 mg/L (Table 1-15). When an equation is developed only with data from waterbodies with wild rice, the median potential sulfate standard increases to 61 mg/L, which would allow much more porewater sulfide to develop. The effect is magnified if an equation is developed with a protective sulfide concentration of 300 μg/L. If developed with all 108 sites, the median potential sulfate standard would be 20 mg/L. But, if the equation is developed with only with data from sites with wild rice, the median potential sulfate standard would be 209 mg/L (Table 1-15). Calculated sulfate standards are clearly influenced by the dataset used to develop the equation.

MPCA staff concludes that it is most defensible to perform the regression that relates sulfate and sulfide with the entire 108-waterbody data set, rather than the data set that only includes sites with wild rice. First, the point of the regression is to develop a mathematical description of the chemical relationship between the three variables that have been demonstrated to control porewater sulfide: sulfate, TOC, and sediment iron (Pollman et al., in press). Second, since it has been shown that elevated sulfide is one of the primary controllers of wild rice presence in a waterbody (Myrbo et al., in press-1), it is evident that excluding waterbodies without wild rice would likely also exclude waterbodies that have high sulfide, which could skew the results of the regression. In fact, excluding sites without wild rice excludes 77% of the highest sulfide concentrations (10 of the 13 waterbodies with the highest sulfide). It is essential to the goal of the analysis to perform a robust regression that accurately predicts elevated sulfide, and not including data with elevated sulfide is counterproductive to that goal.

Table 1-15. Effect on median protective sulfate concentrations (for waters with wild rice) of developing equations with different datasets, and different protective sulfide concentrations.

	Median calculated protective sulfate concentration in waters with wild rice (N=67)	
Protective sulfide concentration used to develop equation	Equation developed with all Class B Waterbodies (N=108)	Equation developed with only Class B Waterbodies with wild rice (N=67)
120 μg/L	14 mg/L*	61 mg/L
300 μg/L	20 mg/L	209 mg/L**

*MPCA proposal **Ramboll (2017) proposal

Protective level of sulfide

Ramboll (2017) asserted that a protective sulfide concentration of 300 μg/L provides a similar level of protection for the wild rice beneficial use as 120 μg/L. The evidence given for this assertion is that there is no statistical difference in average wild rice stem densities below the 120 μg/L and below 300 μg/L (55 and 52 stems/m^2, respectively, p. 3-3) (MPCA's calculations are slightly different, 57 and 53 stems/m^2, a difference that is not important in this discussion).

The appropriate way to determine if 300 μg/L provides a similar level of protection as 120 μg/L is different than the test performed by Ramboll (2017). Rather than compare overlapping ranges of porewater sulfide, which violates the fundamental statistical principle that requires independence between two compared groups, it is more appropriate to compare the under 120 μg/L group to the 120-300 μg/L group. If 300 μg/L is similarly protective, there would not be a significant difference between these two groups.

As shown in Table 1-16, the average and median stem densities of the less-than-120 µg/L group are greater than the 120-300 µg/L group (average density of 57 vs 38, and median density of 47 vs 21 stems/m^2). A nonparametric statistical test (Wilcoxon-Mann-Whitney) finds that the two groups are significantly different at the p= 0.06 level. A more in-depth analysis shows that waterbodies with porewater sulfide less than 120 µg/L are more likely to have dense (> 40 stems/m^2) wild rice than stands with sulfide between 120 µg/L and 300 µg/L (Appendix 9). Wild rice waters with sulfide less than 120 µg/L are 5.6 times as likely as sites with sulfide between 120 and 300 µg/L to have dense wild rice (> 40 stems/m^2) than sparse wild rice (< 10 stems/m^2) ($p<0.5$). This is consistent with the change-point analysis, which found that wild rice density is significantly lower at sulfide concentrations greater than 112 µg/L (Appendix 7).

Table 1-16. Comparison of wild rice occurrence and density between two groups: waterbodies with porewater less than 120 µg/L and waterbodies with sulfide between 120 µg/L 300 µg/L.

Group	Number if waterbodies	Number of waterbodies with wild rice	Proportion with wild rice	Average density of wild rice (stems/m^2)	Median density of wild rice (stems/m^2)
less than 120 µg/L	69	49	71%	57	47
120 to 300 µg/L	22	13	59%	38	21

Therefore, wild rice density is significantly less robust between 120 and 300 µg/L than below 120 µg/L. Because density of wild rice in a waterbody is likely related to persistence of the population and to maintaining the beneficial use of wild rice, MPCA concludes that a porewater sulfide concentration of 300 µg/L is not protective of the wild rice beneficial use.

The MPCA did not make any changes to the equation based on the information provided. The proposal does have a lower error rate than the MPCA-proposed approach; however, it is important to remember that while it is desirable to minimize error rates as much as possible, doing so is a secondary consideration. The primary goal and requirement of the standard is to protect the wild rice beneficial use from the impacts of elevated sulfide. The MPCA's review of the proposal shows that the changes would result in a standard that is not sufficiently protective.

Chapter 2. Evidence that a different standard is needed for some wild rice waters

The basis of the proposed equation to calculate a numeric sulfate standard for wild rice waters is founded upon interrelationships of sulfate, organic carbon, and iron that produces sulfide, which is the toxic agent. With this understanding, there are two probability-based relationships involved in the determination of a sulfate concentration that will protect wild rice from elevated sulfide in the sediment: (1) the choice of a particular sulfide concentration that would be protective of wild rice, and (2) the translation of that sulfide concentration to the corresponding sulfate concentration for that particular waterbody (based on the iron and organic carbon in that waterbody's sediment). As a result, there will be false predictions associated with any sulfate standard, but there will be fewer false predictions when sulfate standards are equation-based, compared to the number of false predictions associated with a fixed standard (as described in part F of Chapter 1).

The first part of this Chapter 2 addresses wild rice waters with false positives and the need for an alternate sulfate standard when sulfide is predicted to be above 120 µg/L but is actually below 120 µg/L. The second part of this Chapter 2 addresses wild rice waters with true positives—sulfide is predicted to be above 120 µg/L and sulfide is actually above that level but the beneficial use is still protected.

Alternate numeric sulfate standard for false positive wild rice waters

A small proportion of wild rice waters in the MPCA-sponsored field study consistently exhibit a porewater sulfide concentration less than 120 µg/L when their ambient sulfate concentrations are greater than their calculated sulfate standards—they are false positives. Such waterbodies are not conforming to the conceptual model on which the equation-based sulfate standard is based, and therefore an appropriate sulfate standard must be determined through an alternative method. Application of an alternate standard could be based on empirical observations.

In the MPCA-sponsored surveys (2011-2013), the dataset that includes all samples in which false negatives and false positives can be identified consists of 222 samples from 115 different natural waterbodies (the dataset termed Class G). In this dataset, at least one false positive was observed in 14 different waterbodies (Fig. 2-1). Thirteen of the 14 waterbodies were sampled more than once, allowing an examination of the consistency of the sulfide predictions. False positives were consistently observed in four of the waterbodies. These four waterbodies consistently had porewater sulfide below 120 µg/L, despite predicted sulfide concentrations above that threshold (Table 2-1). Wild rice was growing in all four of the waterbodies. The most reasonable explanation for unexpectedly low porewater sulfide in these waterbodies is that surface water sulfate was not penetrating downward into the sediment because of upwelling groundwater.

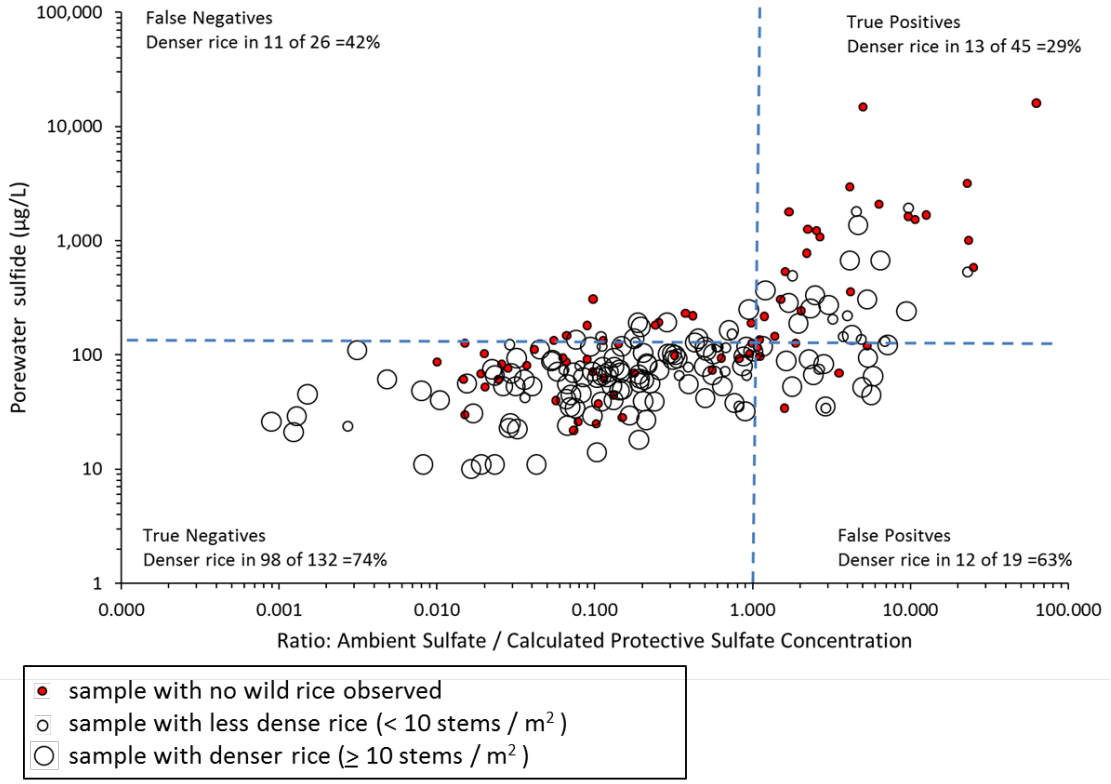


Figure 2-1. Prediction accuracy of the proposed equation for 222 site visits to 115 different waterbodies (data set Class G), plus density of wild rice occurrence, at two levels of density. Of the 222 samples, 19 false positives were found from 14 different waterbodies, of which 12 had wild rice. In 46 site visits to these 14 different waterbodies there were 19 false positives, 10 true negatives, 5 false negatives, and 12 true positives Table 2-1).

False positives (where elevated sulfate does not elevate sulfide) are a concern because they potentially could cause unneeded investment in sulfate control. False positives may merely be the result of random error, especially when ambient sulfate is close to the calculated numeric sulfate standard. Random error is likely the cause of a false positive observed in Second Lake. Second Lake was sampled once in 2011 and once in 2012, and wild rice density was at about the median density of the Class B sites (41 stems per square meter) in both years (37 and 48 stems/m^2, respectively). In 2012, a false positive was observed when the ambient sulfate concentration in Second Lake was 0.7 mg/L, which was slightly greater than the calculated protective level of 0.6 mg/L, and the measured porewater sulfide, 119 µg/L, was slightly lower than the protective threshold of 120 µg/L (Table 2-1). In 2011, a false negative determination for Second Lake was perhaps an example of random error in the other direction. The ambient sulfate concentration was 0.9 mg/L, lower than the calculated protective level of 1.9 mg/L, and the measured porewater sulfide of 139 µg/L was greater than the protective threshold of 120 µg/L (Table 2-1). Four of the ten sites likely exhibit false positives as a result of random error associated with ambient sulfate levels similar to the calculated protective sulfate concentration (Table 2-1).

More importantly, false positives may also be the result of the failure of a waterbody to conform to the conceptual model upon which the equation is based. As described in Chapter 1 of this TSD, the

conceptual model is supported by significant statistical relationships (Pollman et al., in press), so that most of the waterbodies in the field survey act as described by the model. The model is based on the assumption that porewater sulfide is produced by bacteria in the sediment that are utilizing sulfate transported from the surface water downwards into the sediment. However, there may be wild rice waters where groundwater actively moves upward through the sediment, in which case sulfate in surface water would not play a major role in the production of sulfide. In such cases, ambient sulfate in surface water in comparison to the calculated sulfate standard can produce false positives, depending on the sediment concentrations of organic carbon and extractable iron. Wild rice waters with upwelling groundwater might be most often encountered in gaining streams, which receive water from groundwater, and some lakes that receive groundwater. The interaction of groundwater and surface waters is complicated, and is a function of multiple variables such as the texture and depth of soils, topography, and even seasonal growth of plants that transpire large amounts of groundwater, such as willows (Fetter, 2001).

In a study of 46 Wisconsin lakes, Nichols and Shaw (2002) found that wild rice is statistically associated with shallow areas where groundwater was emerging into the lake. The researchers were not sure why there would be such an association, but it is possible that they happened to sample waterbodies where upwelling groundwater allowed porewater sulfide concentrations to be low enough for wild rice to grow. Nichols and Shaw did not discuss the issue of sulfate and sulfide.

Of the 14 waterbodies in the MPCA study with at least one false positive, upwelling groundwater at four waterbodies seems the likely explanation for unexpectedly low porewater sulfide concentrations (Table 2-1). Three of the four waterbodies are small streams that could be receiving base groundwater flows that keep sulfate in the surface water from moving into the sediment.

For instance, Second Creek (not related to Second Lake, discussed above), was sampled five times and porewater sulfide was less than 120 µg/L in each case despite relatively high sulfate concentrations (303 to 838 mg/L; sulfate was not measured for one of the samplings). Only two of the samples were false positives, because calculated protective concentrations are also relatively high (148 to 947 mg/L) as a result of low sediment TOC and high extractable iron (Table 2-1). Because of interest in this site that combined high sulfate, low sulfide, and robust wild rice density, in 2015 researchers from the University of Minnesota conducted an investigation that measured and modeled groundwater and geochemistry at the site (Yourd, 2017). Yourd found that the model of the geochemical relationships corroborated the findings of Pollman et al. (in press) that sulfide accumulation in porewater depends on the levels of iron and organic carbon—but that hydrologic flux can also play a significant role in the geochemistry of porewater. Yourd concluded that porewater sulfide concentrations in an iron-rich environment like Second Creek may only become elevated when high concentrations of sulfate are able to move into the sediment. Yourd only observed sulfate movement into the sediment when stream water levels were unusually high—which occurred because of a temporary obstruction directly downstream of the study area.

Implementation of an alternate sulfate standard to protect wild rice

When porewater sulfide concentrations are consistently below the protective concentration of 120 µg/L, but ambient sulfate concentrations exceed the equation-based standard (false positives), it is apparent that ambient sulfate concentrations are not being efficiently converted into sulfide. The alternate sulfate standard proposal is based on the idea that when porewater sulfide concentrations are protective of wild rice, the ambient sulfate concentration must also be at a protective concentration.

However, even given these satisfactory conditions, it is not immediately obvious what the applicable sulfate standard should be for a wild rice water that consistently exhibits false positives. An obvious

candidate numerical sulfate standard would be the average ambient sulfate concentration that had been observed for a few years, since that concentration had not caused an exceedance of the protective sulfide concentration of 120 µg/L. But the problem with adopting the average ambient sulfate concentration as the standard is that it is likely, given hydrologic variability, that annual average sulfate concentrations will sometimes be higher in coming years (unless the few years of data were unusual and resulted in the highest possible sulfate concentrations).

Therefore, a reasonable alternate sulfate standard would be higher than the average sulfate concentration observed over just two years of monitoring. But how much higher? One approach is to relate the measured porewater sulfide concentration to 120 µg/L, and to calculate how much higher sulfate could be to maintain porewater sulfide at or below 120 µg/L. Such an approach would need to assume (1) that some surface water sulfate might make its way into the sediment and be converted to sulfide, and (2) that waterbodies have differing empirical efficiencies of converting sulfate to sulfide (the molar ratio of sulfide to sulfate, expressed as a percentage). In the MPCA-sponsored field survey, only 17 of the 115 different natural waterbodies had a sample with efficiency exceeding 50%. The median conversion efficiency of the natural waterbodies was 7.7%. The sulfate-addition experiment of Pastor et al. (2017) offers an opportunity to calculate the efficiency of conversion with different sulfate concentrations interacting with a given sediment (and consistent concentrations of TOC and extractable iron). As the sulfate concentrations increased, the efficiency of conversion declined significantly from a maximum of 4% at the lowest sulfate concentration to a maximum of about 2% (Fig. 2-2).

Therefore, it is likely that the maximum increase in porewater sulfide concentrations as a result of increased sulfate would be proportional to the increase in sulfate. The experimental sulfate additions of Pastor et al. (2017), showing a declining efficiency, suggest that the sulfide increase would be less than proportional. With this understanding, a conservative alternate standard would be an increase in the observed ambient sulfate that is proportional to the degree that 120 µg/L is greater than the observed maximum porewater sulfide concentration. For instance, if the observed porewater sulfide were 80 µg/L and observed ambient sulfate were 110 mg/L, a conservative sulfate standard would be 165 mg/L sulfate (120/80 * 110 mg/L).

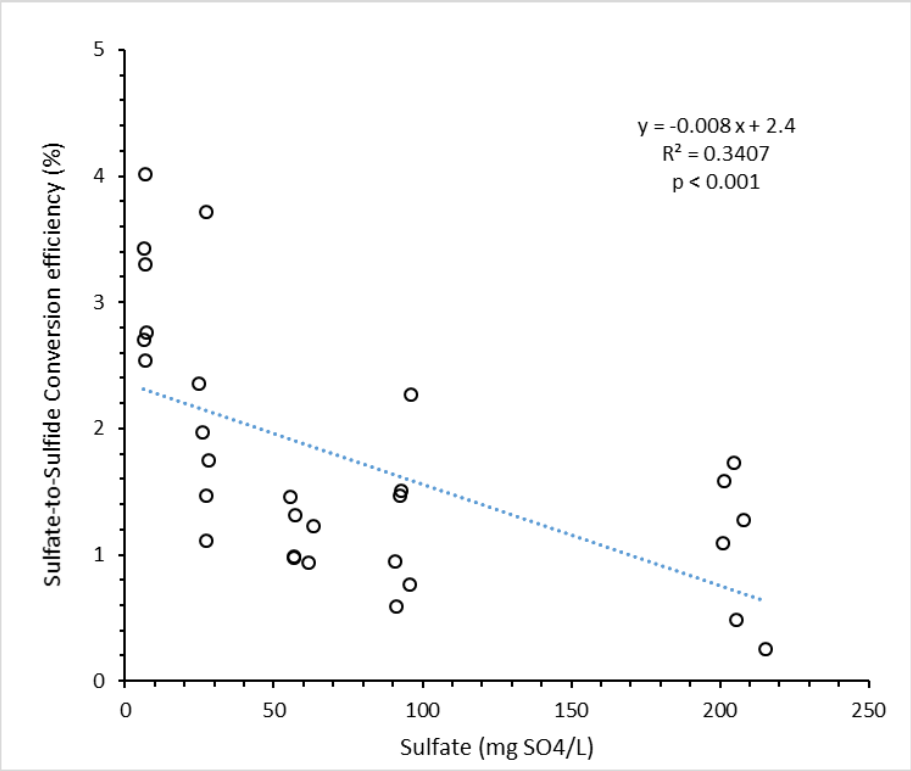


Figure 2-2. Trend in the efficiency of conversion of sulfate in surface water to sulfide in porewater (molar ratio of sulfide to sulfate, as a percentage), after three growing seasons of sulfate additions. The experiment is described in Pastor et al. (2017).

Summary: An alternate sulfate standard to protect wild rice

Implementation of an alternate sulfate standard in a wild rice water would be appropriate when a) ambient sulfate concentrations exceed the equation-based standard and b) porewater sulfide concentrations are demonstrably below the protective concentration of 120 µg/L. The most likely explanation for such observations is the upwelling of groundwater that is lower in sulfate than the surface water. However, sulfate in the surface water may contribute to the production of porewater sulfide if, for instance, groundwater reverses direction seasonally. It might be problematic to set the sulfate standard at the ambient concentration observed over just a few years of monitoring, since natural hydrologic fluctuation may produce an exceedance of the standard. A protective approach to calculating an alternate sulfate standard would be to adjust the observed ambient sulfate concentration by the factor that the protective sulfide concentration of 120 µg/L exceeds the observed ambient porewater sulfide concentration.

Table 2-1. All waterbodies in the field survey that exhibited at least one false positive (Class G dataset) Waterbodies are clustered into three categories in an effort to understand why false positives were produced: 1) Four waterbodies for which the likely explanation is that groundwater was upwelling through the sediment, so that the sites were not accurately modeled by the proposed equation; 2) Four waterbodies for which the likely explanation is random error because sulfate level is only slightly greater than the calculated protective concentration; and 3) Six waterbodies, each of which were sampled at least three times, that exhibited inconsistent behavior, which might be resolved with more extensive sampling. (CPSC120 = Calculated Protective Sulfate Concentration associated with a protective sulfide concentration of 120 µg/L).

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
Waterbodies for which the likely explanation for false positive(s) is upwelling groundwater. Even when sulfate is high relative to CPSC, porewater sulfide is low.												
↓	TN	0.78	0.8	N/A	Second Creek	FS-303	5/30/2013	303	99	13,086	2.20	388.6
↓	TN	0.33	0.8	57.6	S007-220	FS-310	6/14/2013	316	93	31,190	4.22	946.8
↓	FP	2.43	0.6	76.4		FS-323	7/11/2013	405	67	10,036	2.91	166.9
↓	FP	5.66	0.4	66.8		FS-351	8/15/2013	838	45	7,088	1.84	148.0
	N/A	N/A	0.9	27.7		FS-384	9/19/2013	N/A	104	22,634	3.42	657.3
↓	FP	2.64	0.6	0.6	Ox Hide Creek	FS-198	9/7/2012	26.4	75	8,743	24.51	10.0
↓	TN	0.50	0.4	10.5	31-0106-00-203	FS-132	9/7/2012	26.4	42	14,936	14.43	52.7
↓	**FP**	5.30	1.0	0.0		FS-350	8/14/2013	25.9	119	3,889	12.12	4.9
↓	**FP**	2.83	0.7	121.0	Turtle River, ND S007-662	FS-358	8/19/2013	198	83	4,262	1.52	70.0
↓	**FP**	1.78	0.4	56.3	Big Swan Lake	FS-205	8/10/2012	5.47	53	1,719	4.81	3.1
↓	**FP**	2.27	0.8	133.7	77-0023-00-207	FS-204	8/10/2012	5.49	91	1,731	5.94	2.4

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
Waterbodies for which the likely explanation for false positive(s) is random error because the sulfate level was only slightly greater than the calculated protective concentration.												
↑	FN	0.45	1.2	37.3	Second Lake	P-24	9/7/2011	0.87	139	3,813	25.67	1.92
↓	**FP**	**1.16**	1.0	48.4	15-0091-00	FS-105	6/27/2012	0.74	119	2,527	33.3	0.64
↓	TN	0.64	0.8	0.0	Snowball Lake	FS-197	9/4/2012	8.4	94	4,213	6.00	13.2
↓	**FP**	**1.11**	0.8	0.0	31-0108-00-202	FS-347	8/12/2013	8.2	97	1,136	1.19	7.4
↓	**FP**	**1.07**	1.0	0.0	Trout Lake	FS-219	9/13/2012	38.6	117	12,535	15.00	35.9
↓	TN	0.96	0.9	0.0	31-0216-00-212	FS-356	8/14/2013	39.1	103	11,992	12.59	40.7
↑	FN	0.19	1.5	41.4	Mississippi R.	FS-208	8/14/2012	18.0	176	2,178	0.41	92.3
↓	**FP**	**1.01**	0.9	12.7	Pool 8 at Genoa	FS-311	6/20/2013	29.3	107	1,544	0.62	29.0
↓	TN	0.56	0.9	52.8	S007-222	FS-334	7/29/2013	44.2	102	1,969	0.40	78.3
↓	TN	0.19	0.5	17.8		FS-370	9/9/2013	33.3	62	6,558	1.43	172.4

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
The behavior of the six waterbodies below is inconsistent												
↑	FN	0.03	1.03	3.8	Sandy Lake	FS-251	9/21/2012	3.1	123	35,905	33.08	105.5
↓	TN	0.09	0.8	0.0	69-0730-00	FS-306	6/11/2013	11.0	91.8	35,357	28.53	122.3
↑	FN	0.98	1.6	0.0		FS-321	7/9/2013	122	189	36,502	29.51	124.9
↑	TP	1.11	1.1	0.0		FS-382	9/17/2013	67.9	135	26,645	32.28	61.2
↑	TP	2.68	9.0	0.0		FS-305	6/11/2013	135	1080	19,094	22.23	50.4
↑	TP	1.51	2.5	0.0		FS-348	8/13/2013	123	305	13,216	8.23	81.6
↓	**FP**	2.91	0.3	0.6		FS-380	9/17/2013	126	34	17,868	22.7	43.3
↓	**FP**	3.53	0.6	0.0		FS-349	8/13/2013	122	70	14,897	20.46	34.6
↓	**FP**	5.81	0.5	74.4	Unnamed Lake	P-57	9/23/2011	6.42	65	1,946	13.80	1.1
↑	TP	1.69	2.4	74.4	34-0611-00-201	P-57	9/23/2011	6.42	286	2,311	6.48	3.8
↑	TP	4.25	1.3	64.9		FS-183	7/30/2012	16.8	150	2,157	5.61	4.0
↑	TP	4.67	11.4	121.3	Monongalia	FS-77	7/26/2012	21.7	1,370	4,953	18.66	4.6
↓	**FP**	5.38	0.8	50.0	Lake	FS-313	6/23/2013	34.7	94	6,028	19.44	6.4
↑	TP	7.17	1.0	87.9	34-0158-01	FS-340	7/31/2013	33.6	122	5,530	22.10	4.7
↑	TP	9.45	2.0	154.4		FS-379	9/13/2013	34.6	242	5,436	26.42	3.7
↓	TN	0.86	0.7	31.6	Stella Lake	P-30	9/14/2011	7.59	80	2,159	2.88	8.8
↑	TP	4.54	14.9	0.3	47-0068-00	FS-188	8/27/2012	18.1	1,790	1,257	2.34	4.0
↓	**FP**	1.63	0.7	57.6		FS-341	8/1/2013	24.7	88	1,786	1.35	15.1

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Class-ification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
↑	TP	6.86	1.1	3.2	Dark Lake	FS-322	7/10/2013	175	131	2,480	1.48	25.5
↑	TP	4.89	1.1	2.9	69-0790-00-202	FS-352	8/15/2013	173	136	5,120	3.61	35.3
↑	TP	5.31	2.5	11.1		FS-368	9/5/2013	175	305	3,354	1.94	33.0
↓	**FP**	4.97	0.4	11.8		FS-369	9/5/2013	176	52	2,037	0.82	35.4
↓	TN	0.15	0.4	25.9	Little Birch Lake	P-47	9/21/2011	3.2	50	4,503	4.46	21.4
↑	FN	0.19	1.6	25.9	77-0089-00	P-47	9/21/2011	3.2	191	2,236	1.75	17.1
↓	**FP**	2.90	0.3	70.0		FS-54	8/3/2012	7.4	35	1,794	6.02	2.6

Evidence that site-specific standards may be needed for false positive wild rice waters

Some waterbodies will have wild rice when sulfide is greater than 120 µg/L

In the MPCA-sponsored field study, 29 waterbodies out of 115 surveyed (Class G dataset, N=222) had at least one true positive (at least one site visit found sulfide to be above 120 µg/L, consistent with the prediction of the proposed equation). Of the 29, 14 waterbodies had at least some wild rice present (48%), compared to 76% (62 out of 82) of the true negative samples (true negatives are samples conforming with predicted sulfide below 120 µg/L).

In some waterbodies where sulfide exceeds 120 µg/L the wild rice is dense and appears to be thriving. However, when sulfide exceeds 120 µg/L fewer samples have denser rice (for instance, greater than 10 stems/m^2). In the Class G dataset (N=222), only 34% of all samples from natural waterbodies have dense wild rice when sulfide exceeds 120 µg/L, whereas 73% of samples have dense wild rice when sulfide is less than 120 µg/L (calculated form Fig. 2-1). In dataset Class B (N=108), which approximates a probabilistic data set of natural waterbodies (Myrbo et al., in press-1), the proportion of samples with denser wild rice is significantly greater when sulfide is less than 120 µg/L (Chi square test; $p < 0.02$). Only 23% of Class B sites have dense wild rice when sulfide exceeds 120 µg/L, whereas 62% of sites have dense wild rice when sulfide is less than 120 µg/L (Fig. 2-3).

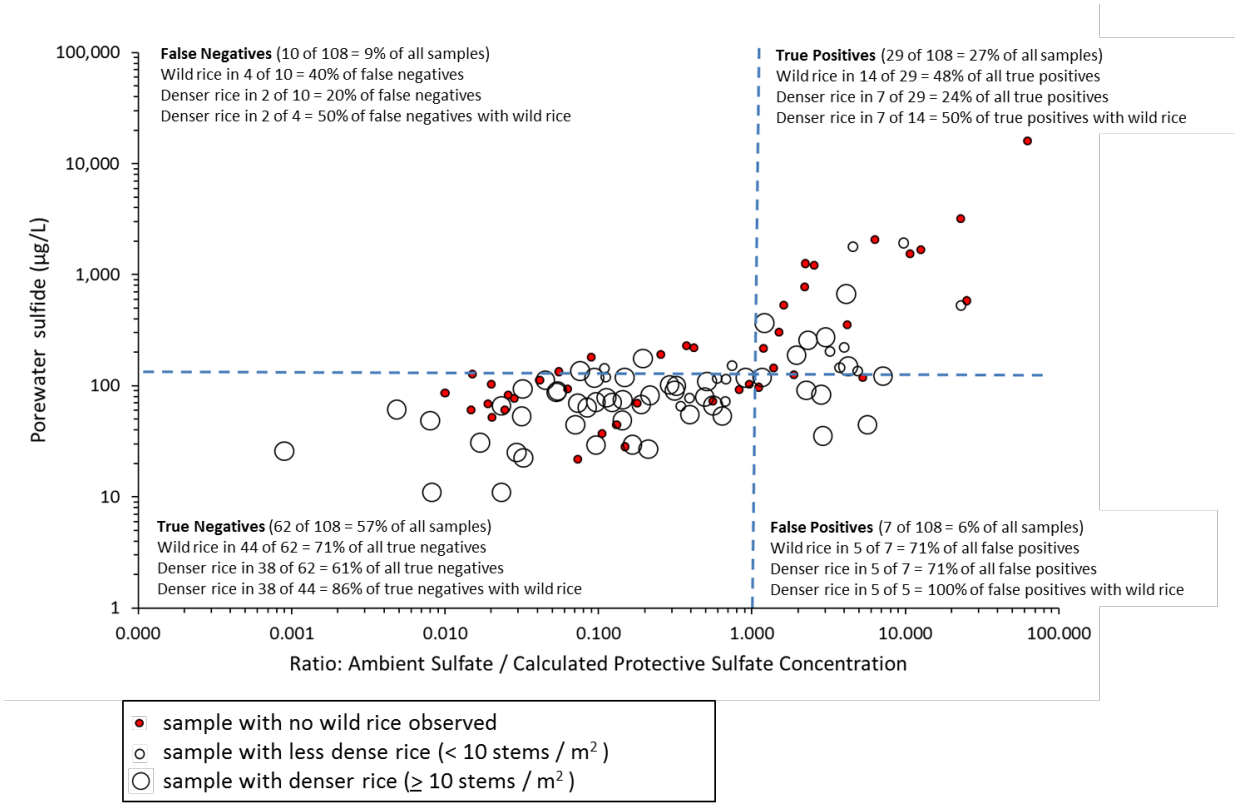


Figure 2-3. Prediction accuracy of the proposed equation for dataset Class B (a subset of 108 different waterbodies in Class G), plus density of wild rice occurrence, at two levels of density.

Possible explanations for wild rice in waterbodies with elevated porewater sulfide

There are several different possible explanations for the observation of wild rice in waterbodies with porewater sulfide in excess of 120 µg/L.

First, 120 µg/L was chosen to be a protective concentration along a gradient of declining probability of wild rice density and occurrence. There is no bright line below which sulfide is not toxic to wild rice and above which sulfide is toxic. As a result, wild rice is sometimes found in waters above the protective concentration, and sometimes in high density (above 40 stems per square meter); however, this occurs much less frequently than at levels of sulfide below the protective concentration. Because no water quality standard is perfect, it is inevitable that there will be some waterbodies where a standard is exceeded even while the beneficial use that the standard is designed to protect is maintained.

Second, it may be that the MPCA survey took too few samples to accurately characterize the specific waterbody. Six of the 14 true positive waterbodies with wild rice are represented by a single sampling (Table 2-2). Additional sampling might reveal that sulfide actually averaged below 120 µg/L. For instance, Stella Lake was sampled three times, and only in one of the three samplings did sulfide exceed 120 µg/L (Table 2-2).

A third possible explanation is that the sampling caught the wild rice population in the process of being extirpated from a waterbody that had sulfide in excess of what could be tolerated by wild rice. Four waterbodies had low densities of wild rice at the time of sampling (Christina, Big Sucker, Dark, and

Sandy, all below 4 stems/m^2; Table 2-2). Pastor et al. (2017) concluded that elevated porewater sulfide concentrations cause wild rice populations to decline by adversely affecting the seed production. Perhaps these sites had reproducing wild rice populations in the past that built up a store of seeds in the sediment that can remain viable for multiple years—a so-called seed bank. Not all viable wild rice seeds germinate in a given year. If sulfate has increased in these sites, increasing porewater sulfide, a possible explanation is that the few plants observed are the remnants of the seed bank produced in lower-sulfide years, and that the waterbody's wild rice population is likely to disappear in coming years.

A fourth possible explanation is that other variables that can control wild rice growth and reproduction, such as water depth, transparency, and nutrients such as nitrogen, are sufficiently optimal so as to offset the negative effects of elevated sulfide. For instance, seven cultivated wild rice paddies were sampled during the MPCA-wild rice study, and sulfide exceeded 120 µg/L at five of the seven sites. Wild rice density is very high in these cultivated operations, and growers have learned to optimize variables such as water depth, transparency, and nitrogen so that wild rice grows and produces seed successfully. Growers keep water depth relatively shallow during early seedling growth, which allows wild rice seedlings to quickly reach the water surface, simultaneously affording the plant more light for photosynthesis and access to the atmosphere. High rates of photosynthesis and emergence from the water into the atmosphere both allow more oxygen to be sent to the developing roots, potentially detoxifying sulfide (as discussed in *How access to oxygen may allow wild rice to detoxify sulfide* in Chapter 1, Part A). Wild rice growers also work to maintain high water transparency for seedlings, sometimes treating the water with copper sulfate to reduce the density of suspended algae. The productivity of wild rice is primarily limited by nitrogen; increased nitrogen availability increases the mean seed weight and number of seeds produced per stem (Pastor et al., 2017). Wild rice growers fertilize the paddies with nitrogen (as urea), which also allows wild rice to grow quickly, accelerating stem length and leaf development, and thereby increasing access to oxygen. There is evidence that wetland plants fertilized with nitrogen can better oxidize sulfide around the roots, reducing the potential toxicity (Howes et al., 1986).

Monongalia Lake is an outlier: dense wild rice despite elevated porewater sulfide

Among the 14 true positive waterbodies with at least some wild rice, Monongalia Lake stands out as having multiple samples that document dense wild rice that occurs simultaneously with elevated porewater sulfide (Table 2-2). Three of four samples were true positives, with porewater sulfide of 122, 242, and an unusually high 1,370 µg/L. Wild rice density was very high in all three samples (88, 154, and 121 stems/m^2, respectively, compared to a Class B median of 41 stems/m^2). Observed surface water sulfate concentrations of 22 to 35 mg/L were much higher than the calculated protective sulfate concentrations of 3.7 to 6.4 mg/L. This large (2,255 acres) but shallow (maximum depth 14 feet) lake lies in the Middle Fork Crow River watershed (Fig. 2-4), which is 46% agricultural, 26% wetland, and 10% developed/urban land use upstream of Monongalia Lake (calculated from MFCRWD, 2007). The Middle Fork Crow River flows through Monongalia Lake. Aside from the occurrence of dense wild rice in the presence of elevated porewater sulfide, the only field study parameters that are slightly atypical, compared to the Class B data set, are that in three of four sediment samples TOC, total sulfur, and total nitrogen are all in the upper quartile of Class B sites. Wild rice may be able to grow and reproduce in Monongalia Lake because environmental variables other than sulfide are sufficiently optimal so as to offset the negative effects of elevated sulfide (the fourth explanation, above), although extensive additional study would be required to evaluate that hypothesis. Monongalia Lake stands out as the least-well understood waterbody in the MPCA-sponsored field study in regards to factors that control wild rice growth and reproduction. The unique nature of this lake points towards the need for site-specific research and standard development.

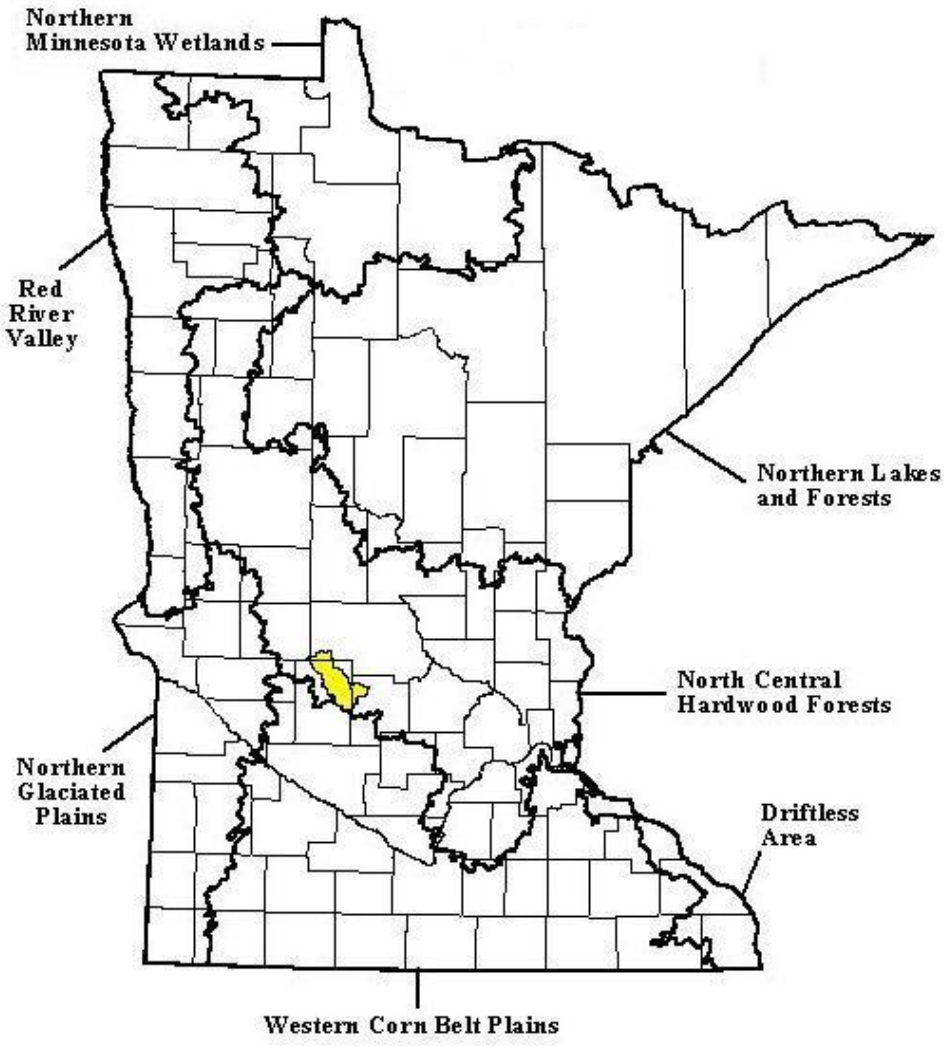


Figure 2-4. Location of the watershed that includes Monongalia Lake (yellow shaded area) (from MFCRWD, 2007).

Table 2-2. All waterbodies in the field survey that exhibited at least one true positive (dataset Class G)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
14 waterbodies with at least one true positive sampling that had wild rice present in at least one survey.												
	↑ TP	1.2	3.1	114.9	Pine Lake 15-0149-00-205	FS-190	8/28/2012	14.7	368	4,477	7.08	12.2
	↑ TP	1.8	4.1	3.0	Anka lake	P-35	9/16/2011	2.23	493	2,170	14.84	1.2
	↑ TP	6.4	5.6	25.9	21-0353-00	P-34	9/16/2011	2.23	671	1,485	23.57	0.3
	↑ TP	23.1	4.4	2.3		FS-192	8/29/2012	8.44	530	1,498	22.85	0.4
	↑ TP	3.0	2.3	30.2	Ina Lake 21-0355-00-202	FS-191	8/29/2012	7.08	274	2,216	9.09	2.3
	↑ TP	9.8	16.1	0.6	Christina Lake 21-0375-00-315	FS-339	7/31/2013	14.6	1,930	1,741	8.96	1.5
	↑ TP	2.5	2.8	12.4	Swan Lake (W Bay)	FS-61	8/30/2012	12.5	332	5,827	22.71	5.0
	↑ TP	4.0	1.8	3.8	31-0067-01	FS-62	8/30/2012	14	221	4,821	22.53	3.5
	↑ TP	3.7	1.2	3.8	Big Sucker L. 31-0124-00-203	FS-216	9/12/2012	7.78	145	3,559	21.45	2.1
	↑ TP	4.7	11.4	121.3	Monongalia	FS-77	7/26/2012	21.7	1,370	4,953	18.66	4.6
↓	FP	5.4	0.8	50.0	Lake	FS-313	6/23/2013	34.7	94	6,028	19.44	6.4
	↑ TP	7.2	1.0	87.9	34-0158-02	FS-340	7/31/2013	33.6	122	5,530	22.10	4.7
	↑ TP	9.4	2.0	154.4		FS-379	9/13/2013	34.6	242	5,436	26.42	3.7
↓	FP	5.8	0.5	74.4	Unnamed Lake	P-57	9/23/2011	6.42	65	1,946	13.80	1.1
	↑ TP	1.7	2.4	74.4	34-0611-00-201	P-57	9/23/2011	6.42	286	2,311	6.48	3.8
	↑ TP	4.2	1.3	64.9		FS-183	7/30/2012	16.8	150	2,157	5.61	4.0

(continued)

Porewater sulfide relative to 120 µg/L		Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
↓		TN	0.9	0.7	31.6	Stella Lake	P-30	9/14/2011	7.59	80	2,159	2.88	8.8
	↑	TP	4.5	14.9	0.3	47-0068-00	FS-188	8/27/2012	18.1	1,790	1,257	2.34	4.0
↓		FP	1.6	0.7	57.6		FS-341	8/1/2013	24.7	88	1,786	1.35	15.1
	↑	TP	2.0	1.6	144.8	West Battle L. 56-0239-00-204	FS-228	8/15/2012	4.03	189	3,108	17.37	2.1
	↑	TP	4.1	5.6	39.8	Bee Lake 60-0192-00-202	FS-87	8/23/2012	11	670	3,054	13.62	2.7
	↑	TP	6.9	1.1	3.2	Dark Lake	FS-322	7/10/2013	175	131	2,480	1.48	25.5
	↑	TP	4.9	1.1	2.9	69-0790-00-202	FS-352	8/15/2013	173	136	5,120	3.61	35.3
	↑	TP	5.3	2.5	11.1		FS-368	9/5/2013	175	305	3,354	1.94	33.0
↓		FP	5.0	0.4	11.8		FS-369	9/5/2013	176	52	2,037	0.82	35.4
	↑	FN	0.0	1.0	3.8	Sandy Lake	FS-251	9/21/2012	3.05	123	35,905	33.08	105.5
↓		TN	0.1	0.8	0.0	69-0730-00	FS-306	6/11/2013	11	92	35,357	28.53	122.3
	↑	TP	2.7	9.0	0.0		FS-305	6/11/2013	135	1,080	19,094	22.23	50.4
	↑	FN	1.0	1.6	0.0		FS-321	7/9/2013	122	189	36,502	29.51	124.9
↓		FP	3.5	0.6	0.0		FS-349	8/13/2013	122	70	14,897	20.46	34.6
	↑	TP	1.5	2.5	0.0		FS-348	8/13/2013	123	305	13,216	8.23	81.6
	↑	TP	1.1	1.1	0.0		FS-382	9/17/2013	67.9	135	26,645	32.28	61.2
↓		FP	1.6	0.3	0.0		FS-381	9/17/2013	126	34	16,172	11.67	79.2
	↑	TP	2.3	2.1	69.7	Bowstring River S007-219	FS-214	9/11/2012	1.34	256	1,974	24.34	0.6

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
15 waterbodies with at least one true positive sampling for which wild rice was not observed in any site visit.												
↑	TP	1.4	1.2	0.0	Rice Lake 02-0008-00-206	FS-231	8/17/2012	3.6	145	2,159	7.98	2.6
↑	TP	62.8	133.3	0.0	Bean Lake 03-0411-00-201	FS-85	8/21/2012	85	16,000	1,967	11.85	1.4
↑	TP	2.5	10.2	0.0	Cromwell Lake 14-0103-00-201	FS-128	8/22/2012	41.2	1,220	2,948	2.85	16.2
↑	TP	10.7	12.8	0.0	North Geneva L. 24-0015-00-209	FS-176	7/24/2012	15.6	1,540	2,212	13.45	1.5
↑	TP	22.9	26.6	0.0	South Geneva L. 24-0015-02-208	FS-177	7/24/2012	14.1	3,190	1,618	16.71	0.6
↑	TP	1.9	1.1	0.0	Upper Panasa L.	FS-59	8/29/2012	29.6	126	895	0.43	15.8
↓	TN	0.1	0.3	0.0	31-0111-00-202	FS-383	9/18/2013	33.6	40	19,148	2.86	590.3
↑	TP	2.0	2.0	0.0	Lower Panasa L.	FS-60	8/29/2012	33.6	243	8,048	14.12	16.5
↑	TP	2.2	10.5	0.0	31-0112-00	FS-357	8/15/2013	28.5	1,260	2,347	2.42	12.7
↑	TP	1.6	4.5	0.0	Little Sucker L. 31-0126-00-202	FS-223	9/14/2012	13.7	534	6,297	16.56	8.5
↑	TP	23.4	8.4	0.0	Holman L.	FS-218	9/13/2012	24.2	1,010	3,035	29.74	1.0
↑	TP	25.1	4.9	0.0	31-0227-00-202	FS-353	8/12/2013	68	583	5,094	30.60	2.7
↑	TP	5.0	123.7	0.0	Lady Slipper L.	P-55	9/22/2011	107.71	14,840	2,814	2.09	21.5
↑	TP	9.7	13.6	0.0	42-0020-00	FS-79	7/27/2012	330	1,630	3,314	1.85	34.1
↑	TP	12.6	14.0	0.0		FS-78	7/27/2012	335	1,680	2,719	1.66	26.5

(continued)

Porewater sulfide relative to 120 µg/L	Accuracy Classification (FP, TP, FN, TN)	Ratio: Surface water SO4 / CPSC120	Ratio: porewater sulfide / 120	Wild Rice (stems/m2)	Waterbody Name, State ID	Field ID	Sample Date	Surface water SO4 (mg/L)	Pore water sulfide (µg/L)	Sediment Extractable Fe (µg/g)	Sediment Total Organic Carbon, TOC (%)	CPSC 120 SO4 (mg/L)
↑	TP	1.7	14.9	0.0	Westport 61-0029-00-204	FS-186	8/1/2012	7.11	1,790	4,917	20.15	4.2
↑	TP	2.2	6.5	0.0	Rice Lake 66-0048-00-203	FS-181	7/27/2012	5.22	777	3,829	21.67	2.4
↑	TP	4.1	24.8	0.0	Rice Lake	FS-184	7/30/2012	2.58	2,970	1,523	15.03	0.6
↑	TP	6.3	17.3	0.0	73-0196-00-216	FS-345	8/7/2013	6.85	2,080	2,012	14.83	1.1
↑	TP	1.2	1.8	0.0	Rice Lake 74-0001-00-201	FS-179	7/25/2012	3.84	217	4,152	19.07	3.2
↑	TP	4.2	3.0	0.0	Gilchrist L. 86-0064-00-201	FS-194	8/31/2012	6.98	355	3,117	20.81	1.7
↑	TP	3.2	1.7	6.7	Westport L. 61-0029-00-205	FS-346	8/8/2013	6.3	205	3,262	19.66	2.0

Chapter 3. Implementation of the wild rice sulfate standard

Sediment Sampling and Analysis

Sediment sampling is conducted to provide the data needed to calculate the numeric sulfate standard for a wild rice water. Sediment total extractable iron (TEFe) and sediment total organic carbon (TOC) concentrations are the two measured variables used in the proposed equation to calculate the numeric sulfate standard. When MPCA developed the draft proposed approach, released in March 2015 (MPCA, 2015), both MPCA and commenters noted that there was spatial variability in the TOC and TEFe measured in the sediment of a wild rice bed. These two parameters are measured in homogenized 10-cm long sediment cores, which represent many years of sediment accumulation. It is not expected that TOC and TEFe change significantly over the near term unless unusual hydrologic events occur. No statistically significant seasonal trend was observed in the field data (Myrbo et al., in press-1). To produce data that are pertinent to the protection of wild rice, sediment sampling must be conducted in the places where wild rice grows. Growth patterns of wild rice vary annually as exemplified in Fig. 3-1. It is important to establish how and where sediment samples will be collected in efforts to be representative of the wild rice water.

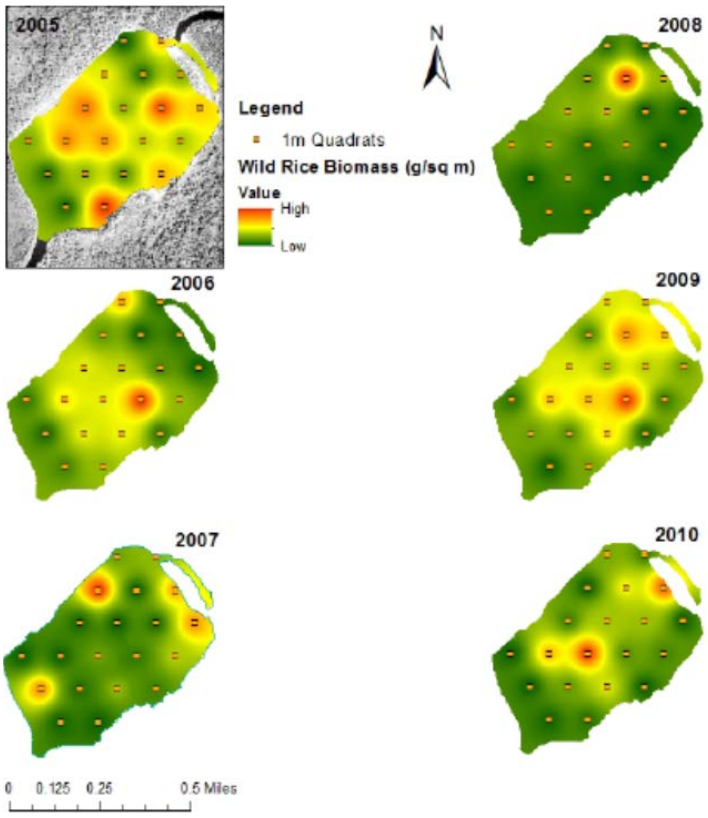


Figure 3-1. Example of variable growth patterns of wild rice biomass across years. Reprinted with permission: Wild Rice Monitoring Handbook by Tonya Kjerland (2015), published by the University of Minnesota Sea Grant College Program; from MN Sea Grant.

To effectively implement an equation-based standard, MPCA must specify how many samples are needed to characterize the sediment of a wild rice water, and how to consider those samples in the calculation of the numeric standard. To inform this approach, in June 2015 a pilot study was conducted to examine spatial variability in sediment TOC and TEFe found in six wild rice waters. In addition, collecting and analyzing a large sample size from different waterbodies is useful for informing decisions about an appropriate sample size for implementation of the water quality standard. The study sampled six different wild rice waters (four rivers and two lakes) identified on the MPCA draft list of wild rice waters, at areas that were sampled during 2011-2013 as part of the MPCA-sponsored field sampling. Within each wild rice water, 25 individual sediment samples (sediment cores) were collected and analyzed for TOC and TEFe.

The 25 sediment sampling locations at each waterbody were selected using the following guidelines:

- The coordinates for each wild rice bed were those used for the wild rice field study. The coordinates all corresponded to an access point adjacent to the waterbody. From that point, a representative bed of wild rice was located and a location was identified to begin sampling. This location was at least fifty meters away from the access point. On streams, the starting point was always upstream of the road crossing, if present. From that point, a transect perpendicular to the shoreline (and to water flow) was followed for collecting samples.
- The first sample was retrieved along the transect line where rice was found growing nearest the shoreline.
- The distance between each sample point was approximately 2 meters.
- Sampling stopped at the point along the transect where wild rice growth was not observed or water depth was too great (approximately 4 feet of water; Kjerland, 2015). In cases where wild rice growth was observed all the way to the opposite shore (e.g., a shallow lake or stream), professional judgment was used to determine a reasonable transect length.
- Additional transects were laid out in the same manner as described, parallel to the first transect, as needed to complete collection of the 25 samples.

All sediment samples were collected in the following manner:

- Sediment was collected using 70 mm diameter polycarbonate core tubes.
- The top ten centimeters of the substrate sampled in each core was collected and placed into a plastic bag. Samples were kept on ice in the field.
- In the lab, each bagged sample was gently mixed by hand. A subsample from each bagged sample was placed into a jar for analysis of TOC and TEFe. These samples were refrigerated until analysis.

Methods for TOC analysis followed EPA method 9060 (EPA, 2004) and analysis of TEFe followed Balogh et al. (2009) as modified by the Minnesota Department of Health (MDH, 2016).

Table 3-1. Summary results from 2015 pilot study, consisting of 25 sediment cores collected and analyzed from each of six wild rice waters. Total organic carbon (TOC) and total extractable iron (TEFe) were quantified in each core. All data is reported in Appendix 3.

Waterbody (abbreviation)	Mean TOC (%)	SD	CV(%)	Minimum	Maximum
Bowstring River (BRT)	16.0	1.9	12	12.6	19.7
Clearwater River (CLRT)	20.3	2.5	12	16.2	25.4
Hesitation Wildlife Management Area (HWMAT)	21.8	2.8	13	16.1	25.9
Mission Creek (MCT)	3.8	0.9	24	1.5	5.3
Monongalia Lake (MLT)	16.9	7.1	42	2.4	27.2
Mississippi River (MRT)	15.2	4.0	26	6.1	22.0

Waterbody (abbreviation)	Mean TEFe (mg/kg)	SD	CV(%)	Minimum	Maximum
Bowstring River	3,827	640	17	2,169	4,680
Clearwater River	13,439	2,652	20	8,370	19,800
Hesitation Wildlife Management Area	38,088	13,850	36	24,300	74,700
Mission Creek	15,707	3,882	25	7,470	22,500
Monongalia Lake	6,041	1,792	30	2,610	9,000
Mississippi River	5,432	436	8	4,680	6,300

As expected, the measured sediment TOC and TEFe concentrations were variable (Table 3-1). Paired values of TOC and TEFe reported from each sediment core analysis were used to calculate a sulfate concentration using the equation being proposed for the water quality standard for sulfate. Analysis of Variance performed on this data set showed significant differences ($p<0.05$) between wild rice waters, which infers that the variability of sulfate values calculated within a waterbody varied less than the variability between wild rice waters. Given this information, it is important to know how sample size affects variability.

One way to examine this is to compare the variability of the data around the mean to increasing sample size. Graphing this for both TOC and TEFe shows that as sample size increases, the variability decreases (Figs. 3-2 and 3-3). Variability is displayed as the width of the confidence interval along the y-axis. The rate of narrowing of the confidence interval levels off at a sample size of about 20 to 25. This suggests that a large amount of the sample variability has been accounted for with a sample size of 25 and that further samples would not greatly improve the estimate of either sediment TEFe or TOC.

Based on this information, the MPCA examined whether this same conclusion could be drawn using composite samples. A composite sample is collected by using the same sampling equipment as described above (core samples), but instead of collecting and analyzing individual samples, a set of samples collected from an area are pooled into a single sample for analysis. MPCA proposes that instead of collecting and analyzing 25 separate core samples from each wild rice water, composites of samples be collected and analyzed from five separate areas within the wild rice water. These five composite samples, each comprised of five individual core samples, would then be analyzed for TOC and TEFe.

An important question to answer is whether the variability of sulfate values calculated from composite samples is similar to the variability seen in values calculated from single core samples. To investigate this, calculated sulfate values from theoretical composite samples were compared to calculated concentrations from individual cores using data from the 2015 pilot study. Cores from the 2015 pilot study were placed into groups of five and average TOC and TEFe values were calculated to simulate a composite sample. The groups were determined by the order that the samples were retrieved from the sample area (i.e. first five samples in first composite group, second five samples in second composite group, etc.) to simulate composite sampling in the field. Using the proposed equation, the protective sulfate value was calculated for each TOC and TEFe pair from individual and composite samples (Appendix 3). Percentile ranks were determined using the full set of individual calculated sulfate values; 10^{th} to 90^{th} percentiles are shown in Table 3-2. The percentiles of each composite calculated sulfate value range from 11% to 93% among the wild rice waters (Appendix 3), showing that the composite samples capture the majority of the variability of the single samples.

To effectively implement an equation-based approach to determining the sulfate standard, a single numeric sulfate standard needs to be determined for each wild rice water. The purpose of sampling sediments in the wild rice bed is to capture the variability of the sediment concentrations of TEFe and TOC to ensure that the single sulfate standard selected from the group of five representative sulfate values calculated is protective of the wild rice beneficial use throughout the wild rice water. The MPCA compared the lowest composite value from each site to the percentile ranks (Table 3-2; Appendix 3), and observed that they all fall within the 10^{th} and 30^{th} percentiles for the six sites. Selecting the lowest value as the sulfate standard for the wild rice water addresses the need to protect for sensitive conditions where sulfide may accumulate, protecting the wild rice.

There are two reasons that it is not reasonable to use the average calculated sulfate concentration rather than the lowest calculated sulfate concentration. First, the goal of developing a sulfate standard is to allow wild rice to grow throughout the suitable locations in a wild rice water, not just in a subset. Use of an average would protect only a portion of the wild rice, given that use of an average implies that about half of the wild rice would need a lower numeric sulfate standard to avoid high porewater sulfide concentrations. Second, while “average” might sound like it would protect half of the wild rice, in fact, protection might be far less than half. The reason that “average” does not necessarily protect half of the wild rice is that calculation of averages is vulnerable to extreme values. For example, if one of the five calculated potential sulfate standards were extremely high, the average could actually be higher than four of the five values. In such a case, the use of an average as the numeric sulfate standard could conceivably protect only a very small proportion of the wild rice in a wild rice water. For the above reasons, use of the lowest calculated sulfate concentration is much more defensible and reasonable than use of a calculated average concentration.

Table 3-2. Lowest calculated sulfate value of composite samples compared to sulfate values at various percentiles calculated from the 25 individual samples analyzed from each waterbody of the pilot study.

		Calculated sulfate values at various percentiles calculated from 25 individual samples (mg/L)				
Waterbody	**Lowest calculated sulfate value from composites (mg/L)**	**10th**	**30th**	**50th**	**70th**	**90th**
Bowstring River	2.1	2.0	3.3	3.6	3.9	5.3
Clearwater River	22.3	19.7	23.5	24.4	32.3	50.1
Hesitation WMA	104.3	85.7	112.7	142.2	217.2	469.4
Mission Creek	240.1	203.1	247.6	294	312.8	397.1
Monongalia Lake	6.6	5.1	6.8	8.6	10.7	13.8
Mississippi River	5.6	4.6	6.0	6.9	9.3	12.8

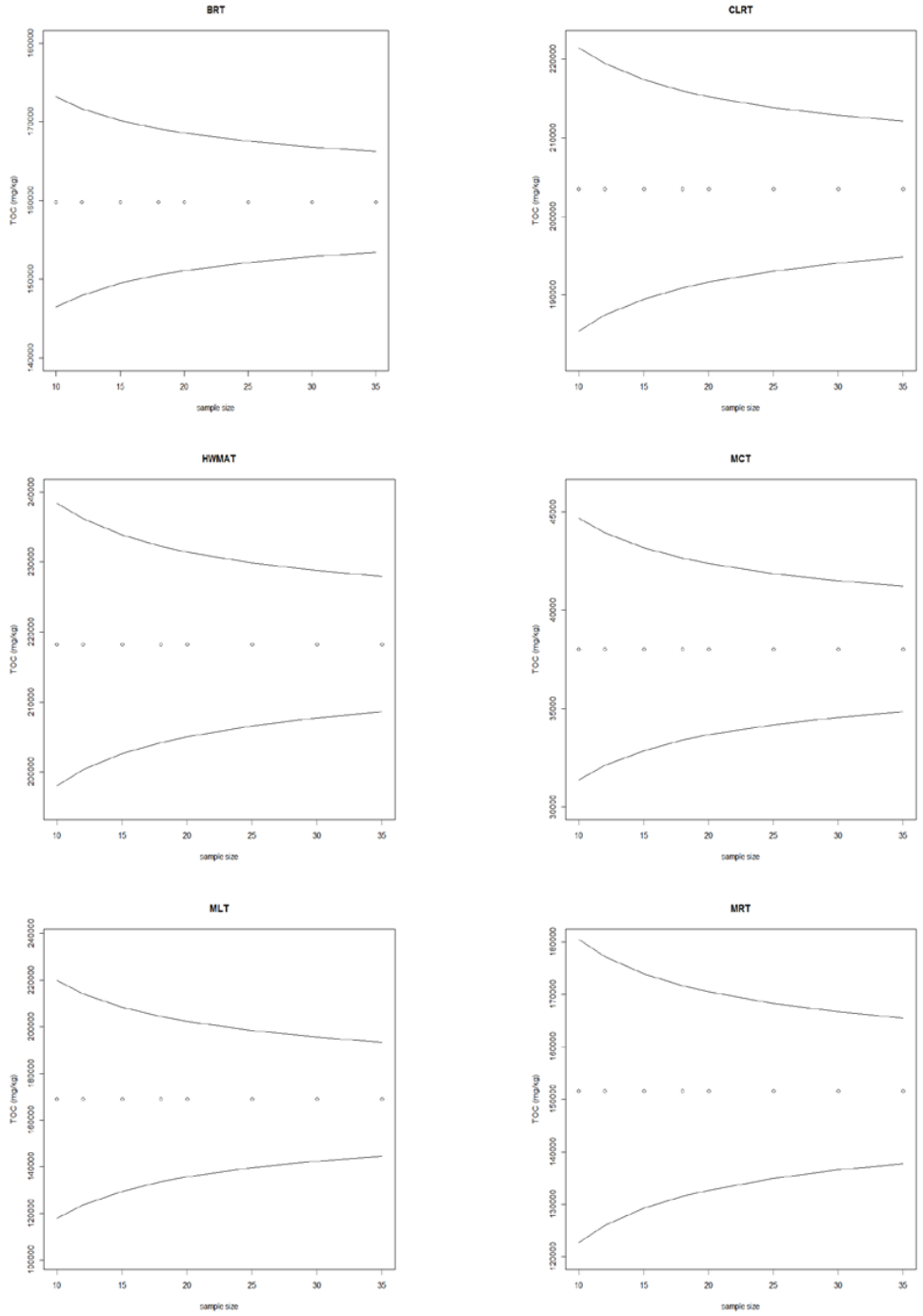


Figure 3-2. Graph of confidence interval widths compared to sample size for sediment TOC for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.

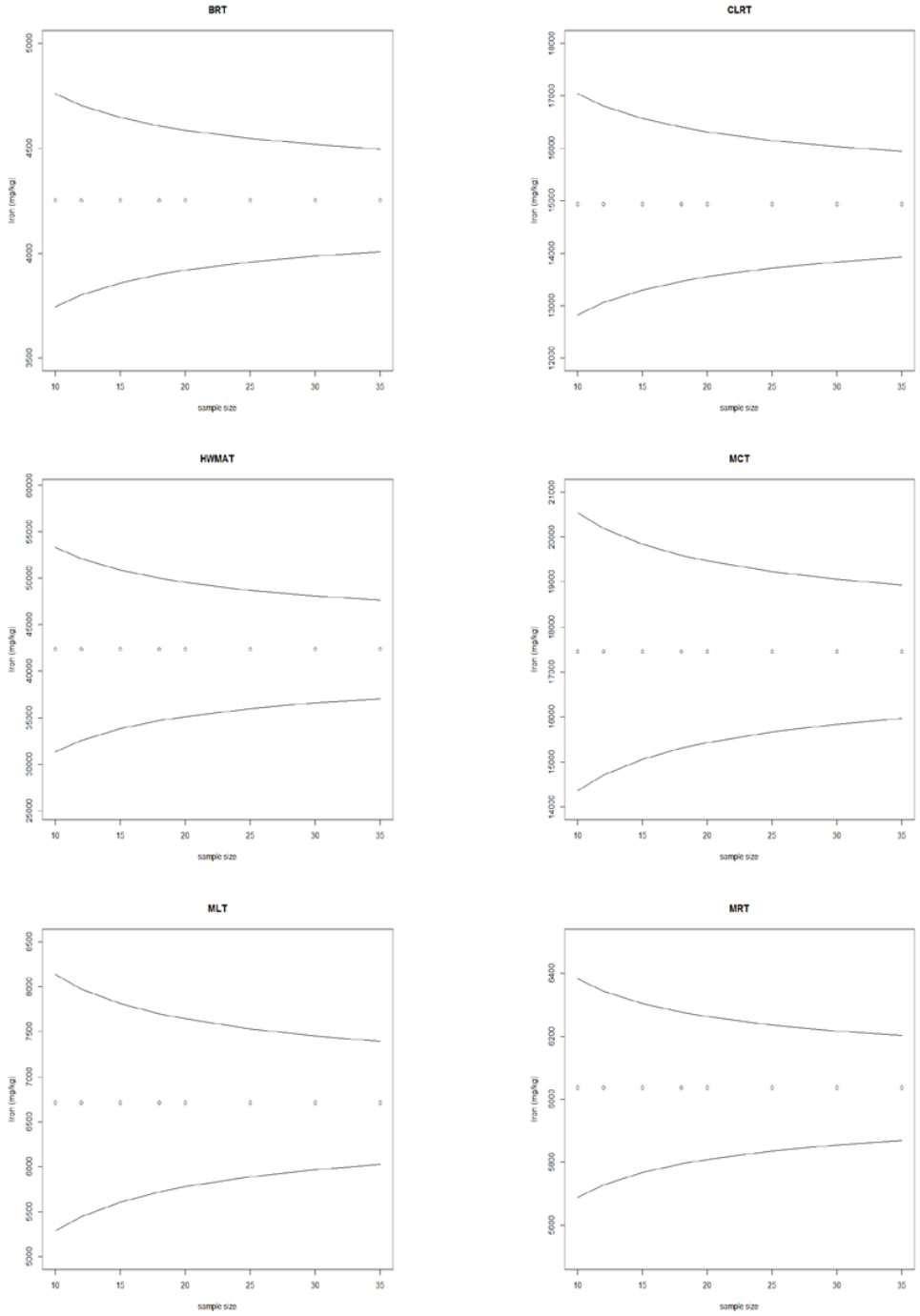


Figure 3-3. Graph of confidence interval widths compared to sample size for sediment iron for each of the waterbodies sampled. See Table 3-1 for an explanation of the waterbody acronyms.

Conforming with the Sulfate Standard

An important part of implementing any water quality standard is determining whether any given waterbody meets the standard. The magnitude, duration, and frequency of a standard not only are the bases for determining how waterbodies are assessed against the standard, but also inform permit requirements.

The magnitude is the level of the standard – in this case the amount of sulfate allowable in the wild rice water to maintain the protective sulfide level. The magnitude will usually be the numeric standard calculated with the equation, using sediment samples collected as described above. It may also be a sulfate concentration derived using the procedures for an alternate standard, or a site-specific standard.

The averaging time of the standard is the duration, and the frequency is how often the magnitude may be exceeded before the standard is considered to be violated. The analysis in Chapter 1 speaks to the magnitude of the standard, while this section discusses the technical information supporting the MPCA's proposed duration and frequency of the standard.

Appropriate duration and frequency are most easily determined for chemicals that are directly toxic to aquatic organisms. Determining duration and frequency for a chemical that has indirect negative effects, such as sulfate, is more challenging.

For this standard, duration is defined as the averaging period for sulfate that was found to be related to observed porewater sulfide concentrations, and frequency is defined as the interval between poor wild rice growth years from which wild rice has the undoubted ability to recover.

Duration (Averaging Time)

Defining duration for a pollutant should reflect the available information about the timeline of impact to the beneficial use. For example, a standard to protect against acutely toxic conditions may be expressed as a "never to exceed" duration, whereas one that protects against impacts over the longer term may be expressed as an annual or even multi-year average.

The MPCA is proposing to apply the standard as an annual average. This means that throughout a year, surface water sulfate concentrations could fluctuate above and below the standard so long as the annual average concentration is below the numeric sulfate standard for that wild rice water.

There are two main factors supporting the use of the annual average. First, sulfate is not directly harmful to wild rice and the conversion of sulfate to sulfide is not instantaneous. Second, the use of an annual average of sulfate concentrations is consistent with the empirical statistical relationships upon which the equation is based.

1. Sulfate is not directly toxic and takes time to convert to sulfide

Expression of the numeric standard as an annual average accounts for the fact that sulfate is not a direct toxicant upon wild rice, but rather that elevated sulfate concentration can lead to elevated sulfide in the sediment porewater, which is the toxicant of concern.

Porewater sulfide is produced biologically under anaerobic conditions throughout the year in the sediment where wild rice grows; accumulation of sulfide in the sediment depends on the sulfate concentration as well as the concentrations of total organic carbon and total extractable iron present in

the sediment. Sulfide can be produced at any time throughout the year (DeRocher and Johnson, 2013). From this understanding, it is reasonable to conclude that the concentration of sulfate in surface water is important throughout the year, not just when wild rice is actively growing.

One possible approach is to implement the standard as a concentration that should never be exceeded. This would be reasonable if sulfate were directly toxic to wild rice. But several studies (Fort et al., 2014; Pastor et al., 2017) demonstrate that sulfate is not directly toxic to wild rice at concentrations encountered in Minnesota. However, over time sulfate can contribute to the buildup of sulfide in the porewater of sediments in which wild rice grows, so it is still important to regulate the concentration of sulfate in surface water.

The effect of elevated sulfate is (a) indirect, and (b) relatively slow. For instance, in a multi-year sulfate addition experiment (treatment sulfate concentrations of 0, 50, 100, 150, and 300-mg/L additions), it was not until the third year of the experiment that wild rice growth and reproduction was significantly affected in the 100 mg/L treatment (Pastor et al., 2017). In this case, the calculated protective sulfate concentration for the sediment used in the experiment was 34 mg/L. Even after five years of sulfate additions the 50 mg/L treatment (which had produced an actual average surface water sulfate concentration of 41 mg/L, less than the target of 50 mg/L because sulfate kept being converted to sulfide in the sediment) had no statistically significant effect on the most sensitive endpoints, seedling survival, seedling germination, and final plant biomass (Pastor et al., 2017). The 41 mg/L average sulfate concentration had not harmed wild rice after five years, which may be because (a) the equation-calculated sulfate concentration of 34 mg/L is sufficiently conservative to be protective of a concentration 20% higher than the calculated standard, or (b) not enough time had passed for the negative impact of elevated sulfate to manifest. It should be noted that the experiment was not a true mimic of likely impacts in the real environment, because the experiment was conducted in plastic tubs that cut off the iron supply from the watershed. Therefore, negative impacts might be observed in the experiment that would not occur in nature, where there is re-supply of iron to the sediment. After five years of sulfate additions, the highest sulfate treatment (300 mg/L) depleted the iron in the experimental tubs, allowing porewater sulfide to increase dramatically (Pastor et al., 2017).

The conversion of sulfate to sulfide is slow because it is a multi-step process. First, sulfate needs to enter the sediment from the overlying water, which in most wild rice sites occurs by diffusion. Diffusion is essentially a consequence of Brownian motion, the vibration of molecules proportional to temperature. While the speed of diffusion is driven by temperature, the direction of diffusion is from areas of high concentrations to areas of low concentration. Diffusion is a slow process, particularly under colder conditions. Second, once sulfate has entered anoxic sediment, the conversion to sulfide is a consequence of the growth of bacteria that respire sulfate instead of oxygen. If the growth of these bacteria is limited by sulfate (they can also be limited by the availability of organic matter), over the long term sulfide production is proportional to the sulfate concentration (Herlihy and Mills, 1985; Urban et al., 1994; Holmer and Storkholm, 2001). Microbial growth is also strongly affected by temperature. Bacteria grow slower under colder conditions.

Not only is the conversion of elevated sulfate to sulfide relatively slow, but the process of sulfate diffusion into the sediment is reversed if there is a decline in the concentration of sulfate in the surface water (DeRocher and Johnson, 2013). After a decline in sulfate concentration, the diffusion gradient is reversed, and unreacted sulfate will diffuse back into the overlying water (until concentrations are equal).

There is limited available information to support the determination of an alternative to a one-year averaging time. A key example is the limited potential to model the effect of varying sulfate

concentrations on porewater sulfide, in order to determine how long sulfate concentrations would need to be elevated to affect sulfide concentrations.

Modeling is one of the most powerful analytical tools available to environmental science. Scientists use models as a way to develop hypotheses, explain complex interrelated processes, and to present their understanding of a particular subject matter. ”Modeling” has a broad definition within environmental science; a model can range from a conceptual diagram to a complex computer model involving days of super-computing processing time.

There are no official rules of “modeling” but there are some best practices, including:

1) Start simple and add complexity later.
2) Add complexity to your model only as necessary.
3) A model is only as good as the quality of the data used to develop and implement the model (a phenomenon often communicated as “GIGO”, or “Garbage In Garbage Out” – a reminder to not put undue faith in the output of an unvalidated model).

The effect of short-term fluctuations in sulfate concentrations on porewater sulfide concentrations is difficult to model mathematically, and therefore difficult to predict. Among other reasons, modeling is difficult because both diffusion and bacterial growth are affected by temperature, and temperature changes rapidly in the spring and fall. For instance, if sulfate concentrations are temporarily high for a time in the winter, less sulfate will diffuse into the sediment than under warmer conditions, and bacterial conversion to sulfide will be slow because of the cold. If the sulfate concentration then declines, spring comes, and the sediment warms, the sulfate will simultaneously begin to diffuse up into the overlying water and conversion to sulfide will accelerate even as the sulfate concentration declines. The MPCA commissioned a study to examine these interactions (DeRocher and Johnson, 2013) that compared the effect of varying sulfate concentrations at two temperatures. The study confirmed many of the expected relationships between temperature, sulfate, sulfide, and iron, but also produced some unexpected results associated with one of the two sediment sources. The unexpected result (continued release of sulfate from the sediment throughout the experiment) was most likely an artifact of exposing the sediment to oxygen while mixing the sediment during the experimental set up. A major lesson of the study is that the development of a general model that predicts porewater sulfide from varying sulfate concentrations and varying temperature would be a major effort.

No published model tries to address the net effect of fluctuating sulfate concentrations and temperature. Rather, modelling efforts to date either assume constant sulfate concentration (for instance, in the marine environment (e.g., Eldridge and Morse, 2000) or step changes from one concentration to either a higher or lower sulfate concentration (for instance, increases or decreases in the atmospheric deposition of sulfate; Nikolaidis et al., 1989). Moreover, there are no published experimental studies in which sulfate concentrations were purposefully varied and compared to the effect of holding the sulfate concentration constant at the average concentration of the varying system. Thus, there is limited information on the effects of short-term fluctuations in sulfate concentrations in order to inform the MPCA’s decision of an averaging time for the standard, beyond the conclusion that sulfate increases act over the longer term—a year or more—rather than days or months.

2. An annual average is consistent with the data and empirical statistical relationships

MPCA developed the equation by using the ambient surface water sulfate concentrations observed in the field survey, which is not significantly different from the annual average concentration (see below). Therefore, the proposed equation is relating the annual average sulfate concentration to porewater sulfide. Myrbo et al. (in press-1) showed that in 14 wild rice waters there is no significant seasonal trend

in porewater sulfide over the wild rice growing season, even though there was a slight increase in surface water sulfate over the summer. If there is an annual cycle in porewater sulfide, it is likely that sulfide is lower in the winter, as studies found (Leonard et al., 1993; Urban et al., 1994), which was attributed to greater winter oxygen penetration, lower sulfate diffusion rates, and decreased bacterial growth rates. The MPCA's equation, which is based on summer porewater sulfide concentrations, is therefore predicting the highest sulfide likely to be encountered in a waterbody.

In addition, Myrbo et al. (submitted-2) found that in experimental mesocosms porewater sulfide was linearly related to annual average sulfate concentration (which varied because the sulfate concentration was readjusted to target concentrations periodically and between adjustments sulfate diffused into the sediment; see Fig. 3-4 in the discussion below).

Implementing the average as an *annual* average is reasonable because of the strong annual temperature and organic matter production cycle in Minnesota, which strongly affect sulfide production. Bacteria only produce sulfide because bacteria are metabolizing decaying plants, which are produced on a strong annual cycle. All wild rice plants die in the fall, producing an abundance of organic matter that drives the production of sulfide, if sulfate is available.

An analysis of repeated samples from 14 different natural wild rice sites showed no significant time trends in sediment total organic carbon or sediment total extractable iron. A slight seasonal increase in sulfate (statistically significant at the p=0.05 level; Myrbo et al., in press-1) was observed, which is likely due to temporary dilution after spring snowmelt (Myrbo et al., in press-1). Because it takes many years to accumulate 10 cm of sediment, it makes sense that the iron and total organic carbon measured in the 10-cm long sediment samples show no change over time. Ten-cm long cores represent about 20 years of sediment accumulation (lead-210 dated age at 9-cm depth of cores from eight wild rice lakes average 21 years, with a median of 19 years; unpublished data from A. Myrbo, University of Minnesota).

For the 14 field study sites for which at least 3 samples were taken in one growing season, the sample used in the development of the equation is not significantly different from the average of the samples (p=0.94, Wilcoxon signed-rank nonparametric test). Conversely, regression analysis shows that the samples used to develop the equation are good estimates of the average of the samples. Regression analysis of log-transformed data (to approximate a normal distribution) with and without Second Creek yields slopes near 1.0 (0.944 to 0.971) and very high R^2 values (both 0.988). Second Creek had relatively high sulfate concentrations, which averaged 466 mg/L in 2013, in contrast to the 13 other sites, which ranged from 0.74 to 174 mg/L.

The equation therefore relates porewater sulfide to average surface water sulfate concentrations, not to maximum sulfate concentrations. Consequently, it is logical to implement the calculated sulfate standard as an annual average. If the calculated sulfate standard were implemented as a maximum value, the associated porewater sulfide concentration would be lower than the protective value of 120 µg/L, which would be over-protective.

Data collected during the sulfate-addition mesocosm experiment of Pastor et al. (2017) provides further evidence that the annual average surface water sulfate concentration is related to the porewater sulfide concentration. In this experiment, sulfate concentrations varied significantly over the year because of conversion to sulfide in the sediment. For instance, in 2013 the highest experimental sulfate treatment averaged 257 mg/L (Fig. 3-4), but ranged from 49 to 308 mg/L. Similarly, the second-highest sulfate treatment averaged 121 mg/L, but ranged from 14 to 151 mg/L. Yet, there is a highly statistically significant relationship between annual average sulfate concentration and porewater sulfide ($p < 0.001$, Fig. 3-4).

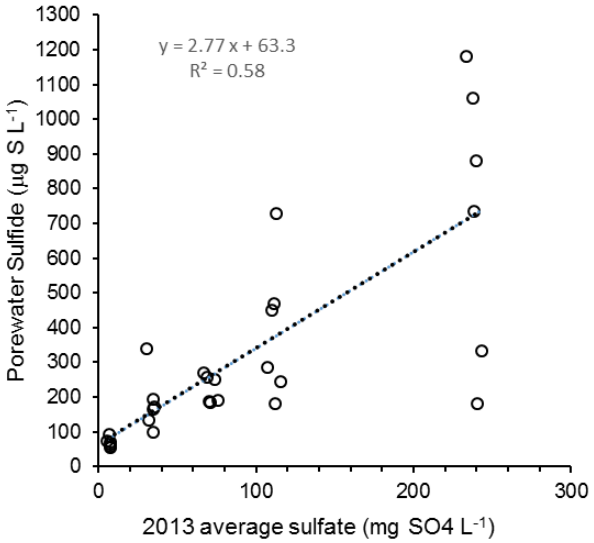


Figure 3-4. Relationship between average sulfate concentration from 2013 (8 measurement dates) and porewater sulfide measured at the end of the 2013 growing season. The regression between average sulfate concentration and porewater sulfide is highly significant ($p < 0.001$).

Frequency (how often the magnitude may be exceeded)

Given natural environmental variability, there is some probability that a water quality standard will be exceeded on occasion, due to factors other than human impacts. For instance, a dry summer can greatly decrease the expected dilution capacity of a receiving water. The question, then, is how frequently the sulfate standard can be exceeded without adversely impacting the beneficial use.

Porewater sulfide concentrations will decrease after an exceedance of a sulfate standard

The level of porewater sulfide is the long-term balance between production and loss of sulfide. If elevated sulfate in one year is followed by a year of lower sulfate, it is expected that porewater sulfide would re-equilibrate to the long-term average. The return to the long-term average sulfide concentration occurs because of a) the un-converted sulfate would diffuse back to the surface water and b) porewater sulfide would be oxidized by oxygen, ferric iron, and other oxidants. The concentration of porewater sulfide in a particular waterbody is, then, the net result of multiple dynamic processes involving sulfide production, sulfide oxidation, and reactions with both ferric (oxidation of sulfide) and ferrous iron (precipitation of sulfide). If sulfate concentrations are temporarily relatively high, producing higher sulfide concentrations, the system will tend to revert to a long-term average porewater sulfide concentration once sulfate is lowered, due to all the processes that affect sulfide—a phenomenon known as "sulfide buffering" (Giordani et al., 2008).

Several studies and reviews have concluded that observed sulfide concentrations are the balance between dynamic sulfide formation and sulfide degradation. Holmer & Storkholm (2001) found that up to 90% of sulfide production is oxidized to sulfate. Leonard et al. (1993) found seasonal variation in sediment sulfide in three lakes in northeastern Minnesota, where there is net loss of sulfide in the

winter. Urban et al. (1994), working in Little Rock Lake in Wisconsin, found that winter oxygen penetration oxidized sulfide in lake sediment, sometimes resulting in sulfate diffusion back into the surface water. The initial spring measurements in the sulfate-addition mesocosms of Pastor et al. (2017) also showed release of sulfate from the sediment into the overlying water (Myrbo et al., submitted-2).

Oxidation of sulfide can occur when sulfide encounters oxygen in the sediment, either through release from plant roots or bioturbation (mixing by benthic animals). Sulfide is also oxidized by the introduction of ferric iron to sediment from the watershed that is mixed downward by bioturbation (sulfide reduces ferric iron, a process that oxidizes the sulfide (Hansel et al., 2015).

As a result of the diffusion of sulfate that never was converted to sulfide back into the surface water, and the multiple processes that oxidize sulfide, temporary high concentrations of sulfate in surface water are not permanently preserved in the sediment as high sulfide. Porewater sulfide concentrations will return to the long-term average after a temporary exceedance of the sulfate standard.

Wild rice populations will recover even if porewater sulfide is temporarily elevated

A waterbody's wild rice population will be able to persist at a high average stem density if the annual average sulfate concentration does not exceed the calculated standard very often. The MPCA had to define what "very often" means in order to define the allowable excursion frequency. Because of the limitations of available environmental knowledge, the severity of an excursion cannot be rigorously related to the impact on a wild rice population. Nevertheless, MPCA expects that a wild rice population will not be significantly harmed by an exceedance that occurs only once in ten years, because that frequency will allow the environmental chemistry and wild rice population to recover between exceedances, thereby providing a high degree of protection.

New findings from the mesocosm experiment described by Pastor et al. (2017) provide some information on potential for wild rice to recover after a decrease in sulfate that had been elevated above the calculated protective concentration. The outdoor experiment grew wild rice in natural sediment at five different levels of sulfate (six replicates of each: control, 50, 100, 150, and 300 mg/L). If a numeric sulfate standard were calculated based on the sediment used in the experiment, it would be 34 mg/L (TOC=8.1%; TEFe=8,300 µg/g). In the fifth year of treatment at the 300 mg/L level, in 2015, no wild rice plants grew in five of the six replicates, and the sixth replicate had just one plant (the control averaged 22 plants/replicate) (Pastor et al., 2017). As an experiment within the experiment, starting in 2016 no sulfate was added to five of the six replicates. In 2016, two of the mesocosms had three plants germinate and produce abundant seeds. Recovery was more widespread in the spring of 2017 (Pastor, 2017a, b). It is informative that wild rice could begin to recover within two years after four years of sulfate concentrations markedly greater than the calculated protective concentration of 34 mg/L. Through 2013, the 300 mg/L sulfate treatment actually averaged 207 mg/L because of ongoing conversion of sulfate to sulfide (Myrbo et al., submitted-2). These observations support the idea that porewater sulfide and wild rice can recover after occasional one-year exceedances in sulfate concentrations above the standard.

Therefore, it is unlikely that one year of elevated sulfate will have a long-term negative effect on wild rice growth and reproduction, so long as sulfate concentrations do not remain elevated above the allowable annual average for multiple years in a row.

Furthermore, the available scientific evidence supports that even a one-year elevation in sulfide levels in the sediment porewater above 120 µg/L would not have a long-term negative effect on wild rice growth and reproduction, so long as sulfide concentrations do not remain elevated above 120 µg/L for multiple sequential years. Relatively poor reproduction in one year out of five or ten years is extremely unlikely

to have a long-term negative effect on the persistence of a wild rice population, because wild rice populations build up a seed bank in the sediment so that only a portion of dormant seeds germinate in any given year. In fact, wild rice is infamous for oscillating between low and high populations under natural conditions on a 3- to 5-year cycle (Pastor and Walker, 2006). The existence of the seed bank allows wild rice to recolonize a waterbody even if all growing plants are eliminated by an environmental disturbance in a given year (MDNR, 2008). For example, a June 2012 precipitation event completely eliminated wild rice in Kettle Lake (Carlton County), but the following year the density of wild rice was above average (55 stems per square meter, compared to a 10-year average of 41 stems per square meter (Vogt, 2017), (not counting two years of zero density, 2012 and 2016).

Based on the foregoing, MPCA is proposing a one in ten year exceedance frequency as reasonable and protective of the beneficial use.

Appendix 1. Other potential consequences of increasing sulfate concentrations

This TSD largely restricts the discussion of negative effects of increased sulfate concentrations to the accumulation of toxic concentrations of sulfide in the sediment porewater of wild rice beds.

There are two other potential pathways of concern regarding elevated sulfate: potential direct toxicity of sulfate to aquatic organisms (e.g. Wang et al., 2016), and negative consequences of sulfide production even if porewater sulfide remains at low levels. These should be kept in mind even if sulfate concentrations could be increased at a site without harming wild rice due to impacts on porewater sulfide. These considerations, while not directly relevant to the refinement of the wild rice sulfate standard, represent additional insight gained from the MPCA-sponsored study that can be used to inform water quality management decision-making.

The scientific literature includes evidence for multiple hypothesized effects associated with the conversion of sulfate to sulfide in sediment. The outdoor mesocosm experiment conducted by Pastor et al. (2) presented an opportunity to evaluate multiple hypotheses simultaneously. Data from the mesocosms were obtained in August 2013 and 2015, the results of which are reported in Myrbo et al. (submitted-2). In addition, the correlations observed in the MPCA-sponsored field survey are consistent with the science summarized below.

Stoichiometric releases associated with sulfate-enhanced decomposition of organic matter

The shallow-water aquatic ecosystems in which wild rice grows usually accumulate significant concentrations of organic matter; in the MPCA field survey the median concentration of organic matter is 25% on a dry weight basis. The organic matter, which is plant litter that has not fully decomposed, accumulates because decomposition by the microbial community is greatly slowed by limited availability of the principal terminal electron acceptors (TEAs) – oxygen, nitrate, oxidized manganese, oxidized iron, sulfate, and carbon dioxide – which are thermodynamically favored in that order (Froelich et al., 1979). A major reason wetlands accumulate organic matter is that oxygen availability, and therefore decomposition, is significantly reduced in water-saturated sediments; oxygen is consumed by bacteria within a few millimeters into sediment, and is supplied at a very slow rate because of its low solubility in water (10 ppm, compared to 210,000 ppm in the atmosphere). The next thermodynamically-favored TEA, nitrate, is not elevated in most wild rice waters, and therefore is not generally available to support decomposition. Manganese concentrations in sediment are usually minor compared to iron, and will not be discussed here. Ferric iron, the next thermodynamically favored TEA, is mostly present as a solid, and therefore of limited availability to bacteria. Even small increases in sulfate availability can increase bacterial activity, increasing decomposition in rough proportion to sulfate concentrations (Perry et al., 1986; Cook et al., 1986). The production of methane, which occurs when carbon dioxide is utilized as a TEA, is the least thermodynamically favorable and generally occurs when the other TEAs are depleted.

Plants have a relatively constant ratio among the important building blocks of carbon (C), nitrogen (N), and phosphorus (P), which are therefore released proportionally during decomposition of sedimentary organic matter. When sulfate is available, release of C, N, and P can be proportional to sulfide production (Froelich et al., 1979; Weston et al., 2006; Myrbo et al., submitted-2). Other plant components, including potassium (Lamers et al., 1998) and silica (Weston et al., 2006), are also released into solution during decomposition of plants. In the controlled outdoor mesocosm experiment, Myrbo et al. (submitted-2) found that porewater sulfide concentrations are significantly related to increases in products of decomposition in the surface water: total phosphorus, total nitrogen, dissolved organic

carbon, and alkalinity. In addition, enhanced decomposition also increased the concentration of total mercury in the overlying water—mercury that presumably had been associated with the solid organic matter that decomposed. Mercury would have been delivered to the sediment from the atmosphere, either directly in precipitation or dry deposition to the emergent plant material, or indirectly from the watershed (Wiener et al., 2006). In the surface water of the mesocosms, total mercury was highly correlated with dissolved organic carbon, which is known to have a high affinity for mercury (Ravichandran, 2004).

It is not surprising that sulfide production is statistically correlated with predicted changes in the controlled mesocosm experiment, where the only change was the sulfate concentration, and sediment and overlying water were initially the same among treatments. That statistically significant correlations with porewater sulfide are observed in the MPCA-sponsored field survey, given the great heterogeneity in landscapes across Minnesota (Moyle, 1956; Heiskary et al., 1987), reinforces the conclusion that sulfide production is an important process that can control the occurrence of wild rice. Although the importance of sulfide is clear to many wetland scientists, this knowledge is not yet widespread among environmental scientists in general. It is pertinent to repeat a quote from Chapter 1 of this TSD from a study in central New York State of the factors that control plant species distribution in a wetland: "…it is puzzling that there has not been more work to investigate the possible role of sulfide as a master variable controlling plant community composition within inland wetland ecosystems." (Simkin et al., 2013).

Among the 108 field sites sampled in the MPCA survey, sulfide production (measured as acid-volatile sulfide or porewater sulfide) is significantly correlated with porewater total phosphorus, ammonia, and silica, and surface water total nitrogen, total phosphorus, potassium, and alkalinity (Myrbo et al., in press-1). These correlations support the hypothesis that production of sulfide is associated with enhanced decomposition of sedimentary organic matter, releasing the nutrients, alkalinity, and mercury to porewater and surface water.

Sulfate-enhanced release of phosphorus from sediment iron through sulfide production

There are two processes through which increased sulfide production is associated with the release of phosphorus from solid phases in the sediment, thereby increasing concentrations in porewater and surface water: 1) enhanced decomposition of organic matter, releasing phosphorus as discussed above, and 2) the release of phosphorus as a result of the interaction of sulfide and iron in the sediment. The addition of sulfate can produce more sulfide, which is thought to interact with iron in several ways that solubilize phosphorus (Caraco et al., 1989; Smolders and Roelofs, 1993; Maynard et al., 2011). When surface water sulfate and sedimentary sulfide concentrations are low, phosphorus is associated with several different phases of iron (Smolders and Roelofs, 1993; Maynard et al., 2011). When sulfate concentrations increase, sulfide production increases in sediment porewater, which reacts with ferrous iron, precipitating as iron-sulfide compounds, which do not sorb phosphate effectively (Roden and Edmonds, 1997).

Potential for sulfate-enhanced increased production of methylmercury

Increased sulfide production, which can result from an increase in sulfate loading or concentration under certain conditions, has long been known to increase the conversion of inorganic mercury to methylmercury (Gilmour et al., 1992), a phenomenon confirmed in an ecosystem-level sulfate addition experiment in Minnesota (Jeremiason et al., 2006). Methylmercury is the form of mercury that bioaccumulates in fish. Increased production of methylmercury is a significant concern, given that bioaccumulation of methylmercury in fish is a major cause of water quality impairments in Minnesota,

resulting in a state-wide effort to reduce mercury contamination in fish (MPCA, 2007). Increased sulfide production not only has been shown to mobilize inorganic mercury from sediment, but also to increase the proportion of that mercury that is converted to methylmercury (Myrbo et al., submitted-2).

Other changes associated with conversion of sulfate to sulfide

The calculated numeric sulfate standard to protect wild rice should not be taken to mean that it is automatically defensible to increase average ambient sulfate concentrations to that level, even if wild rice would not be harmed. Indeed, even if the calculated sulfate concentration is not thought to be directly harmful to aquatic biota, and even if none of the three classes of chemical changes that are associated with increased sulfide production, described above, there may be other concerns about increasing sulfate concentrations and sulfide production.

The produced sulfide has a number of non-exclusive potential fates. The sulfide could 1) remain in the sediment porewater as free sulfide, 2) diffuse into the surface water, to be oxidized to sulfate, 3) be oxidized in the sediment, 4) volatilize to the atmosphere, or 5) react with metals (usually forming iron-sulfide compounds), forming insoluble precipitates in the sediment. The net concentration of sulfide remaining in the sediment can be quantified as acid-volatile sulfide (AVS), which can be a useful indicator of cumulative sulfide production.

Some of the consequences of sulfide production are not necessarily negative, and some have as yet poorly understood ramifications. For instance, one stoichiometric consequence of the conversion of sulfate to sulfide is the production of alkalinity. While it is not clear that additional alkalinity is negative, it is a change in the aquatic ecosystem, and some organisms seem to have different alkalinity optima (e.g., Moyle, 1945; Vestergaard and Sand-Jensen, 2000).

The production of alkalinity is not necessarily permanent, because the conversion of sulfate to sulfide is reversible. In many of the field survey sites most of the sulfide was precipitated as a solid when it reacted with iron, forming iron-sulfide compounds. If the waterbody dries and the sediment is exposed to oxygen in the atmosphere, the sulfide can be oxidized, and upon rewetting release sulfate as sulfuric acid, negating the alkalinity that had been produced earlier when the sulfide was produced. This has been documented in eastern North America (Kerr et al., 2012) and Australia, where the issue of restoration of wetlands with sulfidic sediment has been addressed (Ning et al., 2011). If the alkalinity had been produced gradually and washed downstream, the production of acid might not be buffered. Thus, even though the production of iron-sulfide solids detoxifies the porewater sulfide by removing it from solution, the accumulation of sulfidic sediment, which can be quantified as AVS, also represents the potential for episodic release of sulfate, which could be acidic.

Appendix 2. Wild Rice Seeds and Food Value

Part of the beneficial use established is the use of wild rice as a food source for wildlife. Information on the amount of wild rice needed to support wildlife, especially waterfowl, may be helpful in considering the beneficial use, and is provided here.

Literature estimates of wild rice seed weights and number of seeds per stem

Ranges for wild rice seed weights found in the scientific literature, other reports, and personal communications are shown in the Table A2-1. Similarly, Table A2-2 shows some literature ranges for the values for number of seeds per seed head in wild rice populations in Minnesota and Wisconsin. Although the ranges are quite large, means and medians of number of seeds per stem in natural wild rice waters are about 50 seeds per stem.

Table A2-1. Wild rice seed weight estimates found in the literature.

Seed weight estimates (dry weight in mg)	Description of paper or research	Reference
17.1 mg-42.3 mg (range of lakes and rivers) 35.39-37.81 mg (range in lakes) 20.63-23.77 mg (range in rivers) Seeds in lakes were 11.9 to 18.2 mg larger than their paired riverine populations. Mean seed mass in lake populations was 41 percent larger than in river populations.	Lacustrine and riverine populations of wild rice in northern Minnesota and Wisconsin, four river and four lake pairs	Eule-Nashoba, 2010; Eule-Nashoba et al., 2012
2011 24.6 (1.24) 2012 27.8 (0.9) 2013 29.7 (1.1)	Means of samples from six control tanks with standard errors in parentheses	Pastor, 2013
20-30 mg (dry) River rice may average somewhat lower than lake rice	Personal communication of literature values from natural stands and weighing of hand-harvested rice	David Schimpf, retired associate professor of biology at University of Minnesota Duluth and technical advisor to MPCA wild rice advisory committee

Table A2-2. Literature ranges of number of seeds per head.

# of grains per head	Description of research, study	Source
Range 19-115 grains per head on 14 stands of wild rice (all are lakes)	Survey of 14 stands of wild rice-Table 1 from 1941	Moyle, 1942, Fisheries Research Investigational Report # 40
Seed scars/panicle Range =23.9-132.8	Lacustrine and riverine populations of wild rice in northern MN and WI, four river and four lake pairs	Eule-Nashoba, 2010; Eule-Nashoba, 2012
12.75-102.35 number of seed scars/panicle Mean of seed scars/panicle 46.39 standard deviation of 29.7	17 wild rice populations in northern Wisconsin	Lu et al., 2005

The MPCA is using an estimate of 25 mg for wild rice seed weight and an estimate of 50 seeds per wild rice stem in the calculations to put into context the food value of wild rice. Table A2-3 shows the number of seeds and stems to support the daily energy needs for a dabbling duck.

Table A2-3. Seeds and stem values to support daily energy needs of a dabbling duck.

Mass of wild rice required to meet the daily energy expenditure of a dabbling duck	Daily Intake of seeds corresponding to 85 grams	Number of stems corresponding to 3,400 seeds (assuming 50 seeds per stem)
85 grams	3,400 seeds	68 stems

The MPCA initially considered criteria for identifying a wild rice water of ¼ acre of wild rice with an average density of 8 stems per square meter, or ½ acre of wild rice with 4 stems per square meter would meet at a minimum the food energy needs of a pair of ducks for two months. Although these criteria were not carried forward into the proposed rule, they support the MPCA's proposal that a single stem of wild rice (or other small amount) is insufficient to demonstrate the beneficial use and support identifying a water as a wild rice water.

Food value of wild rice

The nutritional value that the wild rice grain affords to waterfowl is equivalent to or exceeds the caloric energy values provided by many other wetland plants and agricultural grains (Sherfy, 1999; Gray et al., 2013). Wildlife researchers use these food energy values in determining species-specific daily energy expenditures for waterfowl supported by a given amount of food over a given management area. These calculations are often expressed as duck-energy days (or duck days) and represent the number of days a given amount of food will support a duck or group of ducks. These types of calculations have been used to build complex models to estimate the carrying capacity of large regions for a variety of species.

In its simplest form, duck-energy days can be determined by the following equation:

> Duck-energy days = Food available (grams dry weight) x True Metabolizable Energy (kcal/gram dry weight)
>
> Daily Energy Expenditure (kilocalories/day)

This can be simplified to solve for the amount of wild rice needed to meet the daily energy requirements of a single duck.

> Daily Energy Expenditure average dabbling duck (kcal/day) = g of wild rice needed by a duck for a day
>
> True Metabolizable Energy (kcal/g dry weight)

The MPCA is using a value of 294.35 kcal/day as the daily energy expenditure for an average dabbling duck (Reinecke and Kaminski, 2006) and a value of 3.47 kcal/g (Sherfy, 1999) for the True Metabolizable Energy of wild rice.

> 294.35 kcal/day divided by 3.47 kcal/g = 84.82 g wild rice needed to feed an average dabbling duck for one day.

While it is recognized that ducks do not only eat wild rice, the approximate 85 gram per day can be used to estimate the number of ducks that can theoretically feed on a given amount of wild rice. See below for details and discussion of values used for estimates of daily energy expenditure and true metabolizable energy and calculation of grams of wild rice needed to feed a dabbling duck for a day.

Daily Energy Expenditures

Daily Energy Expenditures (DEE) for waterfowl are calculated based on the strong relationship between body mass and basal metabolic rate (BMR) or resting metabolic rate (RMR) within and among species of birds (King, 1974; Prince, 1979; Miller and Eadie, 2006).

The Mississippi Alluvial Valley (MAV) is a waterfowl management area in the Lower Mississippi that is important to migrating and wintering waterfowl. Resource managers of the Mississippi Alluvial Valley Joint Venture have developed a DEE of 294.35 that is based on: 1) the daily energy requirements of an dabbling ducks during fall and winter and 2) the population goals in the MAV for seven dabbling ducks and the wood duck (Reinecke and Kaminski, 2006). Previously, the daily energy expenditures of the mallard were used as a surrogate for all the species found in the joint venture. This value is a reasonable estimate for daily energy expenditure for ducks eating wild rice as the dabbling ducks found in the Mississippi Alluvial Valley include many of the same species found in Minnesota and include species that consume wild rice such as the mallard and blue-winged teal.

True Metabolizable Energy (energy value of waterfowl foods)

True metabolizable energy (TME) is recognized as a valid expression of dietary quality that can be measured rapidly and reliably (Miller and Reinecke, 1984). The TME of waterfowl foods is an important component for accurate assessments of waterfowl energetics. It can be calculated indirectly using a regression model, or measured experimentally by feeding birds a controlled diet and measuring excretory energy.

There is a lack of TME values for common waterfowl food and species. The species studied most frequently include the Mallard, American Black Duck, Northern Pintail, Blue-Wing Teal, Carolina Wood Duck and Canada Goose. Eadie et al., cite values of true metabolizable energy (TME) of white rice as ranging from 3.34 to 3.76 kcal/gram. (Eadie et al., 2008). One study reports a mean value of 3.47 kilocalories/gram (3.07 to 3.92 range) for the TME of wild rice (*Zizania aquatica*)(Sherfy, 1999) and was based on blue-winged teal. Mallard values were not available for wild rice.

The MPCA is planning to use the mean TME of 3.47 kilocalories per gram for wild rice reported in 1999 by Sherfy as the TME for wild rice.

Calculation of amount of wild rice needed to an average dabbling duck for a day

> 294.35 kcal/day divided by 3.47 kcal/g = 84.82 g wild rice needed to feed an average dabbling duck for one day.

Although ducks do not only eat wild rice, this value can be used to estimate the number of ducks that can obtain food from a given amount of wild rice. A wild rice water of 0.25 acres of wild rice with a stem density of eight stems per square meter or 0.50 acres with a stem density of four stems per square meter would meet at a minimum the food energy needs of a pair of ducks for two months.

Appendix 3. Results for the 2015 sediment pilot study

Raw sediment analytical data, selected statistics and calculated values of sulfate for sites sampled during the 2015 sediment pilot study. Individual ID = individual sediment core sample; % TOC = percent sediment Total Organic Carbon; TEFe = sediment Total Extractable Iron; Composite = Average of 5 individual samples composited as one sample; Calculated sulfate = sulfate value calculated using the equation: Sulfate = 0.0000121 x ($Iron^{1.923}$/$Organic\ Carbon^{1.197}$); Organic Carbon = TOC and Iron = TEFe.

Table A3-1. Results for sediments collected from Bowstring River.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	16.8	4230				3.9
1-2	16.9	4050				3.5
1-3	19.1	4230				3.3
1-4	17.2	4140				3.6
1-5	16.3	3600	17.3	4050	3.5	3.0
1-6	15.0	2520				1.6
1-7	14.4	3330				3.0
1-8	13.5	4500				5.7
2-10	13.5	3870				4.3
2-11	16.0	4140	14.5	3672	3.5	4.0
2-12	15.1	4590				5.2
2-13	17.0	4320				4.0
2-14	15.7	4320				4.4
2-15	14.0	3510				3.4
2-16	14.8	3690	15.3	4086	4.1	3.5
2-9	15.6	3870				3.6
3-17	14.2	3780				3.8
3-18	12.6	4320				5.7
3-19	15.6	3870				3.6
3-20	15.1	4680	14.6	4104	4.3	5.4
3-21	19.0	3060				1.8
3-22	16.4	3150				2.3
4-23	17.7	3240				2.2
4-24	18.4	2160				1.0
4-25	19.7	4500	18.2	3222	2.1	3.6
Mean=	16.0	3827				3.6
Std. Dev=	1.9	640				1.2
CV (%)=	12%	17%				34%
min=	12.6	2160				1.0
max=	19.7	4680				5.7

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
2.0	3.3	3.6	3.9	5.3

Table A3-2. Results for sediments collected from Clearwater River.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	19.2	11700				23.4
1-2	21.0	15300				35.3
1-3	16.6	8370				14.6
1-4	21.0	12600				24.3
1-5	16.2	13500	18.8	12294	26.4	37.8
1-6	20.3	18900				55.1
1-7	25.4	13500				22.1
2-10	19.9	14400				33.5
2-11	16.5	10800				24.1
2-12	17.5	11700	19.9	13860	31.1	26.2
2-13	25.3	12600				19.4
2-14	18.9	18000				54.7
2-15	24.5	12600				20.2
2-8	21.2	12600				24.0
2-9	17.7	15300	21.5	14220	29.7	43.3
3-16	21.8	9900				14.6
3-17	21.5	12600				23.6
3-18	21.0	13500				27.7
3-19	22.5	12600				22.3
3-20	18.4	11700	21.0	12060	22.3	24.7
3-21	22.2	13500				25.9
3-22	19.0	19800				65.3
3-23	20.9	12600				24.4
3-24	19.9	11700				22.4
3-25	20.2	16200	20.4	14760	34.0	41.2
Mean=	20.3	13439				30.0
Std. Dev=	2.5	2652				13.0
CV (%)=	12%	20%				43%
min=	16.2	8370				14.6
max=	25.4	19800				65.3

Percentiles for individual calculated sulfate values				
10th	30th	50th	70th	90th
19.7	23.5	24.4	32.3	50.1

Table A3-3. Results for sediments collected from Hesitation Wildlife Management Area.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
T1	22.7	45000				255.7
T10	25.6	27000				82.9
T11	23.9	36000				156.5
T12	23.4	29700				110.9
T13	24.7	26100	24.1	32760	129.5	81.1
T14	25.9	36000				142.2
T15	23.0	29700				113.2
T16	25.4	31500				112.6
T17	23.6	39600				190.9
T18	22.9	39600	24.2	35280	148.6	197.9
T19	21.4	25200				90.0
T2	19.8	48600				349.2
T20	19.3	37800				222.1
T21	22.1	30600				125.8
T22	22.1	34200	20.9	35280	176.4	155.8
T23	19.0	74700				838.5
T24	20.7	58500				472.9
T25	20.0	56700				464.1
T3	18.3	69300				759.2
T4	16.1	41400	18.8	60120	558.6	328.6
T5	17.4	27000				131.6
T6	17.6	24300				106.0
T7	20.9	26100				99.0
T8	24.7	25200				75.8
T9	25.1	32400	21.1	27000	104.3	120.5
Mean=	21.8	38088				231.3
Std. Dev=	2.8	13850				204.5
CV (%)=	13%	36%				88%
min=	16.1	24300				75.8
max=	25.9	74700				838.5

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
85.7	112.7	142.2	217.2	469.4

Table A3-4. Results for sediments collected from Mission Creek.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	3.1	11700				207.0
1-2	3.6	15300				294.0
1-3	5.1	16200				213.7
1-4	4.5	18900				333.7
1-5	3.7	11700	4.0	14760	240.1	170.4
1-6	2.8	10800				203.9
1-7	3.3	13500				255.8
1-8	4.0	12600				175.1
2-10	3.3	17100				400.1
2-11	3.6	18000	3.4	14400	277.0	392.7
2-12	4.2	17100				303.2
2-13	1.5	7470				202.6
2-14	3.2	14400				302.8
2-15	2.6	11700				251.9
2-16	4.5	16200	3.2	13374	259.2	250.1
2-9	4.4	14400				205.4
3-17	4.0	17100				314.0
3-18	3.9	18000				359.3
3-19	5.3	19800				298.1
3-20	5.2	19800	4.6	17820	293.0	307.8
3-21	4.6	20700				391.3
3-22	5.0	22500				413.4
3-23	3.8	22500				582.0
3-24	2.4	10800				247.0
3-25	3.6	14400	3.8	18180	375.0	263.4
Mean=	3.8	15707				293.6
Std. Dev=	0.9	3882				93.6
CV (%)=	24%	25%				32%
min=	1.5	7470				170.4
max=	5.3	22500				582.0

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
203.1	247.6	294.0	312.8	397.1

Table A3-5. Results for sediments collected from Monongalia Lake.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	4.3	3330				12.4
1-2	9.9	5310				11.3
1-3	19.2	6930				8.6
1-4	2.4	2610				15.7
1-5	23.1	8910	11.8	5418	9.6	11.1
2-6	2.5	2610				14.8
2-7	19.1	6750				8.2
2-8	10.6	4950				9.1
3-10	18.7	6210				7.2
3-11	12.5	9000	12.7	5904	10.3	23.6
3-9	17.4	7740				11.9
4-12	14.7	5760				8.3
4-13	20.1	6300				6.7
4-14	24.1	4410				2.7
4-15	24.7	7110	20.2	6264	6.6	6.7
5-16	19.3	7830				10.8
5-17	23.6	8820				10.6
5-18	22.6	6300				5.9
6-19	27.2	6570				5.1
6-20	25.1	4950	23.6	6894	6.6	3.3
7-21	21.6	5760				5.2
7-22	8.5	4050				8.1
8-23	14.8	4950				6.1
8-24	18.3	6750				8.6
8-25	17.9	7110	16.2	5724	7.3	9.8
Mean=	16.9	6041				9.3
Std. Dev=	7.1	1792				4.4
CV (%)=	42%	30%				47%
min=	2.4	2610				2.7
max=	27.2	9000				23.6

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
5.1	6.8	8.6	10.7	13.8

Table A3-6. Results for sediments collected from Mississippi River.

Individual ID	TOC (% Dry)	TEFe (mg/kg)	TOC Composite (% Dry)	TEFe Composite (mg/kg)	Calculated Sulfate Composite (mg/L)	Calculated Sulfate Individual (mg/L)
1-1	19.3	4680				4.0
1-10	22.0	4770				3.5
1-11	15.1	5310				6.8
1-12	12.4	6120				11.4
1-13	16.6	5940	17.1	5364	6.0	7.6
1-14	11.8	5760				10.7
1-15	14.1	6030				9.5
1-16	12.3	5670				9.9
1-17	9.7	5220				11.3
1-18	9.2	5580	11.4	5652	10.8	13.7
1-19	6.1	4860				17.2
1-2	16.0	5130				6.0
1-3	19.4	5130				4.7
1-4	14.0	5400				7.7
1-5	16.9	5220	14.5	5148	6.8	5.8
1-6	20.8	5220				4.5
1-7	17.5	5130				5.4
1-8	17.6	5850				6.9
	16.8	5670				6.8
2-20	18.6	5130	18.3	5400	5.6	5.0
2-21	15.8	5220				6.3
2-22	17.8	5490				6.0
2-23	15.2	5940				8.4
2-24	16.5	6300				8.5
2-25	7.6	5040	14.6	5598	7.9	14.0
Mean=	15.2	5432				8.1
Std. Dev=	4.0	436				3.4
CV (%)=	27%	8%				43%
min=	6.1	4680				3.5
max=	22.0	6300				17.2

Percentiles for individual calculated sulfate values

10th	30th	50th	70th	90th
4.6	6.0	6.9	9.3	12.8

Appendix 4. Statistical characterization of wild rice density in 10 wild rice waters over 12 years

The long-term data collection by the 1854 Treaty Authority (Vogt, 2017) constitutes the best available information on variation over time in average wild rice density among a variety of waterbodies. From 2005 to the present, the 1854 Treaty Authority conducted surveys of wild rice density in ten wild rice waters using a consistent methodology (described in Kjerland, 2015). Surveys were conducted in late August or early September when the rice was standing and reaching maturity. Wild rice density in each waterbody was determined from at least 20 sample plots of an area of 0.5 m^2 each. The annual average density for each of the ten waters (Table A4-1) ranged from a minimum of zero (which occurred three times) to a maximum of 408 stems/m^2, with an average of 46 stems/m^2. The median, or most typical, density was 30 stems/m^2 (Table A4-2).

Table A4-1. Average wild rice density in ten wild rice waters, as monitored by the 1854 Treaty Authority (Vogt, 2017). Density is average stems per square meter from a minimum of 20 fixed sampling points.

Year	Big Rice Lake	Breda Lake	Cabin Lake	Campers Lake	Cramer Lake	Kettle Lake	Little Rice Lake	Round Island Lake	Stone Lake	Vermilion River
2005	58	80	86	29	80	11	61	408	48	88
2006	13	66	56	46	58	36	16	95	17	201
2007	11	53	82	59	46	76	30	40	42	116
2008	11	69	21	67	28	32	14	11	25	153
2009	4	85	26	75	29	19	6	44	10	90
2010	7	76	99	74	74	65	12	88	38	42
2011	5	42	9	27	28	8	11	154	26	87
2012	7	15	20	0	17	0	12	45	5	75
2013	11	25	14	17	17	55	9	60	5	113
2014	5	61	28	35	29	42	4	37	26	97
2015	4	49	27	40	36	64	24	23	25	99
2016	4	11	20	24	85	0	13	24	11	100

Table A4-2. Statistical characterization of wild rice density in 10 wild rice waters over 12 years (data from Table A4-1).

percentile	stems/m^2
10	7
15	11
25	14
50	30
75	65
85	75
90	88

Appendix 5. EC10 values from MPCA-sponsored hydroponic data

Data used

Dr. John Pastor conducted three experiments where his team added sulfate to hydroponic wild rice at concentrations of 0 to 2,880 µg/L, described in Pastor et al. (2017). Pastor's team measured the weight of the plants and the mean sulfide at the beginning of the study and at the end of the study. The variables used for this analysis are:

To measure sulfide concentration:

- Mean initial sulfide concentration (µg/L)
- Arithmetic time weighted mean (TWM) sulfide concentration (µg/L)
- Geometric TWM sulfide concentration (µg/L)

To measure plant growth:

- Weight change (mg)
- Weight gain: the weight change with any weight loss set to 0 (mg)

Curve fitting

Logistic regressions and estimates of EC10, with 95% confidence intervals, were conducted with routine *drc* (R, Ritz et al., 2015).

Predicting weight gain from mean initial sulfide

Fig. A5-1 shows the log logistic curve fits for both predicting the weight change (Fig. A5-1A) and predicting the weight gain (Fig. A5-1B).

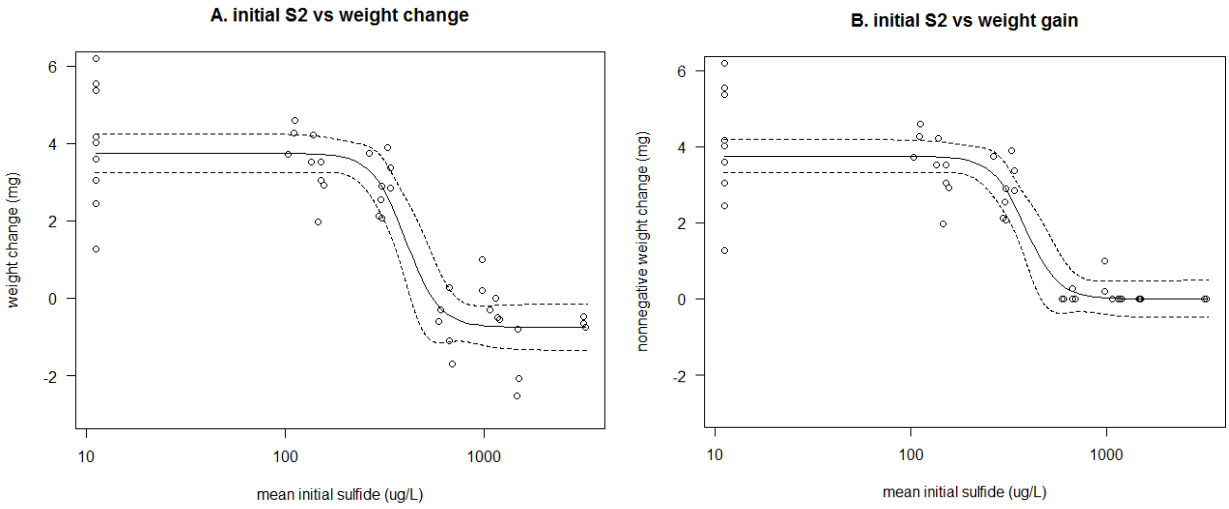


Figure A5-1. Logistic regressions using initial sulfide hydroponic concentration to predict plant growth. A) weight change. B) weight gain.

In both graphs that the weight change stays constant until well over 100 µg/L initial sulfide. The resulting EC10, EC20, and EC50 values are in Table A5-1. While the EC values are lower when using weight gain vs weight change, the numbers are not significantly different. For both, concentrations below 250 µg/L look to be protective.

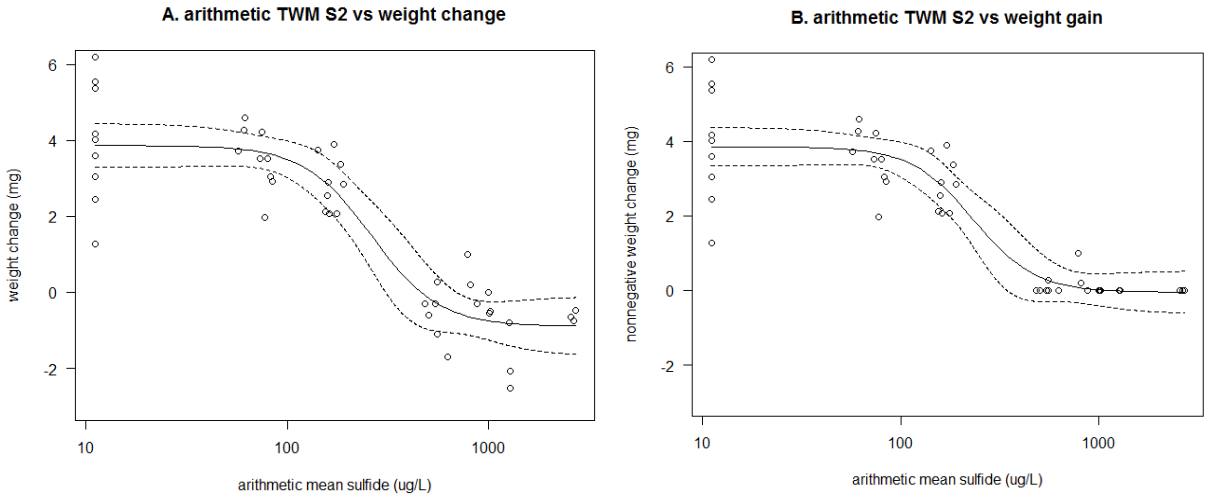
Table A5-1. EC10, EC20, and EC50 values for initial mean sulfide hydroponic concentration.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.37 mg	255 µg/L	<11 µg/L - 322 µg/L	3.465 mg	251 µg/L	<11 µg/L - 285 µg/L
EC20	2.99 mg	296 µg/L	235 µg/L - 363 µg/L	3.080 mg	294 µg/L	234 µg/L - 353 µg/L
EC50	1.87 mg	378 µg/L	324 µg/L - 474 µg/L	1.925 mg	384 µg/L	331 µg/L - 486 µg/L

Predicting weight gain from arithmetic time weighted mean sulfide concentration

Fig. A5-2 shows the log logistic curve fits for both predicting the weight change (Fig. A5-2A) and predicting the weight gain (Fig. A5-2B).

Figure A5-2. Logistic regressions using arithmetic time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.



In both graphs that the weight change starts to decrease around 100 µg/L. The resulting EC10, EC20, and EC50 values are in Table A5-2 below. Once again, the EC values for weight gain (i.e. no negative weight change) are lower than the EC values for weight change (which allows for weight loss). However, the numbers are similar, and the confidence intervals are large enough to confirm they are not significantly different. When the arithmetic TWM sulfide concentration is used, the EC10 is between 100 and 110 µg/L.

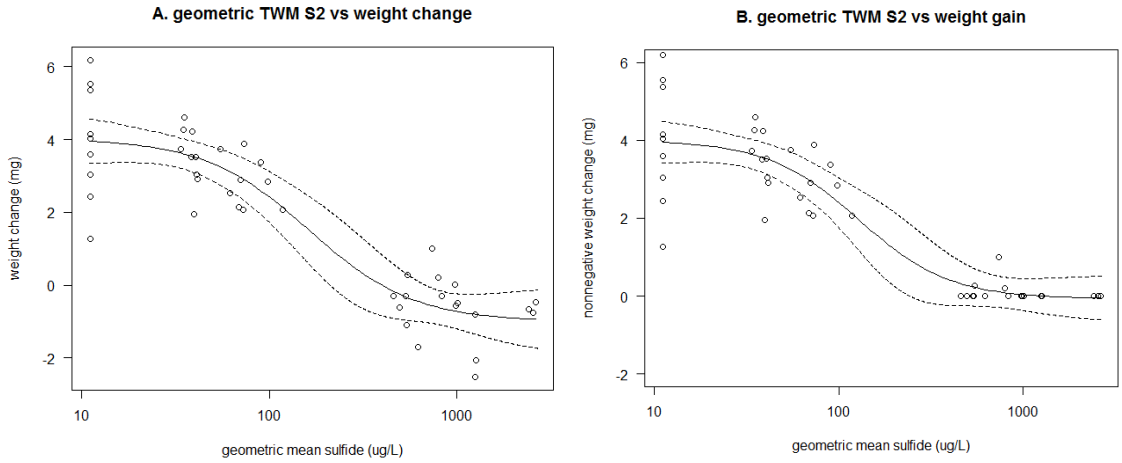
Table A5-2. EC10, EC20, and EC50 values for arithmetic TWM sulfide hydroponic exposures.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.474 mg	103 µg/L	<11 µg/L - 162 µg/L	3.465 mg	106 µg/L	<11 µg/L - 158 µg/L
EC20	3.088 mg	140 µg/L	95 µg/L - 195 µg/L	3.080 mg	141 µg/L	98 µg/L - 188 µg/L
EC50	1.930 mg	227 µg/L	174 µg/L - 312 µg/L	1.925 mg	231 µg/L	182 µg/L - 335 µg/L

Predicting weight gain from geometric time weighted mean sulfide concentration

Fig. A5-3 shows the log logistic curve fits for both predicting the weight change (Fig. A5-3A) and predicting the weight gain (Fig. A5-3B).

Figure A5-3. Logistic regressions using geometric time-weighted mean (TWM) sulfide hydroponic concentrations to predict plant growth. A) weight change. B) weight gain.



The geometric time weighted mean results in lower mean estimates of sulfide, and therefore, the curve has almost no "plateau", but shows an effect on wild rice growth almost immediately. As a result, the EC10 values are very low (around 40 µg/L), and have tighter confidence intervals than the other two estimates of sulfide concentration.

Table A5-3. EC10, EC20, and EC50 values for geometric TWM sulfide hydroponic exposures.

	Wt change	Estimate	In 95% CI	Wt gain	Estimate	In 95% CI
EC10	3.558 mg	38 µg/L	<11 µg/L - 67 µg/L	3.551 mg	39 µg/L	<11 µg/L - 66 µg/L
EC20	3.163 mg	59 µg/L	37 µg/L - 96 µg/L	3.157 mg	60 µg/L	40 µg/L - 90 µg/L
EC50	1.977 mg	128 µg/L	89 µg/L - 216 µg/L	1.973 mg	127 µg/L	91 µg/L - 224 µg/L

Appendix 6. EC10 estimates from experimental mesocosms

Data used

Dr. John Pastor conducted a multi-year experiment where his team added sulfate to outdoor mesocosms in which wild rice grew in natural sediment at five different levels of sulfate (six replicates of each: control, 50, 100, 150, and 300 mg/L). Porewater sulfide was measured in the sediment of each of the 30 mesocosms in August, 2013. The experiment had been initiated in June, 2011.

The variables used for this analysis are:

Porewater sulfide.

To quantify wild rice response:

- Percent of filled (viable) seeds
- Number of plants that emerged from the sediment

Percent filled seeds as a function of porewater sulfide

Using all the data, the EC10 using percent filled seeds and the baseline sulfide of 69.28 µg/L is:
288 µg/L with a 95% confidence interval of (0, 648).
However there was a statistical outlier, mesocosm #29, which had a porewater sulfate of 1180 µg/L but still had 53.5% of the seeds filled. The number of plants that emerged was low. Since this mesocosm had a Cook's distance of 0.6, which was twice as high as the next highest distance (Fig. A6-1), the regression was recalculated without mesocosm #29. Without mesocosm #29, the EC10 is:
228 µg/L with a 95% confidence interval of (0, 414) (Fig. A6-2).

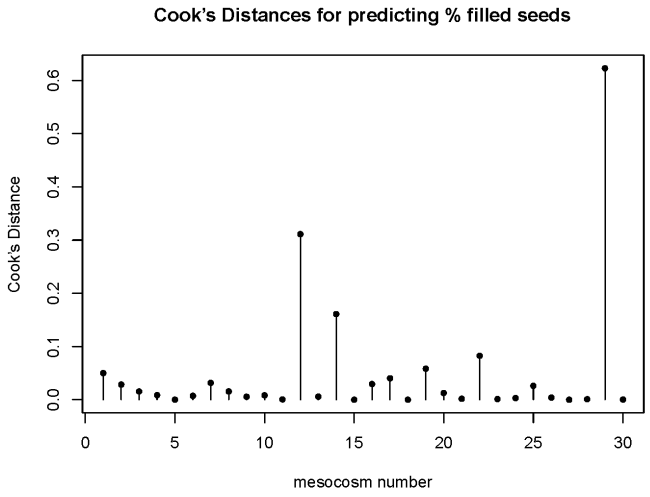


Figure A6-1. Cook's distances for regression of percent filled seeds against porewater sulfide.

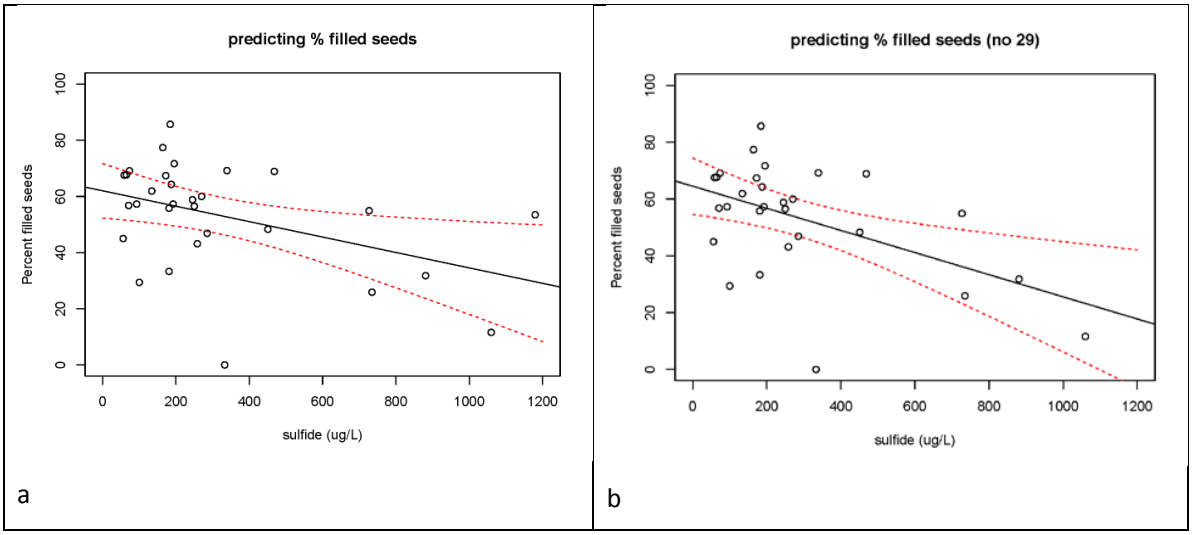


Figure A6-2. Regressions of percent filled seeds against porewater sulfide in wild rice mesocosms sampled August 2013 (Pastor et al., 2017), with all data (a), and without outlier mesocosm #29 (b).

Number of emerged plants as a function of porewater sulfide

The EC10 using number of emerged plants does require a log transformation of sulfide in order to fit a linear model. When this is done, the EC10 is:
121 µg/L with a 95% confidence interval of (6, 241) (Fig. A6-3).

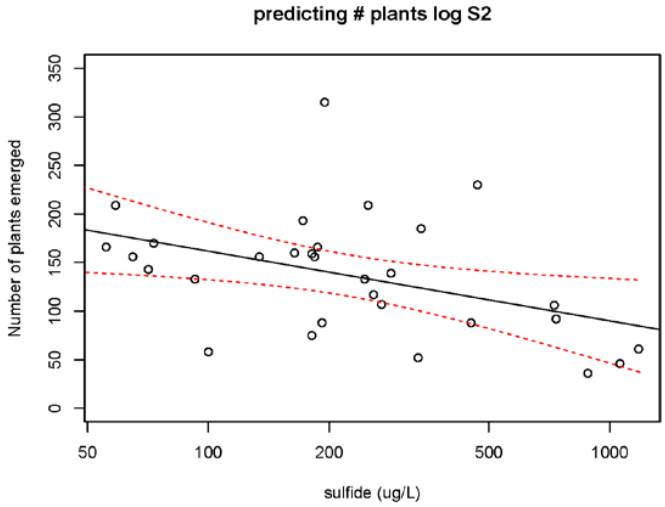


Figure A6-3. Regressions of number of plants emerged against porewater sulfide in wild rice mesocosms sampled August 2013 (Pastor et al., 2017).

Appendix 7. Protective sulfide estimates from MPCA-sponsored field survey

Goal of analysis

Utilizing the large dataset from the MPCA-sponsored wild rice field study, to investigate the relationships between potential protective sulfide levels and error rates, wild rice presence, and wild rice density.

Data used

The Class B data set from the wild rice field study was analyzed to identify potential protective sulfide concentrations. This data set includes 108 different sites measured as close to mid-August as possible. Relevant measurements include:

- Porewater sulfide (in µg/L)
- Wild rice density (stems/m^2)

Potential protective sulfide concentrations

Using the wild rice field survey data, three different methods were used to identify potential protective sulfide concentrations: (1) EC10 estimates from regressions, (2) Visual examination of a graphical representation of the proportion of sites with wild rice present, and (3) Change-point analysis.

1. EC10 estimates from regressions of field data:

The binary logistic regression that relates porewater sulfide to the presence and absence of wild rice can be calculated a number of different ways, producing different EC10 estimates of protective sulfide concentrations. The production of multiple EC10 estimates suggests that the selection of a protective concentration of sulfide should be the outcome of weighing multiple lines of evidence, rather than relying on a single calculation. Each estimate has a range of uncertainty. A reasonable protective sulfide concentration lies within the overlapping uncertainty ranges. An exploration of uncertainty around the estimates was recommended by the independent peer review panel (ERG 2014, p. 6).

Initially, MPCA conducted binary logistic regressions of wild rice presence/absence against linear sulfide concentrations. However, re-examination of the data used in the logistic regression found that porewater sulfide concentrations are skewed (Fig. A7-1a), and that a log-10 transformation would approximate normality (Fig. A7-1b).

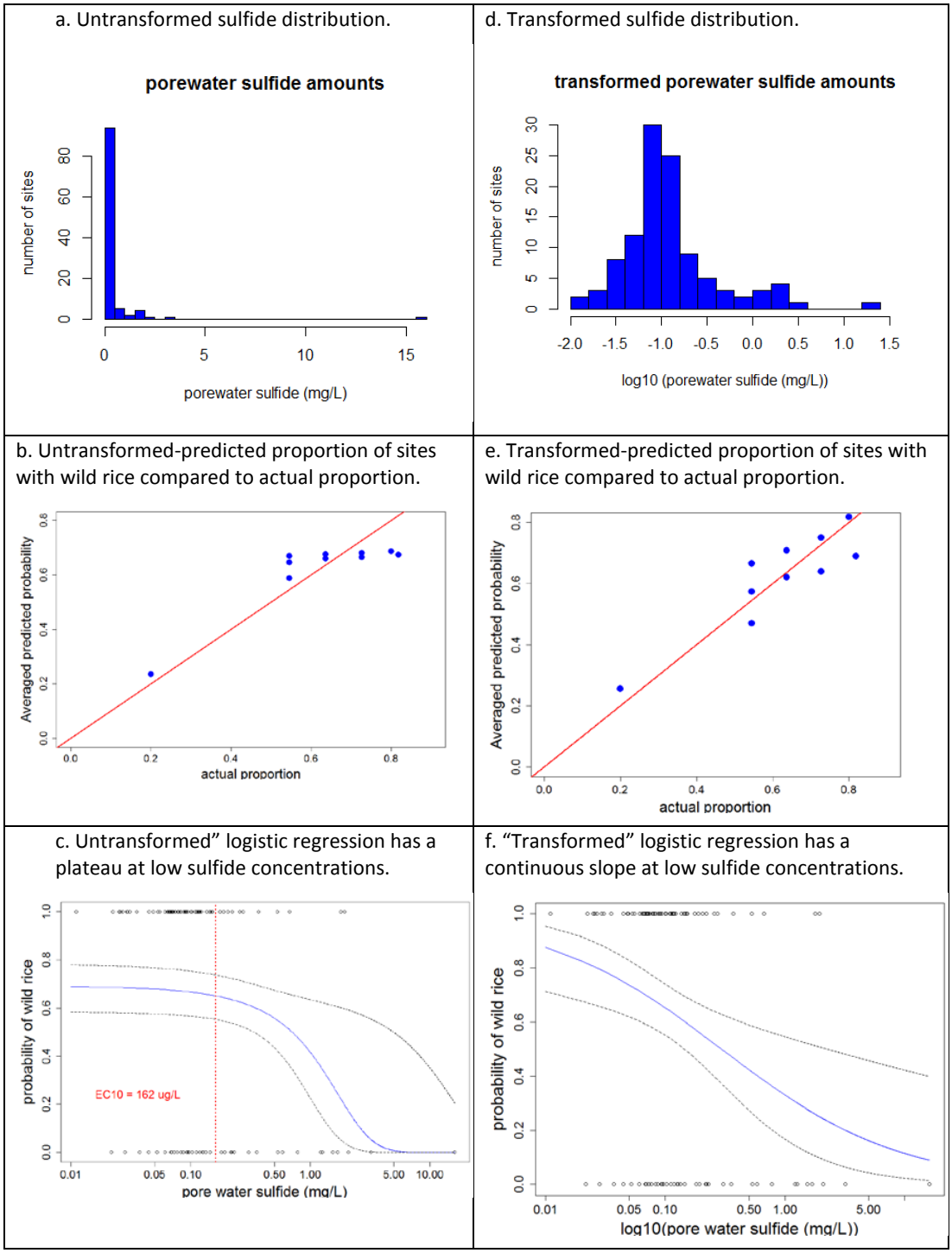


Figure A7-1. Comparison of the use of untransformed data in logistic regression (a-c) to the use of log-10 transformed data (d-f). Sulfide is graphed in both c and f on a log scale, but the modeling used untransformed data in c. In b and e the data are divided into deciles in order to assess the accuracy of the logistic prediction.

It is apparent that transforming the data results in more accurate predictions of the proportion of sites with wild rice (the data follow the 1:1 line better in Fig. A7-1e than in Fig. A7-1b). However, the logistic curve based on transformed sulfide data (Fig. A7-1f) is problematic for the calculation of an EC10

because of the absence of a plateau. Calculation of an EC10 assumes that there is a plateau, or baseline, of "no effect" of a stressor, from which a 10 percent effect adverse can be calculated. But the re-calculated logistic curve (Fig. A7-1 f) exhibits a continuous slope as sulfide declines, all the way down to the analytical reporting limit of 11 µg/L, which makes it difficult to identify a "baseline" probability from which to calculate a 10 percent effect. To complete the exercise of calculating an EC10, MPCA assumed a baseline probability based on the proportion of sites with wild rice for the 10 sites with the lowest sulfide concentration (8 out of 10 sites had wild rice, a baseline probability of 0.80).
Because low-transparency sites (< 30 cm) generally do not support wild rice regardless of how low sulfide is in the porewater, it is likely more accurate to calculate an EC10 for sulfide from a data set that does not include low-transparency sites that did not support wild rice because of low light (Fig. A7-2b).

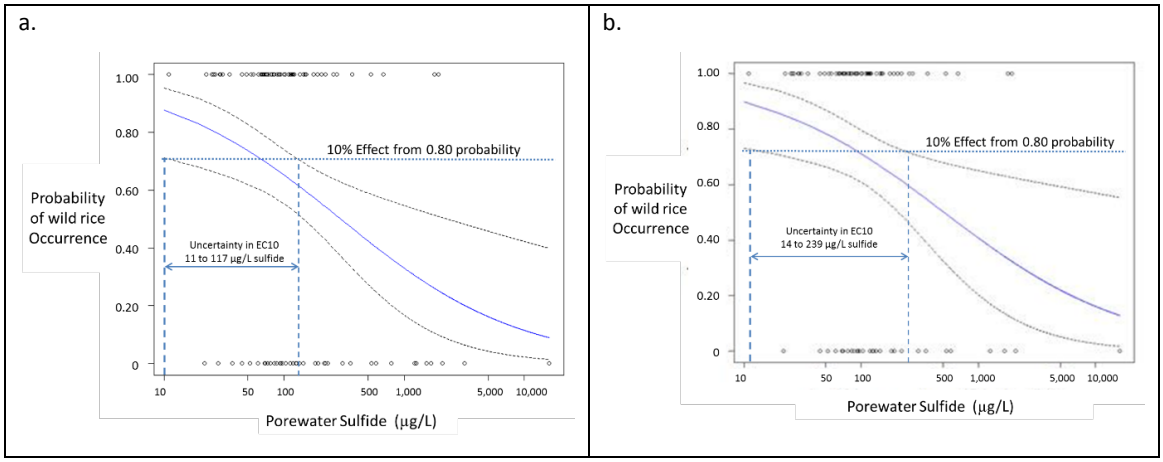


Figure A7-2. a. Logistic regression of all data in Class B, showing the 10% effect horizontal line. b. Logistic regression of all data with transparency > 30 cm. The horizontal 10% effect lines span the distance between the lower and upper 95% confidence intervals (Table A7-1), which is the indication of uncertainty (the confidence intervals shown are calculated for the probability at a given sulfide concentration, not for uncertainty in sulfide for a given effect level).

The calculated EC10 values range from 58 µg/L (all 108 sites) to 93 µg/L (the 96 sites with transparency greater than 30 cm). The uncertainty of the EC10 estimates was quantified by identifying the range of sulfide concentrations that contain a given EC10 wild rice proportion in their 95% confidence interval based on the binary logistic regression (Fig. A7-1; Table A7-1). Note that the uncertainty around the calculated EC10 values is relatively large, ranging from the sulfide reporting limit in the field survey, < 11 µg/L, to 239 µg/L (Table A7-1).

Table A7-1. Calculated sulfide EC10 values, based on wild rice presence/absence.

Data Set	EC_{10} (µg/L)	Uncertainty of sulfide concentrations around the EC10 (µg/L)	
		Lowest value within the 95% CI	Highest value within the 95% CI
All Sites (N=108)	58	< 11	117
Sites with transparency > 30 cm (N=96)	93	14	239

The peer review panel concluded that the MPCA field survey provided some of the best data available to investigate the relationship between wild rice and sulfide, and recommended that MPCA conduct a statistical analysis of the probability of wild rice occurrence as a function of the porewater sulfide levels (ERG, 2014). Binary logistic regression (BLR), described in Part B of Chapter 1, is "binary" in the sense that it classifies field sites as having, or not having, a wild rice population – the density of the wild rice is irrelevant to the classification. It is useful to note that Minnesota lakes with wild rice that are monitored by the 1854 Treaty Authority average 46 stems/m^2 with a median density of 30 stems/m^2 (Appendix 4), and in the MPCA-sponsored field survey, average wild rice density was 51 stems/m^2 with a median density of 41 stems/m^2 (Myrbo et al., in press-1).

MPCA's use of the binary logistic regression for the calculation of an EC10 was questioned in a comment on the March 2015 Draft Proposal as a non-standard statistical technique (MCC, 2015). MPCA found no objections raised in the scientific literature when this same statistical technique was used to assess the effect of selenium on mallard egg viability and duckling mortality (Adams et al., 2003). The Adams et al. (2003) study was subsequently cited favorably in an EPA guidance document (EPA, 2007); this guidance was subsequently explicitly approved by the EPA Science Advisory Board as an exceptional analysis of toxicity in a field setting: "Toxicity in wildlife from metals exposures is generally poorly understood and is rarely quantified in field settings. A few notable exceptions are those mechanisms described in avian waterfowl exposure to [selenium] (Adams et al., 2003)…" Thus, it appears that MPCA's statistical analysis of the wild rice field data, although perhaps not traditional, is supported by the scientific literature, EPA guidance, and the Science Advisory Board.

A protective sulfide concentration was identified as a 10% decrease (a 10% effect concentration, or EC10) from control conditions using a logistic regression where the probability of wild rice presence was predicted against log 10 transformed sulfide concentration (Class B data with water transparency > 30 cm data). The EC10 was 93 µg/L (95% confidence interval of 14 -239 µg/L). However, the "control" condition, or baseline, was difficult to define, since there was no range of sulfide concentrations where the probability of wild rice was constant. Therefore, the EC10 of 93 µg/L is misleadingly precise.

2. Visual examination of a graphical representation of the proportion of field sites with wild rice present against sulfide concentrations:

The visual examination identified a protective sulfide concentration of 120 µg/L. Focusing on sulfide between 20 and 1000 µg/L, the proportion of sites with wild rice present above each measured sulfide concentration was graphed for (1) all sites or (2) for all sites with water transparency greater than 30 cm.

(30 cm is identified as a threshold because 11 of 12 sites with lower transparency did not have wild rice; Myrbo et al. (submitted-2) identified water transparency as one of the environmental variables aside from porewater sulfide that controls wild rice presence in Minnesota waterbodies). The proportions above each sulfide concentration were graphed versus the sulfide concentration. In both graphs, a potential protective sulfide concentration was identified as a dip at 120 µg/L in the proportion of sites with wild rice (Fig. A7-3).

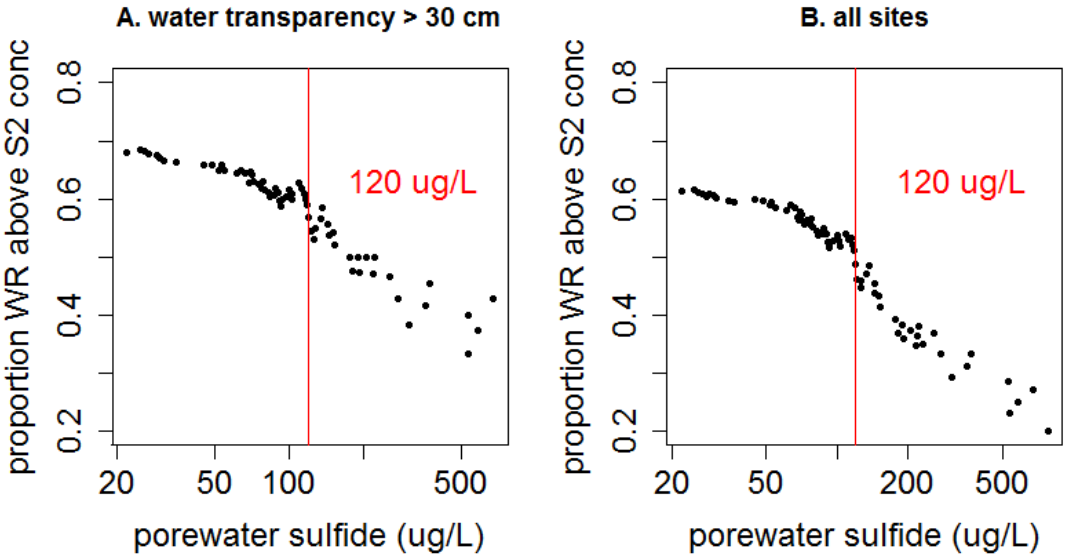


Figure A7-3. Percent of sites above the sulfide concentration that contain wild rice. A. Class B sites where water transparency was greater than 30 cm (N=96). B. All sites in Class B (N=108).

3. Change-point analysis of the field data:

To assess whether the visual identification of 120 µg/L is supported through a recognized objective procedure, change-point analysis was used to identify sulfide concentrations that are associated with changes in the trend in the density of wild rice in relation to porewater sulfide. A potential protective sulfide concentration was found by ordering the data from lowest sulfide to highest sulfide concentration, then performing change-point analysis on the wild rice density (average number of stems per square meter). The resulting sulfide concentrations was 112 µg/L, although the confidence interval is wide (95% confidence interval 25-368 µg/L).

Appendix 8. Empirical demonstration that a MBLR probability of 0.5 is most accurate

Background

The MPCA used multiple binary logistic regression (MBLR) to develop an equation to calculate the surface-water sulfate concentration corresponding to a porewater sulfide concentration of 120 µg/L (given the TOC and extractable iron in the sediment of a particular wild rice water). MPCA chose to use MBLR to avoid re-transformation bias in solving for the sulfate concentration that is incurred with some other statistical models, following the recommendation in Pollman et al. (in press).

However, many people are unfamiliar with the steps necessary to derive an equation that predicts sulfate from a MBLR model. First, the output of a logistic regression cannot be used to directly predict the sulfate concentration of interest. Rather, the MBLR-based model output is an equation that predicts the probability that sulfide would be greater than 120 µg/L (given the TOC and extractable iron in the sediment of the wild rice water). The model can be rearranged to predict the sulfate concentration of interest, but only if a probability is inserted into the model. This TSD asserts that the appropriate probability is 0.5.

Theoretical basis for choosing 0.5

A probability of 0.5 yields sulfate concentration predictions that have an equal chance of being too high or too low. Choosing probabilities that are higher or lower than 0.5 would bias predictions either generally higher than the best sulfate standards (probabilities greater than 0.5) or lower (probabilities less than 0.5). For instance, if a probability of 0.8 is used, a sulfate standard would be calculated that produces an 80% probability of the of the porewater sulfide being over 120 µg/L, which would be under-protective. Conversely, if an equation is developed with a probability of 0.2, the resulting equation would produce an 80% probability of porewater sulfide being below 120 µg/L, which would be over-protective. A probability of 0.5 is balanced between the possibilities of over- and under-protection, and is most likely to accurately calculate a sulfate standard that is related to 120 µg/L.

Choosing a probability of 0.5 therefore has a result similar to the goal of the more familiar statistics that seek to characterize the most likely prediction, by achieving a “best fit” or describing the “central tendency” through calculations of medians, means, or linear regressions. All of these approaches use a variety of methods to identify a value that is neither too high nor too low. MBLR maximizes the probability of choosing an accurate dependent variable (sulfate, in this case), whereas other approaches maximize accuracy through other methods; for instance linear regression minimizes the distance of the best fit line to the data points of the dependent variable.

Empirical demonstration that 0.5 yields the most accurate prediction of a protective sulfate concentration

The MPCA has identified a porewater sulfide concentration of 120 µg/L as the optimal concentration to serve as a regulatory protective threshold. This concentration of 120 µg/L is optimal to protect wild rice because the MPCA-sponsored survey of potential wild rice waters found that the density and probability of occurrence of wild rice decreases at or above that concentration.

If porewater sulfide could be directly regulated, there would be no need to regulate sulfate. However, porewater sulfide is a function of three variables (sulfate, sediment TOC, and sediment iron), and only sulfate makes sense to regulate, since the sediment characteristics are natural landscape features. Therefore, the goal is to choose a model that can predict sulfate concentrations that most accurately correspond to actual porewater sulfide concentrations.

Even though 120 µg/L is the optimal sulfide concentration to serve as a threshold, it is not perfect. That is, wild rice does sometimes occur in waterbodies where the field survey found porewater sulfide at concentrations greater than 120 µg/L. In addition, although the density of wild rice (measured in stems per square meter, stems/m^2) was generally lower when sulfide concentrations were greater than 120 µg/L, some of the waterbodies had dense rice (described here as greater than 40 stems/m^2).

Nevertheless, it is clear that wild rice is more likely to be present, and more likely to be dense, if a waterbody has porewater sulfide less than 120 µg/L (Table A8-1).

Table A8-1. Rates of wild rice presence and density above and below 120 µg sulfide/L. (Class B dataset)

	Sulfide less than 120 µg/L			Sulfide greater than 120 µg/L		
Stems/m^2	Number of sites	% of all sites	% of sites with wild rice	Number of sites	% of all sites	% of sites with wild rice
Over 40	28	41%	57%	6	15%	33%
Between 10 & 40	15	22%	31%	3	8%	17%
Less than 10	6	9%	12%	9	23%	50%
No wild rice observed	20	29%	0%	21	54%	
Total	69	100%	100%	39	100%	100%

In waterbodies with porewater sulfide less than 120 µg/L, it is 2.7 times more likely to encounter dense wild rice (over 40 stems/m^2) than in higher (>120 µg/L) sulfide waters (41% vs. 15%). In addition, wild rice is less likely to be found in waterbodies with higher sulfide; in 54% of high-sulfide waterbodies wild rice was not found, in contrast to 29% of the low-sulfide waterbodies. Furthermore, when wild rice is present in a high-sulfide waterbody, it is 4.1 times more likely to have low density wild rice (less than 10 stems/m^2) (50% vs 12%). A low density of wild rice (less than 10 stems/$m^{2)}$ may indicate that the population is not reproducing or germinating well (Table A8-1).

The goal of choosing an equation that calculates the sulfate concentration corresponding to a sulfide concentration of 120 µg/L is to find an equation that best corresponds to the empirical observations seen in the effect of porewater sulfide on both wild rice density and presence/absence (Table A8-1).

The ability of the MBLR-based equation to reproduce the pattern of empirical observations can be assessed with the data from the MPCA field survey. The proposed equation produces a calculated protective sulfate concentration (CPSC) that corresponds to 120 µg/L, based on the sediment TOC and iron from each waterbody. In principle, sulfate concentrations greater or less than the CPSC should have the same effect on wild rice density and presence/absence as when porewater sulfide is greater or less than 120 µg/L. The question is, then, what probability produces the most accurate equation at reproducing the pattern of measured porewater sulfide?

First, let us examine the effect that using different probability levels to develop sulfate equations have on the number of waterbodies with different levels of wild rice density. If equations are developed with a high probability that sulfide would be greater than 120 µg/L (e.g., 0.9), then CPSCs are very high; only 4 waterbodies would exceed the calculated sulfate standards to protect wild rice, and only one of the four had wild rice, and the density is less than 10 stems/m^2 (Table A8-2). Using a high probability would therefore produce calculated standards that would be severely under-protective. Conversely, if equations are developed with a low probability that sulfide would be less than 120 µg/L (e.g., 0.1), then CPSCs are very low, and 76% of waterbodies would exceed the calculated sulfate standards (Table A8-2). In reality, only 36% of waterbodies have porewater concentrations greater than 120 µg/L, so using a low probability would be severely over-protective.

In Table A8-2, the number waterbodies predicted by the equations developed with a range of probabilities (0.1 to 0.9) that sulfide would exceed 120 µg/L can be compared to the empirically

observed sulfide levels in the wild rice survey of 108 potential wild rice waterbodies. The predicted numbers that most closely match the empirical observations are printed in bold. Densities that have no bold densities are associated with probabilities of 0.1, 0.2, 0.8, and 0.9. Therefore, equations developed with probabilities 0.3 through 0.7 are most likely to reflect the observed relationship between porewater sulfide and wild rice density.

Table A8-2. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies in four levels of wild rice density, compared to the empirically observed relationship of wild rice with porewater sulfide (last line of the table). Calculations are based on the Class B data set, which best approximates a probabilistic data set.

	Sulfate less than CPSC				**Sulfate greater than CPSC**			
Probability that sulfide >120 µg/L	Density over 40 stems/m²	Density between 10 & 40 stems/m²	Density less than 10 stems/m²	No wild rice observed	Density over 40 stems/m²	Density between 10 & 40 stems/m²	Density less than 10 stems/m²	No wild rice observed
~~0.9~~	~~34~~	~~18~~	~~14~~	~~38~~	~~0~~	~~0~~	~~1~~	~~3~~
~~0.8~~	~~32~~	~~18~~	~~13~~	~~35~~	~~2~~	~~0~~	~~2~~	~~6~~
0.7	***29***	***16***	*8*	*33*	***5***	***2***	*7*	*8*
0.6	*26*	***16***	*8*	*29*	*8*	***2***	*7*	*12*
0.5	*24*	***16***	*8*	*24*	*10*	***2***	*7*	*17*
0.4	*24*	***14***	***5***	*22*	*10*	***4***	***10***	*19*
0.3	*21*	*13*	*3*	***19***	*13*	*5*	*12*	***22***
~~0.2~~	~~16~~	~~11~~	~~2~~	~~18~~	~~18~~	~~7~~	~~13~~	~~23~~
~~0.1~~	~~9~~	~~5~~	~~0~~	~~12~~	~~25~~	~~13~~	~~15~~	~~29~~
	Observed sulfide less than 120 µg/L				**Observed sulfide greater than 120 µg/L**			
	28	15	6	20	6	3	9	21

Second, the range of potential probabilities can be further narrowed by comparing the success of the calculated equations in reproducing the pattern that is empirically observed between porewater sulfide and the presence and absence of wild rice, in contrast to comparing against wild rice density (evaluated above). The best matches occur when the probability is set to 0.5 (Table A8-3).

Two logical goals of developing a water quality standard to protect wild rice is to maximize the occurrence of wild rice when a waterbody conforms to the standard, and to minimize the occurrence of wild rice when a waterbody exceeds the standard. While wild rice occurrence is the protection goal, significant occurrence of wild rice at levels above the calculated standards would mean the calculation is inaccurate. These two goals are best met with a probability of 0.5. When sulfate is less than the calculated standard, the maximum proportion with wild rice occurs when the equation is developed with a probability of 0.5 (Table A8-3). Conversely, when sulfate is greater than the calculated standard, the minimum proportion also occurs when the equation is developed with a probability of 0.5 (Table A8-3).

Table A8-3. Effect that different probability levels (that sulfide would exceed 120 µg/L) have on the number of waterbodies with and without wild rice, compared to the empirically observed relationship of wild rice with porewater sulfide (last line of the table). (WR = wild rice) (Class B dataset)

	Sulfate less than CPSC			**Sulfate greater than CPSC**		
Probability that sulfide >120 µg/L	WR vs no WR	total	% with wild rice	WR vs no WR	Total	% with wild rice
0.7	53 vs 33	86	62%	14 vs 8	22	64%
0.6	50 vs 29	79	63%	17 vs 12	29	59%
0.5	**48 vs 24**	**72**	**67%**	**19 vs 17**	**36**	**53%**
0.4	43 vs 22	65	66%	24 vs 19	43	56%
0.3	37 vs 19	56	66%	30 vs 22	52	58%
	Observed sulfide less than 120 µg/L			**Observed sulfide less than 120 µg/L**		
	49 vs 20	69	71%	18 vs 21	39	46%

In conclusion, use of a probability of 0.5 produces an equation that produces sulfate concentrations that correspond most accurately to the sulfide threshold of 120 µg/L. Use of probabilities less than 0.5 produce equations that are over-protective; the calculated sulfate standards are lower, and would classify more waterbodies as exceeding standards that have porewater sulfide less than 120 µg/L and dense wild rice populations. Conversely, use of probabilities greater than 0.5 produce equations that are under-protective; the calculated standards are higher, and would classify fewer waterbodies as exceeding standards that actually have porewater sulfide greater than 120 µg/L and less dense wild rice populations. Use of a probability of 0.5 is therefore the best approach for developing an equation that calculates protective sulfate concentrations.

Appendix 9. Examination of a proposed protective sulfide of 300 µg/L

Goal of analysis

Other scientists have presented evidence in favor of 300 µg/L as a potential protective sulfide concentration threshold. The goal here is to examine 300 µg/L versus 120 µg/L as potential protective sulfide thresholds by looking at how each concentration is associated with wild rice occurrence and density.

Data analyzed

The field data from 2012-2013 was analyzed, during which time 108 different waterbodies were sampled. If there was more than one sample from a waterbody in this time, the sample that was closest to August 11th was used to ensure consistency. This dataset, which approximates a probabilistic survey of potential wild rice waters, is referred to as the Class B dataset. Wild rice density (in stems per square meter) and porewater sulfide (in µg/L), among other variables, were measured at each site.

Wild rice presence

In order to examine the pattern of wild rice occurrence and density associated with the two potential sulfide concentrations thresholds, three groups were created: a group with sulfide below the MPCA proposed standard of 120 µg/L, a group with sulfide concentrations between 120 µg/L and the proposed 300 µg/L threshold, and, finally, a group with sulfide concentrations above 300 µg/L. If the 300 µg/L sulfide concentration is protective, then it would be expected to only find a significant difference between the group under 300 and the group over 300, but not between the group under 120 and the group over 120 but less than 300. If the 120 µg/L is the better choice for a protective sulfide threshold, it would be expected that wild rice presence is significantly greater below 120 than above 120 (both the 120-300 group and the above 300 group). However, it would not be expected to see a significant difference between the 120-300 µg/L group and >300 µg/L group. The number of waters for each group are in Table A9-1:

Table A9-1. Number of Class B waters in three different groups, separated by porewater sulfide concentration.

	Under 120 µg/L	between 120 and 300 µg/L	Over 300 µg/L
Wild rice present	49	13	5
Wild rice absent	20	9	12

First, the question is if there are any differences between the three groups. A chi square test for independence was performed to see if any of the three sulfide level groups were different with respect to wild rice presence. With a p value of 0.0063, there is evidence of a significant difference among the three sulfide level groups with regards to presence or absence of wild rice.

To find out where the difference lies, the odds of wild rice presence for any two sulfide concentration groups were compared, and a two sample proportion test with continuity correction was conducted to obtain the p value (Table A9-2).

Table A9-2. Comparison of the odds of wild rice presence between any two sulfide concentration groups. Significant differences are in bold.

Group 1	Group 2	Odds ratio	95% Confidence Interval	P value
Under 120	Between 120 & 300	1.70	(0.63, 4.59)	0.434
Under 120	**Over 300**	**5.88**	**(1.83, 18.86)**	**0.0037**
Between 120 & 300	Over 300	3.47	(0.90, 13.31)	0.1286
Under 120	**Between 120 & 300 plus Over 300**	**2.86**	**(1.26, 6.47)**	**0.0334**
Under 120 plus between 120 & 300	**Over 300**	**5.13**	**(1.65, 15.93)**	**0.0060**

One advantage of expressing statistical results with odds ratios is that they are relatively easy to put into words. For example, looking at the second line of Table A9-2, the odds of having wild rice when the sulfide is under 120 (group 1) is 5.88 times the odds of having wild rice when the sulfide is over 300 (group 2). The 95% confidence interval for this odds ratio of 5.88 ranges from 1.83 to 18.86. Since this interval does not include 1.0 (a value of 1.0 means the odds are the same), there is a significant difference between the under 120 and the over 300 groups. Since the interval is entirely over 1.0, the under 120 µg/L group (group 1) has significantly higher odds of having wild rice than the over 300 group (group 2). The p value for a proportion test based on these numbers is 0.0037.

Based on these odds ratios and p values, there is not a significant difference between the under 120 and the between 120 & 300 groups with regards to wild rice presence (Table A9-2). However, there is not a significant difference between the between 120 & 300 group and the over 300 group, either. Only the under 120 is significantly different from the over 300 group. Since there is not a significant difference between the 120 to 300 group versus the over 300 group, it is not possible to definitively say that 300 is protective. However, this could be largely due to the small number of sites in each group—the odds ratio is a not-statistically-significant 3.47 (p=0.13), which means that the odds of having wild rice when the sulfide is between 120 and 300 µg/L would be almost 3.5 times the odds of having wild rice when the sulfide is above 300 µg/L. Further, there is not a significant difference between under 120 and between 120 & 300 (p=0.43), so even though wild rice is significantly more likely to be present under 120 µg/L sulfide than above 300 µg/L, based on presence/absence, it is not possible to conclude that 120 is better than 300 as a protective sulfide value.

The final two lines of Table A9-2 are calculated in an attempt to merge groups to look at 120 as a cutoff versus 300 in hopes it is possible to derive a more definitive answer. If the two groups are merged together and compared to the third group, there are significant differences between the groups. The odds of having wild rice when sulfide is under 120 µg/L are 2.86 times the odds of having wild rice when the sulfide is over 120 µg/L. Since the confidence interval is entirely above 1.0, the sites with sulfide under 120 µg/L are significantly more likely to have wild rice than those over 120 µg/L sulfide (p=0.03). Moreover, the odds of having wild rice below 300 µg/L sulfide are 5.13 times the odds of finding wild rice above 300 µg/L, and this is also statistically significant. Therefore, based on presence/absence data it is not possible to determine whether the 120 µg/L or 300 µg/L sulfide concentration threshold is more protective.

Wild rice density

The analysis was then expanded to examine the density of the wild rice stands above and below these potential protective sulfide concentrations. Because wild rice density fluctuates from year to year in natural wild rice waters, low density is not necessarily indicative of an unhealthy population in any single waterbody. However, any condition that is statistically associated with lower density among a variety different waterbodies should be suspected of impairing the reproduction and/or germination of wild rice populations, and therefore decreasing the probability that a population will successfully persist over the long term. Conversely, conditions statistically associated with higher wild rice density can be interpreted as associated with a higher probability that a wild rice population will persist over the long term.

To statistically associate porewater sulfide with low and high wild rice densities, reasonable thresholds for identification of relatively low density and high density were identified. Low and high density thresholds of 10 and 40 stems/m^2 were assessed against the long-term wild rice surveys conducted by the 1854 Treaty Authority on ten relatively un-impacted wild rice waters over 12 years (Appendix 4). The long-term data have a median, or most typical density, of 30 stems/m^2 among the ten waterbodies. The "high" density of 40 stems/m^2 corresponds to the 56th percentile (44% of annual visits to these ten waterbodies had wild rice density greater than 40 stems/m^2). The "low" density of 10 stems/m^2 corresponds to the 14th percentile; in the long-term survey, 86% of the visits to these ten waterbodies were greater than 10% stems/m^2. For comparison, the sites with wild rice in the Class B dataset have a median density of 41 stems/m^2. In other words, Class B sites rated as "high density" had greater wild rice density than 50% of the Class B sites with any wild rice, and 56% of the surveys conducted by the 1854 Treaty Authority.

Therefore, the number of sites in the Class B dataset with wild rice density was examined in three categories: over 40 stems/m^2, below 10 stems/m^2, and in between those two ranges. Therefore, there are four groups for analysis: the dense group (over 40 stems/m^2), the intermediate group (between 10 and 40 stems/m^2), the sparse group (under 10 stems/m^2), and the group with no wild rice. The information is in Table A9-3, below. There are not only differences in the proportion with wild rice, there are also differences among the density groups.

Table A9-3. Number of Class B sites of different density in three different sulfide concentration groups.

	Under 120 µg/L	between 120 and 300 µg/L	Over 300 µg/L
Over 40 stems/m^2	28	5	1
Between 10 and 40 stems/m^2	15	2	1
Below 10 stems/m^2	6	6	3
Wild rice absent	20	9	12

There are enough groups with a low number of observations per group that a Fisher's test (nonparametric) is preferred over a Chi square test (parametric) to confirm statistical differences. A Fisher's test of independence resulted in a p value of 0.002, which indicates that at least two groups differ.

The odds ratios and confidence intervals were then calculated to compare the under 120 to the 120-300 group. In addition, a two-sample proportion test with continuity correction was calculated to find the probability that the groups have the same proportion in each group. The use of the continuity correction on small sample sizes per group results in a slightly different test than the odds ratio, so a confidence interval that does not include 1 (and indicates a significant difference)

may occur when the p value is over the standard cutoff of 0.05. For the sites with sulfide over 300 µg/L, there are not enough lakes per group in the 3 wild rice groups to run odds ratios or proportion tests. Therefore, the odds ratios all compare the under 120 group to the 120-300 group (Table A9-4).

Table A9-4. Results of a two-sample proportion test to find the probability of groups having the same proportion in each group. Comparisons that are significantly different are in bold.

Group 1	Group 2	Stem density	Odds ratio	95% Confidence Interval	P value
Under 120	Between 120 & 300	>40 vs 10-40	0.75	(0.13, 4.32)	1.0
Under 120	***Between 120 & 300***	***>40 vs <10***	***5.60***	***(1.28, 24.56)***	***0.044***
Under 120	Between 120 & 300	>40 vs no WR	2.13	(0.73, 7.46)	0.235
Under 120	Between 120 & 300	>40 vs <40	3.47	(0.90, 13.31)	0.375
Under 120	**Between 120 & 300**	**>40 vs <10 & no WR**	**3.23**	**(1.03, 10.45)**	**0.072**
Under 120	**Between 120 & 300**	**10-40 vs <10**	**7.50**	**(1.17, 48.15)**	**0.065**
Under 120	Between 120 & 300	10-40 vs no WR	3.38	(0.63, 17.97)	0.262
Under 120	***Between 120 & 300***	***10-40 vs <10 & no WR***	***3.54***	***(1.28, 9.84)***	***0.024***
Under 120	***Between 120 & 300***	***>10 vs <10***	***6.14***	***(1.54, 24.54)***	***0.018***
Under 120	Between 120 & 300	>10 vs no WR	2.76	(0.90, 8.48)	0.127
Under 120	***Between 120 & 300***	***>10 vs <10 & no WR***	***3.55***	***(1.28, 9.84)***	***0.024***
Under 120	Between 120 & 300	<10 vs no WR	0.45	(0.11, 1.79)	0.429

The comparisons in Table A9-4 show that the groups with wild rice density at or above 10 stems/m^2 (whether they are over 40, between 10 and 40, or 10 and up) are significantly different from the groups with wild rice density below 10 stems/m^2 (whether they are between 0 and 10, or both no wild rice and between 0 and 10). Since the confidence intervals are all over 1.0, there is significantly higher odds of observing dense wild rice if the sulfide concentration is below 120 µg/L than if the sulfide concentration is between 120 and 300 µg/L.

Discussion

Based on wild rice presence versus absence, it is not possible to find a statistically significant difference between those sites with sulfide below 120 µg/L and those with sulfide between 120 µg/L and 300 µg/L. However, when wild rice density is examined, there is significantly higher density for those sites with sulfide below 120 µg/L compared to those with sulfide between 120 µg/L and 300 µg/L. Therefore, while sulfide concentrations between 120 µg/L and 300 µg/L do not produce a significant difference in the proportion of sites with wild rice, sites with porewater sulfide less than 120 µg/L are more likely to have dense wild rice than stands with sulfide between 120 µg/L and 300 µg/L. Wild rice waters with sulfide less than 120 µg/L are 5.6 times as likely as sites with sulfide between 120 and 300 µg/L to have dense (>40 stems/m^2) than sparse wild rice (<10 stems/m^2).

It should be noted that the statistical tests described in this report were conducted in accordance with a fundamental assumption of statistical analysis, which is that groups being compared are independent of

each other. That is, there was no overlap in the waterbodies between groups; no waterbody was in more than one group in any given statistical test. For instance, it would not be appropriate to statistically compare under 300 µg/L to under 120 µg/L, since 79% of the data in the under 300 µg/L group is the data from the under 120 µg/L group.

Conclusions

Based on a statistical analysis of the MPCA field survey, a protective sulfide concentration of 300 µg/L would not be as protective of wild rice as a concentration of 120 µg/L. The 22 waterbodies in the survey with sulfide concentrations between 120 and 300 µg/L were just as likely to have wild rice as waterbodies with sulfide below 120 µg/L, but were significantly less likely to have dense wild rice (greater than 40 stems/m^2). Waterbodies in the <120 µg/L sulfide group are 5.6 times as likely to have dense wild rice as waterbodies in the 120-300 µg/L sulfide group.

Appendix 10. Educational credentials and qualifications of TSD and SONAR authors

The following MPCA staff conducted research, analyzed data, and authored the documents that are the basis for the MPCA proposal for establishing the level of sulfide and sulfate to protect wild rice.

Name	Qualifications
David Bael Economic Policy Analyst	Ph.D. Candidate, Applied Economics (University of Minnesota); Masters of Public Policy (University of Minnesota); B.S. Biology and B.S. Management Science (Massachusetts Institute of Technology); 5 years experience in the analysis of issues related to environmental economics and finance
Baishali Bakshi Economist, Water Quality Standards	Ph.D. Economics (University of California-Irvine); 8 years experience in data analysis and natural resources policy.
Gerald Blaha Research Scientist	University of Minnesota / Century College – coursework in biology / air and water analysis; 40 years experience in water quality standards and water use classifications
William Cole Supervisor	M.Aq. Aquaculture (Auburn University); B.S. Biology (John Brown University); 10 years experience developing and implementing water quality standards
Elise Doucette Policy Specialist	B.S. Biology (University of Minnesota-Duluth); 15 years experience in water quality regulations.
Patricia Engelking Planner Principal	B.A. Chemistry (Washington University); 28 years of experience in water quality
Stephanie Handeland Hydrologist	B.S. Geology (Winona State University); 22 years experience in water quality (NPDES) wastewater permitting; 9 years experience mining permitting
Elizabeth Kaufenberg Research Scientist	M.S. Water Resources Science (University of Minnesota); B.S. Environmental Science (UW-River Falls); 8 years experience in water quality (co-authored 4 papers)
Scott Kyser Wastewater Engineer	M.S. Civil and Environmental Engineering (University of Minnesota-Twin Cities); B.A. Biology (Gustavus Adolphus College); Registered Professional Engineer in Minnesota; 6 years experience in environmental engineering
Shannon Lotthammer Director	M.S. Ecology (University of Minnesota-Twin Cities); B.S. Biology (University of Minnesota-Duluth); 23 years experience in state and local environmental management.
Phillip Monson Research Scientist	M.S. Entomology (University of Maine); B.S. Biology (University of Minnesota-Duluth); 15 years experience developing and implementing water quality standards
Carol Nankivel Planner Principal	B.S. Soil Science (University of Minnesota); 35 years experience in preparation of administrative rules
Catherine Neuschler Manager	Master of Public Affairs (School of Public and Environmental Affairs, Indiana University); B.A. Environmental Studies (Macalester College); 11 years experience at the MPCA.

(continued)

Emily Peters Data Analyst (now at MDNR)	Ph.D. Ecology (University of Minnesota); B.S. Ecology (University of California Santa Cruz); 12 years experience analyzing ecological data and publication of peer-reviewed findings (authored or co-authored 15 papers)
Michael Schmidt	J.D. (University of Minnesota); 8 years experience in the Clean Water Act and state water law
Marta Shore Data Analyst (now teaching in the biostatistics program at the University of Minnesota)	M.S. Statistics with supporting work in Biostatistics (University of Minnesota); B.A. Biology (University of Chicago); 10 years experience performing statistical analyses
Edward Swain Research Scientist	Ph.D. Ecology (University of Minnesota); B.A. Biology (Carleton College); 33 years experience in aquatic ecology research and publication of peer-reviewed findings (authored or co-authored 30 papers)

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NMW Exh. 9

JGR Biogeosciences

RESEARCH ARTICLE
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Sulfur Geochemistry Destabilizes Population Oscillations of Wild Rice (*Zizania palustris*)

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Key Points:

- Populations exposed to elevated sulfate went extinct in 6 years, overriding interannual biomass oscillations
- Iron addition and litter removal slightly alleviated sulfide toxicity but did not prevent population extinction
- Stability of oscillating populations can be evaluated with only a few years' data using an eigenvalue

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Abstract Elevated inputs of sulfate to freshwater systems can increase sulfide concentrations in anoxic soils and subsequently destabilize aquatic plant populations, but the interactions between sulfate, other geochemical cycles, and interannual plant population cycles are poorly understood. Increased sulfate loading increases mineralization of nitrogen from litter, but the sulfide produced during this process can limit nitrogen uptake by plants. In some cases, iron may mitigate sulfide's impacts on plants by precipitating iron sulfide. We examined the interannual effects of sulfate loading on mesocosm populations of wild rice, an emergent aquatic plant that undergoes population oscillations and is sensitive to sulfide. Using experimental mesocosms with self-perpetuating populations, we investigated how population dynamics respond to manipulations of surface water sulfate (10 mg L^{-1} or 300 mg L^{-1}), sediment iron (4.3 mg g^{-1} or 10.9 mg g^{-1} dry weight), and shoot litter (present or removed). Populations exposed to constant 10 mg L^{-1} sulfate concentrations had stable biomass oscillations of approximately 3-year periods, consistent with previous studies that demonstrated litter-driven oscillations in nitrogen availability. Populations exposed to 300 mg L^{-1} sulfate concentrations produced fewer and smaller seeds and declined to extinction in 6 years or less. We did not find a strong effect of iron loading or litter removal on wild rice biomass or seed production. Our observations show the potential of elevated surface water sulfate to rapidly destabilize wild rice populations under varying iron and organic carbon concentrations.

Plain Language Summary Plants that naturally grow in freshwater do not survive well if the water contains elevated concentrations of sulfate. Sulfate reduction produces sulfide that subsequently inhibits the uptake of nitrogen, an essential plant nutrient. Some annual plants go through boom-bust cycles with years alternating between high and low biomass because nitrogen takes more than a year to be released from dead plant matter. We investigated the combined effect of sulfate and natural biomass cycles on the stability of wild rice populations by growing plants in large tanks and exposing them to high-sulfate and low-sulfate concentrations, high and low iron concentrations, and with plant matter from the previous growing season either returned or removed. Nearly all plant populations exposed to high sulfate had died by 6 years into the experiment, regardless of iron concentration or litter removal. We show a method to analyze population stability with just a few years of data.

1. Introduction

Northern wild rice (*Zizania palustris*) is one of four species in the genus *Zizania*, which are the only native aquatic grains in North America. The range of northern wild rice (hereafter wild rice) is centered across the Great Lakes region and is most abundant in the rivers and lakes of the watersheds of Lakes Superior and Michigan in northern Minnesota, Wisconsin, and Ontario. Wild rice beds are usually very large (tens or hundreds of hectares) and monotypic. Because of its widespread distribution and tendency to form large monotypic stands, wild rice has great potential to control the quality of waters draining into Lakes Superior and Michigan and influence the food supply for waterfowl, muskrats, and other members of the food web. In addition, harvesting and eating wild rice are essential traditional practices that provide food sovereignty and well-being for the native Ojibway people of the watersheds of Lakes Superior and Michigan (Fond du Lac Band of Lake Superior Chippewa, 2018). Therefore, the productivity, perpetuation, and restoration of wild rice are of great ecological and cultural significance.

Production of wild rice biomass is limited by the supply of nitrogen from decomposing plant litter, sediment organic matter, and hydrologic inputs (Pastor & Walker, 2006; Sims et al., 2012; Walker et al., 2006, 2010). Because it is an annual plant, wild rice's nitrogen requirements must be fully supported by uptake during each year. Over 60% of nitrogen uptake happens during a 2-week window in early summer (Grava & Raisanen, 1978;

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Sims et al., 2012). Nitrogen, however, is not released from the previous year's litter until later in summer or even the following year (Hildebrandt et al., 2012; Sain, 1984; Walker et al., 2010). In fact, there is considerable microbial immobilization of nitrogen into fresh litter during the period when the demands of wild rice growth for nitrogen are greatest (Hildebrandt et al., 2012; Walker et al., 2010). The coincidence of microbial nitrogen immobilization with the period of rapid nitrogen uptake causes wild rice biomass and litter production to cycle with a period of approximately 4 years (Pastor & Walker, 2006; Walker et al., 2010).

Inputs of sulfate from bedrock weathering, mine drainage, and agriculture enhance sulfide production in natural wild rice ecosystems (Bailey et al., 2017; Lamers et al., 2013; Myrbo et al., 2017a). Wild rice production appears to be adversely impacted by sulfide in the vicinity of its rooting zone. The survival of juvenile seedlings and weights of seeds decrease with increased hydrogen sulfide concentrations in wild rice's rooting zone in aquatic sediments (Pastor et al., 2017). The production of sulfide may be coupled to increased litter deposited in sediment during productive years of the wild rice population cycle. These large litter cohorts could reduce sediment redox potential (Eh) by providing additional labile carbon to support additional bacterial growth and hence oxygen demand the following year, thereby enhancing the potential for reduction of sulfate to sulfide (Azam et al., 1991; Gao et al., 2003, 2004).

Other biogeochemical reactions in the sediments may impede the bioavailability of sulfide to wild rice roots. The most important reaction is precipitation of sulfide with reduced iron (Morse et al., 1987). In both mesocosm and lake studies (Bailey et al., 2017; Myrbo et al., 2017a), iron in sediments appear to exert a strong control on the accumulation of dissolved sulfide in sediments. Bulk sediment iron content is strongly associated with lower porewater sulfide in field conditions and mitigates sulfide toxicity to macrophytes in other aquatic ecosystems (Lamers et al., 2002; Ruiz-Halpern et al., 2008; Van der Welle et al., 2007).

However, iron sulfide can precipitate on roots of mature plants and is associated with impaired nitrogen uptake and inhibited seed production (LaFond-Hudson et al., 2018, 2020a). Plant-mediated gas transport of oxygen from the atmosphere into the rhizosphere allows formation of iron oxides on root surfaces, and oxygen fluxes are typically highest when plants are photosynthetically active (Blossfeld et al., 2011; Han et al., 2018; Marzocchi et al., 2019). As observed on many emergent macrophytes, iron oxide forms on wild rice roots as the plant grows (Jorgenson et al., 2012; Mendelssohn et al., 1995; Sundby et al., 1998). At maturity and the start of seed production, however, root plaques transition from iron oxide to iron sulfide if porewater sulfate is abundant (LaFond-Hudson et al., 2018). We have imaged iron sulfide plaques and quantified plaque iron and sulfide concentrations from plants grown in mesocosms with 300 mg L^{-1} sulfate (Pastor et al., 2017) and have visually observed black root plaques in the field at lower sulfate concentrations (unpublished data). Plants that accumulate greater concentrations of iron sulfide plaques have lower seed nitrogen mass (LaFond-Hudson et al., 2018, 2020a).

There are, therefore, complex and as yet poorly understood couplings among biomass and litter cycles, nitrogen availability, sulfide inhibition of seed production, control of sulfide concentrations in sediments by iron and litter, and precipitation of iron sulfide on roots during seed production. Here, we investigate how litter-driven population oscillations interact with sulfate geochemistry in wild rice using controlled mesocosm experiments that allow us to scale rhizosphere geochemistry-plant physiology interactions from individual plants to an entire population for several generations. In our mesocosms, we elevated geochemical inputs of sulfate and iron and manipulated carbon through the presence or absence of litter. We investigated: (a) the patterns of biomass oscillations in high-sulfate and low-sulfate conditions, and (b) whether litter and iron enhance or alleviate sulfate's effects on biomass oscillations through the production and precipitation of sulfide.

2. Methods

2.1. Experimental Design

The interactions of sulfate, iron, and litter in wild rice sediment and their effect on wild rice population dynamics were studied using 40 mesocosms. Polyethylene stock tanks (High Country Plastics 400 L, 132 × 78 × 61 cm) were used to assemble the mesocosms (Figure 1). Sediment in the tanks was taken from Rice Portage Lake (MN Lake ID 09003700, 46.7038, −92.6829) on the Fond du Lac Band of Lake Superior Ojibway Reservation in Carlton County, Minnesota (Table 1). This lake is a productive wild rice lake with little surrounding development and its sediment has been used successfully to grow wild rice in previous experiments (Pastor et al., 2017). Sediment was homogenized before it was added to the tanks. Clean sand (10 cm) was added to the bottom of the

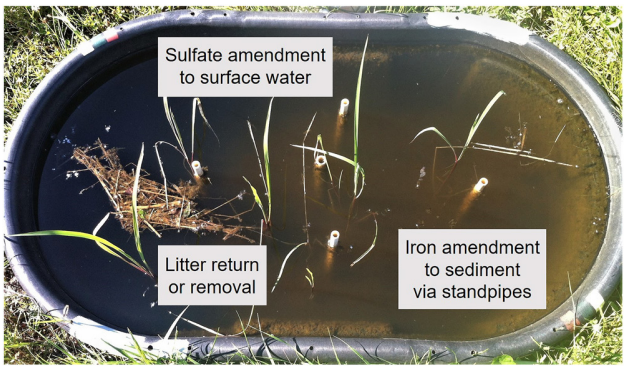


Figure 1. Picture of a mesocom showing the application of sulfate, iron, and litter treatments. The center standpipe was used to moderate the water level after rain events. The other four standpipes connect to a ring of perforated PVC pipes in the sediment to allow release of iron(II) chloride. Sulfate was applied as sodium sulfate several times over each growing season to maintain surface water concentrations near 300 mg L^{-1} sulfate. Litter was weighed at the end of each growing season, and either returned to the mesocosms, or permanently removed.

tanks before 50 L of lake sediment were placed in each tank, resulting in a sediment depth of about 10 cm on top of 10 cm of sand. Water levels were maintained at 22 cm with a drain standpipe during precipitation and well water additions to account for evaporation. These depths of sand, sediment, and water represent typical wild rice rooting depth and water column heights in the field and have been used in several wild rice mesocosm experiments previously (Hildebrandt et al., 2012; Pastor et al., 2017; Walker et al., 2010).

To test the effects and interactions of sulfate, iron, and litter, we used a factorial design with five replicates for each of eight combinations of elevated or background sulfate, elevated or background iron, and the presence or absence of litter (Figure 1). These combinations were randomly assigned to the tanks in the first year of the experiment. For high-sulfate treatments, enough sodium sulfate was added to the surface water to bring the sulfate concentration to a target level of 300 mg L^{-1}. Surface water sulfate concentrations were tested weekly and sodium sulfate was added as required to maintain concentrations at the target level of 300 mg L^{-1} throughout the growing season for the duration of the experiment. The low-sulfate tanks were filled with water from an on-site well with concentrations around 10 mg L^{-1} and received no additional sulfate additions except for precipitation, which averaged 2.3 ± 1.5 mg L^{-1} sulfate. The sulfate concentrations in the low-sulfate tanks averaged around 7 mg L^{-1} over several years (Pastor et al., 2017). The low-sulfate conditions for our experiment are still higher than the median sulfate concentration of Minnesota wild rice waters, 1.8 mg L^{-1} (Myrbo et al., 2017a), but is just below Minnesota's protective sulfate standard for wild rice waters. At 300 mg L^{-1}, our high-sulfate treatment is close to the EPA's secondary standard for sulfate in drinking water (250 mg L^{-1}) and represents surface water concentrations of a few lakes and rivers in Minnesota that contain wild rice (Myrbo et al., 2017a). Prior to both sulfate and iron amendment, sediment iron was extracted from homogenized sediment samples using 1 M HCl and quantified on a Varian fast sequential flame atomic absorption spectrometer with an acetylene torch (Federation & Association, 2005). The sediment initially contained 77 µmol Fe g^{-1} dry weight (Table 1), 85% of which was Fe(II) (Phenanthroline method, see Section 2.2). Each iron-amended tank received 96 g Fe^{2+}, bringing total iron concentrations up to approximately 196 µmol Fe g^{-1}, or 10.9 mg g^{-1} dry weight. This amendment level aimed to noticeably increase iron concentrations without causing iron toxicity (Kinsman-Costello et al., 2015). Iron was applied gradually in four separate aliquots during the first growing season. For each addition, 75 g $FeCl \cdot 4H_2O$ was dissolved in 400 mL well water and added directly into the sediment through PVC standpipes connected to a buried perforated PVC ring. The standpipes and ring were flushed with 100 mL of tank water immediately after the iron(II) chloride was injected. Samples of pore water iron were taken several times over the course of the first growing season in 10 points distributed across the tank to ensure that the iron loading was distributed evenly. Noniron tanks did not receive additional iron except for occasional well water that contained 0.17 mg L^{-1} Fe. Noniron tanks did not have a buried PVC ring, but all mesocosms had a center standpipe draining into an external bucket to restore the water level after rain events.

Table 1
Initial Bulk Sediment Physical and Chemical Characteristics

Sediment property	Value
Porosity	0.87
Bulk density	0.29 g cm^{-3}
Percent solids	30
Solid-phase acid volatile sulfide (AVS)	0.346 ± 0.054 µmol g^{-1}
Solid-phase extractable iron	76.7 ± 5.1 µmol g^{-1}
Solid-phase ferrous iron	65.2 ± 4.8 µmol g^{-1}
Porewater sulfide	0.659 ± 0.239 µmol L^{-1}
Porewater ferrous iron	435 ± 200 µmol L^{-1}
Initial solid phase S:Fe ratio	0.00450
Initial porewater ΣS^{2-}:Fe^{2+} ratio	0.0015

Note. Sulfide was measured from initial sediment and porewater iron is an average from fall measurements in tanks unamended with iron.

To test the effect of shoot litter cohorts on sulfide production, shoot litter produced by the wild rice population was retained in half the tanks and removed from the remaining tanks. Litter removal was chosen as the experimental treatment rather than litter addition because in typical freshwater, organic-rich wild rice habitats, sulfide production is generally limited by sulfate rather than organic carbon, so increasing litter may have had little effect. Only aboveground litter was removed for two reasons: (a) to minimize sediment disturbance, and (b) to focus on litter effects on sulfide production. Litter-driven biomass oscillations are driven primarily by recalcitrant root litter (Walker et al., 2010), so aboveground litter manipulation was not

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expected to disrupt interannual cycles. Initial organic carbon content of the sediment was 14.8 ± 1.7% by dry weight (Pastor et al., 2017).

2.2. Geochemical Sampling and Analysis

In 2019 (year 5 of study), passive diffusion samplers (peepers) were installed in the tanks during vegetative growth (July) and seed production (September) to obtain porewater measurements from discrete depths in the top six cm of sediment. The peepers were placed in deionized water that was bubbled with nitrogen for 1 week prior to installation (Johnson et al., 2019). The peepers were transported to mesocosms in degassed water and installed in three tanks for each treatment. Each peeper contained four wells, the top of which was in the flocculant litter layer at the sediment surface and the bottom of which was approximately six cm below the sediment surface. Two weeks after peepers were installed, each peeper was removed and quickly placed in a large, resealable plastic bag purged with nitrogen gas to keep porewater anoxic during porewater extraction. Approximately 6 mL of porewater from each well was extracted with a syringe and allocated for immediate sulfide and iron measurements in vials preloaded with reagents. A separate aliquot was used to measure pH within 30 s.

Iron(II) and sulfide were quantified colorimetrically using the phenanthroline and methylene blue methods, respectively, on a HACH DR5000 UV-Vis spectrophotometer (Federation & Association, 2005). The pH of the pore water was measured by placing a calibrated ThermoScientific Orion pH electrode in porewater immediately after it was extracted from peepers.

2.3. Biological Sampling and Analysis

Seedlings usually began to germinate around mid-May. When seedlings grew to the water surface, populations in each mesocosm were thinned to approximately 30 plants per tank, which is the optimal density to limit competition and minimize overlapping rhizospheres (Lee, 2002). In August, as plants began to flower, six plants from each tank were randomly selected and tagged. Seed data were collected from these six plants and extrapolated to the total number of plants in the tank. Seeds from the remaining plants were left in the tank to reseed the sediment for the next year. Seeds were sorted as filled or unfilled by visual inspection, counted, and dried at 65 °C and weighed to determine seed mass. After all plants had completely senesced in October, all aboveground biomass was removed and weighed with a small subsample dried at 65 °C and weighed for moisture correction. The litter that was not dried was returned to the mesocosms assigned to retain litter within a few days.

2.4. Data Analysis

The data collected in this experiment are available at the Digital Repository for University of Minnesota (LaFond-Hudson et al., 2020b). Porewater data collected from peepers were examined to understand how porewater sulfide to iron ratios and saturation with respect to FeS were changed 5 years after geochemical manipulation. A three-way ANOVA was used to test the effect of each geochemical manipulation (sulfate, iron, litter). Repeated measures ANOVAs were used to determine the effect of time, sulfate addition, iron addition, and litter removal on biological traits and porewater measurements. Data were checked for normality and heteroscedasticity using R's standard diagnostic plots and for sphericity using the ez R package (Lawrence, 2016), which provides sphericity corrections in the case of violated assumptions. For porewater data, nondetects (12% of samples) and lost samples (11%) were removed. Zeros were not removed in biological data, as these were true absences of plant or seed tissue. Including zeros in biological data resulted in large variance under high-sulfate conditions, making it harder to detect differences between treatments using ANOVA tests. However, averages calculated including zeros (extinction of the population) represent the effects of high sulfate more completely than removing zeros.

To test the propensity of the population to oscillate in high-sulfate and low-sulfate conditions, we regressed the change in vegetative biomass ($B(t) - B(t-1)$) against the vegetative biomass from the previous year ($B(t-1)$). A negative slope of this regression indicates that high productivity 1 year leads to lower productivity the following year and, conversely, that years with low productivity are followed by years with higher productivity (Walker et al., 2010). The slope of this line is $\partial(\mathrm{d}B/\mathrm{d}t)/\partial B$, which is effectively an eigenvalue of system. A critical value of −1 of this eigenvalue defines a Hopf bifurcation giving birth to stable limit cycles (Pastor & Walker, 2006; Strogatz, 1994; Walker et al., 2010). A slope of −1 or more negative indicates propensity for stable oscillations,

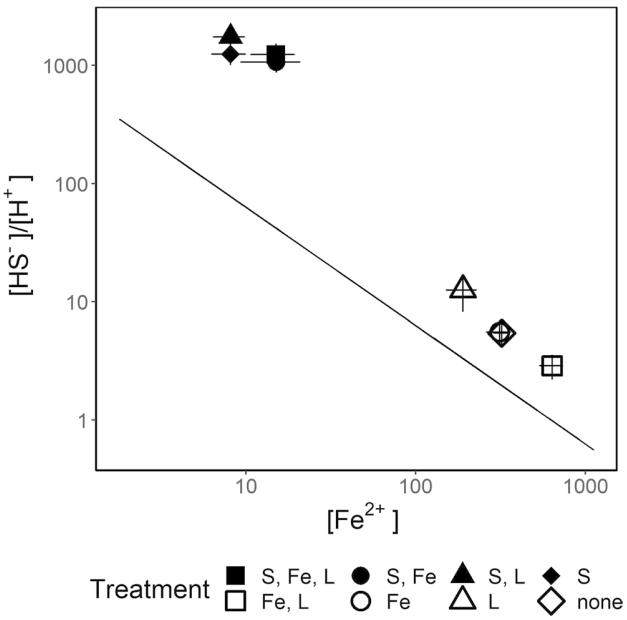


Figure 2. Comparison of ferrous iron (μmol L^{-1}) and sulfide concentrations (both sulfide and H+ in mol L^{-1}) in sediment porewater after 5 years of sulfate amendment (S), iron amendment (Fe), and litter retention (L). Data points represent average porewater measurements for each treatment (n = 12–30; most n = 24). Error bars represent the standard error around the mean. Porewater was collected 2, 4, and 6 cm below the sediment surface using peepers. Open symbols are for mesocosms with background sulfate concentrations and closed symbols are for mesocosms with nominal sulfate concentrations of 300 mg L^{-1} in overlying water. The line depicts the ion activity product at saturation for iron and bisulfide at pH 7.0 (log K_{sp} = −3.2). Porewater pH in elevated sulfate mesocosms ranged from 6.85 to 8.56 with a mean of 7.33 while porewater pH in low-sulfate mesocosms averaged ranged from 6.18 to 8.83 with a mean of 6.67.

while a slope between −1 and 0 indicates dampened oscillations (Walker et al., 2010). Dampened oscillations will eventually converge on a stable value of biomass, which may include zero (extinction). When no biomass is produced the following year, the population is at the boundary line $y = -x$ and the population is extinct. Vegetative biomass, rather than total biomass (vegetative + seeds), was used in this analysis because asynchrony in nitrogen mineralization and nitrogen uptake is expected to affect plants most during vegetative growth (Grava & Raisanen, 1978; Walker et al., 2010).

3. Results

3.1. Geochemical Context

Both high-sulfate and low-sulfate conditions contained sulfide and iron concentrations that favored precipitation of FeS (ion activity product > Ksp, Figure 2) in the fifth year of the study. Sulfate amendments to the surface water increased porewater sulfide and pH, and decreased porewater ferrous iron concentrations ($p < 0.001$ for all, Table 2). Sulfate addition raised porewater sulfide from an average of 4 μmol L^{-1} in unamended tanks to 110 μmol L^{-1} and lowered porewater ferrous iron concentrations by a similar order of magnitude, from 309 to 12 μmol L^{-1}. The pH in high-sulfate mesocosms was 0.65 units higher than in low-sulfate conditions (7.32, 6.67). Iron amendment increased porewater ferrous iron concentrations by approximately 70–80% ($p < 0.001$, Table 2) but did not notably change sulfide or pH. Litter removal did not significantly change sulfur or iron geochemistry.

3.2. Geochemical Effects on Biomass and Reproduction

All measured traits of wild rice growth and reproduction changed with time (Table 3). Sulfate addition strongly and consistently decreased all measured traits of wild rice growth (Table 3). The main effects of iron addition and litter removal were much weaker and inconsistent relative to sulfate addition, therefore we examined how iron and litter affected wild rice growth and reproduction using separate repeated measures ANOVAs for the high and low sulfur populations, respectively.

Total aboveground biomass density (hereafter referred to as biomass) was similar between high-sulfate and low-sulfate populations during the first year of the experiment (100 g m^{-2}), but biomass in high-sulfate populations declined to less than 5 g m^{-2} during the subsequent 5 years (Figure 3). In 2017, 4 years into the experiment, 8 out of the 20 populations receiving high sulfate loads produced no biomass regardless of iron addition and litter removal. Seven populations recovered partially in 2018, possibly from the germination of seeds from previous years buried in the sediment, but by 2019, 16 out of these 20 populations produced no biomass. In low-sulfate

Table 2
The Effect of Five Years of Sulfate, Iron, and Litter Additions on Porewater Concentrations of Sulfide and Iron, Porewater pH, and the Saturation Index With Respect to FeS Calculated From the Former Three Measurements in 2019 (Three-Way ANOVA)

Variable	Sulfate	Iron	Litter	Significant interactions
Porewater sulfide	**$p < 0.001$**	$p = 0.53$	$p = 0.64$	S × Fe, Litter × Fe, S × Litter × Fe
Porewater iron	**$p < 0.001$**	**$p < 0.001$**	$p = 0.16$	
Porewater pH	**$p < 0.001$**	$p = 0.64$	$p = 0.57$	S × Litter × Fe
Saturation index of FeS	**$p < 0.001$**	$p = 0.19$	$p = 0.96$	

Note. Significant interactions (in bold) refer to combinations of sulfate, iron, and/or litter that are significant at $p < 0.05$.

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Table 3
Repeated Measures ANOVA Testing the Influence of Sulfate Amendment, Iron Amendment, and Litter Removal on Wild Rice Growth and Reproduction for 2014–2020

Trait	Year	Sulfate	Iron	Litter	Significant interactions
Vegetative biomass	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$p = 0.81$	$p = 0.44$	S × L × Fe × Year, S × L × Year, S × Year, Fe × Year
Population seed mass	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$p = 0.59$	$p = 0.09$	S × L × Year, S × Year
Filled seed ratio	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$p = 0.25$	S × Year
Mass per filled seed	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$\mathbf{p = 0.002}$	$p = 0.40$	S × Year, Fe × Year
Total biomass	$\mathbf{p < 0.001}$	$\mathbf{p < 0.001}$	$p = 0.71$	$p = 0.23$	S × Fe × L × Year, S × L × Year, S × Year
High-sulfate conditions					
Vegetative biomass	$\mathbf{p < 0.001}$	–	$p = 0.82$	$p = 0.83$	L × Year, Fe × L × Year Fe × Year
Population seed mass	$\mathbf{p < 0.001}$	–	$p = 0.28$	$p = 0.67$	
Filled seed ratio	$\mathbf{p < 0.001}$	–	$\mathbf{p = 0.01}$	$p = 0.42$	
Mass per filled seed	$\mathbf{p < 0.001}$		$\mathbf{p = 0.04}$	$p = 0.20$	
Total biomass	$\mathbf{p < 0.001}$	–	$p = 0.74$	$p = 0.97$	L × Year, Fe × L × Year
Low-sulfate conditions					
Vegetative biomass	$\mathbf{p < 0.001}$	–	$p = 0.68$	$p = 0.30$	Fe × Year
Population seed mass	$\mathbf{p < 0.001}$	–	$p = 0.82$	$p = 0.08$	
Filled seed ratio	$\mathbf{p < 0.001}$	–	$\mathbf{p = 0.01}$	$p = 0.41$	Fe × Year
Mass per filled seed	$\mathbf{p < 0.001}$	–	$\mathbf{p = 0.02}$	$p = 0.86$	Fe × Year
Total biomass	$\mathbf{p < 0.001}$	--	$p = 0.82$	$p = 0.18$	

Note. Bold values highlight p-values < 0.05. Separate tests for high-sulfate and low-sulfate conditions are also included. Interactions are listed if significance is $p < 0.05$.

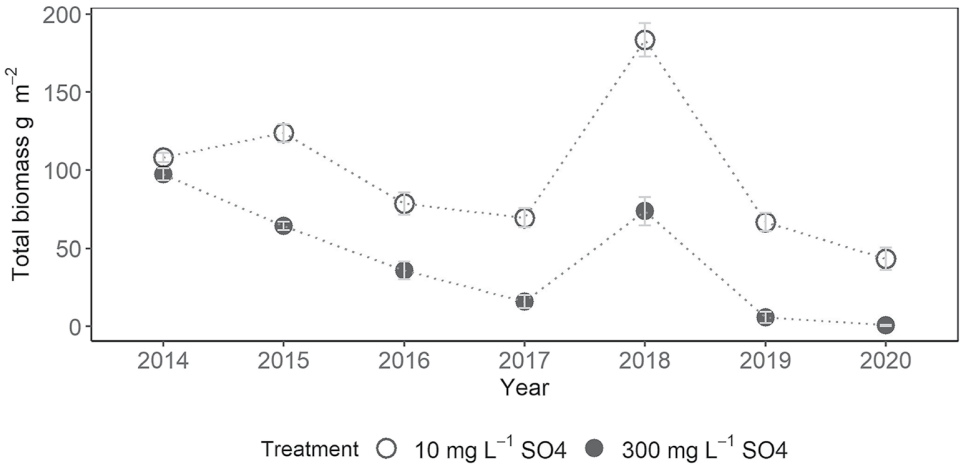


Figure 3. Annual average aboveground biomass (total) density (g m^{-2}) in populations grown in high sulfate (300 mg L^{-1}, filled circles) and low sulfate (10 mg L^{-1}, empty circles) in the overlying water. Error bars depict the standard error around the mean ($n = 20$).

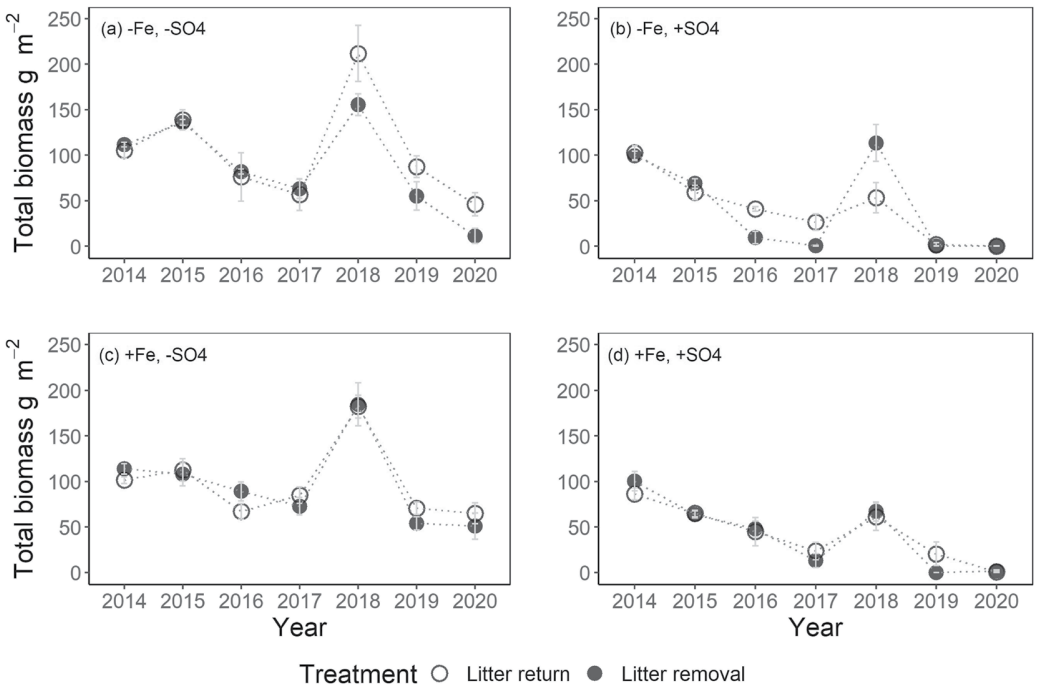


Figure 4. Annual average aboveground biomass (total) density (g m^{-2}) in populations grown with the previous year's aboveground litter returned (empty circles) or removed (filled circles). Populations were treated with combinations of low sulfate ($-SO_4$, 10 mg L^{-1}; a, c) or high sulfate ($+SO_4$, 300 mg L^{-1}; b, d) and low iron (−Fe, 4.3 mg g^{-1}; a, b) or high iron (+Fe, 10.9 mg g^{-1}; c, d). Error bars depict the standard error around the mean ($n = 5$).

conditions, populations showed a stable 3-year cycle of biomass (Figure 3) that oscillated between about 50 and 175 g m^{-2} on average, with peaks in the second and fifth growing season (2015 and 2018).

When biomass was examined separately in high-sulfate and low-sulfate treatments, populations grown in low sulfate were not affected by iron, litter, or their interaction (Figures 4a and 4c). Populations grown in high sulfate were not consistently influenced by iron and litter but were affected by interactions between iron, litter, and year (Table 3). In high-sulfate conditions, litter removal amplified oscillations in the treatments that did not receive iron addition, with lower biomass in years 3–4 of the experiment and higher biomass in year 5 compared to the populations with litter return (Figure 4b). In high-sulfate treatments that received additional iron, no differences in biomass were observed between litter removal and litter return (Figure 4d).

The proportion of filled seeds, individual seed mass, and population seed mass approached zero after six generations in high-sulfate conditions, while the same seed traits remained constant in low-sulfate conditions (Figure 5, seed mass not shown). Sulfate-amended populations that received iron amendment had a 20% higher filled seed ratio for 2015–2017 ($p = 0.01$, Table 3 and Figure 5b), but the effect of iron diminished in 2018 and 2019. In high-sulfate conditions, iron increased the average individual seed mass by approximately 40% in 2016 and by 20–30% in 2017–2018 ($p = 0.04$, Table 3 and Figure 5d). By 2020, only two sulfate-amended populations produced seeds; both also received iron amendment. In low-sulfate conditions, the proportion of filled seeds and average mass per filled seed were slightly increased by iron amendment (Figures 5a, 5c and Table 3). Litter removal did not affect any seed traits (Table 3).

3.3. Stability of Population Cycles

Changes in vegetative biomass from 1 year to the next were regressed against the biomass of the previous year to examine population oscillations for stability (Walker et al., 2010). All treatments with low sulfate were aggregated to compare to all treatments exposed to elevated sulfate since iron and litter manipulations had little effect in comparison to sulfate. In low-sulfate conditions, biomass oscillations are stable, as indicated by the slope of −1.3 for years 2015–2018 (Figure 6a) and a consistent annual biomass production between 50 and 125 g m^{-2}.

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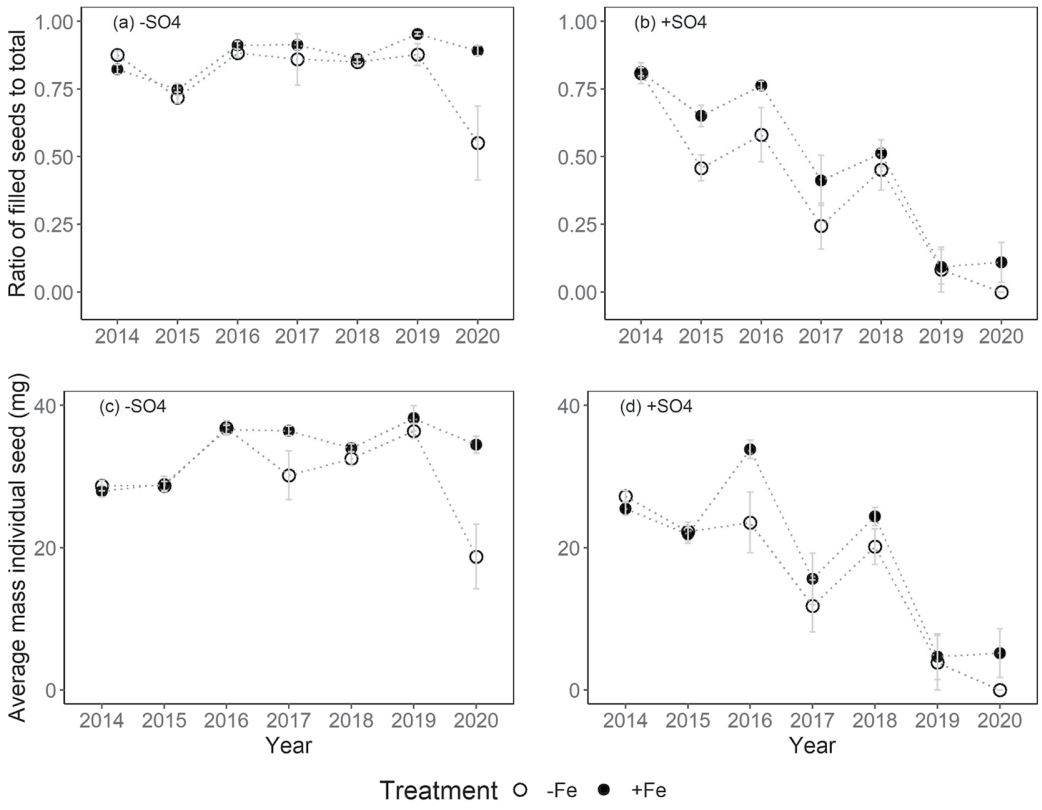


Figure 5. Effects of iron addition (+Fe, 10.9 mg g^{-1}; −Fe, 4.3 mg g^{-1}) on the ratio of filled seeds to total seeds (includes empty husks; a, b) and on average individual seed mass (c), (d) in 10 and 300 mg L^{-1} sulfate. Error bars depict the standard error around the mean (n = 10).

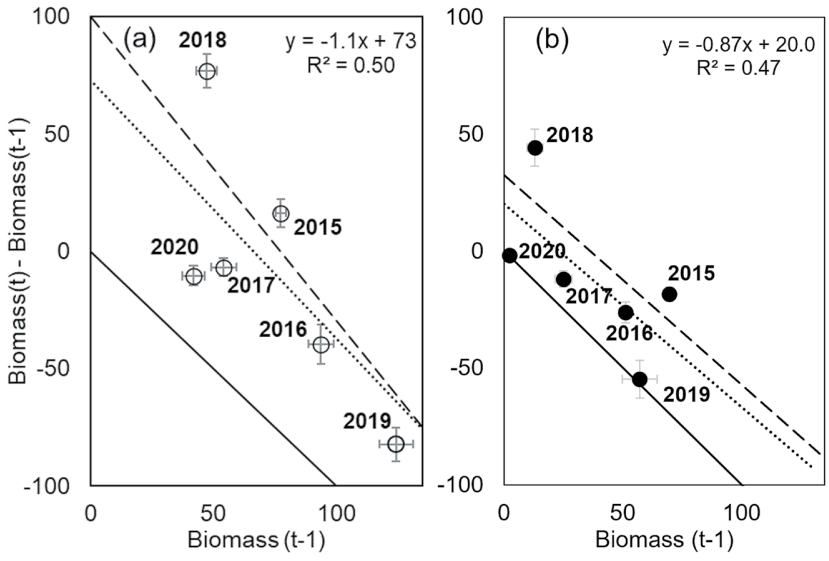


Figure 6. The relationship between average year-on-year change in vegetative biomass (g m^{-2}) to the previous year's vegetative biomass in (a) 10 mg L^{-1} sulfate and (b) 300 mg L^{-1} sulfate. Error bars show the standard error. The slope of the dashed line represents $\partial(dB/dt)/\partial B$ and is calculated from populations in 2015–2018, representing approximately one population cycle. The dotted line also represents $\partial(dB/dt)/\partial B$ and is calculated from 2015 to 2020, to show the stability after 1.5 population cycles. The solid line represents extinction, when all biomass from the previous year is lost.

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This slope is nearly identical to the slopes found a previous experiment (−1.04 to −1.20) that showed wild rice undergoes litter-driven productivity cycles (Walker et al., 2010). When data from 2019 to 2020 are included in this analysis, the slope is −1.1, still representing stable oscillations. The present experiments and those of Walker et al. (2010) were done during different time periods and in different wild rice populations. Therefore, the two experiments provide independent corroboration of one another.

In high-sulfate conditions, a slope of −0.9 for year 2015–2018 indicates dampened oscillations (Figure 6b). Biomass decreased every year except for 2018, when the populations partially recovered. From 2018 to 2019, almost all points from sulfate-amended mesocosms fell on the boundary line $y = -x$, indicating that populations lost the entirety of the biomass produced during 2018 and produced no new biomass in 2019 (Figures 6b and 3). The data point for 2020 is near zero, indicating two consecutive years of extinct populations. The trajectory of mean biomass over time decreases in the presence of sulfate under our experimental conditions (Figure 3) and oscillations were dampened. In low-sulfate concentrations, biomass peaked in 2015 and 2018, demonstrating a 3-year cycle. Elevated sulfide produced in tanks with high-sulfate concentrations extinguished the population cycle which persisted in tanks with low-sulfate concentrations.

4. Discussion

Elevating surface water sulfate to 300 mg L^{-1} led mesocosm populations of wild rice to extinction in six growing seasons. This study adds to the growing body of literature describing the impacts of sulfate loading and subsequent sulfide exposure on wild rice, including lower rates of seedling survival, delayed phenology, impaired seed production, and declining biomass (Johnson et al., 2019; LaFond-Hudson et al., 2020a; Pastor et al., 2017). In conjunction, field observations both recently as well as decades ago show that wild rice presence becomes increasingly unlikely with elevated surface water sulfate and porewater sulfide (Moyle, 1945; Myrbo et al., 2017a). Statistical modeling of environmental parameters associated with wild rice presence has suggested that iron and organic carbon play the strongest role in controlling sulfide's effects on wild rice populations (Pollman et al., 2017), and similar conclusions have been reached about other sensitive aquatic plant species growing in freshwater wetlands with increasing sulfate loads (Lamers et al., 2002; Van der Welle et al., 2007). In our study, iron addition and organic carbon removal did not limit sulfide accumulation in sediment enough to stabilize wild rice populations.

In anoxic freshwater systems, sulfide production is generally limited by the supply of sulfate, whereas in marine systems or other systems with high sulfate, sulfide production is generally limited by the supply of organic matter (Ruiz-Halpern et al., 2008). Wild rice naturally grows in low-sulfate, organic-rich habitats (Myrbo et al., 2017a), meaning sulfate is the likely limiting factor for sulfide production. In mesocosms with elevated sulfate, litter removal alone did not appear to decrease sulfide concentrations and instead led to slightly faster population decline. It is possible that litter removal decreased the availability of macronutrients or micronutrients, such as nitrogen, potassium, or iron that might be replenished in natural ecosystems with more hydrologic connectivity. Notably, for populations with sulfate added and litter removed, population biomass declined faster in populations with ambient iron compared to populations receiving iron addition (Figures 4b and 4d). Another possibility is that in the litter return treatments, sulfate addition increased rates of litter decomposition and nutrient availability (Myrbo et al., 2017b). Under the conditions in this study and previous studies (Pastor et al., 2017), sulfide's inhibition of nitrogen uptake influenced population biomass more strongly than sulfate-enhanced nutrient mineralization, but the interactions of these two processes warrant further study.

In the presence of excess uncomplexed Fe(II), sulfide reacts quickly with iron, forming relatively stable iron sulfide solid phases. The ratio of sulfur to iron or similar metrics related to the degree of pyritization can be used to determine the capacity of sediment to precipitate iron sulfide and keep porewater sulfide concentrations low (Johnson et al., 2019; Julian et al., 2017). We added iron only during the first growing season of the experiment, making this treatment a pulse (one time) rather than a press (ongoing). In contrast, our sulfate and litter manipulations were maintained throughout the experiment. This decision was made to avoid Fe^{2+} toxicity to the plants (Kinsman-Costello et al., 2015; Sahrawat, 2005) and likely contributed to our findings of little alleviation of sulfide toxicity by iron compared to other studies with freshwater vegetation in environments with natural differences in groundwater upwelling of iron (Lamers et al., 2002) or seagrasses in experiments with monthly iron addition (Ruiz-Halpern et al., 2008). We observed some mitigation of sulfide's effects on seed production by

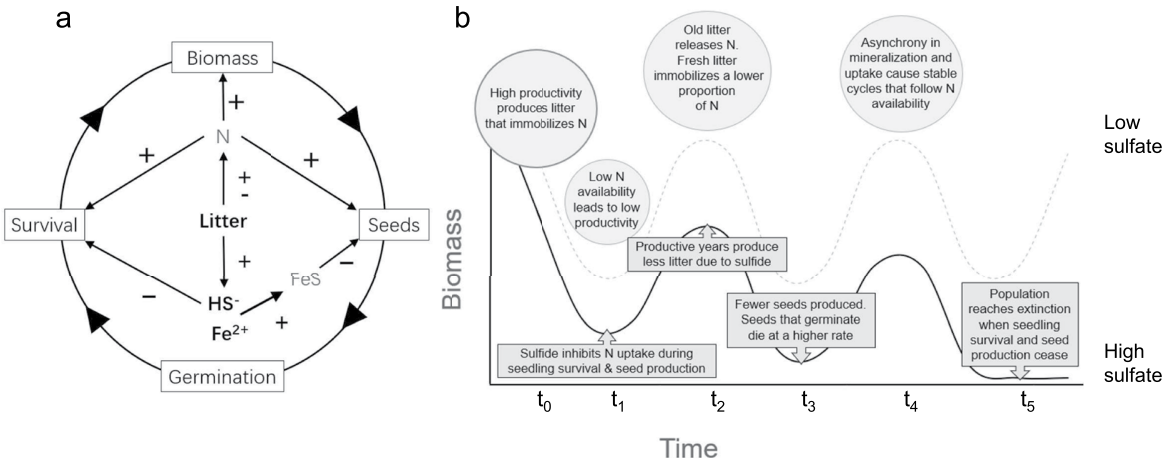


Figure 7. A conceptual model synthesizing the interactions of sulfur, iron, and litter during the life cycle of wild rice (a), and the effect of sulfur on interannual biomass cycles of wild rice (b) based on this study, Walker et al. (2010), and LaFond-Hudson et al. (2018). In (a), the (+) and (−) symbols in (a) represent positive or negative relationships between geochemical constituents and plant traits. In (b), the top, dashed line represents low-sulfate conditions, and the lower, solid line represents conditions with elevated sulfate loading. Time intervals ($t_0 - t_5$) do not represent consecutive years, because time between peaks is typically 3–5 years for wild rice.

iron in the first 4 years of the experiment, but iron's effect diminished by 2018 and 2019 (Figure 5) and porewater measurements in 2019 confirmed that iron concentrations were low in all elevated sulfate treatments, regardless of whether the mesocosms received additional iron (Figure 2).

The effect of sulfur on wild rice population dynamics is further complicated by oscillations due to a lag in nitrogen mineralization from litter relative to the timing of nitrogen uptake during the life cycle (Walker et al., 2010). We present a conceptual model built on the synthesis of previous work elucidating the connection between nitrogen and biomass cycles (Pastor & Walker, 2006; Walker et al., 2010); connections between sulfate, biomass, and seed production (Johnson et al., 2019; LaFond-Hudson et al., 2018; Pastor et al., 2017); and the present study which explores the role of sulfate and biomass cycles (Figure 7). Within each life cycle, nitrogen controls plant growth, and seed production (Grava & Raisanen, 1978). Litter immobilizes then slowly mineralizes nitrogen, leading to alternating negative and positive effects on nitrogen availability (Walker et al., 2010, Figure 7a). When sulfate is added to the system, production of sulfide directly decreases seedling survival and seed production (Figure 7a and Pastor et al., 2017). Iron precipitates sulfide into less-reactive iron sulfide, potentially alleviating some of the effects of sulfide on seedling survival and seed production, although iron sulfide also accumulates on root surfaces concomitant with decreased seed nitrogen (LaFond-Hudson et al., 2018). As the life cycle repeats each year (Figure 7a), these geochemistry-plant interactions lead to different interannual trajectories depending on the level of sulfate present in the system (Figure 7b). In low-sulfate conditions, high biomass production 1 year leads to an above average amount of litter that immobilizes nitrogen and decreases nitrogen availability in the following year(s) (Figure 7b, t_0, t_1). The lower nitrogen availability creates competition among seedlings for scarce nitrogen, leading to diminished biomass and seed production. As litter slowly decays, it releases nitrogen during subsequent years (Figure 7b t_2), and thereby increases seedling survival, biomass, and seed production. Alternating nitrogen availability leads to stable biomass cycles in low-sulfate environments (Figures 3 and 7b, t_4 etc.). At the levels of experimental sulfate addition we used, sulfur impacts overwhelmed the effects of litter-controlled N availability, causing the populations to quickly decrease (Figures 3 and 7b, t_3). When constant sulfate loading sustains declines in seedling survival and seed production, both germination and population biomass decline, eventually to extinction (Figure 7b, t_5).

Although we examined complicated interactions among sulfur, iron, organic carbon, nitrogen, and plant cycles, the mesocosms we used in this study are a relatively simple system containing only one species, homogenized sediment across mesocosms, and little hydrological mixing or external nutrient inputs. Projects that manage or restore plant populations in aquatic ecosystems occur in systems affected by interactions between geochemistry, surface and groundwater hydrology, and competing ecological communities. Many such projects monitor biomass annually, but traditional time series analyses require lengthy monitoring periods. When the monitoring period is

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too short to detect population oscillations with a time series analysis, it may be insightful to use annual biomass data from one or two population cycles to calculate and analyze eigenvalues that describe the population's stability. In our experiment, we calculated and analyzed eigenvalues from less than two population cycles and our eigenvalue for populations in low-sulfate conditions corresponded closely to the results of Walker et al. (2010). Even though these were different experiments done during nonoverlapping time periods, the two experiments corroborate one another and strongly suggest that wild rice populations unimpeded by sulfate loadings oscillate stably with an approximate period of 4 years. Additionally, these findings are consistent with observations in some regional lakes and rivers containing wild rice stands (Vogt, 2021). This method is general enough to be applied analogously to data from other field studies and may be useful for identifying at-risk populations or determining whether management and restoration of an oscillating population is effective.

Only some aquatic plants experience population oscillations; however, many are limited by nitrogen and experience sustained or occasional exposure to sulfide, for example, the salt marsh species *Spartina alterniflora* (Mendelssohn & Morris, 2000). Estuarine wetlands receive sulfate from tidal inputs and interannual changes in precipitation can lead to wide variations in interannual nitrogen loading and sulfate intrusion (Sinha & Michalak, 2016). Understanding how plants respond to sulfate and sulfide under fluctuating nitrogen availability may be critical for understanding wetland vegetation dynamics. Perhaps species with different patterns of nitrogen uptake compared to wild rice can be more resilient to sulfide exposure, or plants with greater sulfide tolerance benefit more from increased sulfur-mediated nutrient availability.

5. Conclusion

This study demonstrates the importance of both litter-driven biomass oscillations and sulfate concentrations to population trajectories over several generations and corroborates studies that investigated these processes separately (Johnson et al., 2019; Pastor et al., 2017; Walker et al., 2010). Our observations show predictable and stable biomass oscillations in systems with less than 10 mg L^{-1} sulfate in surface water and rapid population declines in systems with surface water sulfate elevated to 300 mg L^{-1}. Although population biomass did oscillate in elevated sulfate conditions, oscillations were unstable and less predictable than in low-sulfate conditions. This work aggregates well-understood rhizosphere and geochemical processes to interpret the effects realized at a population scale. We did not find consistent or sustained contributions of iron or litter to stability of wild rice populations. Instead, interactions between sulfate, litter, and iron in this study point to complex couplings among plant life cycles, nutrient availability, and iron and sulfur cycling that become manifest over several generations through at least one population cycle. We conclude that both geochemical context and plant life cycle patterns play a considerable role in determining the stability of oscillating plant populations.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Data are publicly available at the Digital Repository for University of Minnesota at https://doi.org/10.13020/cq0g-r486 and are cited within the data analysis section of the methods.

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NMW Exh. 10

Water body name	Water body description	Water body	Year added	Basin	AUID	Use Class	County	HUC 8	Watershed name	Partial tribal designation	Affected designated use	Pollutant or stressor	TMDL Commitment	EPA category	Mercury TMDL	Year TMDL plan	TMDL ID	AUID previously listed	Changes since last List	Progress status
Embarrass River	Esquagama Lk to St Louis R	Stream	2024	Lake Superior	04010201-B00	2Bg	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Cannon River	Belle Cr to split near mouth	Stream	2024	Upper Mississippi River, Lower Portion	07040002-501	2Bg	Goodhue	2 07040002	Cannon River		Wild Rice Production	Sulfate		2	5					FINAL
Buffalo River	Unnamed ditch to Hay Cr	Stream	2024	Red River of the North	09020106-594	2Bg	Becker	6 09020306	Buffalo River		Wild Rice Production	Sulfate		2	5					FINAL
Poplar River	Spring Lk to Highway 59	Stream	2024	Red River of the North	09020305-518	2Bg	Polk	5 09020305	Clearwater River		Wild Rice Production	Sulfate		2	5					FINAL
Hill River	Hill River Lk to Lost R	Stream	2024	Red River of the North	09020305-539	2Bg	Red Lake	5 09020305	Clearwater River		Wild Rice Production	Sulfate		2	5					FINAL
Dunka River	Unnamed ditch to Birch Lk	Stream	2024	Rainy River	09030001-987	1B, 2Ag	St. Louis	1 09030006	Rainy River - Headwaters		Wild Rice Production	Sulfate		2	5					FINAL
Sturgeon River	Headwaters (Little Sturgeon Lk 69-1290-00) to E Br Sturgeon R	Stream	2024	Rainy River	09030005-527	2Bg	St. Louis	5 09020201	Little Fork River		Wild Rice Production	Sulfate		2	5					FINAL
Rice	Lake or Reservoir	Lake	2024	Minnesota River	10-0078-00	2B	Carver	2 07020012	Lower Minnesota River		Wild Rice Production	Sulfate		2	5					FINAL
Tilde	Lake or Reservoir	Lake	2024	Red River of the North	14-0004-00	2B	Clay	8 09020106	Wild Rice River		Wild Rice Production	Sulfate		2	5					FINAL
Little Rabbit	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	18-0139-00	2B	Crow Wing	4 07010104	Mississippi River - Brainerd		Wild Rice Production	Sulfate		2	5					FINAL
Bear	Lake or Reservoir	Lake	2024	Cedar River	24-0028-00	2B	Freeborn	3 07080201	Winnebago River		Wild Rice Production	Sulfate		2	5					FINAL
North Twin	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	31-0190-00	2B	Itasca	3 07010104	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Trout	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	31-0216-00	1B, 2A	Itasca	3 07010104	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Elizabeth (Main Lake)	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	34-0022-02	2B	Kandiyohi	5 07010205	South Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Green	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	34-0079-00	2B	Kandiyohi	4 07010205	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Orwell	Lake or Reservoir	Lake	2024	Red River of the North	56-0945-00	2B	Otter Tail	3 09020107	Otter Tail River		Wild Rice Production	Sulfate		2	5					FINAL
Birch	Lake or Reservoir	Lake	2024	Rainy River	69-0003-00	2B	Lake	1 09030006	Rainy River - Headwaters		Wild Rice Production	Sulfate		2	5					FINAL
Pearl	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	73-0037-00	2B	Stearns	2 07010205	Sauk River		Wild Rice Production	Sulfate		2	5					FINAL
Rice	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	73-0196-00	2B	Stearns	4 07010205	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Clearwater (West)	Lake or Reservoir	Lake	2024	Upper Mississippi River, Upper Portion	86-0252-02	2B	Stearns	3 07010206	Mississippi River - St. Cloud		Wild Rice Production	Sulfate		2	5					FINAL
Dark	Lake or Reservoir	Lake	2024	Rainy River	69-0790-00	2B	St. Louis	5 09030006	Little Fork River		Wild Rice Production	Sulfate		2	5					FINAL
Long Prairie River	Spruce Cr to Eagle Cr	Stream	2022	Upper Mississippi River, Upper Portion	07010108-505	2Bg	Todd	8 07010108	Long Prairie River		Wild Rice Production	Sulfate		2	5					FINAL
Stella	Lake or Reservoir	Lake	2022	Upper Mississippi River, Upper Portion	47-0068-00	2B	Meeker	4 07010205	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Goodners	Lake or Reservoir	Lake	2022	Upper Mississippi River, Upper Portion	73-0076-00	2B	Stearns	2 07010205	Sauk River		Wild Rice Production	Sulfate		2	5					FINAL
Partridge River	Headwaters to St Louis R	Stream	2020	Lake Superior	04010201-552	2Bg	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Embarrass River	Headwaters to Embarrass Lk	Stream	2020	Lake Superior	04010201-579	2Bg	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Second Creek (First Creek)	First Cr to Partridge R	Stream	2020	Lake Superior	04010201-952	2Bg	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Embarrass River	Embarrass Lk thru Esquagama Lk	Stream	2020	Lake Superior	04010201-A99	2Bg	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Swan River	Swan Lk to Trout Cr	Stream	2020	Upper Mississippi River, Upper Portion	07010103-753	2Bg	Itasca	3 07010103	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Long Prairie River	Fish Trap Cr to Crow Wing R	Stream	2020	Upper Mississippi River, Upper Portion	07010108-501	2Bg	Morrison	8 07010108	Long Prairie River		Wild Rice Production	Sulfate		2	5					FINAL
Crow River, Middle Fork	Headwaters to Monongalia (Mud) Lk	Stream	2020	Upper Mississippi River, Upper Portion	07010204-537	2Bg	Kandiyohi	4 07010204	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Stanchfield Creek	Tres Cr (Stanchfield Bk) to Rum R	Stream	2020	Upper Mississippi River, Upper Portion	07010207-518	2Bg	Isanti	7 07010207	Rum River		Wild Rice Production	Sulfate		2	5					FINAL
Trott Brook	CD 51 to Rum R	Stream	2020	Upper Mississippi River, Upper Portion	07010207-680	2Bg	Anoka	7 07010207	Rum River		Wild Rice Production	Sulfate		2	5					FINAL
Mississippi River	Chippewa R (WI) to L & D #6	Stream	2020	Upper Mississippi River, Lower Portion	07040003-627	2Bg	Wabasha	3 07040003	Mississippi River - Winona		Wild Rice Production	Sulfate		2	5					FINAL
Mississippi River	Root R to MN/IA border	Stream	2020	Upper Mississippi River, Lower Portion	07060001-509	2Bg	Houston	1 07060001	Mississippi River - Reno		Wild Rice Production	Sulfate		2	5					FINAL
Clearwater River	Ruffy Bk to JD 1	Stream	2020	Red River of the North	09020305-647	2Bg	Clearwater	5 09020305	Clearwater River		Wild Rice Production	Sulfate		2	5					FINAL
Sand River	Headwaters (Sandy Lk 69-0730-00) to Pike R	Stream	2020	Rainy River	09030002-501	2Bg	St. Louis	2 09030006	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Pike River	Sand R to Vermilion Lk	Stream	2020	Rainy River	09030002-503	2Bg	St. Louis	2 07040002	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Sturgeon	Lake or Reservoir	Lake	2020	Upper Mississippi River, Lower Portion	25-0017-01	2B	Goodhue	1 07040001	Mississippi River - Lake Pepin	Prairie Island	Wild Rice Production	Sulfate		2	5					FINAL
Hay	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	31-0037-00	2B	Itasca	3 07010104	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Swan Lake Southwest Bay	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	31-0067-03	2B	Itasca	3 07010104	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Ox Hide	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	31-0106-00	2B	Itasca	3 07010104	Mississippi River - Grand Rapids		Wild Rice Production	Sulfate		2	5					FINAL
Lake Monongalia - main basin	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	34-0158-01	2B	Kandiyohi	4 07010204	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
Lake Monongalia - Middle Fork Crow River	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	34-0158-02	2B	Kandiyohi	4 07010204	North Fork Crow River		Wild Rice Production	Sulfate		2	5					FINAL
East Vermilion	Lake or Reservoir	Lake	2020	Rainy River	69-0378-01	1C, 2Bd	St. Louis	2 09030006	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Vermilion - Pike Bay	Lake or Reservoir	Lake	2020	Rainy River	69-0378-03	1C, 2Bd	St. Louis	2 09030006	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Wynne	Lake or Reservoir	Lake	2020	Lake Superior	69-0434-02	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Embarrass	Lake or Reservoir	Lake	2020	Lake Superior	69-0496-00	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Esquagama	Lake or Reservoir	Lake	2020	Lake Superior	69-0565-00	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Cedar Island (N.Portion)	Lake or Reservoir	Lake	2020	Lake Superior	69-0568-01	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Cedar Island (S.Portion)	Lake or Reservoir	Lake	2020	Lake Superior	69-0568-02	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Perch	Lake or Reservoir	Lake	2020	Lake Superior	69-0688-00	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Little Sandy	Lake or Reservoir	Lake	2020	Rainy River	69-0729-00	2B	St. Louis	2 09030006	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Sandy	Lake or Reservoir	Lake	2020	Rainy River	69-0730-00	2B	St. Louis	2 09030006	Vermilion River		Wild Rice Production	Sulfate		2	5					FINAL
Upper Estuary	Lake or Reservoir	Lake	2020	Lake Superior	69-1291-04	2B	St. Louis	1 04010201	St. Louis River		Wild Rice Production	Sulfate		2	5					FINAL
Rice	Lake or Reservoir	Lake	2020	Upper Mississippi River, Upper Portion	71-0142-00	2B	Sherburne	3 07010206	Mississippi River - St. Cloud		Wild Rice Production	Sulfate		2	5					FINAL

NMW Exh. 11



Minnesota's Total Maximum Daily Load Studies Prioritization Framework 2022–2032

April 2024

MINNESOTA POLLUTION CONTROL AGENCY

CLEAN WATER LAND & LEGACY AMENDMENT

Document Number: wq-iw1-82

1. Introduction

The U.S. Environmental Protection Agency (EPA) requires states, territories, and authorized tribes to submit a Total Maximum Daily Load (TMDL) prioritization framework. The framework is a planning document that describes long-term priorities, a rationale for selecting those priorities, and a general strategy for implementing the goals of the EPA's 2022–2032 Vision.

This document is Minnesota's TMDL Prioritization Framework for 2022–2032 (Framework), and it provides supporting documentation to Minnesota's impaired waters lists[1] over the Framework time period. The draft Framework was made available for public review during the public notice period for the 2024 impaired waters list and is posted on the Minnesota Pollution Control Agency's (MPCA) TMDL and WRAPS guidance[2] web page.

The EPA also requires two-year commitments for completing TMDLs starting with federal fiscal year (FY) 2025–2026. The Framework must be shared with EPA by April 1, 2024, and the first set of two-year commitments must be submitted to EPA through its ATTAINS (Assessment, TMDL, Tracking, and Implementation System) database by September 30, 2024.

The Framework describes Minnesota's process for selecting water bodies on Minnesota's impaired waters list for TMDL development and describes how the MPCA sets priorities and commitments for TMDLs under the EPA's Vision process.

2. EPA's Vision Process and Minnesota's TMDL Commitments

The EPA developed a Vision for the Clean Water Act Section 303(d) program[3] beginning in 2013. Section 303(d) requires states, territories, and authorized tribes to list impaired waters and develop TMDLs for water bodies in need of a TMDL. A TMDL establishes the maximum amount of a pollutant allowed in a water body and serves as the starting point or scientific planning tool for restoring water quality.

The EPA's 2013 Vision was a long-term planning and prioritization process, and states and territories were asked to put together a TMDL priority framework report and TMDL priorities and commitments for 2016–2022.

Minnesota's TMDL Priority Framework Report[4] (2015, with 2022 updates) included a list of priority impairments for 2016–2022. This long-term framework reflected Minnesota's priorities for development of TMDLs and included impairments for which MPCA committed to develop TMDLs—1,225 impairments on Minnesota's 2014 impaired waters list. Of these, 280 impairments were removed during EPA's "open season" for priority list modification. At the end of the 2013 Vision period in 2022, the MPCA had completed 98% of the final priority list of TMDL commitments.

[1] https://www.pca.state.mn.us/air-water-land-climate/minnesotas-impaired-waters-list
[2] https://www.pca.state.mn.us/business-with-us/tmdl-and-wraps-guidance
[3] https://www.epa.gov/tmdl#:~:text=Section%20303(d)%20of%20the%20Clean%20Water%20Act%20authorizes%20EPA,(TMDLs)%20for%20these%20waterbodies.
[4] MPCA 2015, with February 2022 update. Minnesota's TMDL Priority Framework Report. Document #wq-iw1-54.

The bridge metric TMDL commitments—*Minnesota's TMDL Priorities for FY23–24*[5]—were developed for federal FY23–24 as a bridge between the original 2013 Vision and the 2022 Vision. Minnesota committed to complete and have EPA-approved TMDL reports for 70 impairments in FY23–24. The EPA expects states and tribes to achieve at least 85% of these TMDL commitments, using the EPA's watershed area-based tracking metric.

The **EPA's 2022 Vision** covers 2022–2032 and builds on the experience gained from implementing the 2013 Vision. The *2022–2032 Vision for the Clean Water Act Section 303(d) Program*[6] "is intended to encourage flexible and innovative approaches for states, territories, and authorized tribes … to implement CWA Section 303(d), as well as to identify ways to use limited resources to lead to restoration and protection, to leverage partnerships, and to encourage development of solutions to emerging and difficult water quality issues."

2.1 Minnesota's 2022–2032 TMDL Commitments

Every two years, the MPCA will commit to developing TMDLs for specific impairments, using this Framework as a guide. The TMDL commitments for each two-year period reflect the components of Minnesota's Watershed Approach and other statewide strategies and initiatives (described in Section 3).

The TMDL commitment list may also take into account degree of impairment, local water plans, development pressure, aquatic recreation significance, needs of other state programs such as wastewater and stormwater, and data availability. The MPCA consults with other state agencies, local partners, tribal nations, and other interested stakeholders to decide for which impairments TMDLs will be developed.

This biennial TMDL commitment list includes TMDLs that MPCA commits to being EPA-approved and TMDLs that MPCA commits to being in progress during the two-year period. This list of TMDL commitments is posted on MPCA's TMDL and WRAPS guidance[7] web page.

The two-year periods for which MPCA will develop TMDL commitment lists are federal FY 2025–2026, 2027–2028, 2029–2030, and 2031–2032, and the lists will be finalized by September 30 of every even year. The federal fiscal years are from October 1 of the first year through September 30 of the following year. For example, the first TMDL commitment list after the bridge metric period will cover 10/1/2024–9/30/2026 and will be finalized by 9/30/2024.

Minnesota expects to complete additional TMDLs for impairments on the impaired waters list as part of our ongoing Watershed Approach. The MPCA will report on progress on the federal FY 2025–26 TMDL commitments and additional TMDLs completed through ATTAINS and as part of our Environmental Performance Partnership Agreement (EnPPA) and Performance Partnership Grant (PPG) workplan.

2.2 Integration of TMDL Commitments with the Biennial Impaired Waters List and Integrated Report

Federal regulations require states and tribes to include a priority ranking for impaired waters that require TMDLs (40 CFR § 130.7 (b)(4)). The *TMDL Commitment Grouping* in Minnesota's 2024 impaired

[5] MPCA 2022. Minnesota's TMDL Priorities for FY23-24. Document #wq-iw1-8.
[6] https://www.epa.gov/system/files/documents/2022-09/CWA%20Section%20303d%20Vision_September%202022.pdf
[7] https://www.pca.state.mn.us/business-with-us/tmdl-and-wraps-guidance

waters list indicates MPCA's priority ranking and reflects the Watershed Approach and other statewide strategies and initiatives as described in this Framework (Section 3). The *TMDL Commitment Grouping* identifies waters targeted for TMDL development in the two-year period following the release of the impaired waters list and consists of two groups:

- TMDL Commitment Grouping 1—All impairments on the MPCA's TMDL commitment list, which includes impairments for which TMDLs will be EPA-approved ("completed") during the two-year period and TMDLs that will be in progress during the two-year period. As noted above, the list of TMDL Commitments in Grouping 1 can be found in the *TMDL Commitment Grouping* field in the latest impaired waters list.[8]
- TMDL Commitment Grouping 2—All remaining impairments that need a TMDL (i.e., category 5 in the *EPA category* field of the impaired waters list)

This Framework does not preclude development of TMDLs for impairments in TMDL Commitment Grouping 2.

3. Minnesota's Prioritization Framework to meet the 2022 Vision

3.1 Foundation for the Prioritization Framework

The 2022–2032 Prioritization Framework reflects Minnesota's values and strategies to protect its abundant water resources.

Minnesota's Governor Walz proclaimed October 2022 the Month of Clean Water on the 50th anniversary of the Clean Water Act.[9] The State of Minnesota's proclamation[10] states the importance of clean water to the state of Minnesota:

- Minnesota's abundant lakes, rivers, and streams provide its residents with clean drinking water and year-round recreational opportunities that greatly enhance their quality of life and support healthy stands of wild rice, aquatic species, and wildlife.
- Clean water plays a vital role in the economic prosperity of Minnesota by supporting a robust tourism industry, providing a sustainable fishing industry, and sustaining our industrial and agricultural sectors.
- Minnesota voters passed a historic amendment to the state constitution in 2008, the Clean Water and Land and Legacy Amendment, which provided the financial support for state programs to protect, enhance, and restore water quality in lakes, rivers, and streams, protect groundwater from degradation, and protect drinking water sources.
- This work relies on continued collaboration between federal, state, tribal, and local governments, to protect and improve our waterways.

[8] https://www.pca.state.mn.us/air-water-land-climate/minnesotas-impaired-waters-list
[9] https://www.pca.state.mn.us/business-with-us/clean-water-act-50th-anniversary
[10] https://mn.gov/governor/assets/10.01.22%20Month%20of%20Clean%20Water_tcm1055-544257.pdf

- In the last 50 years, the health of our lakes, rivers, streams, and wetlands have dramatically improved for many pollutants.

Clean Water, Land and Legacy Amendment

As noted in the Governor's proclamation, the resources supporting the Framework largely come from the Clean Water, Land, and Legacy Amendment, approved by the voters in 2008, which supports the "Clean Water Fund."[11] The Fund has been the most important factor in accelerating the breadth and pace of Minnesota's clean water work by creating a stable funding source for a watershed-based approach to protect and restore our water resources.

The Legacy Amendment increases the state sales tax by three-eighths of one percent continuing until 2034, and 33% of the sales tax revenue from the Legacy Amendment is allocated to the Clean Water Fund. Those funds may only be spent to protect, enhance, and restore water quality in lakes, rivers, and streams and to protect groundwater from degradation.

The Clean Water Fund amounts to over $300 million biennially. The *Clean Water Fund Performance Report*[12] summarizes the funds invested, action taken, and outcomes achieved. Past and current investments include monitoring, assessment and characterization, nonpoint source implementation, groundwater/drinking water implementation, and research, evaluation and tool development activities.

Because of the significant resources provided by the Clean Water Fund, the MPCA's prioritization of TMDLs is not so much about applying scarce resources to a limited number of projects, but rather applying those resources to TMDLs across the entire state in a systematic fashion.

3.2 MPCA's Watershed Approach

Protecting Minnesota's waters is a joint effort among seven partner agencies who collaborate and partner on Minnesota's water resource management activities under the Clean Water Fund.

Minnesota adopted *The Minnesota Water Management Framework*,[13] which is a watershed-based management approach that promotes increased collaboration and a common vision for planning and implementation activities. Partnerships among state agencies, tribes, local governments, and stakeholders play a key role in successful resource management as they prioritize, target, and measure Clean Water Fund activities.

As part of the framework, MPCA follows a "Watershed Approach"[14] in which the MPCA and its partners systematically evaluate waters in each major watershed in Minnesota every 10 years, following the *Watershed Lake and Stream Monitoring Schedule* (2018–2028).[15] This process begins with lake and

[11] https://www.legacy.mn.gov/clean-water-fund
[12] https://www.pca.state.mn.us/sites/default/files/lrp-f-1sy22.pdf
[13] https://bwsr.state.mn.us/sites/default/files/The%20Minnesota%20Water%20Management%20Framework%202023.pdf
[14] https://www.pca.state.mn.us/air-water-land-climate/watershed-approach-to-water-quality
[15] https://www.pca.state.mn.us/sites/default/files/wq-swm1-12.pdf

stream water quality and biological monitoring. Once completed, the MPCA and its partners assess the monitoring data to determine if the water bodies meet state water quality standards.

Waters not meeting water quality standards are considered "impaired" and are added to Minnesota's impaired waters list for potential TMDL development. The MPCA develops its list of two-year TMDL Commitments from the impaired waters list to meet the requirements of EPA's Vision process.

The Watershed Approach rotates every year through the state's watersheds, completing the evaluation of waters through the whole state in a 10-year cycle, evaluating 6 to 8 major watersheds each year. While intensive watershed monitoring closely follows the 10-year schedule to maintain the data record, TMDL and Watershed Restoration and Protection Strategy (WRAPS) development timing is more flexible based largely on local partner timing needs. The following is an overview of the first two cycles.

- First cycle of the Watershed Approach: Working with local and regional partners, and funded by the Clean Water, Land, and Legacy Amendment, the MPCA completed the first cycle of the Watershed Approach in June 2023 (see "The power of partnerships" at Water quality resources[16]), when the last of the WRAPS reports were completed for all 80 major watersheds in the state. Intensive watershed monitoring was completed for all 80 watersheds in 2019.

  The first round of watershed monitoring and assessment provided a baseline for determining where waters need protection and restoration, and for informing selection of management strategies. Each WRAPS report uses monitoring and modeling data, along with information from stressor identification and TMDL studies in the watershed, and develops ideas for local strategies needed on the ground to protect and restore waters. This informs local water planning called One Watershed, One Plan[17] (1W1P) to develop comprehensive local water plans that target local implementation activities to see improvement in water quality.

- Second cycle of the Watershed Approach: The MPCA began its second cycle of the Watershed Approach in 2019. The second round of watershed-based lake and stream monitoring continues to include biological, fish contaminant, water quality, and pollutant load sampling. This monitoring is essential to measure progress in restoring and protecting lakes and streams. Additionally, the monitoring will fill gaps to guide local planning and implementation efforts and track long-term changes in water quality and biological communities over time. This second cycle will start to show the effects of restoration and protection efforts that were implemented based on first-cycle WRAPS reports. "WRAPS Updates" (see more below in Section 3.2.1) are underway in many watersheds, and several are scheduled for completion each year during the Vision period.

- Future cycles: Going forward, the MPCA will update WRAPS reports, coordinate with local partners to understand local conditions, and revise targets for successive phases of restoration and protection efforts.

[16] https://www.pca.state.mn.us/business-with-us/water-quality-resources
[17] https://bwsr.state.mn.us/one-watershed-one-plan

Throughout the 2022 Vision period, the MPCA will continue to use the Watershed Approach as the basis for the Framework, and to select impairments for 2022–2032 TMDL commitments. The following sections describe these key components of the MPCA's Watershed Approach in more detail: WRAPS, TMDLs, protection studies, and public participation.

3.2.1 Watershed Restoration and Protection Strategies

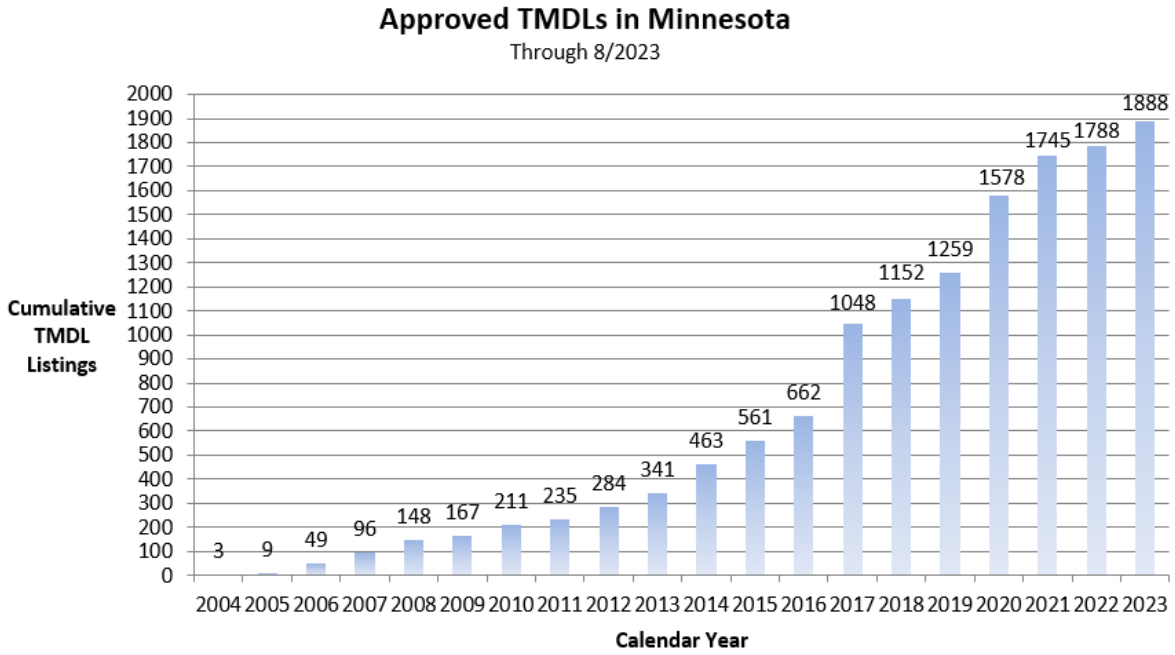
The MPCA develops WRAPS reports, which are required by the state Clean Water Legacy Act. WRAPS use TMDL reports, monitoring results, stressor identification, modeling, and other information to develop strategies for restoring polluted waters and protecting healthy ones. Local partners use this information to develop and set priorities for Comprehensive Watershed Management Plans and implementation projects. As of June 2023, WRAPS have been approved for all 80 watersheds.

"WRAPS Updates" tell the story of what has changed in each watershed since the original WRAPS was completed and focus on new issues and watershed information and action needs. As part of WRAPS Updates, watersheds will have monitoring and assessment and stressor identification report updates, new TMDLs, and many will have additional studies or source assessments depending on the priorities of local partners. Watershed Updates will vary in scale as appropriate. The focus will be on what has changed, trends, what work has been done, new strategies needed, and what additional work is most needed to advance water quality goals in that watershed. The WRAPS Update is a vehicle to synthesize the work that has been done and document ongoing or future work needs. WRAPS Updates add value to the Watershed Approach and include work that is deemed useful to restoration and protection efforts.

3.2.2 Total Maximum Daily Loads

As noted above, TMDLs support the restoration components of WRAPS projects, and are the measure for MPCA's two-year TMDL Commitments to EPA for the 2022 Vision. As explained in Section 2.1, impaired waters are identified as commitments based on the expectation that a TMDL will be completed or in-progress for that impaired listing.

Following the Watershed Approach, watershed TMDLs are organized based on a rotating 10-year schedule, and provide source assessments and pollutant load allocations for permitted and nonpermitted pollutant sources. There have been significant gains in efficiency over the past decade (see figure below) as the agency has evolved the approach to TMDL development to a watershed scale.



To view the status of WRAPS and TMDLs, including that of select 2022–2032 TMDL Commitments, see MPCA's *Healthier Watersheds* webpage.[18]

3.2.3 Protection Studies

The MPCA has created a process for developing "protection studies" using the Watershed Approach. A protection study establishes water quality targets and goals for a water body that already meets water quality standards:

- Targets for water body condition. The target may be a numeric target that is equal to or more restrictive than a state water quality standard (e.g., lake phosphorus concentration, stream TSS concentration), or may be for a parameter for which a numeric state water quality standard does not exist (e.g., frequency of nuisance algae blooms, stream temperature).
- Goals to meet the condition targets (e.g., load reduction goal, vegetation enhancement goals).

The approach is similar to a TMDL study; the difference is that in a TMDL study the numeric target is typically a state water quality standard, whereas in a protection study the numeric target may be lower than the water quality standard and is developed with input from local partners. To provide meaningful load reduction goals in a protection study, it is important to already have data on the water body condition and pollutant sources.

The following general steps make up a protection study:

- Characterize water body and watershed
- Establish target for water body condition

[18] https://www.pca.state.mn.us/business-with-us/healthier-watersheds-tracking-the-actions-taken

- Source assessment
- Establish goals to meet the condition targets
- Strategy development (optional)

3.2.4 Public Participation and Partner Engagement

Public participation and engagement refer to education, outreach, marketing, training, technical assistance, and other methods of working with local and state partners to achieve water resource management goals. The MPCA also works with tribal nations to seek their involvement in projects of mutual interest.

During the first cycle of the Watershed Approach, the MPCA made an important early commitment to support this work at the local level. The goals of MPCA's investment were to support partner participation in WRAPS development and to encourage more active participation by individual stakeholders and groups in dialogue and actions that lead to cleaner water. Funds provided by a partner agency, the Board of Water and Soil Resources (BWSR), are now used to support public participation in planning and implementation projects.

WRAPS and TMDL reports are available on MPCA's Watershed Information web page[19] and typically have a 30-day public notice period to gather comments and suggestions before the reports are finalized. Public meetings may also be held to share information on projects, or to get input on draft reports during the public notice period. MPCA's project managers work closely with local partners throughout the development of WRAPS and TMDLs, and local priorities are a key element in determining TMDL Commitments for the 2022 Vision.

3.3 Other Statewide Strategies and Initiatives Informing Minnesota's Priority Framework

In addition to the specific components of the Watershed Approach mentioned above, the MPCA is also working on other key strategies and initiatives that inform Minnesota's Priority Framework and TMDL commitments. Here are some notable examples of the work that will have an ongoing influence on how the agency prioritizes its work.

3.3.1 Nutrient Reduction Strategy

The Minnesota Nutrient Reduction Strategy (NRS)[20] outlines how Minnesota will reduce nutrient pollution in its lakes and streams and limit impacts downstream. Excessive nutrients, particularly phosphorus and nitrogen, pose a significant problem for Minnesota's lakes and rivers, as well as downstream waters including the Great Lakes, Lake Winnipeg, and the Gulf of Mexico. Nutrients are important for human and aquatic life. However, when levels exceed normal conditions, they can cause excessive algae growth, low levels of oxygen, toxicity to aquatic life, and unhealthy drinking water.

The NRS was first published in September 2014, and called for nutrient level reductions in major rivers by 10% to 20%, with 45% to 50% reductions by 2040, compared to baselines in the 1990s. The MPCA has developed estimates of how much the nutrient load must be reduced in each watershed to lower Minnesota's nutrient contribution to waters outside of the state, and many WRAPS and WRAPS Updates

[19] https://www.pca.state.mn.us/business-with-us/watershed-information
[20] https://www.pca.state.mn.us/air-water-land-climate/reducing-nutrients-in-waters

contain strategies to meet these reduction goals. The information will help determine which and how many management practices are needed to achieve nutrient load reductions and will require ongoing work with partners to select and implement effective best management practices.

The MPCA, working with 10 other organizations, has begun a process to update and improve the NRS by 2025. The revised strategy will include the most up-to-date science on practices to reduce nutrients, remaining river nutrient load reductions, and priority areas. It will also include improved approaches to accelerate adoption of best management practices, track and show progress, and work in partnership with local watersheds.

3.3.2 Environmental Justice

All Minnesotans deserve to live in conditions that support a healthy and fulfilling life. One important part of this is living in an environment with clean air, clean water, and unpolluted land. Working to achieve these conditions is at the heart of the MPCA's mission to protect and improve our environment and human health. As with other work in the WRAPS process, the nature of environmental justice work will vary as widely as the context and conditions vary from watershed to watershed.

The MPCA will continue to work on ways to better incorporate environmental justice considerations into the WRAPS Update process. For example, the agency actively seeks to partner with tribal nations on monitoring, assessments, WRAPS Updates, or other watershed activities. The agency invites tribal staff to help identify priority areas within a watershed to inform monitoring site selection and assessment and can provide funding for tribes to conduct monitoring. Tribes are also invited to participate in local working groups, and to provide data, information, Indigenous Knowledge, and/or Traditional Ecological Knowledge about a watershed to include in a monitoring and assessment report, a TMDL report, and/or a WRAPS Update report.

3.3.3 Climate Change

The MPCA is a leading state agency working to address the impacts of climate change on water resources in Minnesota. As a member of the Governor's subcabinet on Climate Change, the MPCA helped create the "Climate Action Framework"[21] and is leading initiatives to meet its goals for the reduction of greenhouse gas emissions and increased resiliency in communities, businesses, agriculture, waterways, contaminated sites, and infrastructure.

The MPCA also participated with state water management agencies to develop the "2020 State Water Plan: Climate and Water,"[22] coordinated by the Minnesota Environmental Quality Board. The MPCA is leading the implementation of the state water plan's action steps, including climate resiliency planning and local water infrastructure grants for local governments and tribal nations**,** water-quality trading permits, tools for tracking greenhouse gas and nutrient reductions from agricultural practices, and new requirements to reduce runoff from feedlots.

Incorporating climate change into WRAPS Update work is a complex challenge. Work will continue to implement these and other strategies using the best scientific tools available to understand and mitigate climate change impacts in Minnesota's lakes and streams. As an example, in the Duluth Urban Area

[21] https://climate.state.mn.us/minnesotas-climate-action-framework
[22] https://www.eqb.state.mn.us/state-water-plan

WRAPS,[23] MPCA considered in-stream temperature sensitivity to prioritize streams in the Lake Superior Watershed that are most vulnerable to climate change, and to implement protection practices.

3.3.4 Mercury

Mercury emitted into the atmosphere deposits in lakes and streams and accumulates in fish, and eating fish with high levels of mercury can be toxic. The MPCA developed a plan to reduce mercury releases[24] by 2025 based on Minnesota's statewide mercury TMDL,[25] which was approved by the EPA in 2007. The plan includes input and recommendations from a broad range of stakeholders. Every two years, the MPCA updates the statewide mercury TMDL to include new impairments that are covered by the statewide TMDL.

The statewide TMDL does not cover mercury impairments in fish where exceptionally high mercury concentrations preclude those waters from meeting the water quality standard even with the mercury source reductions called for in the statewide TMDL. The MPCA has begun development of individual mercury TMDLs for these waters. Mercury concentrations in surface waters can be influenced by multiple environmental factors, including mercury air deposition, dissolved oxygen, dissolved organic matter, and sulfate among other factors.

3.3.5 PFAS

Per- and polyfluoroalkyl substances, commonly known as PFAS, are a family of nearly 5,000 chemicals now pervasive in the environment. Called "forever chemicals," they do not break down and can bioaccumulate in both humans and other living organisms. Some PFAS are toxic.

Minnesota state agencies developed a strategic, coordinated approach to protecting families and communities from these ubiquitous substances. *Minnesota's PFAS Blueprint*[26] takes a three-pronged approach:

- Prevent PFAS pollution wherever possible.
- Manage PFAS pollution when prevention is not feasible or pollution has already occurred.
- Clean up PFAS pollution at contaminated sites.

Minnesota's PFAS Blueprint identifies short- and long-term opportunities, as well as legislative actions, to manage PFAS in our environment and protect families and communities. Over the coming months and years, state agencies will further develop these strategies and engage Minnesotans on how best to implement them.

Future needs and opportunities are complex and resource intensive. State agencies and community partners will need to work together to undertake projects that most strategically advance the collective goal to protect human health and the environment from the impacts of PFAS.

[23] https://www.pca.state.mn.us/sites/default/files/wq-ws4-42a.pdf
[24] https://www.pca.state.mn.us/air-water-land-climate/reducing-mercury-releases
[25] https://www.pca.state.mn.us/business-with-us/statewide-mercury-tmdl
[26] https://www.pca.state.mn.us/air-water-land-climate/minnesotas-pfas-blueprint

3.3.6 *Sulfate*

Wild rice is an important part of the ecosystem in many Minnesota lakes and streams. Wild rice is also a cultural and spiritual resource to many, particularly members of Minnesota's Dakota and Ojibwe tribal communities, and is an important economic resource to those who harvest and market it.

In 1973, Minnesota adopted a sulfate standard to protect wild rice[27] based on studies showing that wild rice was found primarily in low sulfate waters. The MPCA has preliminarily determined a list of approximately 2,400 waters that can reasonably be considered waters that could potentially be used for production of wild rice.

As of the 2022 impaired waters list, 35 water bodies are listed with wild rice production impairments due to high sulfate. Sulfate impairments are predominantly point source driven, and the MPCA is primarily addressing these impairments through discharge permits. The MPCA is continuing to work with the public and state, local, federal, and tribal nations to develop ways to protect this important Minnesota resource through implementation of the long-standing sulfate standard in monitoring, assessment, and permitting.

3.3.7 *Chloride*

Minnesota has a growing salty water problem that threatens its freshwater fish and other aquatic life. The Statewide Chloride Management Plan (CMP)[28] outlines a comprehensive strategy to reduce salt (chloride) use from a variety of sources to protect our lakes, rivers, and other water resources. The CMP incorporates water quality conditions, sources of chloride, salt reduction strategies, protection strategies, and monitoring recommendations as well as measurement and tracking of results.

The plan was developed by the MPCA in partnership with municipalities, counties, watershed districts, and other state experts. As outlined in the CMP, the MPCA and partners will collaborate on a number of salt reduction and prevention efforts:

- Develop chloride TMDLs to address the 54 waters (as of the 2022 impaired waters list) with aquatic life impairments due to chloride.
- Monitor, evaluate, and better understand the level of chloride in lakes, streams, wetlands, and groundwater.
- Develop statewide tools and materials for partners and stakeholders interested in minimizing the impact of chloride on Minnesota lakes, rivers, and groundwater.
- Conduct MPCA's Smart Salting training[29] to help organizations that apply road salt improve operator effectiveness and reduce chloride pollution, while keeping roads, parking lots, and sidewalks safe.
- Offer chloride reduction grants from the MPCA in selected communities to work with residents, local businesses, institutions, and industries to identify chloride sources and decrease or eliminate their use.

[27] https://www.pca.state.mn.us/air-water-land-climate/protecting-wild-rice-waters
[28] https://www.pca.state.mn.us/business-with-us/statewide-chloride-resources
[29] https://www.pca.state.mn.us/business-with-us/smart-salting-training

3.4 Process moving forward and contact information

The Framework is intended to describe the broad, long-term direction for the agency's TMDL program that will fulfill its TMDL Commitments throughout the 2022–2032 Vision period.

This document will remain on the agency's TMDL and WRAPS guidance[30] web page. Any significant updates will be made available for review with the appropriate edition of the impaired waters list. The most recent two-year commitments for TMDLs that will be completed, or are in development, can also be found on the web page.

For questions or comments on the Framework, please see contact information on the MPCA's TMDL and WRAPS guidance web page.

[30] https://www.pca.state.mn.us/business-with-us/tmdl-and-wraps-guidance

NMW Exh. 12

Trump officials blocked US Steel from stopping work at plant

Bloomberg News | September 19, 2025 | 3:51 pm Top Companies USA Iron Ore



Credit: US Steel

The US blocked United States Steel Corp.'s plan to stop production at a plant in Illinois, according to a Trump administration official, a move highlighting the federal government's continued influence over an iconic American firm after approving its takeover by Japan-based Nippon Steel Corp.

The action, first reported by the *Wall Street Journal* on Friday, was confirmed by an official on condition of anonymity.

"US Steel will continue to supply slabs to Granite City," the company said in a statement Friday. "As we shared earlier, our goal was to maintain flexibility, and we are pleased to have found a solution to continue to slab consumption at Granite City."

Nippon Steel clinched its $14.1 billion deal for US Steel in June after a year and a half of negotiations over a contentious deal became a political flashpoint in the 2024 presidential election.

The move, which created the world's second-largest steelmaker, was approved by President Donald Trump after concessions made by Nippon Steel, including a "golden share" scheme that gives the administration a say in certain business actions.

The *Journal* reported Friday that Commerce Secretary Howard Lutnick informed US Steel chief executive officer Dave Burritt that Trump was invoking the golden share to keep operations running at the Granite City plant, citing a person familiar with the matter.

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Anglo-Teck merger to unlock Chile mine synergies, if Glencore signs off
Glencore is an equal partner with Anglo at Collahuasi, one of the world's largest copper deposits.

September 19, 2025 | 03:08 pm
"We're in project Manhattan 2.0", uranium CEO says
In 2024, the US consumed 50 million pounds of uranium, but only produced 677,000 pounds, according to the EIA.

September 19, 2025 | 08:54 am
BHP eyes Geraldine Slattery for CEO role, FT reports
BHP could appoint Slattery, who leads its Australia operations, as the company's first female chief executive in 140 years.

September 19, 2025 | 07:54 am
China's rare earth product exports surge to highest since 2012
Despite the surge in Chinese exports, end-users of rare earth magnets in Europe remain undersupplied.

September 18, 2025 | 09:59 am
Cobalt's EV battery comeback as prices nearly double
The size of the electric vehicle battery cobalt market in August jumped to the highest since December 2022.

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Trump and his Democratic rivals in the 2024 election campaigned against the deal, but the president reversed his position once in office, saying that the agreement would help preserve steel jobs in the US. To help clinch the deal, the Japanese company also agreed to invest an additional $11 billion by 2028. Nippon had previously raised its pledged additional investment in an effort to win Trump's backing.

Nippon Steel settles disputes with USW, Cliffs over US Steel deal



The companies also dropped claims involving Cleveland-Cliffs and its CEO Lourenco Goncalves, who had opposed the deal.

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US Steel last week said it would focus on producing and processing steel slabs at two other facilities while reducing slab consumption at the Granite City plant in Illinois. It said the Granite City facility would be maintained in an operational state and that no employee there would be laid off or have their pay adjusted, casting the decision as one that would allow US Steel to "maintain future flexibility, while maximizing our domestic production footprint."

The United Steelworkers, in a statement Friday, accused US Steel of trying to "wiggle out of its commitments and make the kind of changes we warned were coming" before backing down.

"In addition to our mills, there are two idled blast furnaces in Granite City that could easily help our nation meet its steel needs," the union said in its statement.

(By Doug Alexander and Josh Wingrove)

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2024
UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549
FORM 10-K

(Mark One)

☒ **ANNUAL REPORT PURSUANT TO SECTION 13 OR 15(d) OF THE SECURITIES EXCHANGE ACT OF 1934**

For the Fiscal Year Ended December 31, 2024
Or

☐ **TRANSITION REPORT PURSUANT TO SECTION 13 OR 15(d) OF THE SECURITIES EXCHANGE ACT OF 1934**

For the transition period from to .

Commission file number 1-16811



United States Steel Corporation

United States Steel Corporation
(Exact name of registrant as specified in its charter)

Delaware	**25-1897152**
(State or other jurisdiction of incorporation)	(I.R.S. Employer Identification No.)

600 Grant Street, Pittsburgh, PA 15219-2800
(Address of principal executive offices) (Zip Code)
Registrant's telephone number, including area code: (412) 433-1121
Securities registered pursuant to Section 12(b) of the Act:

Title of Each Class	Trading Symbol(s)	Name of Exchange on which Registered
United States Steel Corporation Common Stock, par value $1.00	**X**	**New York Stock Exchange**
United States Steel Corporation Common Stock, par value $1.00	**X**	**Chicago Stock Exchange**

Securities registered pursuant to Section 12(g) of the Act: None

Indicate by check mark if the registrant is a well-known seasoned issuer, as defined in Rule 405 of the Securities Act. Yes _☑_ No __

Indicate by check mark if the registrant is not required to file reports pursuant to Section 13 or Section 15(d) of the Act. Yes ___ No __☑__

Indicate by check mark whether the registrant (1) has filed all reports required to be filed by Section 13 or 15 (d) of the Securities Exchange Act of 1934 during the preceding 12 months (or for such shorter period that the registrant was required to file such reports), and (2) has been subject to such filing requirements for the past 90 days. Yes __☑__ No _____

Indicate by check mark whether the registrant has submitted electronically every Interactive Data File required to be submitted pursuant to Rule 405 of Regulation S-T (§232.405 of this chapter) during the preceding 12 months (or for such shorter period that the registrant was required to submit such files). Yes __☑__ No _____

Indicate by check mark whether the registrant is a large accelerated filer, an accelerated filer, a non-accelerated filer, a smaller reporting company, or an emerging growth company. See the definitions of "large accelerated filer," "accelerated filer," "smaller reporting company," and "emerging growth company" in Rule 12b-2 of the Exchange Act.

Large accelerated filer ☒ Accelerated filer ☐ Non-accelerated filer ☐ Smaller reporting company ☐ Emerging growth company ☐

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Indicate by check mark whether the registrant has filed a report on and attestation to its management's assessment of the effectiveness of its internal control over financial reporting under Section 404(b) of the Sarbanes-Oxley Act (15 U.S.C. 7262(b)) by the registered public accounting firm that prepared or issued its audit report. ☑

If securities are registered pursuant to Section 12(b) of the Act, indicate by check mark whether the financial statements of the registrant included in the filing reflect the correction of an error to previously issued financial statements. ☐

Indicate by check mark whether any of those error corrections are restatements that required a recovery analysis of incentive-based compensation received by any of the registrant's executive officers during the relevant recovery period pursuant to §240.10D-1(b). ☐

Indicate by check mark whether the registrant is a shell company (as defined in Rule 12b-2 of the Act). Yes ☐ No ☒

Aggregate market value of Common Stock held by non-affiliates as of June 30, 2024, (the last business day of the registrant's most recently completed second fiscal quarter): $8.5 billion. The amount shown is based on the closing price of the registrant's Common Stock on the New York Stock Exchange composite tape on that date. Shares of Common Stock held by executive officers and directors of the registrant are not included in the computation. However, the registrant has made no determination that such individuals are "affiliates" within the meaning of Rule 405 under the Securities Act of 1933.

There were 225,216,524 shares of United States Steel Corporation Common Stock outstanding as of January 27, 2025.

Documents Incorporated By Reference:

Portions of the Proxy Statement for the 2025 Annual Meeting of Stockholders are incorporated into Part III.

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FORWARD-LOOKING STATEMENTS

This report contains information regarding the Company that may constitute "forward-looking statements," as that term is defined under the Private Securities Litigation Reform Act of 1995 and other securities laws, that are subject to risks and uncertainties. We intend the forward-looking statements to be covered by the safe harbor provisions for forward-looking statements in those sections. Generally, we have identified such forward-looking statements by using the words "believe," "expect," "intend," "estimate," "anticipate," "project," "target," "forecast," "aim," "should," "plan," "goal," "future," "will," "may" and similar expressions or by using future dates in connection with any discussion of, among other things, statements expressing general views about future operating or financial results, operating or financial performance, trends, events or developments that we expect or anticipate will occur in the future, anticipated cost savings, potential capital and operational cash improvements and changes in the global economic environment, anticipated capital expenditures, the construction or operation of new or existing facilities or capabilities and the costs associated with such matters, statements regarding our greenhouse gas emissions reduction goals, as well as statements regarding the merger between the Company and Nippon Steel Corporation (the "Merger"), including the timing of the completion of the Merger. However, the absence of these words or similar expressions does not mean that a statement is not forward-looking. Forward-looking statements include all statements that are not historical facts, but instead represent only the Company's beliefs regarding future goals, plans and expectations about our prospects for the future and other events, many of which, by their nature, are inherently uncertain and outside of the Company's control. It is possible that the Company's actual results and financial condition may differ, possibly materially, from the anticipated results and financial condition indicated in these forward-looking statements. Management of the Company believes that these forward-looking statements are reasonable as of the time made. However, caution should be taken not to place undue reliance on any such forward-looking statements because such statements speak only as of the date when made. In addition, forward looking statements are subject to certain risks and uncertainties that could cause actual results to differ materially from the Company's historical experience and our present expectations or projections. Risks and uncertainties include without limitation: the ability of the parties to consummate the Merger on a timely basis or at all; the occurrence of any event, change or other circumstances that could give rise to the termination of the definitive agreement and plan of merger relating to the Merger (the "Merger Agreement"); risks arising from litigation related to the Merger, either brought by or against the parties; the risk that the parties to the Merger Agreement may not be able to satisfy the conditions to the Merger in a timely manner or at all; risks related to disruption of management time from ongoing business operations due to the Merger and related litigation; certain restrictions during the pendency of the Merger that may impact the Company's ability to pursue certain business opportunities or strategic transactions; the risk that any announcements relating to the Merger could have adverse effects on the market price of the Company's common stock; the risk of any unexpected costs or expenses resulting from the Merger; the risk that the Merger and its announcement could have an adverse effect on the ability of the Company to retain customers and retain and hire key personnel and maintain relationships with customers, suppliers, employees, stockholders and other business relationships and on its operating results and business generally; and the risk the pending Merger could distract management of the Company. The Company directs readers to Item 1A of this Annual Report on Form 10-K, and the other documents it files with the SEC for other risks associated with the Company's future performance. These documents contain and identify important factors that could cause actual results to differ materially from those contained in the forward-looking statements. All information in this report is as of the date above. The Company does not undertake any duty to update any forward-looking statement to conform the statement to actual results or changes in the Company's expectations whether as a result of new information, future events or otherwise, except as required by law.

References in this Annual Report on Form 10-K to (i) "U. S. Steel," "the Company," "we," "us" and "our" refer to United States Steel Corporation and its consolidated subsidiaries unless otherwise indicated by the context, (ii) "Big River Steel" refers to Big River Steel Holdings LLC and its direct and indirect subsidiaries unless otherwise indicated by the context and (iii) "Transtar" refers to Transtar LLC and its direct and indirect subsidiaries unless otherwise indicated by the context.

Non-Generally Accepted Accounting Principles (non-GAAP) Financial Measures

This report contains the non-GAAP financial measure cash conversion cycle. We believe the cash conversion cycle is a useful measure in providing investors with information regarding our cash management performance and is a widely accepted measure of working capital management efficiency. The cash conversion cycle should not be considered in isolation or as an alternative to other Generally Accepted Accounting Principles (GAAP) metrics as an indicator of performance.

PART I

Item 1. BUSINESS

United States Steel Corporation, with operations in the United States of America and Central Europe, is a steelmaker that is pursuing a customer-centric, competitive, Best for All®, differentiated strategy by investing in our competitive advantages. We are executing on our strategy by investing where we have distinct cost and capability advantages so that we are a superior steel solutions provider for our customers. By offering the new steels that our customers are increasingly demanding, we aim to achieve world-competitive positioning in strategic, high-margin end markets and deliver high-quality, value-added products and innovative solutions utilizing a lower carbon footprint than previously available through our traditional integrated steelmaking model.

U. S. Steel has annual raw steel production capability of 25.4 million net tons (20.4 million tons in North America and 5.0 million tons in Europe). U. S. Steel performs a wide range of applied research, development and technical support functions at facilities in Pennsylvania, Michigan, Texas and Slovakia. U. S. Steel supplies customers throughout the world primarily in the automotive, construction, consumer (packaging and appliance), electrical, industrial equipment, service center/distribution, structural tubing and energy (oil country tubular goods (OCTG) and line pipe) markets. According to the worldsteel Association's latest published statistics, U. S. Steel is the third largest U.S. based steel producer and the twenty-fourth largest steel producer in the world. U. S. Steel is a Delaware corporation established in 1901.

Agreement and Plan of Merger with Nippon Steel Corporation

On December 18, 2023, the Company entered into an Agreement and Plan of Merger (such agreement, as it may be amended, modified or supplemented from time to time, the "Merger Agreement") by and among the Company, Nippon Steel North America, Inc., a New York corporation ("Purchaser"), 2023 Merger Subsidiary, Inc., a Delaware corporation and a wholly owned subsidiary of Purchaser ("Merger Sub"), and solely as provided in Section 9.13 therein, Nippon Steel Corporation, a Japanese corporation ("NSC"). Pursuant to the Merger Agreement, and upon the terms and subject to the conditions described therein, Merger Sub will merge with and into the Company, with the Company continuing as the surviving corporation and a wholly owned subsidiary of Purchaser (the "Merger"). On April 12, 2024, the Company obtained the approval of its stockholders required to adopt the Merger Agreement. The Company's stockholders approved the Merger with 98.8% approval of shares voted, satisfying a condition to closing. Subject to the terms and conditions set forth in the Merger Agreement, each share of the Company's common stock, par value $1.00 per share, outstanding immediately prior to the effective time of the Merger (the "Effective Time") will, at the Effective Time, automatically be converted into the right to receive $55.00 per share in cash, without interest, subject to any required tax withholding. The parties have substantially complied with the Second Requests issued on April 3, 2024 and the statutory waiting period under the Hart-Scott-Rodino Antitrust Improvements Act of 1976 has now expired. In addition, all required regulatory approvals outside of the United States related to the Merger have been received. The closing conditions to the Merger include clearance by the Committee on Foreign Investment in the United States ("CFIUS") under the Defense Production Act of 1950, as amended. Following referral of the transaction by CFIUS to the President of the United States, on January 3, 2025, the President of the United States issued an order prohibiting the Merger and requiring the parties to abandon the Merger Agreement within thirty days. On January 10, 2025, CFIUS granted an extension of that deadline to June 18, 2025.

On January 6, 2025, the Company, NSC and Purchaser jointly filed a lawsuit in the United States Court of Appeals for the District of Columbia Circuit alleging that the President's order blocking the Merger was issued for purely political reasons, which are irrelevant to U.S. national security, challenging the decision by the President of the United States and the CFIUS process as violating the constitutional due process rights of the Company, NSC and Purchaser, as well as the applicable statute governing CFIUS and the Administrative Procedure Act, and as an exercise of power that exceeded the authority provided to the President of the United States under the applicable statute. On the same day, the Company, NSC and Purchaser also filed a lawsuit in the United States District Court for the Western District of Pennsylvania against Cleveland-Cliffs Inc. ("Cliffs"), Cliffs' Chief Executive Officer Lourenco Goncalves, and the President of the United Steel, Paper and Forestry, Rubber, Manufacturing, Energy, Allied Industrial and Service Workers International Union (the "USW"), David McCall, for engaging in a coordinated series of anticompetitive and racketeering activities illegally designed to prevent any party other than Cliffs from acquiring the Company as part of an illegal campaign to monopolize critical domestic steel markets.

Segments

U. S. Steel has four reportable segments: North American Flat-Rolled (Flat-Rolled), Mini Mill, U. S. Steel Europe (USSE) and Tubular Products (Tubular). The Tubular segment includes the electric arc furnace at our Fairfield Tubular Operations in Fairfield, Alabama. The Other category includes the results of our real estate business.

Flat-Rolled

The Flat-Rolled segment includes the operating results of U. S. Steel's integrated steel plants and equity investees in North America involved in the production of slabs, strip mill plates, sheets and tin mill products, as well as all iron ore, direct reduced grade pellets, pig iron, and coke production facilities in the United States. These operations primarily serve North American

customers in the automotive, appliance, construction, container, pipe and tube, sheet converter, solar, industrial equipment and service center markets.

During 2024, Flat-Rolled had aggregate annual raw steel production capability of 13.2 million tons at our Gary Works, Mon Valley Works, and Granite City Works facilities. Raw steel production was 8.4 million tons in 2024, 9.4 million tons in 2023, and 8.8 million tons in 2022. Raw steel production averaged 63 percent of capability in 2024, 71 percent of capability in 2023, and 67 percent of capability in 2022.

Mini Mill

The Mini Mill segment includes the operating results of U. S. Steel's Big River Steel facility and a second mini mill facility, Big River Steel 2 (Big River 2 or BR2), which produced its first coil in October 2024. Both facilities are located in Osceola, Arkansas. The Mini Mill segment produces hot-rolled, cold-rolled, coated sheets and electrical steels. This operation primarily serves North American customers in the automotive, construction, pipe and tube, sheet converter, electrical, solar, industrial equipment and service center markets.

Mini Mill had aggregate annual raw steel production capability of 3.3 million tons at our Big River Steel facility through the third quarter of 2024, which increased to 6.3 million tons at our Big River Steel and BR2 facilities during the fourth quarter of 2024. Raw steel production was 2.8 million tons in 2024, 3.0 million tons in 2023, and 2.7 million tons in 2022. Raw steel production averaged 80 percent of capability in 2024, 89 percent of capability in 2023, and 80 percent of capability in 2022.

European Operations

The USSE segment includes the operating results of U. S. Steel Košice (USSK), U. S. Steel's integrated steel plant and coke production facilities in Slovakia, and its subsidiaries. USSE conducts its business mainly in Central and Western Europe and primarily serves customers in the European transportation (including automotive), construction, container, appliance, electrical, service center, conversion and oil, gas and petrochemical markets. USSE produces and sells slabs, hot-rolled, cold-rolled, hot-dip galvanized, and color-coated coils, electrical steel, strip mill plate, sheet, tin mill products and spiral welded pipe.

USSE has annual raw steel production capability of 5.0 million tons. USSE's raw steel production was 3.8 million tons in 2024, 4.4 million tons in 2023, and 3.8 million tons in 2022. USSE's raw steel production averaged 77 percent of capability in 2024, 88 percent of capability in 2023, and 77 percent of capability in 2022.

Tubular

The Tubular segment includes the operating results of U. S. Steel's tubular production facilities and an equity investee in the United States. These operations can produce and sell rounds, seamless and electric resistance welded (ERW) steel casing and tubing (commonly known as OCTG), and standard and line pipe and mechanical tubing and primarily serve customers in the oil, gas and petrochemical markets. U. S. Steel Tubular Products LLC (USSTP), a wholly owned subsidiary of U. S. Steel, designs and develops a range of premium and semi-premium connections to address our customers' needs. The Tubular segment has annual raw steel production capability of 900 thousand tons. Raw steel production was 575 thousand tons in 2024, 568 thousand tons in 2023, and 634 thousand tons in 2022. Raw steel production averaged 64 percent of capability in 2024, 63 percent of capability in 2023, and 70 percent of capability in 2022. Tubular has total production capability of 1.9 million tons.

For further information, see "Item 7. Management's Discussion and Analysis of Financial Condition and Results of Operations" and Note 4 to the Consolidated Financial Statements.

Steel Shipments by Market and Segment

The following table, except as noted in Footnote 1 below, does not include shipments to end customers by joint ventures and other equity investees of U. S. Steel. Shipments of materials to these entities are included in the "Further Conversion – Joint Ventures" market classification. During 2024, sales to one customer of the Flat-Rolled segment were approximately $2.0 billion which exceeded 10 percent of U. S. Steel's total consolidated net sales. During 2023 and 2022, no single customer accounted for more than 10 percent of U. S. Steel's total consolidated net sales.

(Thousands of Tons)	Flat-Rolled	Mini Mill	USSE	Tubular	Total
Major Market – 2024					
Steel Service Centers	**1,039**	**941**	**877**	**—**	**2,857**
Further Conversion – Trade Customers	**1,709**	**673**	**245**	**—**	**2,627**
– Joint Ventures [1]	**193**	**—**	**—**	**—**	**193**
Transportation and Automotive [1]	**3,389**	**14**	**559**	**—**	**3,962**
Construction and Construction Products	**584**	**591**	**1,224**	**11**	**2,410**
Containers and Packaging	**358**	**6**	**223**	**—**	**587**
Appliances and Electrical Equipment	**441**	**82**	**176**	**—**	**699**
Oil, Gas and Petrochemicals	**—**	**—**	**1**	**465**	**466**
All Other	**132**	**—**	**273**	**—**	**405**
TOTAL	**7,845**	**2,307**	**3,578**	**476**	**14,206**
Major Market – 2023					
Steel Service Centers	1,506	1,116	848	—	3,470
Further Conversion – Trade Customers	1,940	729	293	—	2,962
– Joint Ventures [1]	211	—	—	—	211
Transportation and Automotive [1]	2,876	17	636	—	3,529
Construction and Construction Products	908	483	1,319	31	2,741
Containers and Packaging	570	1	312	—	883
Appliances and Electrical Equipment	429	78	172	—	679
Oil, Gas and Petrochemicals	—	—	—	447	447
All Other	266	—	319	—	585
TOTAL	8,706	2,424	3,899	478	15,507
Major Market – 2022					
Steel Service Centers	1,128	1,080	839	—	3,047
Further Conversion – Trade Customers	2,163	772	289	—	3,224
– Joint Ventures [1]	256	—	—	—	256
Transportation and Automotive [1]	2,611	20	619	—	3,250
Construction and Construction Products	922	310	1,052	30	2,314
Containers and Packaging	693	13	423	—	1,129
Appliances and Electrical Equipment	416	93	225	—	734
Oil, Gas and Petrochemicals	—	—	3	494	497
All Other	183	—	309	—	492
TOTAL	8,372	2,288	3,759	524	14,943

[1] PRO-TEC automotive substrate shipments are included in the Transportation and Automotive category.

Steel Industry Background and Competition

The global steel industry is cyclical, highly competitive and has historically been characterized by global overcapacity.

U. S. Steel's competitive position may be affected by, among other things, differences among U. S. Steel's and its competitors' cost structure, labor costs, environmental remediation and compliance costs, global capacity, achievement of innovations in new technologies and sustainable products and the existence and magnitude of government support.

U. S. Steel competes with many North American and international steel producers. Competitors include 1) integrated producers, which use iron ore and coke as the primary raw materials for steel production, 2) Electric Arc Furnace (EAF) producers, which primarily use steel scrap and other iron-bearing feedstocks as raw materials and 3) slab re-rollers, who purchase mostly imported, but some domestic, semi-finished products and convert them into sheet products. In addition, other materials, such as aluminum, plastics and composites, compete with steel in several applications. According to worldsteel Association, global steel production in 2024 of approximately 1.88 billion metric tons was consistent compared to 2023. Among the top ten steel producing countries, steel production in China, Japan, the United States, and Russia decreased, while production in India, Germany, and Brazil increased. The top five steel producing countries accounted for approximately 74 percent of the world's steel production in 2024.

See "International Trade" below for a discussion of global overcapacity and the Company's efforts to mitigate the competitive impact.

EAF producers typically require lower capital expenditures for construction and operation of facilities and may have lower total employment costs. Some EAF producers utilize thin slab casting technology to produce flat-rolled products and are increasingly able to compete directly with integrated producers in many flat-rolled product applications previously produced only by integrated steelmakers. Slab re-rollers do not incur the cost of melting steel; their input costs are largely driven by the market price of slabs.

U. S. Steel provides defined benefit pension and/or other post-employment benefits to approximately 55,000 current employees, retirees and their beneficiaries. Many of our competitors do not have comparable retiree obligations. Participation in U. S. Steel's main defined benefit pension plan was closed to new entrants on July 1, 2003 and benefit accruals for all non-represented participants were frozen effective December 31, 2015. Participation in U. S. Steel's retiree medical and life insurance programs for USW-represented employees was closed to employees hired or rehired (except in limited circumstances) on or after January 1, 2016. For non-represented employees, retiree medical benefits were eliminated December 31, 2017, and retiree life insurance benefits were eliminated for those who retired after December 31, 2017.

We believe that our major North American and many European integrated steel competitors are confronted with substantially similar environmental regulatory conditions and therefore do not believe that our relative position with regard to such competitors will be materially affected by the impact of environmental laws and regulations. However, if future regulations do not recognize that the integrated steel process involves a series of chemical reactions involving carbon that create carbon dioxide (CO_2) emissions without linking these emissions to steel scrap as well, the competitive position of our integrated operations will be adversely impacted compared to mini mills. Our competitive position compared to producers in developing nations such as China, Russia, Brazil and India will be harmed unless such nations require commensurate reductions in CO_2 emissions or there are policies to adjust for the carbon emissions disparities. Competing materials such as plastics may not be similarly impacted. The specific impact on each competitor will vary depending on a number of factors, including the age and location of its operating facilities and its production methods. U. S. Steel is also responsible for remediation costs related to former and present operating locations and disposal of environmentally sensitive materials. Many of our competitors, including North American producers, or their successors, that have been the subject of bankruptcy relief have no or substantially lower liabilities for such environmental remediation matters.

In 2025, we expect additional steelmaking capacity will enter the domestic steel market as competitors' growth projects come on-line or ramp up to full production in North America throughout the year.

Business Strategy

We are executing on our Best for All® strategy to provide customers with profitable steel solutions for people and planet. Our strategy is focused on developing quality products and customer process solutions by investing where we have distinct cost or capability competitive advantages. We are expanding our competitive advantages in low-cost iron ore, mini mill steelmaking, and best-in-class finishing assets with innovative solutions and commercial acumen. These competitive advantages are built on a foundation of research, innovation and deep customer relationships. In executing our strategy, we aim to enhance our earnings profile, deliver long-term cash flow through industry cycles and reduce our cost, capital, and carbon intensity. By offering the product capabilities, including the more sustainable steels (steels made with lower greenhouse gas emissions), our customers are increasingly demanding, we can achieve more competitive positioning in strategic, high-margin end markets, and deliver high-quality, sustainable, value-added products and innovative solutions.

Our strategy is informed by our critical success factors, which are the bedrock of the Best for All® strategy: (1) Win in Strategic Markets; (2) Move Up the Talent Curve; and (3) Move Down the Cost Curve.

We are focused on strategic markets, where there is the greatest opportunity to provide differentiated, innovative and value-added solutions that will help our customers succeed. We are investing in new technologies to improve our cost position and increase our capabilities, including our mini mill steelmaking and best-in-class finishing capabilities. Beginning in 2021, we began investing nearly $4 billion in the Mini Mill segment to enhance our finishing and EAF capabilities. In 2023, we completed construction of a 200-thousand-ton, non-grain oriented (NGO) electrical steel line at our Big River Steel facility, which produced its first coil in September 2023, and shipped the first prime coil in early 2024. This construction project was completed on-time and on-budget. Construction of BR2 near Osceola, Arkansas continued throughout 2024 and BR2 produced its first coil in October 2024. This new mini mill has about 3 million tons per year of steelmaking capability and combines two state-of-the-art EAFs with differentiated steelmaking and finishing technology, including endless casting and rolling equipment and an advanced high-strength steel (AHSS) finishing line. Total capital spend for BR2 is expected to be approximately $3.6 billion. In addition, the construction of a 325-thousand-ton dual Galvalume®/galvanized coating line at Big River Steel was completed in the second quarter of 2024. We are enhancing our competitive advantage in low-cost iron ore by expanding this advantage to serve our growing fleet of EAF producers. In our Flat-rolled segment, construction of a direct reduced (DR) grade pellet facility at the Company's Keetac ore operations was completed on-time and on-budget in December 2023. We also completed our 500-thousand-ton, pig iron machine at Gary Works in 2022 and ramped to full run-rate production in 2023. Capital expenditures for strategic projects were $1.6 billion during the year ended December 31, 2024.

We know that to accomplish our objectives, we also need to continue to move up the talent curve. We are investing in our employees and providing the training and resources they need to succeed. This will help reinforce our culture of caring, where accountability, fairness and respect are foundational, and high performance and inclusion in all its forms are valued and celebrated. See "Human Capital Management" below for additional information on our talent attraction, development, and retention initiatives.

From time to time, U. S. Steel evaluates potential strategic and organizational opportunities, which may include the acquisition, divestiture or consolidation of assets. Given the cyclicality of our industry, we are focused on strategically deploying our capital, in-line with our capital allocation framework, in order to invest in areas consistent with the execution of our Best for All® strategy and are considering various possibilities, including exiting lines of business and the sale of certain assets, that we believe would ultimately result in greater stockholder value. The Merger Agreement contains customary limitations on U. S. Steel's ability to engage in such transactions without the consent of Purchaser.

As the Company advances and expands its mini mill capability, it seeks to become better, not bigger and will adjust its footprint accordingly by re-evaluating cost and capability advantages within its evolving footprint. In December 2021 and June 2022, the Company permanently idled the steelmaking and ironmaking operations, respectively, at its Great Lakes Works facility. In addition, in March 2022, the Company permanently idled the finishing facilities at its East Chicago Tin operations, which had been idled on a temporary basis during 2019. The coil finishing process at Great Lakes Works continues to operate and remains a component of the Company's operating plans. In December 2022, the Company indefinitely idled the majority of tin operations at our Gary Works facility. In November 2023, the steel and ironmaking production facilities at Granite City Works were indefinitely idled. The plant continues to process slabs to produce hot-rolled, cold-rolled and coated sheets. Production at USS-UPI, LLC (UPI) was indefinitely idled in December 2023.

Commercial Strategy

Our commercial strategy is focused on providing customer-centric solutions with differentiated and value-added steel products, which includes AHSS such as our newer grades of generation 3 (GEN3) steel, coated sheets for the automotive and appliance industries, electrical steel sheets for the manufacture of motors and electrical equipment, a wide variety of bare, laminated and pre-painted corrosion-resistant sheets for construction and related applications, hot rolled coils used in the production of construction and agricultural-related heavy machinery as well as skelp for line pipe used for energy transmission as well as

extraction, tin mill products for the packaging industry and OCTG pipe, connections, accessories and rig site services for use in drilling for oil and gas. In addition, our portfolio of customers serves a variety of different traditional and emerging industries meeting the needs of numerous markets.

U. S. Steel is committed to leveraging our Best for All® strategy to develop and commercialize our low-carbon footprint and high value-add steels for our current and future customers. The Company's current five-year plan includes objectives to develop and commercialize numerous differentiated grades of low-carbon, high rate of recycled-content steels, providing compelling new options for customers in automotive, appliance, industrial equipment, construction, renewable energy and other markets to enhance the sustainability of their products. For example, in April 2021, we announced a new sustainable steel product line, verdeX™, which is made with up to 90% recycled steel content and a reduced carbon footprint - as much as 70-80% less carbon emissions than traditional integrated steelmaking methods. After launching our verdeX™ brand of sustainable steel products in 2021, we have worked closely with customers on their own sustainability goals. In 2022, we achieved our first agreements with customers on the sale of verdeX™ products moving forward, and more agreements have been put in place for use in various industries, setting the stage for increased adoption of verdeX™ in existing and developing steel products produced by our customers. In 2023, with the completion of the NGO electrical steel line at our Big River Steel facility, the Company began production of its InduX™ electrical steel products which have the magnetic properties necessary for electric vehicles as well as generators. InduX™ electrical steel products are wide, ultra-thin, light-weight and infinitely recyclable. In addition, we continue to work with customers in numerous industries to help them implement AHSS solutions in the products they manufacture. While the automotive industry has been most active in the adoption of these products in vehicle platforms, and continues to accelerate the deployment of AHSS solutions in new vehicle launches, U. S. Steel is successfully introducing AHSS in other industries as well. In January 2023, we also enhanced our construction product portfolio by offering a best-in-class warranty of up to 60 years for our Galvalume® products, and in October 2023 launched Coastalume®, the first carbon steel solution designed for use in coastal construction applications, offering a combination of superior corrosion resistance and durability in a more cost-effective product than other widely used options. Most recently, U. S. Steel launched a new coated steel product in September 2024 called ZMAG™, a product designed to offer more strength, corrosion resistance, and sustainable steel benefits in numerous industries, but developed specifically for the growing solar segment.

We are responsive to our customers' changing needs by developing new steel products and uses for steel that meet their evolving markets and regulatory demands. We have research centers in Munhall, Pennsylvania, Košice, Slovakia, and Houston, Texas, as well as an automotive technology center in Troy, Michigan. The focus of these centers is to engineer new products and to co-create innovative solutions that meet our customers' toughest challenges to reduce carbon emissions, increase strength, improve longevity and serve the needs of their customers.

For automotive customers leveraging AHSS, we commissioned a first of its kind GEN3 hot dipped galvanize line at our PRO-TEC Coating Company (PRO-TEC) joint venture in 2020, and have embedded application engineers at original equipment manufacturers (OEMs) to demonstrate how to best utilize the strong, yet highly formable, cost effective material in body design to meet passenger safety requirements while significantly reducing weight to meet future vehicle fuel efficiency standards.

In our tubular markets, we continue development of premium and semi-premium tubular connections designed for our customers that operate in challenging drilling environments. These connections optimize well construction activities and provide outstanding sealing capabilities for onshore and offshore oil and gas drilling in North America. An example is the USS-TALON HTQ™, which was introduced in 2020 for customers that are constructing onshore natural gas and oil wells with long laterals requiring best-in-class torque capacity and optimized well-bore clearances.

Commercial Sales of Product

U. S. Steel characterizes sales as contract sales if sold pursuant to an agreement with a defined volume and pricing and a duration of longer than three months, and as spot if sold without a defined volume and pricing agreement, typically three months or less. In 2024, approximately 76 percent, 56 percent, 50 percent and 82 percent of sales by Flat-Rolled, Mini Mill, USSE and Tubular, respectively, were contract sales. Some contract pricing agreements include fixed prices while others are adjusted periodically based upon published prices of steel products or cost components.

Human Capital Management

At U. S. Steel, we are focused on attracting and retaining the top talent needed to support our strategic transformation and meet our customers' evolving needs as a sustainable steel solutions provider. The support and development of our people is foundational to achieving our Best for All® strategy. We refer to this strategic talent pillar as "Moving Up the Talent Curve."

Our focus on people extends to our current and future employees. We aim to have an engaged workforce and foster an environment that promotes new ideas and innovation, reflects the communities where we operate, and delivers exceptional customer service. To achieve the Best for All® strategy, we must have the "Best from All."

Active Employees as of December 31, 2024	
North America	14,341
Slovakia	7,712
Total	22,053

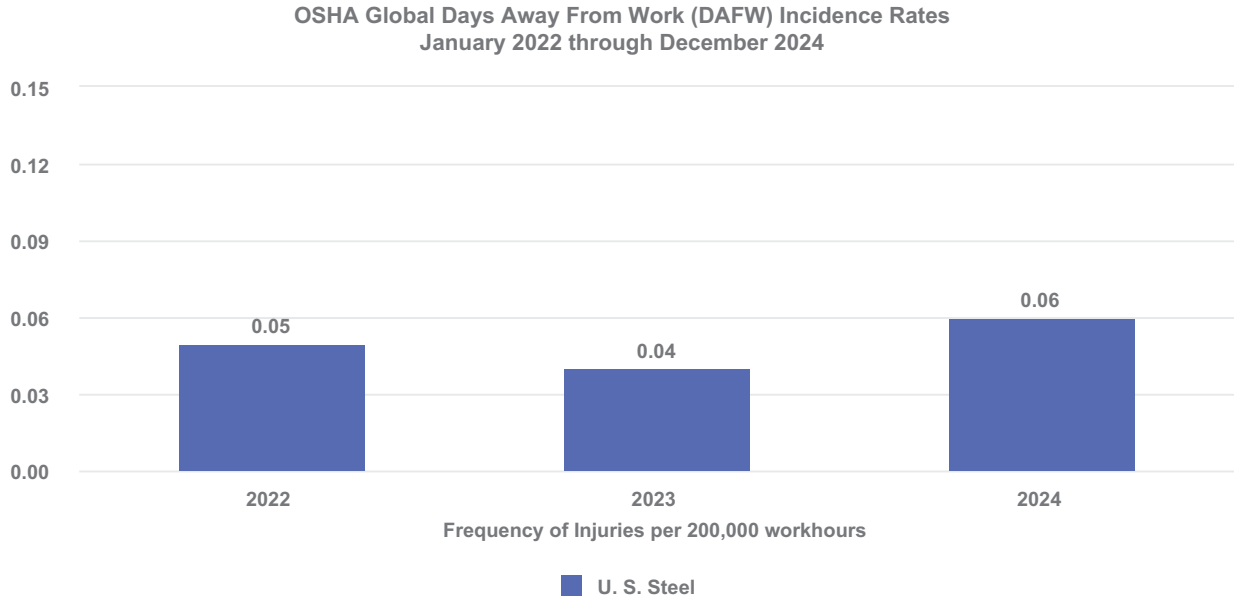
Ethics & Compliance

Our culture is based on our S.T.E.E.L. Principles: **S**afety First; **T**rust and Respect; **E**nvironmental Stewardship; **E**xcellence and Accountability; and **L**awful and Ethical Conduct. We expect our employees and members of our board of directors to take personal responsibility to "do what's right," and our Code of Ethical Business Conduct serves as the foundation for the actions of our employees and directors. To further ensure that employees understand the Company's expectations and all applicable rules, we provide annual formal ethics and compliance training to our employees and have frequent communications with information about key compliance topics, which include messages from senior management underscoring the importance of doing business with integrity. Employees also receive summaries of current events that demonstrate the need to do business lawfully and ethically that include reminders of the Company's expectations for all employees. In addition, through our annual policy certification process, employees of USSK, non-represented employees in the United States, and members of our board of directors certify their ongoing compliance with our Code of Ethical Business Conduct.

Employee Health & Safety

At U. S. Steel, we have a long-standing commitment to the safety and health of every person who works in our facilities. Every employee deserves to return home safely at the end of every day, and we are working to eliminate all injuries and incidents. In addition, the psychological safety of all employees is important to us. We have combined physical safety and psychological safety into the construct of 360° safety. Ensuring a safe workplace also improves productivity, quality, reliability and financial performance. By making safety and health a personal responsibility, our employees are making a daily commitment to follow safe work practices, look out for the safety of co-workers and ensure safe working conditions for everyone. A "Safety First" mindset is as essential to our success as the tools and technologies we rely on to do business.

Our objective is to attain a sustainable zero harm culture supported by leadership and owned by an engaged and highly skilled workforce, empowered with the capabilities and resources needed to assess, reduce and eliminate workplace risks and hazards. In support of these objectives, we have developed an enhanced Safety Management System, initiated new safety communication methods and enhanced contractor safety processes. One of our most important safety protocols is our fatality prevention audit program. These proactive assessments of the processes and protocols we have in place, and adherence to them, to avoid fatalities and severe injuries are conducted annually at the enterprise level and more frequently at each of our facilities. We assess our safety performance through a variety of lagging and leading indicators, including OSHA Days Away From Work (DAFW). This measurement allows us to evaluate the frequency of injuries sustained at our facilities requiring an employee to stay at home for more than one day. U. S. Steel has achieved record-safety performance in this measurement in the last several years, routinely achieving performance better than industry benchmarks.



For 2024, we had a corporate DAFW rate of 0.06, which is 10 times better than the U.S. Bureau of Labor Statistics' Iron and Steel benchmark DAFW rate of 0.60.

Talent Attraction, Development and Retention

Attracting, developing, and retaining a workforce of talented people with unique experiences and skillsets is essential to having high-performing teams that drive results for our Company's stakeholders. As part of our commitment to cultivating a culture of caring, we have inclusive benefits available for our U.S. non-represented workforce, including expanded parental leave, back-up dependent care, infertility coverage and healthcare continuation for the families of employees who suffered work-related or military service fatalities. We also support several employee resource groups (ERGs) to enhance employee engagement and promote a culture of belonging. Our ERGs also provide training and education, mentorship and networking opportunities for their members.

We believe that attraction, development and retention of talent is essential to our success, especially in today's competitive labor market. We offer internship programs, partner with universities, community colleges and technical schools, and collaborate with community employment centers and economic development nonprofit organizations to build strong and diverse internal and external sources of potential employees and opportunities for our existing employees' growth and development.

Once at U. S. Steel, we seek to provide opportunities for continuous learning and development. All of our employees at a director-level and above have a formal professional development plan that is assessed at least annually. In addition, we proactively monitor our attrition rates and take targeted actions to ensure our highest potential and performing employees are motivated to remain with the Company. Over the past five years, our regrettable voluntary turnover rate has been at or below 5.5 percent.

We offer a competitive total rewards package of compensation and benefits that we regularly evaluate and benchmark across the manufacturing industry to ensure that we position U. S. Steel as an employer of choice.

Labor Relations

Approximately 80% of our employees in North America and Slovakia are covered by collective bargaining agreements. We work closely with union representatives to provide safe and productive workplaces that enable our employees to deliver high-quality products and meet the needs of our customers. Our relationship with the USW includes not only a commitment to safety programs, but also a common approach to combating the unfairly traded imports that threaten our industry, our company and ultimately the jobs of our employees.

Certain hourly employees of U. S. Steel's flat-rolled, tubular, cokemaking and iron ore operations in the United States are covered by collective bargaining agreements with the USW entered into effective September 1, 2022 (the 2022 Labor Agreements) that expire on September 1, 2026. The 2022 Labor Agreements include a signing bonus for each eligible USW-represented employee and annual 5% wage increases effective September 1, 2022, 2023, 2024, and 2025. The 2022 Labor Agreements also provide for certain increases to pension and retirement benefits, including increases in our defined benefit pension plan, retiree healthcare account contributions, and to the contribution rate to the Steelworkers Pension Trust from $3.50 to $4.00 per hour, effective January 1, 2023. During the fourth quarter of 2022, U. S. Steel recorded a charge of approximately $67 million for the 2022 Labor Agreements signing bonus and related costs.

In addition, as part of the collective bargaining process, U. S. Steel and the USW agreed to leverage the overfunded Other Post-Employment Benefit (OPEB) plans to support the benefits provided to active represented employees. The OPEB plans were modified to allow the Company to utilize a certain amount of surplus assets to pay additional legally permissible benefits previously paid by the Company. The arrangement permits the Company to utilize a target of $75 million annually for active and retiree employee benefits, with an annual minimum of $50 million, beginning in 2023 and continuing through December 31, 2026. For additional information, see Note 18 to the Consolidated Financial Statements in Part II Item 8 of this Form 10-K.

Capital Structure, Liquidity and Capital Allocation

Our strategy's primary financial goal is to enhance stockholder value by utilizing our capital structure, liquidity and enhanced capital allocation priorities to advance the Company's strategic objectives, generate long-term value and reward stockholders. Our cash deployment strategy is aligned with our corporate strategy and includes executing on strategic projects and portfolio moves, maintaining a strong balance sheet and a healthy pension plan, and delivering sustainable growth with a focus on core values such as safety and environmental stewardship and rewarding stockholders for the continued progress we make. Cash deployment is also performed with a customer-centric focus on improving safety, our environment, quality, delivery and cost.

Our liquidity supports our ability to satisfy short-term obligations, fund working capital requirements and provides a foundation to execute key strategic priorities. We are focused on maintaining a strong balance sheet and may proactively refinance or repay our debt from time to time to protect our capital structure from unforeseen external events and re-financing risks.

In 2024, we repaid approximately $128 million in debt, and we ended the year with $3.62 billion of total liquidity.

On May 18, 2023, U. S. Steel closed on an offering consisting of an aggregate principal amount of $240 million unsecured Arkansas Development Finance Authority environmental improvement revenue bonds, which carry a green bond designation. The bonds, issued through Arkansas Development Finance Authority, have a coupon rate of 5.700% and carry a final maturity of 2053 (2053 ADFA Green Bonds). U. S. Steel received net proceeds of approximately $238 million after fees of approximately $2 million related to the underwriting and third-party expenses and will pay semiannual interest. The net proceeds from the issuance of the 2053 ADFA Green Bonds were used to partially fund work related to the BR2 facility.

The Merger Agreement contains customary limitations on U. S. Steel's ability to incur additional indebtedness without the consent of Purchaser.

On July 25, 2022, following the completion of previously authorized $800 million share repurchase programs, the Board of Directors authorized a share repurchase program for the repurchase of up to $500 million of the Company's outstanding common stock from time to time in the open market or privately negotiated transactions at the discretion of management. The Company's share repurchase program does not obligate it to acquire any specific number of shares.

U. S. Steel repurchased 7.1 million shares of its common stock for approximately $175 million during the year ended December 31, 2023. No share repurchases were completed in 2024, and there is approximately $126 million remaining under the current stock repurchase authorization. We do not expect to utilize the remainder of this authorization. In addition, the Board of Directors declared quarterly dividends of five cents per common share for each of the quarters in 2024. The Merger Agreement contains a customary prohibition on U. S. Steel's ability to engage in additional share repurchases without the consent of Purchaser.

Facilities and Locations as of December 31, 2024

Location Overview

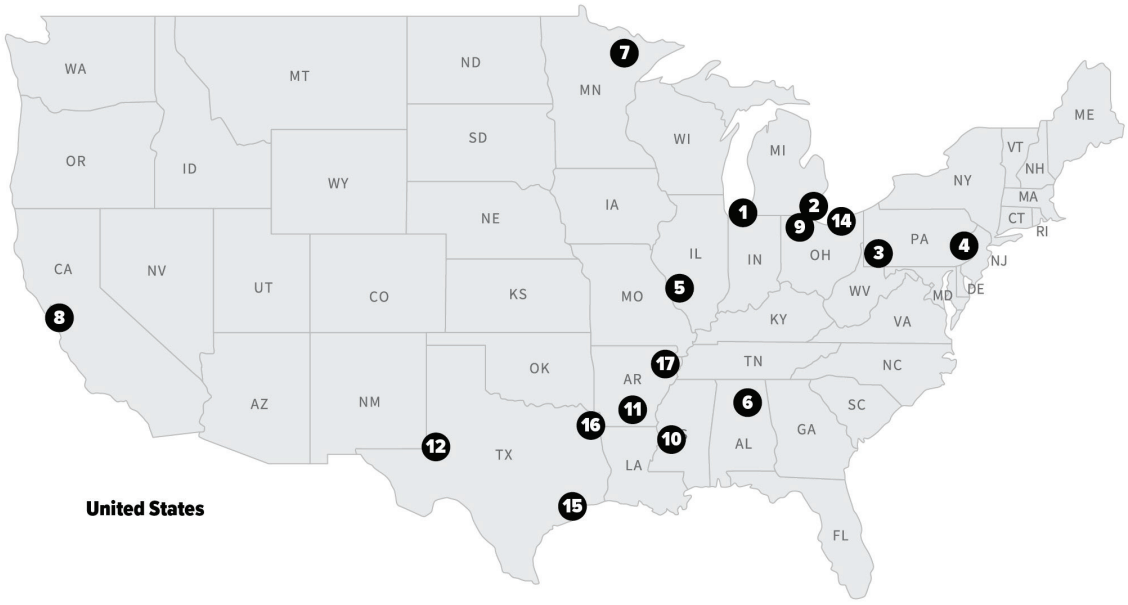
Flat-Rolled Segment
- **1** Gary Works
- **2** Great Lakes Works
- **3 4** Mon Valley Works
- **5** Granite City Works
- **6** Fairfield Sheet
- **7** Minntac
- **7** Keetac
- **7** Hibbing Taconite
- **8** USS-UPI, LLC
- **9** PRO-TEC Coating Company
- **10** Double G Coatings Company
- **1** Chrome Deposit*
- **2** Automotive Center

Mini Mill Segment
- **17** Big River Steel
- **17** Big River Steel 2

Tubular Segment
- **6** Fairfield Tubular
- **14** Lorain Tubular
- **15** Offshore Operations Houston
- **16** Lone Star Tubular
- **11 16** Wheeling Machine Products
- **12** Patriot Premium Threading Services

USSE Segment
- **13** U. S. Steel Košice

Administrative and Research
- **3** Corporate Headquarters
- **3** Research and Technology Center
- **15** U. S. Steel Tubular Products Innovation
- **13** USSE Research



*Chrome Deposit locations are near major steel mills and are not reflected on the map above.



Maps not drawn to scale.

Flat-Rolled

The operating results of all of U. S. Steel's domestic-integrated steel and sheet plants, coke and iron ore operations and ore and sheet production joint ventures are included in Flat-Rolled. Also included within Flat-Rolled is a research and technology center located in Munhall, Pennsylvania (near Pittsburgh) and an automotive technology center in Troy, Michigan. The research and technology center carries out a wide range of applied research, development and technical support functions. The automotive technology center brings automotive sales, service, distribution and logistics services, product technology and applications research into one location and much of U. S. Steel's work in developing new grades of steel to meet the demands of automakers for high-strength, light-weight and formable materials is carried out at this location.

Flat-Rolled Operations Table

Operations, (Property Location)	Annual Production Capability	Principal Products and/or Services
Gary Works, (Gary, Indiana) [(a)]	7.5 million tons of raw steel which includes 0.5 million tons of pig iron capability	strip mill plate in coil; hot-rolled, cold-rolled and coated sheets; tin mill products and pig iron
Midwest, (Portage, Indiana)	finishing facility	hot-rolled, cold-rolled and coated sheets; tin mill products
Great Lakes Works [(b)], (Ecorse, River Rouge and Dearborn, Michigan)	finishing facility	cold-rolled and coated sheets
Mon Valley Works [(c)]: Edgar Thompson, (Braddock, Pennsylvania), Irvin, (West Mifflin, Pennsylvania), Fairless, (Fairless Hills, Pennsylvania), and Clairton, (Clairton, Pennsylvania)	2.9 million tons of raw steel and 3.6 million tons of coke	hot-rolled, cold-rolled and coated sheets; coke and coke by-products
Granite City Works [(d)], (Granite City, Illinois)	2.8 million tons of raw steel	hot-rolled, cold-rolled and coated sheets
USS-UPI, LLC (UPI) [(e)], (Pittsburg, California)	finishing facility	cold-rolled and coated sheets; tin mill products
Fairfield Works, (Fairfield, Alabama)	finishing facility	coated sheets
Minnesota Ore Operations: Minntac, (Mt. Iron, Minnesota) and Keetac, (Keewatin, Minnesota)	22.4 million tons of iron ore pellets which includes 4.0 million tons of DR-grade pellet capability	iron ore pellets and DR (direct reduced) grade pellets

[(a)] The majority of tin operations were indefinitely idled as of December 31, 2022. Pig iron is sold on an intercompany basis to Big River Steel and BR2.

[(b)] The steel and ironmaking production facilities were permanently idled in December of 2021 and June of 2022, respectively. Great Lakes Works' pickle line, cold mill and CGL continue to operate, while the DESCO and electrolytic galvanizing lines are indefinitely idled.

[(c)] From time to time, we may swap coke with other domestic steel producers or sell on the open market. Coke by-products are sold to the chemicals and raw materials industries.

[(d)] In November 2023, the steel and ironmaking production facilities at Granite City Works were indefinitely idled. The plant continues to process slabs to produce hot-rolled, cold-rolled and coated sheets.

[(e)] In December 2023, production at UPI was indefinitely idled.

Joint Ventures Within Flat-Rolled

U. S. Steel participates in a number of joint ventures that are included in Flat-Rolled, most of which are conducted through subsidiaries. All of these joint ventures are accounted for under the equity method. The significant joint ventures and other investments are described below.

Joint Ventures [(a)] Within Flat-Rolled Table

Joint Venture, (Property Location)	U. S. Steel's Ownership Percentage	Annual Production Capability
Hibbing Taconite Company (Hibbing); (Hibbing, Minnesota)	14.7%	9.0 million tons of which U. S. Steel's share is 1.3 million tons
PRO-TEC Coating Company (PRO-TEC), (Leipsic, Ohio)	50.0%	2.0 million tons [(b)]
Double G Coatings Company (Double G) [(c)]; Jackson, Mississippi	50.0%	315 thousand tons
Chrome Deposit Corporation (CDC), (six locations near major steel plants)	50.0%	not applicable

[(a)] See further information about our equity investees in Note 12 to the Consolidated Financial Statements.

[(b)] U. S. Steel's domestic production facilities supply PRO-TEC with cold-rolled sheets and U. S. Steel markets all of PRO-TEC's products.

[(c)] Each partner supplies its own steel to Double G and markets what is processed by Double G.

Mini Mill

The operations of Big River Steel and Big River Steel 2 are included in Mini Mill. The Big River Steel and BR2 facilities, located in Osceola, Arkansas, are EAF sheet steel production facilities.

Mini Mill Operations Table

Operations, (Property Location)	Annual Production Capability	Principal Products and/or Services
Big River Steel, (Osceola, Arkansas)	3.3 million tons of raw steel	hot-rolled, cold-rolled and coated sheets; electrical steels
Big River Steel 2, (Osceola, Arkansas)	3.0 million tons of raw steel	hot-rolled, cold-rolled and coated sheets; electrical steels

USSE

USSE operates in Košice, Slovakia an integrated facility and a research laboratory, which, in conjunction with our Research and Technology Center, supports efforts in coke making, electrical steels, other value-added products and design and instrumentation.

USSE Operations Table

Operations, (Property Location)	Annual Production Capability	Principal Products and/or Services
U. S. Steel Košice, (Košice, Slovakia)	5.0 million tons of raw steel	coke; slabs; strip mill plate: hot, cold and coated sheets; tin mill products; spiral welded pipe

Tubular

Tubular manufactures seamless and welded OCTG, standard pipe, line pipe and mechanical tubing.

Tubular Operations Table

Operations, (Property Location)	Production Capability	Principal Products and/or Services
Fairfield Tubular Operations, (Fairfield, Alabama)	0.9 million tons of raw steel [(a)] and 750 thousand tons of tubular	seamless tubular pipe
Lorain Tubular Operations [(b)], (Lorain, Ohio)	380 thousand tons of tubular	seamless tubular pipe
Lone Star Tubular [(b)], (Lone Star, Texas)	#1 electric-weld pipe mill (EWPM) 400 thousand tons and #2 EWPM 380 thousand tons of tubular	welded tubular pipe
Wheeling Machine Products [(c)], (Pine Bluff, Arkansas and Hughes Springs, Texas)	not applicable	tubular couplings
Offshore Operations, (Houston, Texas)	not applicable	tubular threading, inspection, accessories and storage services and premium connections
Tubular Processing [(d)], (Houston, Texas)	not applicable	tubular processing

[(a)] Based on the rounds caster capacity which is its constraining production unit.

[(b)] In April 2020, the Lorain Tubular and Lone Star Tubular operations were indefinitely idled.

[(c)] In April 2020, the Wheeling Machine Products at Hughes Springs, Texas was indefinitely idled.

[(d)] Tubular Processing has been indefinitely idled since 2015.

Joint Ventures [(a)] Within Tubular Table

Operations, (Property Location)	U. S. Steel's Ownership Percentage	Production Capability	Principal Products and/or Services
Patriot Premium Threading Services, (Midland, Texas)	50%	not applicable	Tubular threading, accessories and premium connections

[(a)] See further information about our equity investees in Note 12 to the Consolidated Financial Statements.

Other

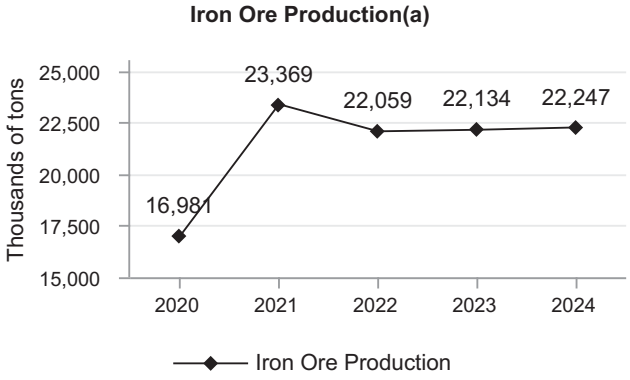
U. S. Steel's Other category includes the operating results relating to our real estate operations. The Company owns approximately 43,000 acres of real estate assets, either held for development or managed, in Alabama, California, Illinois, Michigan, Minnesota, Ohio, Pennsylvania and Texas.

Raw Materials and Energy

As a predominantly integrated producer, U. S. Steel's primary raw materials are iron units in the form of iron ore pellets and sinter ore, carbon units in the form of coal and coke (which is produced from coking coal) and steel scrap. For our EAF production, our primary raw material is scrap. U. S. Steel's raw materials supply strategy consists of acquiring and expanding captive sources of certain primary raw materials and entering into flexible supply contracts for certain other raw materials at competitive market prices that are subject to fluctuations based on market conditions at the time.

The amounts of such raw materials needed to produce a ton of steel will fluctuate based upon the specifications of the final steel products, the quality of raw materials and, to a lesser extent, differences among steel producing equipment. In broad terms, the Company's integrated steel process consumes approximately 1.4 tons of coal to produce one ton of coke and then it consumes approximately 0.3 tons of coke, 0.3 tons of steel scrap (approximately 60 percent of which is internally generated) and 1.3 tons of iron ore pellets to produce one ton of raw steel. In addition, we consume approximately 10 mmbtu's of natural gas per ton produced. Generally, the Company's mini mill operations consume approximately 0.8 tons of steel scrap, 0.3 tons of pig iron, and 0.1 tons of HBI to produce one ton of raw steel. In addition, the mini mill operations consume approximately 0.6 MKWH of electricity per ton of raw steel produced. While we believe that these estimated consumption amounts are useful for planning purposes, and are presented to give a general sense of raw material and energy consumption related to steel production, substantial variations may occur.

Iron Ore



[(a)] Includes our share of production from Hibbing through December 31, 2024.

The iron ore facilities at Minntac and Keetac contain approximately 900 million short tons of indicated resources and probable reserves and our share of recoverable reserves at the Hibbing joint venture is approximately 4 million short tons. Refer to Mining Properties in Item 2 of this Form 10-K for additional information. Recoverable reserves are defined as the tons of product that can be used internally or delivered to a customer after considering mining and beneficiation or preparation losses. Minntac and Keetac's annual capability and our share of annual capability for the Hibbing joint venture total approximately 23 million tons. We have iron ore pellet production capability that exceeds our steelmaking capability in the U.S.

We historically have sold iron ore pellets to third parties, including in 2024, 2023, and 2022. The Company has agreements to supply iron ore pellets to third-party customers over the next several years.

All of USSE's iron ore requirements are purchased from outside sources, primarily Ukrainian mining companies. Prices are determined in long-term contracts with strategic suppliers or as spot prices negotiated monthly or quarterly. We believe that supplies of iron ore adequate to meet USSE's needs are available at competitive market prices.

During 2024, USSE protected approximately 25 percent of its operation's iron ore purchases with financial swaps to manage exposure to iron ore price fluctuations.

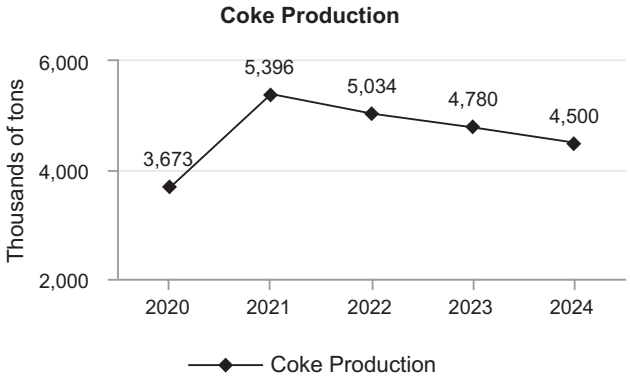
Coking Coal

All of U. S. Steel's coal requirements for our cokemaking facilities are purchased from outside sources. Pricing for Flat-Rolled's coking coal contracts is typically negotiated on a yearly basis, and from time to time we have entered into multi-year agreements for a portion of our coking coal requirements.

Prices for European contracts are negotiated quarterly, annually or determined as index-based prices.

We believe that supplies of coking coal adequate to meet our needs are available from outside sources at competitive market prices. The main source of coking coal for Flat-Rolled is the United States, and sources for USSE include Poland, Ukraine, Canada, Australia and the United States.

Coke



In North America, the Flat-Rolled segment operates a cokemaking facility at the Clairton Plant of Mon Valley Works. At our Granite City Works, we have a coke supply agreement with Gateway that expires on June 30, 2025, with a U. S. Steel option to extend the supply agreement for an additional six months to December 31, 2025. Blast furnace injection of coal, and self-generated coke oven gas is also used to reduce coke usage.

With Flat-Rolled's cokemaking facilities, it has the capability to be nearly self-sufficient with respect to its annual coke requirements at normal operating levels. Coke from time to time has been purchased from, sold to, or swapped with suppliers and other end-users to adjust for production needs and reduce transportation costs.

In Europe, the USSE segment operates cokemaking facilities at USSK. While USSE is self-sufficient for coke at normal operating levels, it periodically purchases coke from Polish and Czech coke producers to meet production needs. Volume and price are negotiated quarterly.

Steel Scrap and Other Materials

We believe that supplies of steel scrap and alloys that are adequate to meet our needs are readily available from outside sources at competitive market prices for the Flat-Rolled, Mini Mill and USSE segments. Generally, approximately 38 percent of our steel scrap requirements were internally generated through normal operations for these segments.

Limestone

All of Flat-Rolled's limestone requirements and USSE's lime and limestone requirements are purchased from outside sources. We believe that supplies of limestone and lime adequate to meet our needs are readily available from outside sources at competitive market prices.

Zinc and Tin

We believe that supplies of zinc and tin required to fulfill the requirements for Flat-Rolled, Mini Mill and USSE are available from outside sources at competitive market prices. For Flat-Rolled and Mini Mill the main sources of zinc are Canada, Mexico and the United States and the main sources of tin are Bolivia, Brazil and Peru. For USSE, the main sources of zinc are Poland and Finland, and the main sources of tin are Peru, Indonesia, and Brazil.

During 2024, Flat-Rolled protected approximately 70 percent of its operation's zinc and tin requirements with financial swap derivatives to manage exposure to zinc and tin price fluctuations. For further information, see Note 16 to the Consolidated Financial Statements.

Natural Gas

All of U. S. Steel's natural gas requirements are purchased from outside sources.

We believe that adequate supplies to meet Flat-Rolled's, Mini Mill's and Tubular's needs are available at competitive market prices. For 2024, approximately 70 percent of our natural gas purchases in Flat-Rolled were based on bids solicited on a monthly basis from various vendors; the remainder were made daily or with term agreements.

We believe that adequate natural gas supplies to meet USSE's needs are available at competitive market prices. During 2024, due to the uncertainty in the energy market, the procurement of natural gas in USSE was based on bids solicited primarily on a quarterly or monthly basis, with the remainder balanced on a daily basis.

Flat-Rolled and USSE use self-generated coke oven and blast furnace gas to reduce consumption of natural gas. USSE uses self-generated coke oven, converter and blast furnace gas to reduce consumption of steam coal that results in lower CO_2 emissions production.

Additionally, Russian supply of natural gas to Europe has decreased significantly in response to enacted sanctions. However, Slovakia has natural gas storage and access to additional supply from other locations including Norway, the U.S. and Africa. Together, these sources are enough to support the country's expected consumption through the 2025 winter season, which includes demand for natural gas for our USSE segment operations.

Industrial Gases

U. S. Steel purchases industrial gas in the U.S. under long-term contracts with various suppliers, with an exception being at our Fairfield Works where the Company's air separation plant provides for the facility's industrial gas needs. USSE owns and operates its own industrial gas facility, but also may purchase industrial gases from time to time from third parties.

International Trade

U. S. Steel continues to face import competition, much of which is unfairly traded and fueled by massive global steel overcapacity, currently estimated to be over 630 million net tons per year—more than six times the entire U.S. steel market and over twenty times total U.S. steel imports. These imports and overcapacity negatively impact the Company's operational and financial performance. U. S. Steel continues to lead efforts to address these challenges that threaten the Company, our workers, our stockholders and our country's national and economic security.

As of the date of this filing, pursuant to a series of Presidential Proclamations issued in accordance with Section 232 of the Trade Expansion Act of 1962, U.S. imports of certain steel products are subject to a 25 percent tariff, except imports from: (1) Argentina, Brazil, and South Korea, which are subject to restrictive quotas; (2) the European Union (EU) that are melted and poured in the EU, within tariff rate quota (TRQ) limits through December 2025; (3) Japan that are melted and poured in Japan within TRQ limits; (4) United Kingdom (UK) that are melted and poured in the UK within TRQ limits; (5) Canada; (6) Mexico, if melted and poured in North America; (7) Ukraine, if melted and poured in Ukraine or the EU, until June 1, 2025; and (8) Australia.

The U.S. Department of Commerce (DOC) is managing a process in which U.S. companies may request and/or oppose temporary product exclusions from the Section 232 tariffs and quotas. U. S. Steel opposes exclusion requests for imported products that are the same as, or substitutes for, products manufactured by U. S. Steel.

Multiple legal challenges to the Section 232 action continue before the U.S. Court of International Trade (CIT) and the U.S. Court of Appeals for the Federal Circuit (CAFC), the latter of which has consistently rejected constitutional and statutory challenges to the Section 232 action. Several challenges to the Section 232 action and retaliation thereto continue at the World Trade Organization (WTO).

Since its implementation in March 2018, the Section 232 action has supported the U.S. steel industry's and U. S. Steel's investments in advanced steel production capabilities, technology, and skills, strengthening U.S. national and economic security. The Company continues to actively defend the Section 232 action.

In February 2019, the European Commission (EC) implemented a definitive safeguard on global steel imports in the form of TRQs that impose 25 percent tariffs on steel imports that exceed the TRQ limit. The EC's safeguard is currently set to expire in June 2026.

Antidumping duties (AD) and countervailing duties (CVD or antisubsidy duties) apply in addition to the Section 232 tariffs, quotas, TRQs and the EC's safeguard, and AD/CVD (antidumping and countervailing duty) orders may continue beyond the Section 232 action and the EC's safeguard. U. S. Steel continues to actively defend and maintain the 69 U.S. AD/CVD orders and 14 EU AD/CVD orders covering U. S. Steel products in multiple proceedings before the DOC, U.S. International Trade Commission (ITC), CIT, CAFC, the EC and European courts, and the WTO.

In May 2024, the ITC voted to continue the AD order on tin mill products from Japan for another five years in the fourth "sunset" review of that order.

Also in May 2024, the EC initiated a new AD investigation on tin plate from China.

In August 2024, the EC initiated new AD investigations on EU imports of hot-rolled steel from Egypt, India, Japan, and Vietnam, with a preliminary decision expected by March 2025.

In September 2024, the DOC initiated new AD/CVD investigations on U.S. imports of corrosion-resistant steel (CORE) from ten countries based on petitions filed by U. S. Steel, Wheeling-Nippon, the USW, Nucor, and Steel Dynamics. On October 18, 2024, the ITC made affirmative preliminary determinations on all ten countries. The DOC will issue preliminary determinations in the first half of 2025.

Additional tariffs of 7.5 to 25 percent continue to apply to certain U.S. imports from China, including certain raw materials used in steel production, semi-finished and finished steel products, and downstream steel-intensive products, pursuant to Section 301 of the Trade Act of 1974. In September 2024, the Office of the United States Trade Representative (USTR) revised certain Section 301 tariffs on U.S. imports from China, including increasing tariffs on steel from 7.5 to 25 percent; batteries from 7.5 to 25 percent; solar cells/modules from 25 to 50 percent; and electric vehicles from 25 to 100 percent.

The United States and EU are currently negotiating a global sustainable steel arrangement to restore market-oriented conditions and address carbon intensity. In June 2023, to inform these ongoing discussions with the EU, USTR requested that the ITC conduct a Section 332 investigation to assess greenhouse gas emissions intensity of steel produced in the United States. The ITC initiated the Section 332 proceeding in July 2023, held a hearing on December 7, 2023, collected information from domestic producers through mid-2024 and will issue a report in the first quarter of 2025. U. S. Steel is actively participating in this Section 332 investigation. In the fourth quarter of 2023, the U.S. agreed to continue the Section 232 TRQs on U.S. imports from Europe through December 2025 and the EU agreed to continue to suspend retaliation on U.S. exports through March 2025.

U. S. Steel will continue to execute a broad, global strategy to maximize opportunities and navigate challenges presented by imports, global steel overcapacity, and international trade law and policy developments.

Environmental Stewardship

U. S. Steel is committed to effective environmental stewardship. We have implemented and continue to develop business practices that are designed to reduce negative environmental impacts of our operations. We believe part of being a good corporate citizen requires a dedicated focus on how our industry affects the environment. We have taken the actions described below in furtherance of that goal. U. S. Steel's environmental expenditures totaled $354 million in 2024, $345 million in 2023, and $334 million in 2022. Overall, environmental compliance expenditures represented approximately 2 percent of U. S. Steel's total costs and expenses in 2024, 2023, and 2022. For further information, see "Item 7. Management's Discussion and Analysis of Financial Condition and Results of Operations – Environmental Matters."

We continue to work on the promotion of cost-effective environmental strategies by supporting the development of appropriate air, water and waste laws and regulations at the local, state, national and international levels. We are committed to reducing our emissions and are investigating, creating and implementing innovative, best practice solutions throughout our operations to improve our environmental performance and to manage and reduce energy consumption.

U. S. Steel's North America operations recycled 4.8 million and 4.9 million tons of purchased and produced steel scrap annually in 2024 and 2023, respectively. USSK recycled approximately 787 thousand tons and 895 thousand tons of produced steel scrap in 2024 and 2023, respectively. Because of steel's physical properties, our products can be recycled at the end of their useful life without loss of quality, contributing to steel's high recycling rate and affordability. In 2024, our North America operations recycled approximately 2.5 million tons of blast furnace slag, 26 thousand tons of Basic Oxygen Process steel slag, and 114 thousand tons of electric arc furnace slag by selling it for use as aggregate and in highway and other construction. In 2024, USSK recycled approximately 1.0 million tons of blast furnace slag, and 287 thousand tons of Basic Oxygen Process steel slag.

Many of our major production facilities have Environmental Management Systems that are certified to the ISO 14001 Standard. This standard, published by the International Organization for Standardization (ISO), provides the framework for the measurement and improvement of environmental impacts of the certified facility.

In 2019, and in each succeeding year since, we published the Clairton Operating and Environmental Report related to our Clairton Plant of Mon Valley Works. While U. S. Steel agreed to publish an annual report as part of the 2019 Allegheny County Health Department Settlement Order and Agreement, we took the opportunity to enhance the report by including detailed descriptions of our operations, our safety and environmental performance and community involvement in order to provide easily accessible information for the public. The report details battery combustion stack and fugitive emission performance at Clairton and Clairton's continued commitment to environmental stewardship. In 2021, we published a similar report for the Edgar Thomson facility.

Reduction of Greenhouse Gas Emissions

In 2019, the Company announced its commitment to reduce greenhouse gas emissions intensity across its global footprint by 20 percent, as measured by the rate of CO_2 equivalents emitted per ton of finished steel shipped, by 2030 based on 2018 baseline levels. Then, in 2021, the Company announced its goal to achieve net-zero emissions by 2050, as measured by the rate of CO_2 equivalents emitted per ton of finished steel shipped. These targets apply to U. S. Steel's global operations.

U. S. Steel plans to achieve its greenhouse gas emissions intensity reduction goals through the execution of multiple initiatives. These include the use of EAF steelmaking technology at U. S. Steel's Fairfield Works, at Big River Steel, the first LEED-certified steel mill in the United States and the first steel mill in North America to receive ResponsibleSteel™ site certification, and at Big River Steel 2. EAF steelmaking primarily relies on recycled scrap, rather than iron ore, to produce new steel products, which is a less carbon intensive process and leverages the ability to continuously recycle steel. Further carbon intensity reductions are expected to come from the implementation of ongoing energy efficiency measures, continued use of renewable energy sources and other process improvements to be developed.

The carbon reduction targets reflect our continued commitment to improvement in production efficiency and the manufacture of products that are environmentally friendly. In addition to a commitment to reduce its own greenhouse gas emissions intensity, U. S. Steel is committed to helping its customers achieve their environmental goals. Our industry-leading XG3™ advanced high-strength steel enables automakers to manufacture lighter weight vehicles that meet federal Corporate Average Fuel Economy (CAFE) standards with reduced carbon emissions. As part of our innovation efforts, we continue to look at new steelmaking technologies so that we can produce steels made with low greenhouse gas emissions intensity (green steels) and further reduce carbon emissions.

Environmental Matters, Litigation and Contingencies

Some of U. S. Steel's facilities were in operation before 1900. Although the Company believes that its environmental practices have either led the industry or at least been consistent with prevailing industry practices, hazardous materials have been and may continue to be released at current or former operating sites or delivered to sites operated by third parties.

Our U.S. facilities are subject to environmental laws applicable in the U.S., including the Clean Air Act (CAA), the Clean Water Act (CWA), the Resource Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), as well as state and local laws and regulations.

U. S. Steel has incurred and will continue to incur substantial capital, operating and maintenance and remediation expenditures as a result of environmental laws and regulations, related to release of hazardous materials, which in recent years have been mainly for process changes to meet CAA obligations and similar obligations in Europe.

EU Environmental Requirements and Slovak Operations

Phase IV of the EU Emissions Trading System (the EU ETS) commenced on January 1, 2021, and will finish on December 31, 2030. The European Commission issued final approval of the updated 2021-2025 Slovak National Allocation table in February 2022. The Slovak Ministry of Environment allocated 6.2 million metric tons of European Union Emission Allowances (EUA) at no charge (free allowances or free allocation) to USSE in March 2024. As of December 31, 2024, we have pre-purchased approximately 2.22 million EUA totaling €161 million (approximately $167 million) via spot purchases or settled forwards to cover the 2024 shortfall of emission allowances and expected shortfall in subsequent years.

The EU's Industrial Emissions Directive requires implementation of EU determined best available techniques (BAT) for Iron and Steel production to reduce environmental impacts as well as compliance with BAT associated emission levels. Total capital expenditures for projects to go beyond BAT requirements were €138 million (approximately $143 million). These costs were partially offset by the EU funding received and may be mitigated over the next measurement periods if USSK complies with certain financial covenants, which are assessed annually. If we are unable to meet these covenants in the future, USSK might be required to provide additional collateral (e.g., bank guarantee) to secure 50 percent of the EU funding received. USSK complied with these covenants as of December 31, 2024, and no additional collateral will be required by the end of June 30, 2025. By this next assessment date, we expect that nine of the fifteen total projects will pass the sustainability monitoring and will be excluded from further assessment to provide additional collateral if the covenants are not met. The last assessment of financial covenants will be performed as of June 30, 2026.

For further discussion of laws applicable in Slovakia and the EU and their impact on USSE, see Note 26 to the Consolidated Financial Statements, "Contingencies and Commitments, Environmental Matters, EU Environmental Requirements."

Minnesota Mining Operations - Water

The State of Minnesota has a sulfate wild rice water quality standard (WQS) set at 10mg/L, which was established in 1973. Since then, industry has been working with the legislature and the Minnesota Pollution Control Agency (MPCA) to reevaluate the environmental protection and science behind the 10 mg/L standard. In 2011, the legislature passed a law requiring MPCA to revise the sulfate standard. MPCA started the process to revise the rulemaking for the sulfate WQS, but it was never completed. During the interim the Keetac National Pollutant Discharge Elimination System (NPDES) permits were issued in November 2011,

with a sulfate standard of 14 mg/L and a compliance schedule. Then in 2015, the Minnesota legislature passed a law that MPCA could not require businesses to expend funds to comply with the sulfate limit until the rulemaking was revised by MPCA as directed by the legislature in 2011. To date the sulfate WQS rulemaking has not been revised.

Keetac has been working to determine the best options to address sulfate. One of the options in process is a site-specific standard (SSS) request to MPCA for Keetac, first applied for in 2014 and then updated in 2022. The SSS presents data specific to the waterbody and explains the actual impact of sulfate. MPCA denied the Keetac SSS based on new agency guidance. U. S. Steel appealed the denial of the SSS and oral argument occurred on January 22, 2025. The United States Environmental Protection Agency (the U.S. EPA) partially rejected the CWA 303(d) list for impaired waters submitted by MPCA for 2021. The MPCA's impaired waters list was in part rejected to add Hay Lake as being impaired for wild rice sulfate. In February 2022, the U.S. EPA Region V sent a letter to MPCA recognizing the conflict between state law and the CWA. In October 2023, MPCA indicated that Dark Lake is now a designated Wild Rice Water, carrying a 10 mg/L sulfate limit. Also in October, MPCA and U. S. Steel entered into a voluntary Sulfate Monitoring Plan related to operational changes with the Keetac DR pellet project. U. S. Steel is currently in discussions with U.S. EPA Region V concerning sulfate compliance at Keetac. This is based upon required monitoring submitted to the MPCA and the interpretation of the WQS for sulfate related to wild rice, even though there is an acknowledged conflict. It is not possible to accurately predict the outcome of this matter at this time.

U. S. Steel is continuing to work to determine the most efficient and effective options to meet the applicable sulfate standards. However, if MPCA does not revise the sulfate standard of 10mg/L for the receiving waters of Keetac or approve the SSS, an impact on mining operations is likely as a result of extensive changes to water collection and treatment that will be required.

New and Emerging Environmental Regulations

United States and European Greenhouse Gas Emissions Regulations

The Phase IV EU ETS period spans 2021-2030 and is divided into two sub periods (2021-2025 and 2026-2030). Currently, the overall EU ETS target is a 40 percent reduction of 1990 emissions by 2030. Free allocation of CO_2 allowances is based on reduced benchmark values and historical levels of production from 2014-2018. Allocations to individual installations may be adjusted annually to reflect relevant increases and decreases in production. The threshold for adjustments is set at 15 percent and is assessed based on a rolling average of two precedent years. In 2023 and 2024, USSK received an allocation for hot metal in the amount corresponding to its historical average, while the allocation for sinter was slightly lower due to lower production.

Lower production in 2019 through 2023 will have an impact on the future free allocation for 2026-2030, where the historical production median for the years 2019-2023 is assessed. During the third quarter of 2024, USSK submitted a request for free allocation for the second sub period 2026-2030 to the European Commission via the Slovak Ministry of Environment. The decision on volume of free allocation is expected at the end of 2025.

In order to achieve the EU political goal of carbon emissions neutrality by 2050, on July 14, 2021, the European Commission released a package of legislative proposals called Fit for 55. The proposals contain significant changes to current EU ETS functions and requirements, including: a new carbon border adjustment mechanism (CBAM) to impose carbon fees on EU imports, further reduction of free CO_2 allowance allocation to heavy industry and measures to strengthen the supply of carbon allowances. The initial phase started on October 1, 2023, with only a reporting obligation without financial impact. The full scale of CBAM will commence on January 1, 2026. CBAM will have an impact on USSK's free allocation starting in 2026 where initial reduction to 97.5% starts until 2035 with no free allocation. Another implication of CBAM is the customs duty that will require USSK to cover all its imports from third parties with CBAM Certificates representing embedded emissions in goods imported. The legislative process is being impacted by the ongoing Russia-Ukraine conflict. The proposals are subject to the EU legislative process, and we cannot predict their future impact.

U. S. Steel continues to monitor emerging regulations on Per- and Polyfluoroalkyl Substances (PFAS). The U.S. EPA has issued regulations on PFAS under several environmental statutes and continues to introduce additional regulations. The Company does not knowingly introduce PFAS in its manufacturing processes, but U. S. Steel continues to review new regulations related to PFAS and their potential impact to the Company.

United States - Air

The CAA imposes stringent limits on air emissions with a federally mandated operating permit program and civil and criminal enforcement sanctions. The CAA requires, among other things, the regulation of hazardous air pollutants through the development and promulgation of National Emission Standards for Hazardous Air Pollutants (NESHAP) and Maximum Achievable Control Technology (MACT) Standards. The U.S. EPA has developed various industry-specific MACT standards pursuant to this requirement. The CAA requires the U.S. EPA to promulgate regulations establishing emission standards for each category of Hazardous Air Pollutants. The U.S. EPA also must conduct risk assessments on each source category that is already subject to MACT standards and determine if additional standards are needed to reduce residual risks.

While our operations are subject to several different CAA rules and categories of NESHAP and MACT standards, the principal impact of these standards on U. S. Steel's operations includes those that are specific to coke making, iron making, steel making

and iron ore processing. The U.S. EPA has several rules under consideration that will impact our operations, as described in the sections below. While many of these rules are not finalized and the impacts are not estimable at this time, the overall cumulative impact could be material.

On July 13, 2020, the U.S. EPA published a Residual Risk and Technology Review rule for the Integrated Iron and Steel MACT category in the Federal Register. Based on the results of the U.S. EPA's risk review, the agency determined that risks due to emissions of air toxics from the Integrated Iron and Steel category are acceptable and that the current regulations provided an ample margin of safety to protect public health. Under the technology review, the U.S. EPA determined that there are no developments in practices, processes or control technologies that necessitate revision of the standards. In September 2020, several petitions for review of the rule, including those filed by the Company, the American Iron and Steel Institute (the AISI), Clean Air Council and others, were filed with the United States Court of Appeals for the D.C. Circuit (D.C. Circuit). The cases were consolidated and are being held in abeyance until the U.S. EPA reviews and responds to administrative petitions for review. The U.S. EPA proposed a revised iron and steel rule on July 31, 2023. U. S. Steel and other entities submitted extensive comments to the U.S. EPA on September 28, 2023. The U.S. EPA signed the final integrated iron and steel rule on March 11, 2024, and it was published in the Federal Register on April 3, 2024. U. S. Steel filed a Petition for Reconsideration with the U.S. EPA and a Petition for Review in the D.C. Circuit challenging the final iron and steel rule on June 6, 2024. U. S. Steel sought a stay of the rule pending the legal challenges. The motion for stay was denied by the D.C. Circuit in November 2024 and parties filed a request for En Banc review on December 6, 2024. Any impacts are not estimable at this time.

For the Taconite Iron Ore Processing category, based on the results of the U.S. EPA's risk review, the agency promulgated a final rule on July 28, 2020, in which the U.S. EPA determined that risks from emissions of air toxics from this source category are acceptable and that the existing standards provide an ample margin of safety. Furthermore, under the technology review, the agency identified no cost-effective developments in controls, practices, or processes to achieve further emissions reductions. Petitions for Review of the rule were filed in the D.C. Circuit, in which the Company and the AISI intervened. The U.S. EPA signed the Taconite Rule on January 31, 2024 and it was published in the Federal Register on March 6, 2024. U. S. Steel filed a Petition for Reconsideration with the U.S. EPA and Petitions for Review with the United States Court of Appeals for the Eighth Circuit and the D.C. Circuit on May 3, 2024, challenging the final Taconite Rule. The Petition for Review is now with the D.C. Circuit and briefing is ongoing. A stay was sought and denied by the D.C. Circuit. Any impacts are not estimable at this time.

The U.S. EPA published the final Coke MACT residual risk and technology rule in the Federal Register on July 5, 2024. The final rule imposes lower emission limits as well as new emission limits and work practices for many emission sources at by-product and heat recovery coke plants. U. S. Steel is reviewing the final version of the rule to determine next steps. On August 30, 2024, the Company, through the American Coke and Coal Chemicals Institute (ACCCI) and Coke Environmental Task Force (COETF), filed a Petition for Review of the coke rule with the D.C. Circuit. On September 3, 2024, the Company separately and jointly with the ACCCI and COETF submitted the Petitions for Reconsideration and Administrative Stay of the final coke rule with the U.S. EPA. The Company through ACCCI and COETF filed a motion to stay the final coke rule with the D.C. Circuit which was denied. Briefing will be in 2025. Any impacts are not estimable at this time.

In response to Court orders that invalidated prior U.S. EPA determinations regarding ozone attainment interference, on April 6, 2022, the U.S. EPA proposed a Federal Implementation Plan (that would replace several pending or disapproved State Implementation Plans) for Regional Ozone Transport for the 2015 Ozone National Ambient Air Quality Standard. The proposed rule would affect electric generating units (EGUs) in 26 states and certain non-EGU industries, including, among several others, coke ovens, taconite production kilns, boilers, blast furnaces, basic oxygen furnaces, reheating furnaces, and annealing furnaces in 23 states, including those where U. S. Steel has operations. The U.S. EPA announced the final rule on March 15, 2023. The final rule only included regulation of boilers and reheat furnaces for the iron and steel industry limiting the potential impacts on the Company. U. S. Steel filed an administrative petition for review and a petition for judicial review to the rule on August 4, 2023. The matter remains before the U.S. EPA Administrator (administrative) and the D.C. Circuit (judicial). While U. S. Steel's and others' petitions to stay the effectiveness of the rule were denied by the D.C. Circuit, the Company, as well as other petitioners, have filed applications to stay the effectiveness of the rule with the Supreme Court of the United States (U.S. Supreme Court). The U.S. Supreme Court granted the stay on June 27, 2024. The effective dates of the rule will be stayed during the pendency of the lower court challenges. Litigation in the D.C. Circuit, in which U. S. Steel is a petitioner, remains ongoing. The U.S. EPA requested the D.C. Circuit remand the administrative record, which was granted, and the record was remanded on December 4, 2024. Any impacts are not estimable at this time.

The CAA also requires the U.S. EPA to develop and implement National Ambient Air Quality Standards (NAAQS) for criteria pollutants, which include, among others, particulate matter (PM) - consisting of PM_{10} and $PM_{2.5}$, lead, carbon monoxide, nitrogen dioxide, sulfur dioxide (SO_2) and ozone.

In October 2015, the U.S. EPA lowered the NAAQS for ozone from 75 parts per billion (ppb) to 70 ppb. On November 6, 2017, the U.S. EPA designated most areas in which we operate as attainment with the 2015 ozone standard. In a separate ruling, on June 4, 2018, the U.S. EPA designated other areas in which we operate as "marginal nonattainment" with the 2015 ozone standard. On December 6, 2018, the U.S. EPA published a final rule regarding implementation of the 2015 ozone standard. Because no state regulatory or permitting actions to bring the ozone nonattainment areas into attainment have yet to be proposed or developed for U. S. Steel facilities, the operational and financial impact of the ozone NAAQS cannot be reasonably estimated at this time. On December 31, 2020, the U.S. EPA published a final rule pursuant to its statutorily required review of

NAAQS that retains the ozone NAAQS at 70 ppb. In January 2021, New York, along with several states and non-governmental organizations, filed petitions for judicial review of the action with the D.C. Circuit. Several other states and industry trade groups intervened in support of the U. S. EPA's action. The case remains in abeyance before the court as the U.S. EPA voluntarily reconsiders the ozone NAAQS. On January 3, 2024, the U.S. EPA filed an unopposed motion to voluntarily remand without vacatur the 2020 rulemaking. In its motion, the U.S. EPA advised the court that it intends to conduct the voluntary remand simultaneously as it conducts an entirely new review of the ozone standard; and that it intends to complete the new review (which is already underway) "as expeditiously as possible". Any impacts related to the U.S. EPA's consideration to revise the ozone NAAQS are not estimable at this time.

On December 18, 2020, the U.S. EPA published a final rule pursuant to its statutorily required review of NAAQS that retains the existing $PM_{2.5}$ standards without revision. In early 2021, several states and non-governmental organizations filed petitions for judicial review of the action with the D.C. Circuit. Several industry trade groups intervened in support of the U.S. EPA's action. The case remains in abeyance before the court as the U.S. EPA voluntarily reconsiders the $PM_{2.5}$ NAAQS. On January 6, 2023, the U.S. EPA proposed to lower the annual $PM_{2.5}$ NAAQS from the current 12 ug/m^3 standard to within the range of 9.0 to 10.0 ug/m^3. On March 6, 2024, the U.S. EPA published a final rule to significantly lower the primary annual $PM_{2.5}$ NAAQS standard from 12 ug/m^3 to 9.0 ug/m^3. In the rule, the U.S. EPA retained the primary 24-hour $PM_{2.5}$ standard at the level of 35 ug/m^3. The rule is being challenged by 24 states as well as trade groups in the D.C. Circuit. Because area designations and State Implementation Plans have not yet been made, any impacts to the Company are not estimable at this time.

Environmental Remediation

For further discussion of relevant environmental matters, including environmental remediation obligations, see "Item 3. Legal Proceedings, Environmental Proceedings."

Property, Plant and Equipment Additions

For property, plant and equipment additions, including finance leases, see "Item 7. Management's Discussion and Analysis of Financial Condition and Results of Operations – Liquidity and Capital Resources" and Note 13 to the Consolidated Financial Statements.

Available Information

U. S. Steel's Internet address is ***www.ussteel.com***. We post our Annual Report on Form 10-K, our quarterly reports on Form 10-Q, our proxy statement, our current reports on Form 8-K, amendments to those reports and our interactive data files to our website free of charge as soon as reasonably practicable after such reports are filed with, or furnished to, the Securities and Exchange Commission (SEC). We also post all press releases and earnings releases to our website.

All other filings with the SEC are available via a direct link on the U. S. Steel website to the SEC's website, *www.sec.gov*.

Also available on the U. S. Steel website are U. S. Steel's Corporate Governance Principles, Code of Ethical Business Conduct and the charters of the Audit Committee, the Compensation & Organization Committee and the Corporate Governance & Sustainability Committee of the Board of Directors. These documents and the Annual Report on Form 10-K and proxy statement are also available in print to any stockholder who requests them. Such requests should be sent to the Office of the Corporate Secretary, United States Steel Corporation, 600 Grant Street, Suite 1844, Pittsburgh, Pennsylvania, 15219-2800 (telephone: 412-433-1121).

U. S. Steel does not incorporate into this document the contents of any website or the documents referred to in the immediately preceding paragraphs.

Other Information

Information on net sales, depreciation, capital expenditures, earnings (loss) before interest, taxes, depreciation and amortization and assets by reportable segment and for business in the Other category and on net sales and assets by geographic area are set forth in Note 4 to the Consolidated Financial Statements.

For significant operating data for U. S. Steel for each of the last five years, see "Five-Year Operating Summary (Unaudited)" within this document.

Item 1A. RISK FACTORS

Merger-Related Risk Factors

Failure to complete the Merger on a timely basis, or at all, would negatively impact our business and financial condition, as well as the price of our common stock.

We may not be able to complete the Merger on a timely basis, or at all, including due to the failure to receive required regulatory approvals. The closing conditions to the Merger include clearance by CFIUS. Following referral of the transaction by CFIUS to the President of the United States, on January 3, 2025, the President of the United States issued an order prohibiting the Merger and requiring the parties to abandon the Merger Agreement within thirty days. On January 10, 2025, CFIUS granted an extension of that deadline to June 18, 2025.

The Company, NSC and Purchaser jointly filed a lawsuit in the United States Court of Appeals for the District of Columbia Circuit alleging that the President's order blocking the Merger was issued for purely political reasons, which are irrelevant to U.S. national security, challenging the decision by the President of the United States and the CFIUS process as violating the constitutional due process rights of the Company, NSC and Purchaser, as well as the applicable statute governing CFIUS and the Administrative Procedure Act, and as an exercise of power that exceeded the authority provided to the President of the United States under the applicable statute. There is no assurance that this lawsuit will be successful, or will ultimately permit the Merger to be consummated. The Company, NSC and Purchaser also filed a lawsuit in the U.S. District Court for the Western District of Pennsylvania against Cliffs, Cliffs' Chief Executive Officer Lourenco Goncalves, and the President of the USW, David McCall, for engaging in a coordinated series of anticompetitive and racketeering activities illegally designed to prevent any party other than Cliffs from acquiring the Company as part of an illegal campaign to monopolize critical domestic steel markets. There is no assurance that this lawsuit will be successful.

If the Merger is not completed, our ongoing business may be adversely affected as follows: (i) we may experience negative reactions from the financial markets, including negative impacts on the market price of our common stock; (ii) some of management's attention will have been directed to the Merger instead of being directed to our own operations and the pursuit of other opportunities that could have been beneficial to us; (iii) the manner in which customers, suppliers and other third parties perceive us may be negatively impacted, which in turn could affect our ability to compete for business; (iv) we may experience negative reactions from employees and there may be further negative impact on the relationship with the leadership of the USW; (v) we will have expended time and resources that could otherwise have been spent on our business; and (vi) we will not obtain the benefits of NSC's commitments to the Company, including the commitments to invest at least $2.7 billion of capital and to share technology to protect and grow the Company. In addition, further delay in consummating the Merger could have an adverse effect on our operating results and adversely affect our relationships with customers and suppliers and would likely lead to a significant diversion of management and employee attention.

Additionally, in approving the Merger Agreement, the Board of Directors considered a number of factors and potential benefits, including the fact that the merger consideration to be received by holders of common stock represented a significant premium over the unaffected trading price, including a premium of 142% to the Company's unaffected closing stock price of $22.72 on August 11, 2023, the last trading day before the Company's announcement of the strategic alternatives review process and a premium of 40% to the Company's closing stock price of $39.33 on December 15, 2023, the last trading day before public announcement of the Merger Agreement. If the Merger is not completed, neither the Company nor the holders of our common stock will realize this benefit of the Merger. Moreover, we would also have nevertheless incurred substantial transaction-related fees and costs and the loss of management time and resources.

Expenses related to the pending Merger are significant and will adversely affect our operating results.

We have incurred and expect to continue to incur significant expenses in connection with the pending Merger, including legal and investment banking fees and fees incurred in connection with the Merger-related litigation. We expect these costs to have an adverse effect on our operating results.

We are subject to business uncertainties and contractual restrictions while the Merger is pending, which could adversely affect our business.

The Merger Agreement requires us to operate in the ordinary course of business and restricts us, without the consent of Purchaser, from taking certain specified actions agreed by the parties to be outside the ordinary course of business until the pending Merger occurs or the Merger Agreement terminates. These restrictions may prevent us from pursuing otherwise attractive business opportunities and making other changes to our business before completion of the Merger or, if the Merger is not completed, termination of the Merger Agreement. In addition, matters relating to the Merger (including integration planning) will require substantial commitments of time and resources by our management, which could divert their time and attention.

Litigation and union grievances and disputes could result in substantial costs or other negative impacts on the Company.

In connection with the Merger, we (along with our directors and officers) may face lawsuits, disputes and/or other actions (including union grievances or actions), in addition to the lawsuits jointly filed by the Company, NSC and Purchaser described above. We may face additional lawsuits, disputes and/or other actions, including those brought by stockholders of the Company or those arising from the Merger-related litigation filed by the Company and NSC, which may seek to enjoin, prevent, and/or delay us from consummating the Merger or may seek damages or other relief from the Company.

One of the conditions to the closing of the Merger is the absence of any injunction or similar order issued by any government entity in the U.S. or other specified jurisdiction or law in the U.S. or other specified jurisdiction that has the effect of prohibiting the consummation of the Merger or that makes consummation of the Merger illegal. Accordingly, if any plaintiff is successful in obtaining an injunction prohibiting the consummation of the Merger, then such injunction may prevent the Merger from becoming effective, or delay its becoming effective within the expected time frame. The ultimate resolution of any such proceedings cannot be predicted, and defending against such claims, even those without merit, could result in substantial costs (including costs in connection with the defense or settlement of stockholder litigation in connection with the Merger and costs associated with our indemnification obligations to our directors and officers), delay, and diversion of management's time and resources, which may negatively impact our financial condition and adversely affect our business and results of operations. We may also file actions to assert our rights in connection with the Merger.

The announcement and pendency of the Merger may adversely affect our business, financial condition and results of operations.

Whether or not the Merger is consummated, the Merger may disrupt our current plans and operations, which could have an adverse effect on our business and financial results. We cannot predict how our customers, distributors, suppliers and strategic partners will view or react to the pendency of the Merger or any of the actions being taken in pursuit of consummating the Merger. If we are unable to reassure our customers, distributors, suppliers and strategic partners to continue transacting business with us, our sales, financial condition, results of operations, cash flows and stock price may be adversely affected.

In addition, uncertainty about the effect of the Merger on our employees may have an adverse effect on our business. These uncertainties may impair our ability to attract, retain and motivate key personnel until the Merger is completed and for a period of time thereafter. Employee retention may be particularly challenging during the pendency of the Merger. If key employees depart and as we face additional uncertainties relating to the Merger, our business relationships may be subject to disruption as customers, suppliers and other third parties attempt to negotiate changes in existing business relationships or consider entering into business relationships with parties other than the Company. If key employees depart or if our existing business relationships suffer, our results of operations may be adversely affected. The adverse effects of such disruptions could be exacerbated by further delay in the completion of the Merger.

The Merger Agreement contains provisions that eliminate our ability to consider alternative transaction proposals.

The Merger Agreement contains non-solicitation provisions that, subject to limited exceptions which applied prior to obtaining the requisite stockholder approval of the Merger, restrict our ability to solicit, initiate, or knowingly encourage or induce competing third-party proposals (or engage in, continue or otherwise participate in negotiations or discussions regarding such third-party proposals) for the acquisition of our stock or assets.

While the Merger Agreement remains in effect, these provisions would prevent us from being able to consider, negotiate or accept a transaction with a potential third-party acquirer that might have an interest in acquiring all or a significant portion of us, even if the acquirer was prepared to pay consideration with a higher per share cash or market value than the market value proposed to be received or realized in the Merger.

If the Merger Agreement is terminated and we decide to seek another business combination, we may not be able to negotiate or consummate a transaction with another party on terms comparable to, or better than, the terms of the Merger Agreement.

All of the matters described above, alone or in combination, could materially and adversely affect our business, financial condition, results of operations and stock price.

Economic and Market Risk Factors

The changing global economic climate is having adverse impacts on our business, which may create new risks and exacerbate certain other risks set forth below.

Changes in the global economic environment, inflation, elevated interest rates, recessions or prolonged periods of slow economic growth, and global instability and actual and threatened geopolitical conflict, could have an adverse effect on our industry and business, as well as those of our customers and suppliers.

Overall economic conditions in the U.S. and globally, including in Europe, including adverse factors such as inflation, rising or sustained elevated interest rates, supply chain disruptions and geopolitical conflicts, including the impacts of the war in Ukraine, significantly impact our business. Periods of economic downturn or continued uncertainty could result in difficulty increasing or maintaining our level of sales or profitability and we may experience an adverse effect on our business, results of operations, financial condition and cash flows.

Our U.S. operations are subject to economic conditions, including credit and capital market conditions, inflation, prevailing interest rates, and political factors, which if changed could negatively affect our results of operations, cash flows and liquidity. Political factors include, but are not limited to, changes to tax laws and regulations resulting in increased income tax liability,

changes in administration resulting in increased or newly imposed tariffs, increased regulation, such as carbon emissions limitations or trading mechanisms, limitations on exports of energy and raw materials, and trade remedies. Actions taken by the U.S. government could affect our results of operations, cash flows and liquidity.

USSE is subject to economic conditions and political factors associated with the EU, Slovakia and neighboring countries, and the euro currency. Changes in any of these economic conditions or political factors could negatively affect our results of operations, cash flows and liquidity. Political factors include, but are not limited to, taxation, nationalization, inflation, government instability, regional conflict, civil unrest, increased regulation and quotas, tariffs, sanctions and other market-distorting measures. Continued uncertainty and economic downturn in the European market throughout 2024, as well as the ongoing war in Ukraine, have had a broad range of adverse impacts on global economic conditions, many of which have had and are likely to continue to have adverse impacts on our USSE business, including increased raw material and energy costs, softer customer demand and lower steel prices, which led to the temporary idling of a portion of USSE's raw steel capability at the end of 2024 until the demand environment improves. These uncertain conditions in the European market could lead to adverse effects on the valuation of USSE's long-lived assets, which could negatively affect our results of operations through potential impairment charges. USSE also purchases a significant portion of its iron ore and coal from suppliers based in Ukraine.

Additionally, we are also exposed to risks associated with the business success and creditworthiness of our suppliers and customers. If our customers or suppliers are negatively impacted by a slowdown in economic markets, we may face the reduction, delay or cancellation of customer orders, delays or interruptions of the supply of raw materials, and increased risk of insolvency and other credit related issues of customers or suppliers, which could delay payments from customers, result in increased customer defaults and cause our suppliers to delay filling, or to be unable to fill, our needs at all or on a timely or cost-effective basis. The occurrence of any of these events may adversely affect our business, results of operations, financial condition and cash flows.

The steel industry, as well as the industries of our customers and suppliers upon whom we are reliant, is highly cyclical, which may have an adverse effect on our customer demand and results of operations.

Steel consumption is highly cyclical and generally follows economic and industrial conditions both worldwide and in regional markets. Price fluctuations are impacted by the timing, magnitude and duration of these cycles, and are difficult to predict. This volatility makes it difficult to balance the procurement of raw materials and energy with global steel prices, our steel production and customer product demand. U. S. Steel has implemented strategic initiatives to produce more stable and consistent results, even during periods of economic and market downturns, but this may not be enough to mitigate the effect that the volatility inherent in the steel industry has on our results of operations.

Additionally, our business is reliant on certain other industries that are cyclical in nature. We sell to the automotive, service center, converter, energy and appliance and construction-related industries. Some of these industries are highly sensitive to general economic conditions and may also face meaningful fluctuations in demand based on a number of factors outside of our control, including regulatory factors, supply chain disruptions, changing customer demand, economic conditions and raw material and energy costs. As a result, downturns or volatility in any of the markets we serve could adversely affect our financial position, results of operations and cash flows.

U. S. Steel has been and continues to be adversely affected by unfairly traded imports and global overcapacity, which may cause downward pricing pressure, lost sales and revenue and decreased market share, production, investment, and profitability.

Currently, global steel production capacity significantly exceeds global steel demand, which adversely affects U.S. and global steel prices. Global overcapacity continues to result in high levels of dumped and subsidized steel imports into the markets we serve. Domestic and international trade laws provide mechanisms to address the injury caused by such imports to domestic industries. Excessive steel imports have resulted and may continue to result in downward pricing pressure and lost sales and revenue, which adversely impacts our business, operations, financial condition and cash flows.

Although U. S. Steel currently benefits from 69 U.S. AD and CVD or anti-subsidy duty orders and 14 EU AD/CVD orders, petitions for trade relief are not always successful or effective. When implemented, such relief is generally subject to periodic reviews and challenges, which can result in revocation of AD/CVD orders or reduction of effective duty rates. There can be no assurance that any relief will be obtained or continued in the future or that such relief will adequately combat unfairly traded imports.

As of the date of this filing, pursuant to a series of Presidential Proclamations issued in accordance with Section 232 of the Trade Expansion Act of 1962, U.S. imports of certain steel products are subject to a 25 percent tariff, except imports from: (1) Argentina, Brazil, and South Korea, which are subject to restrictive quotas; (2) the European Union (EU) that are melted and poured in the EU, within TRQ limits through December 2025; (3) Japan that are melted and poured in Japan within TRQ limits; (4) United Kingdom (UK) that are melted and poured in the UK within TRQ limits; (5) Canada; (6) Mexico, if melted and poured in North America; (7) Ukraine, if melted and poured in Ukraine or the EU, until June 1, 2025; and (8) Australia. The Section 232 national security action on steel imports currently provides U. S. Steel and other domestic steel producers critical relief from imports. With no scheduled end date, the future coverage and duration of the Section 232 action is not known. Further, the U.S.

government may negotiate alternatives to the Section 232 tariffs for certain countries, similar to TRQ agreements with the EU, Japan, and the UK.

USTR's review of additional tariffs of 7.5 to 25 percent on certain U.S. imports from China, including certain raw materials used in steel production, semi-finished and finished steel products, and downstream steel-intensive products, pursuant to Section 301 of the Trade Act of 1974 could change the coverage and levels of such tariffs. In September 2024, USTR revised certain Section 301 tariffs on U.S. imports from China, including increasing tariffs on steel from 7.5 to 25 percent; batteries from 7.5 to 25 percent; solar cells/modules from 25 to 50 percent; and electric vehicles from 25 to 100 percent.

In February 2019, the EC implemented a definitive safeguard on global steel imports in the form of TRQs. The TRQs, which impose 25 percent tariffs on steel imports that exceed the TRQ limit, are currently effective through June 2026.

All of the above factors present a degree of uncertainty to our financial and operational performance, our customers, and overall economic conditions, all of which could impact steel demand and our performance. Faced with significant import competition and overcapacity in various markets, we will continue to evaluate potential strategic and organizational opportunities, which may include exiting lines of business and the sale of certain assets, temporary shutdowns or closures of facilities.

Shortages of skilled labor, increased labor costs or our failure to attract and retain other highly qualified personnel in the future could disrupt our operations and adversely affect our financial results.

We depend on skilled labor for the manufacture of our products. Some of our facilities are located in areas where demand for skilled labor often exceeds supply. Shortages of some types of skilled labor, such as electricians and qualified maintenance technicians, could restrict our ability to maintain or increase production rates, lead to production inefficiencies and increase our labor costs. Our strategic investments also require a set of job skills that is different from our prior needs. Our continued success executing on our strategy, and constructing and commercializing depends on the active participation of our key employees and ability to attract employees with different skills. We have recently observed an overall tightening and increasingly competitive labor market. The competitive nature of the labor markets in which we operate, the cyclical nature of the steel industry and our resulting needs for skilled employees increase our risk of not being able to recruit, train and retain the employees we require at efficient costs and on reasonable terms, and could lead to increased costs, such as increased overtime to meet demand and increased compensation to attract and retain employees. In addition, many companies, including U. S. Steel, have had employee layoffs as a result of reduced business activities during industry downturns. The loss of our key people or our inability to attract new key employees could adversely affect our operations. Additionally, layoffs or other adverse actions could result in an adverse relationship with our workforce or third-party labor providers. If we are unable to recruit, train and retain adequate numbers of qualified employees and third-party labor providers on a timely basis or at a reasonable cost or on reasonable terms, our business and results of operations could be adversely affected. Additionally, an overall labor shortage, lack of skilled labor, increased turnover, labor strikes or labor inflation as a result of general macroeconomic factors that affect our customers or suppliers could have a material adverse impact on the Company's operations, results of operations, liquidity or cash flows.

Strategic Risk Factors

Commercialization of our strategic investments in new technologies and products may not be fully successful.

Execution of our Best for All® strategy depends, in part, on the successful commercialization of a number of investments we have made in new facilities, technologies and products and successfully transitioning our footprint to a lower-cost, carbon and capital intensive model. Our Best for All® strategy is centered around expanding our competitive advantages in low-cost iron ore, mini mill steelmaking, and best-in-class finishing capabilities. These competitive advantages are built on a foundation of research, innovation and deep customer relationships. We have expanded our low-cost iron ore competitive advantage by investing in ways to translate the advantage to feed our growing EAF footprint. This includes investments in a pig iron caster at the Gary Works facility in the fourth quarter of 2022, and DR-grade pellet capabilities in Keetac, Minnesota, completed in December 2023. We have expanded our mini mill steelmaking capabilities through the construction of a second mini mill facility in Osceola, Arkansas, which produced its first coil in October 2024. We also expanded our best-in-class finishing capabilities through investments in a non-grain oriented electrical steel line and galvanizing construction line at Big River Steel. In executing our strategy, we aim to enhance our earnings profile, deliver long-term cash flow through industry cycles and reduce our cost, capital, and carbon intensity. By offering the product capabilities, including the more sustainable steels our customers are increasingly demanding, we believe that we can achieve more competitive positioning in strategic, high-margin end markets, and deliver high-quality, sustainable, value-added products and innovative solutions.

Successful commercialization of our strategic investments and execution of our strategy are subject to changing market conditions and demand for our new products, capability and cost performance, and customer adoption of new technologies. New product development or modification is costly, may be restricted by regulatory requirements, involves significant research, development, time, expense and human capital and may not necessarily result in the successful commercialization of new products, customer adoption of new technologies or products or new technologies may not perform as intended or expected. Unsuccessful commercialization and execution of these strategic investments, underperformance of any of these assets, failure of new products to gain market acceptance, delays or inability to achieve customer qualification standards, or limited or reduced demand in the marketplace could adversely affect our business, results of operations and financial condition and may limit the benefits of our stockholder value creation strategy.

From time to time, we engage in acquisitions, divestitures and joint ventures and may encounter difficulties in integrating and separating these businesses and therefore we may not realize the anticipated benefits.

As we pursue our Best for All® strategy, we may seek growth opportunities through strategic acquisitions as well as evaluate our portfolio for potential divestitures to optimize our business footprint and portfolio. The success of these transactions will depend on our ability to integrate or separate, as applicable, assets and personnel in these transactions and to cooperate with our strategic partners. We may encounter difficulties in integrating acquisitions with our operations as well as separating divested businesses, and in managing strategic investments. Furthermore, we may not realize the degree, or timing, of benefits we anticipate when we first enter into a transaction.

Additionally, we seek opportunities to monetize non-core and excess iron assets, including through real estate sales, third party agreements and option agreements. These opportunities may not materialize or generate the financial benefits expected. For example, Stelco Inc. (Stelco), a subsidiary of Cliffs, holds an option (Option) to acquire an undivided 25 percent interest in a to-be-formed entity that will own the Company's current iron ore mine located in Mt. Iron, Minnesota. There is a possibility that Stelco may not exercise its Option in the anticipated timeframe or at all. If the proposed joint venture with Stelco is not successful, fails to provide the benefits we expect, or is not created at all, we may in the future have more iron ore than we need to support the business. Additionally, the existence of the Option may deter future potential opportunities to monetize the iron ore assets. Any of the foregoing could adversely affect our business and results of operations.

Operational and Commercial Risk Factors

Our operational footprint, unplanned equipment outages and other unforeseen disruptions may adversely impact our results of operations or result in idle facility costs or impairment charges.

U. S. Steel has adjusted its operating configuration to advance its Best for All® strategy, in response to market conditions, including global economic volatility, declining steel prices, oil and gas industry disruption, global overcapacity and unfairly traded imports, and to optimize capability and cost performance, by idling and restarting production at certain facilities. Due to our existing operational footprint, the Company may not be able to respond in an efficient manner to fully realize the benefits from changing market conditions that are favorable to integrated steel producers or most efficiently mitigate the negative impacts of such changes. Our decisions concerning which facilities to operate and at what levels are made based upon execution of our Best for All® strategy, market conditions, our customers' orders for products as well as the capabilities and cost performance of our locations. We may concentrate production operations at several plant locations and not operate others, and as a result we may incur idle facility costs or impairment charges.

Our steel production depends on the operation of critical structures and pieces of equipment, such as blast furnaces, electric arc furnaces, steel shops, casters, hot strip mills and various structures and operations, including information technology systems, that support them, as well as finishing lines at our facilities and certain of our joint ventures. While we invested in operational and reliability enhancements to our assets through the asset revitalization program, launched in 2017, and continue to implement initiatives focused on proactive maintenance of key machinery and equipment at our production facilities, we may experience prolonged periods of reduced production and increased maintenance and repair costs due to equipment failures at our facilities or those of our key suppliers.

It is also possible that operations may be disrupted due to other unforeseen circumstances such as power outages, explosions, fires, floods, earthquakes, pandemics, terrorism, accidents, severe weather conditions, changes in U.S., European Union and other foreign tariffs, free trade agreements, trade regulations, laws and policies. We are also exposed to similar risks involving major customers and suppliers such as force majeure events of raw materials suppliers that have occurred and may occur in the future. Availability of raw materials and delivery of products to customers could be affected by logistical disruptions, such as shortages of barges, ocean vessels, rail cars or trucks or unavailability of rail lines or of the locks on the Great Lakes or other bodies of water. To the extent that lost production could not be compensated for at unaffected facilities and depending on the length of the outage, our sales and our unit production costs could be adversely affected.

We are subject to outbreaks of infectious disease, such as risks related to the global COVID-19 pandemic, which had adverse impacts on economic and market conditions and our business. Public health crises, including COVID-19, have created and may create significant volatility, uncertainty and economic disruption in the regions in which we operate.

The physical impacts of climate change may also have a material adverse effect on our results of operations. Climate change may be associated with increased occurrence of extreme weather conditions, which could include, among other things, increased risk of flooding, extreme cold or potential heat stress at facilities and other natural disasters that may lead our customers to curtail or shut down production or to supply chain and operational disruptions.

We face increased competition within our industry and from alternative materials and risks concerning innovation, new technologies, products and increasing customer demand for lower-carbon products.

As a result of increasingly stringent regulatory requirements and increased market and technological changes driven by broader trends such as decarbonization and electrification efforts in response to climate change, designers, engineers and industrial manufacturers, especially those in the automotive industry, are increasing their use of lighter weight, less carbon intense and alternative materials, such as aluminum, composites, plastics and carbon fiber. Use of such materials could reduce the demand for our steel products or steel products generally, which may reduce our profitability and cash flow. Additionally, the trend toward light weighting in the automotive industry, which requires lighter gauges of steel at higher strengths, could result in lower steel volumes required by that industry over time.

Additionally, technologies such as direct iron reduction, oxygen-coal injection and experimental technologies such as molten oxide electrolysis and hydrogen flash smelting may be more cost effective than our current production methods. However, we may not have sufficient capital to invest in such technologies and may incur difficulties adapting and fully integrating these technologies into our existing operations. We may also encounter production restrictions, or not realize the cost benefit from such capital intensive technology adaptations to our current production processes.

Finally, we may face increased competition due to the rapid development and rising use of digital, artificial intelligence (AI) and machine learning technologies. Failure to early adopt and incorporate such technologies to improve productivity, yields, manufacturing technology or support functional teams may put us at a long-term competitive disadvantage.

Limited availability, or volatility in prices of raw materials, scrap and energy may constrain operating levels and reduce profit margins.

U. S. Steel and other steel producers have periodically faced problems obtaining sufficient raw materials and energy in a timely manner due to delays, defaults, severe weather conditions, or force majeure events, shortages or transportation problems (such as shortages of barges, ore vessels, rail cars or trucks, or disruption of rail lines, waterways, or natural gas transmission lines), resulting in production curtailments. As a result, we may be exposed to risks concerning pricing and availability of raw materials and energy resources from third parties as well as logistics constraints moving our own raw materials and scrap to our plants. USSE purchases substantially all of its iron ore and coking coal requirements from outside sources. Any curtailments or escalated costs may further reduce profit margins.

U. S. Steel has agreed, and may continue to agree, to purchase raw materials and energy at prices that have been, and may be, above future market prices or in greater volumes than required in the future. Additionally, any future decreases in iron ore, scrap, natural gas, electricity and oil prices may place downward pressure on steel prices. If steel prices decline, our profit margins on indexed contracts and spot business could be reduced.

A failure of our information technology infrastructure and cybersecurity threats may adversely affect our business operations.

Despite efforts to protect confidential business information, personally identifiable information (PII), and the control systems of manufacturing plants, U. S. Steel systems and those of our joint ventures and third-party service providers have been and may be subject to cyberattacks or system breaches. System breaches can lead to theft, unauthorized disclosure, modification or destruction of proprietary business data, PII, or other sensitive information, to defective products, production downtime and damage to production assets, and the inaccessibility of key systems, with a resulting impact to our reputation, competitiveness and operations. We have experienced cybersecurity attacks that have resulted in unauthorized persons gaining access to our information technology systems and networks, and we could in the future experience similar attacks. To date, no cybersecurity attack has had a material impact on our financial condition, results of operations or liquidity.

While the Company continually works to safeguard our systems and mitigate potential risks, there can be no assurance that such actions will be sufficient to prevent cyberattacks or security breaches or mitigate all potential risks to our systems, networks and data, particularly with the recent proliferation and sophistication of cyberattacks and cyber intrusions around the world. The number of threats and events has increased substantially every year, which is expected to continue, particularly as the use of artificial intelligence makes these attempts look more legitimate. The potential consequences of a material cybersecurity attack include reputational damage, investigations and/or adverse proceedings with government regulators or enforcement agencies, litigation with third parties, disruption to our systems, including production capabilities, unauthorized release of confidential, personally identifiable or otherwise protected information, corruption of data, diminution in the value of our investment in research, development and engineering and increased cybersecurity protection and remediation costs, which in turn could adversely affect our competitiveness, results of operations and financial condition. The amount of insurance coverage we maintain may be inadequate to cover claims or liabilities resulting from a cybersecurity attack.

We depend on third parties for transportation services and increases in costs or the availability of transportation may adversely affect our business and operations.

Our business depends on the transportation of a large number of products, both domestically and internationally. We rely primarily on third parties, including the divested Transtar business, for transportation of the products we manufacture as well as delivery of our raw materials. Any increase in the cost of the transportation of our raw materials or products, as a result of increases in fuel or labor costs, higher demand for logistics services, consolidation in the transportation industry or otherwise, may adversely affect our results of operations as we may not be able to pass such cost increases on to our customers.

Our transportation service providers may face disruptions due to weather conditions or events, strikes, labor shortages or other constraints. If any of these providers were to fail to deliver raw materials to us or deliver our products in a timely manner, we may be unable to manufacture and deliver our products in response to customer demand. In addition, if any of these third parties were to cease operations or cease doing business with us, we may be unable to replace them at a reasonable cost. Such failure of a third-party transportation provider could harm our reputation, negatively affect our customer relationships and have a material adverse effect on our financial position and results of operations.

Our 2022 Labor Agreements with the USW contain provisions that may impact certain business activities.

Our 2022 Labor Agreements with the USW contain provisions that grant the USW a limited right to bid on the Company's sale of a facility (or sale of a controlling interest in an entity owning a facility) covered by the 2022 Labor Agreements, excluding public equity offerings and/or the transfer of assets between U. S. Steel and its wholly owned subsidiaries. The 2022 Labor Agreements also require a minimum level of capital expenditures (subject to approval of the Board of Directors) to maintain the competitive status of the covered facilities, and place certain limited restrictions on our ability to replace product produced at a covered facility with product produced at other than Company facilities or affiliates or U.S. or Canadian facilities with employee protections similar to the protections found in the 2022 Labor Agreements when the Company is operating covered facilities below capacity. The provisions in the 2022 Labor Agreements, as well as current or future proposed labor legislation or regulations, could unfavorably impact certain business activities including pricing, operating costs, margins and/or our competitiveness in the marketplace.

Financial Risk Factors

Our business and execution of our strategy require substantial expenditures for capital investments, debt service obligations, operating leases and maintenance that we may be unable to fund, which may require other actions to satisfy our obligations under our debt.

We have approximately $4.1 billion of long-term debt (see Note 17 to the Consolidated Financial Statements). If our cash flows and capital resources are insufficient to fund our planned capital expenditures or debt service obligations, we may face substantial liquidity problems and may be forced to reduce or delay investments and capital expenditures, terminate strategic projects, or to dispose of material assets or operations or issue additional debt or equity. We may not be able to take such actions, if necessary, on commercially reasonable terms or at all. The Credit Facility Agreement, the documents governing the USSK Credit Facility, the documents governing the Big River Steel ABL (Asset Based Loan) Facility and Big River Steel notes, and the indentures governing our existing senior unsecured notes may restrict our ability to dispose of assets and may also restrict our ability to raise debt or equity capital to be used to repay other debt when it becomes due. We may not be able to consummate those dispositions or to obtain proceeds in an amount sufficient to meet any debt service obligations then due. Our inability to generate sufficient cash flows to satisfy our debt obligations, or to refinance our indebtedness on commercially reasonable terms or at all, would materially and adversely affect our financial position and results of operations and may place us at a competitive disadvantage with competitors who may have less indebtedness and other obligations or greater access to financing. In addition, the availability under our Credit Facility Agreement and Big River ABL Facility may be reduced if we have insufficient collateral, or if we do not meet a fixed charge coverage ratio test. Availability under the USSK Credit Agreement could be limited if USSK does not meet certain financial covenants.

Our ability to service or refinance our debt or fund investments and capital expenditures required to maintain or expand our business operations depends on our financial condition and operating performance, which are subject to prevailing economic and competitive conditions and to certain financial, business, legislative, regulatory and other factors beyond our control, such as supply and demand conditions, inflation, prevailing interest rates, supply chain disruptions and the impacts of the war in Ukraine. We may not be able to maintain a level of cash flows from operating activities sufficient to permit us to satisfy our liquidity needs. In addition, the availability under certain of our debt instruments may be limited if we do not meet certain financial covenants. Furthermore, the agreements governing the BRS (Big River Steel) ABL Facility and other outstanding indebtedness of Big River Steel LLC and its subsidiaries limit their ability, subject to certain exceptions, to pay dividends or distributions or make other restricted payments, such that we may not be able to access the cash generated by these subsidiaries to fund our other expenditures.

If we cannot make scheduled payments on our debt, we will be in default and holders of our senior unsecured notes could declare all outstanding principal and interest to be due and payable, the lenders under the Sixth Amended and Restated Credit

Agreement, the USSK Credit Facility and the Export Credit Agreement could terminate their commitments to loan money, accelerate full repayment of any or all amounts outstanding (which may result in the cross acceleration of certain of our other debt obligations) and the lenders could foreclose against the assets securing their borrowings and we could be forced into bankruptcy or liquidation. All of these events would materially and adversely affect our financial position and results of operations.

Furthermore, ratings agencies could downgrade our ratings either due to factors specific to our business, a prolonged cyclical downturn in the steel industry, macroeconomic trends such as global or regional recessions and trends in credit and capital markets more generally. Ratings agencies also may lower, suspend or withdraw ratings on the outstanding securities of U. S. Steel or Big River Steel. Any lowering, suspension or withdrawal of such ratings may have an adverse effect on the market prices of such securities.

Any decline in our operating results or downgrades in our credit ratings may make raising capital or entering into any business transaction more difficult, lead to reductions in the availability of credit or increased cost of credit, adversely affect the terms of future borrowings, may limit our ability to take advantage of potential business opportunities, may have an adverse effect on the terms under which we purchase goods and services, and lead to reductions in the availability of credit.

We have significant retiree health care, retiree life insurance and pension plan costs, which may negatively affect our results of operations and cash flows.

We maintain retiree health care and life insurance and defined benefit pension plans covering many of our domestic employees and former employees upon their retirement. Some of these benefit plans are not fully funded, and thus will require cash funding in future years. Minimum contributions to domestic qualified pension plans (other than contributions to the Steelworkers Pension Trust (SPT) described below) are regulated under the Employee Retirement Income Security Act of 1974 (ERISA) and the Pension Protection Act of 2006 (PPA).

The level of cash funding for our defined benefit pension plans in future years depends upon various factors, including voluntary contributions that we may make, future pension plan asset performance, actual interest rates under the law, the impact of business acquisitions or divestitures, union negotiated benefit changes and future government regulations, many of which are not within our control. In addition, assets held by the trusts for our pension plan and our trust for retiree health care and life insurance benefits are subject to the risks, uncertainties and variability of the financial markets. Future funding requirements could also be materially affected by differences between expected and actual returns on plan assets, actuarial data and assumptions relating to plan participants, the discount rate used to measure the pension obligations and changes to regulatory funding requirements. See “Item 7. Management’s Discussion and Analysis of Financial Condition and Results of Operations” and Note 18 to the Consolidated Financial Statements for a discussion of assumptions and further information associated with these benefit plans.

U. S. Steel contributes to a domestic multiemployer defined benefit pension plan, the SPT, for USW-represented employees formerly employed by National Steel and represented employees hired after May 2003. We have legal requirements for future funding of this plan should the SPT become significantly underfunded or we decide to withdraw from the plan. Either of these scenarios may negatively impact our future cash flows. The 2022 Labor Agreements increased the contribution rate for most steelworker employees. Collectively bargained company contributions to the plan could increase further as a result of future changes agreed to by the Company and the USW.

The accounting treatment of goodwill, equity method investments and other long-lived assets could result in future asset impairments, which would reduce our earnings.

We periodically test our goodwill, equity method investments and other long-lived assets to determine whether their estimated fair value is less than their value recorded on our balance sheet. The results of this testing for potential impairment may be adversely affected by uncertain market conditions for the global steel industry and general economic conditions. If we determine that the fair value of any of these assets is less than the value recorded on our balance sheet, and, in the case of equity method investments the decline is other than temporary, we would likely incur a non-cash impairment loss that would negatively impact our results of operations. We have incurred asset impairment charges in recent years, including during the year ended December 31, 2024, and there can be no assurances that continued market dynamics or other factors may not result in future impairment charges.

We are subject to foreign currency risks, which may negatively impact our profitability and cash flows.

The financial condition and results of operations of USSE are reported in euros and then translated into U.S. dollars at the applicable exchange rate for inclusion in our financial statements. The appreciation of the U.S. dollar against the euro negatively affects our Consolidated Results of Operations. International cash requirements have been and in the future may be funded by intercompany loans, which may create intercompany monetary assets and liabilities in currencies other than the functional currencies of the entities involved, which can have a non-cash impact on income when they are remeasured at the end of each period. Procurement of equipment of announced strategic projects may be denominated in foreign currencies, which could adversely affect the costs of these projects.

In addition, foreign producers, including foreign producers of subsidized or unfairly traded steel with foreign currency denominated costs may gain additional competitive advantages or target our home markets if the U.S. dollar or euro exchange rates strengthen relative to those producers' currencies. Volatility in the markets and exchange rates for foreign currencies and contracts in foreign currencies could have a significant impact on our reported financial results and condition.

Financial regulatory frameworks introduced by U.S. and EU regulators may limit our financial flexibility or increase our costs.

We use swaps, forward contracts and similar agreements to mitigate our exposure to volatility, which entails a variety of risks. The Commodity Future Trading Commission's Dodd Frank and the EU's European Market Infrastructure Regulation and other government agencies' regulatory frameworks can limit the Company's ability to hedge interest rate, foreign exchange (FX), or commodity pricing exposures, which could expose us to increased economic risk. These frameworks may introduce additional compliance costs or liquidity requirements. Some counterparties may cease hedging as a result of increased regulatory cost burdens, which in turn may reduce U. S. Steel's ability to hedge its interest rate, FX or commodity exposures.

We are a party to various legal proceedings, the resolution of which could negatively affect our profitability and cash flows in a particular period.

We are involved at any given time in various litigation matters, including administrative and regulatory proceedings, governmental investigations, environmental matters and commercial disputes. Our profitability and cash flows in a particular period could be negatively affected by an adverse ruling or settlement in any legal proceeding or investigation. While we believe that we have taken appropriate actions to mitigate and effectively manage these risks, due to the nature of our operations, these risks will continue to exist and additional legal proceedings or investigations may arise from time to time.

Additionally, we may be subject to product liability claims that may have an adverse effect on our financial position, results of operations and cash flows. Events such as well failures, line pipe leaks, blowouts, bursts, fires and product recalls could result in claims that our products or services were defective and caused death, personal injury, property damage or environmental pollution. The insurance we maintain may not be adequate, available to protect us in the event of a claim, or its coverage may be limited, canceled or otherwise terminated, or the amount of our insurance may be less than the related impact on our enterprise value after a loss. We establish reserves based on our assessment of contingencies, including contingencies for claims asserted against us in connection with litigation, arbitrations and environmental issues. Adverse developments in litigation, arbitrations, environmental issues or other legal proceedings may affect our assessment and estimates of the loss contingency recorded as a reserve and require us to make payments in excess of our reserves, which could negatively affect our operations, financial results and cash flows. See "Item 3. Legal Proceedings" and Note 26 to the Consolidated Financial Statements for further details.

Regulatory Risk Factors

Compliance with existing and new environmental regulations, environmental permitting and approval requirements may result in delays or other adverse impacts on planned projects, our results of operations and cash flows.

Steel producers in the U.S., along with their customers and suppliers, are subject to numerous federal, state and local laws and regulations relating to the protection of the environment. These laws and regulations concern the generation, storage, transportation, treatment, disposal, release, emission or discharge of pollutants, contaminants, petroleum, and hazardous substances into the environment, the reporting of such matters, and the general protection of public health and safety, natural resources, wildlife and the environment. Steel producers in the EU are subject to similar laws. These laws and regulations continue to evolve and are becoming increasingly stringent. For example, the U.S. EPA has proposed to lower the NAAQS for fine particulate matter ($PM_{2.5}$), which would result in stringent and costly requirements to control emissions from existing operations and restrictions on new projects. The ultimate impact of complying with such laws and regulations is not always clearly known or determinable because regulations under some of these laws have not yet been promulgated, and the subject of ongoing litigation or are undergoing revision, and new versions or interpretations of existing laws may take effect. Additionally, compliance with certain of these laws and regulations, such as the CAA and state and local requirements governing air emissions, could result in substantially increased capital requirements and operating costs and could require changes to the equipment or facilities we operate. Compliance with current or future laws and regulations could entail substantial costs and could have a negative impact on our results of operations and cash flows. The amount and timing of environmental expenditures is difficult to predict.

In addition, the Company must obtain, maintain and comply with numerous permits, leases, approvals, consents and certificates from various governmental authorities in connection with the construction and operation of new production facilities or modifications to existing facilities. In connection with such activities, the Company may need to make significant capital and operating expenditures to detect, repair and/or control air emissions, to control water discharges or to perform certain corrective actions to meet the conditions of the permits issued pursuant to applicable environmental laws and regulations.

There can be no assurance that future approvals, licenses and permits will be granted or that we will be able to maintain and renew the approvals, licenses and permits we currently hold. Failure to do so could have a material adverse effect on our results of operations and cash flows. Furthermore, compliance with environmental permitting and approval requirements may be costly and time consuming and could result in delays or other adverse impacts on planned projects, our results of operations and cash flows.

Failure to comply with legal requirements, including permits, leases, approvals, consents and certificates, may result in administrative, civil and criminal penalties, and revocation of permits and our authority to conduct business or construct certain facilities, substantial fines or sanctions, or citizens' suits or enforcement actions (including orders limiting our operations or requiring corrective measures), among other consequences.

We have significant environmental remediation costs that negatively affect our results of operations and cash flows.

Various environmental laws impose liability on a current or former owner or operator of real property for investigation or cleanup of hazardous substances or petroleum products at, on, under or migrating from our currently or formerly owned or leased real property. Some of U. S. Steel's current and former facilities were in operation before such environmental regulations were in place. Hazardous materials associated with those facilities have been and may continue to be encountered at current or former operating sites or delivered to sites operated by third parties. In some cases, liability may be imposed without regard to contribution or to whether we knew of, or caused, the release of hazardous substances. Liability under these laws may be joint and several, meaning that we could be required to bear 100% of the liability even if other parties are also liable. U. S. Steel is involved in numerous remediation projects at currently operating facilities, facilities that have been closed or sold to unrelated parties and other sites where material generated by U. S. Steel was deposited. In addition, there are numerous other former operating or disposal sites that could become subject to remediation, which may negatively affect our results of operations and cash flows. Contamination at our current properties could result in limitations on or interruptions to our operations and liens in favor of the government for costs the government incurs in cleaning up contamination. In addition, we may be liable for the costs of investigating or remediating contamination at off-site waste facilities where we have arranged for the disposal, or treatment of hazardous substances, without regard to whether we complied with environmental laws in doing so.

Reducing greenhouse gas (GHG) emissions from steelmaking operations to meet corporate targets or comply with new regulations as well as stakeholder expectations and mitigate potential physical impacts of climate change could significantly increase costs to manufacture future materials or reduce the amount of materials being manufactured.

Iron and steel producers around the world are facing mounting pressure to reduce greenhouse gas emissions from operations. The majority of greenhouse gas emissions from the production of iron and steel are caused by the combustion of fossil fuels, the use of electrical energy, and the use of coal, lime, and iron ore as feedstock. The two main production processes are the integrated route of blast furnace ironmaking in combination with basic oxygen furnace steelmaking (BOF) and the alternative route of electric arc furnace steelmaking. Both routes generate greenhouse gas emissions with the latter process, involving the electric arc melting of a majority of steel scrap, generating less than half that of the traditional integrated steelmaking process.

Federal, state and local governmental agencies within the United States may introduce regulatory changes in response to the potential impacts of climate change, including the introduction of carbon emissions limitations or trading mechanisms, and changes in U.S. administration may result in new or significantly different regulations than those currently impacting or likely to impact the Company. Any such regulation regarding climate change and GHG emissions could impose significant costs on our operations and on the operations of our customers and suppliers, including increased energy, capital equipment, emissions controls, environmental monitoring and reporting and other costs in order to comply with current or future laws or regulations concerning climate change and GHG emissions. Any adopted future climate change and GHG regulations could negatively impact our ability, and that of our customers and suppliers, to compete with companies situated in areas not subject to or not complying with such limitations. Inconsistency of regulations may also change the attractiveness of the locations of some of the Company's assets and investments. In addition, changes in certain environmental regulations, including those that may impose output limitations or higher costs associated with climate change or greenhouse gas emissions, could substantially increase the cost of manufacturing and raw materials to us and other steel producers. For additional details, see "Part I – Item 1, Business – New and Emerging Environmental Regulations – United States and European Greenhouse Gas Emissions Regulations" above.

Additionally, the European Union has established aggressive CO_2 reduction targets of 40 percent by 2030, against a 1990 baseline, and full carbon neutrality by 2050. As part of the European Green Deal the Commission proposed in September 2020 to raise the 2030 reduction target to at least 55 percent compared to 1990. The new target has since been endorsed by the European Parliament. An emission trading system (ETS) was established to encourage compliance with set emissions reduction targets. These aggressive targets require drastic measures within the steel industry to comply. The transition to EAF technology, as well as incremental gains in energy reduction, use of renewable energy, hydrogen-based steelmaking and continued asset and process improvements are expected to reduce our GHG footprint. However, the development of breakthrough technologies is likely required to continue the path of low to no carbon footprint in the steel industry. Implementation of new technologies will most likely require significant amounts of capital and an abundant source of low-cost hydrogen and/or green power, most likely leading to an increase in the cost of future steelmaking. In addition, the cost of emission allowances is forecast to increase, along with the number of allowances decreasing in the next several years. The price of CO_2 emission allowances was 71 euro per metric ton as of December 31, 2024, and forecasts call for potential prices exceeding 100 euro per metric ton in future years.

Environmental, social and governance matters may impact our business and reputation.

In addition to the changing rules and regulations related to environmental, social and governance (ESG) matters imposed by governmental and self-regulatory organizations such as the SEC and the New York Stock Exchange, a variety of third-party organizations, institutional investors and customers evaluate the performance of companies on ESG topics, and the results of these assessments are widely publicized. These changing rules, regulations and stakeholder expectations have resulted in, and are likely to continue to result in, increased general and administrative expenses and increased management time and attention spent complying with or meeting such regulations and expectations. Reduced access to or increased cost of capital may occur as financial institutions and investors increase expectations related to ESG matters.

Developing and acting on initiatives within the scope of ESG, and collecting, measuring and reporting ESG-related information and metrics can be costly, difficult and time consuming and is subject to evolving reporting standards. We may also communicate certain initiatives and goals, regarding environmental matters, diversity, social investments and other ESG-related matters, in our SEC filings or in other public disclosures. These initiatives and goals within the scope of ESG could be difficult and expensive to implement, the technologies needed to implement them may not be cost effective and may not advance at a sufficient pace, and we could be criticized for the accuracy, adequacy or completeness of the disclosure. Furthermore, statements about our ESG-related initiatives and goals, and progress against those goals, may be based on standards for measuring progress that are still developing, internal controls and processes that continue to evolve and assumptions that are subject to change in the future. In addition, we could be criticized for the scope or nature of such initiatives or goals, or for any revisions to these goals. If our ESG-related data, processes and reporting are incomplete or inaccurate, or if we fail to achieve progress with respect to our goals, including our previously announced commitments to reduce greenhouse gas emissions, within the scope of ESG on a timely basis, or at all, our reputation, business, financial performance and growth could be adversely affected. In addition, in recent years “anti-ESG” sentiment has gained momentum across the U.S., with several states and Congress having proposed or enacted “anti-ESG” policies, legislation, or initiatives or issued related legal opinions, and the President having recently issued an executive order opposing diversity equity and inclusion (“DEI”) initiatives in the private sector. Such anti-ESG and anti-DEI-related policies, legislation, initiatives, litigation, legal opinions, and scrutiny could result in U. S. Steel facing additional compliance obligations, becoming the subject of investigations and enforcement actions, or sustaining reputational harm.

New and changing data privacy laws and cross-border transfer requirements could have a negative impact on our business and operations.

Our business depends on the processing and transfer of data between our affiliated entities, to and from our business partners, and with third-party service providers, and of our employees, which may be subject to data privacy laws and/or cross-border transfer restrictions. In North America and Europe, new legislation and changes to the requirements or applicability of existing laws, as well as evolving standards and judicial and regulatory interpretations of such laws, may impact U. S. Steel’s ability to effectively process and transfer data both within the United States and across borders in support of our business operations and/or keep pace with specific requirements regarding processing and safeguarding personal information. While U. S. Steel takes steps to comply with these legal requirements, non-compliance could lead to possible administrative, civil, or criminal liability, as well as reputational harm to the Company and its employees. The costs of compliance with privacy laws and the potential for fines and penalties in the event of a breach may have a negative impact on our business and operations.

Item 1B. UNRESOLVED STAFF COMMENTS

None.

Item 1C. CYBERSECURITY

Risk Management and Strategy

U. S. Steel maintains robust processes for identifying, assessing, and managing material risks from cybersecurity threats. U. S. Steel’s cybersecurity program is based on the National Institute of Standards and Technology (“NIST”) Cybersecurity Framework, and the risk of cybersecurity threats is integrated into the Company’s Enterprise Risk Management (“ERM”) program, led by the Chief Risk Officer. The ERM program includes an annual risk prioritization process to identify key enterprise risks. Each key risk is assigned risk owners to establish action plans and implement risk mitigation strategies. The cybersecurity threat risk action plan is managed at the enterprise level by the Senior Vice President – Business Development and President, USSE, the Vice President & Chief Information Officer (the “CIO”) and the Chief Information Security Officer (the “CISO”). Each quarter, the risk owners review and update the cybersecurity threat risk action plan to provide the status on specific risk mitigation actions and to identify new threats. U. S. Steel works closely with its internal and external auditors to assess, plan for, prevent and mitigate cybersecurity risks.

The Company maintains a Cybersecurity Incident Response Plan (CSIRP), which establishes an organizational framework and guidelines intended to facilitate an effective response and handling of cybersecurity incidents that could jeopardize the availability, integrity, or confidentiality of U. S. Steel’s assets. The CSIRP outlines roles and responsibilities, criteria for measuring the severity of a cybersecurity incident, and an escalation framework. The CSIRP also addresses senior management responsibility with respect to disclosure determinations related to a cybersecurity incident and provides for Audit Committee and Board briefings as appropriate. Along with the CSIRP, management sustains numerous programs and processes to stay

informed about and monitor the prevention, detection, mitigation and remediation of cybersecurity incidents. The list of cybersecurity programs and processes described below is not meant to be exhaustive, but to provide examples of such programs and processes.

The Company engages with third party cybersecurity specialists to provide an independent assessment of U. S. Steel's cybersecurity programs and to prepare a 5-year plan to maintain compliance and operational excellence. Management periodically reviews the 5-year plan and modifies it in response to changes in the threat landscape and otherwise as needed. Management employs in-depth defense mechanisms throughout the enterprise, including, but not limited to, employee training, vulnerability management, multi-factor authentication, cybersecurity insurance and table top exercises to mitigate and/or prevent cybersecurity incidents. The Company conducts security assessments of certain third party providers before and periodically throughout their engagement and has established procedures intended to mitigate risks related to security incidents originating from third parties. For example, management assesses the cybersecurity proficiency of potential third party cloud suppliers before utilizing their services. The assessment identifies cybersecurity-related risks and makes recommendations to enhance the security of new cloud computing services.

A cybersecurity incident may be detected in a number of ways, including, but not limited to, through automated reporting mechanisms, network and system indicators, intrusion detection systems, internal investigations, employee reports, law enforcement reports, or other third party notification. To oversee and identify cybersecurity threat risks on a day-to-day basis, including from third party service providers, the Company maintains a security operations center with round-the-clock monitoring, and the CIO receives regular reports on industry activity.

Upon receiving notification of a cybersecurity incident, the cybersecurity operations team acts to isolate and contain the threat. The CISO, along with the Chief Safety & Security Officer, will consult and determine the incident severity level, which determines whether the incident should be escalated. Critical and high severity incidents must be reported to the General Counsel. The Company may engage third party experts for assistance with crisis management, including forensic investigations, ransom negotiation, or crisis communication. During this process, the cybersecurity operations team will take steps to preserve evidence as soon as possible, including, but not limited to, memory dumps, log preservation and forensic hard drive collection. In addition, the General Counsel, in consultation with the CISO and others as necessary and appropriate, will promptly evaluate whether the incident requires legal notifications or disclosure, including whether the incident requires disclosure under the U. S. securities laws.

Following a cybersecurity incident, the General Counsel will direct the development of documentation regarding lessons learned in the response, including evaluation of preparedness capability, to continuously strengthen the cybersecurity posture of the Corporation.

Management has not identified risks from cybersecurity threats, including as a result of any previous cybersecurity incidents, that have materially affected or are reasonably likely to materially affect U. S. Steel, including its business strategy, results of operations or financial condition. See "Item 1A. Risk Factors, *A failure of our information technology infrastructure and cybersecurity threats may adversely affect our business operations.*" above for more information. While we continually work to safeguard the information systems we use, and the proprietary, confidential and personal information residing therein, and mitigate potential risks, there can be no assurance that such actions will be sufficient to prevent cybersecurity incidents or mitigate all potential risks to such systems, networks and data or those of our third party providers.

Governance

The Board of Directors is responsible for overseeing the assessment and management of enterprise-level risks that may impact U. S. Steel. The Audit Committee has primary responsibility for overseeing risk management, including oversight of risks from cybersecurity threats. Management, including the CIO and CISO, reports on cybersecurity matters regularly to the Board, primarily through the Audit Committee, including an annual report regarding specific risks and mitigation efforts within U. S. Steel and a 5-year cybersecurity threat assessment conducted by third party experts. Management provides benchmarking information and updates on key operational and compliance metrics to the Board. In addition, cybersecurity training is provided to the full Board of Directors, including training by third party experts, to educate directors on the current cybersecurity threat environment and measures companies can take to mitigate risk and impact of cyberattacks.

As described above, management is actively involved in assessing and managing U. S. Steel's material cybersecurity risks. The CIO and the CISO primarily lead these efforts. The CIO is responsible for the oversight of U. S. Steel's entire global IT operation, including the cybersecurity program, and holds a Bachelor of Science in operations research and industrial engineering and a Bachelor of Arts in economics from Cornell University, as well as a Master of Science in engineering and Master of Business Administration from Massachusetts Institute of Technology. The CIO's previous work experience includes holding the position of CIO at a $25 billion manufacturing conglomerate and a $7 billion energy company. The CISO reports directly to the CIO and has responsibility for leadership of U. S. Steel's global cybersecurity program. She holds a bachelor's degree in Information Systems Management, a master's degree in Internet Information Systems, and a doctorate in Instructional Management and Leadership. The CISO also completed the Chief Information Security Officer Certificate program at Carnegie Mellon University, where she now serves as a coach for the program, and holds a Certified Information Systems Security Professional (CISSP) certification.

She is an active member of the Greater Pittsburgh CISO Group and the Steel City OTSEC Information Sharing Group, and serves on the Editorial Review Board for two peer-reviewed journals (International Journal of Cyber Research and International Journal of Information and Communication Technology Education).

Item 2. PROPERTIES

See Item 1. Business, Facilities and Locations for listings of U. S. Steel's main properties, their locations and their products and services.

U. S. Steel and its predecessors have owned their properties for many years with no material adverse title claims asserted. In the case of Great Lakes Works, Granite City Works, the Midwest Plant and Keetac iron ore operations, U. S. Steel or its subsidiaries are the beneficiaries of bankruptcy laws and orders providing that properties are held free and clear of past liens and liabilities. In addition, U. S. Steel or its predecessors obtained title insurance, local counsel opinions or similar protections when significant properties were initially acquired or since acquisition.

At the Midwest Plant in Indiana, U. S. Steel has a supply agreement for various utility services with a company that owns a cogeneration facility located on U. S. Steel property. The Midwest Plant agreement expires in 2028.

U. S. Steel leases its headquarters office space in Pittsburgh, Pennsylvania.

For property, plant and equipment additions, including finance leases, see "Item 7. Management's Discussion and Analysis of Financial Condition and Results of Operations, Liquidity and Capital Resources" and Note 13 to the Consolidated Financial Statements.

Mining Properties

Summary Overview of Mining Operations

U. S. Steel operates two surface iron ore mining complexes in Minnesota consisting of the Minntac Mine and Pellet Plant and the Keetac Mine and Pellet Plant, which are wholly owned by the Company. As of December 31, 2024, U. S. Steel owns a minority interest in the iron ore mining assets of Hibbing Taconite Company.

The following table provides a summary of the net book value of the land and PP&E at the Minntac and Keetac mines as of December 31, 2024:

(in millions)	Gross Value	Accumulated Depreciation	Net Book Value
Minntac Mine and Pellet Plant			
Land	$ 35	$ —	$ 35
Other property, plant and equipment	1,780	1,359	421
Total	**$ 1,815**	**$ 1,359**	**$ 456**
Keetac Mine and Pellet Plant			
Land	$ 7	$ —	$ 7
Other property, plant and equipment	505	224	281
Total	**$ 512**	**$ 224**	**$ 288**

The following table provides a summary of our mineral production by mining complex for each reportable period:

Iron Ore Pellets	Production		
(Millions of short tons)	**2024**	**2023**	**2022**
Iron Ore Pellets			
Minntac Mine and Pellet Plant	**15.5**	15.5	15.1
Keetac Mine and Pellet Plant	**5.8**	5.5	5.9
Hibbing Taconite Company [(1)]	**0.9**	1.1	0.9
Total	**22.2**	22.1	21.9

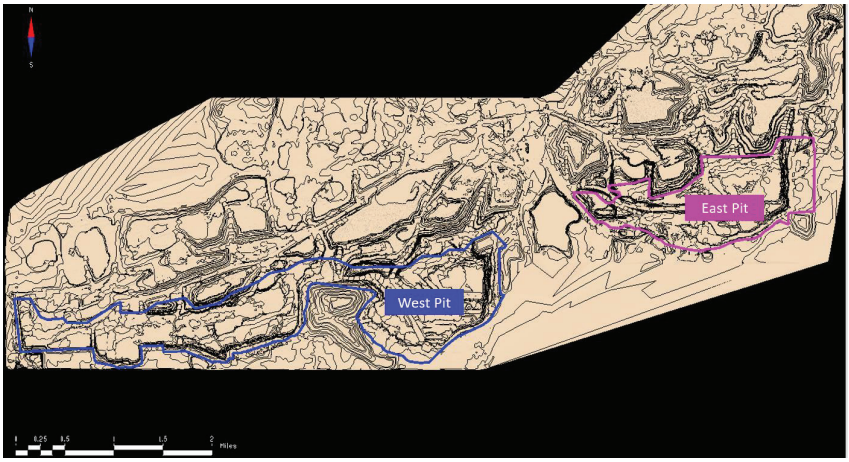
[(1)] Represents U. S. Steel's proportionate share of production as these investments are unconsolidated equity affiliates.

We engaged DRA Global and Barr Engineering Co. ("DRA") to provide feasibility studies and technical report summaries for our material mining operations at Minntac and Keetac in 2023. Indicated and inferred mineral resources and probable mineral reserve information for the Minntac and Keetac mines were updated based on those DRA estimations. Changes as a result of this study were due to improved resource and reserve estimation methodology. The majority shareholders of the Hibbing Taconite Company separately engaged qualified persons to perform the same procedures at the Hibbing Taconite Mine in 2024. Accordingly, the figures below for the Hibbing Taconite Mine were provided by the majority shareholders using the reports provided by the qualified persons. The tables showing resources and reserves by mining property were prepared using the results of the procedures performed by the qualified persons designated by each organization, which have no affiliation with or interest in our material mining properties.

Item 302 of Regulation S-K requires registrants to file the technical report summary as an exhibit to the Form 10-K filing when disclosing for the first time mineral reserves or resources or when there has been a material change in the mineral reserves or resources since the last technical report summary filed for the properties. The Company did not have material changes to its reserves or resources during 2024, nor is it disclosing mineral reserves or resources for the first time. Accordingly, the technical report summary is not filed as an exhibit with this Form 10-K.

Minntac Mine and Pellet Plant

The Minntac Mine and Pellet Plant is located in Mountain Iron, Minnesota and is wholly owned and operated by U. S. Steel. On April 30, 2020, the Company granted Stelco Inc. (Stelco), a subsidiary of Cleveland-Cliffs Inc., a purchase option to acquire a 25 percent interest in the Minntac mining operations. The option can be exercised at any time before January 31, 2027. For more information regarding the purchase option, please see Note 20 to the Consolidated Financial Statements. The Minntac Mine has 25,420 acres of surface rights. The surface mine is in the production stage whereby taconite iron ore is mined using the Truck-Shovel method. The mine is approximately 57 years old and has been operated by U. S. Steel since 1967. For discussions regarding encumbrances, violations, fines, etc. related to the Minntac Mine, see Item 3. Legal Proceedings.



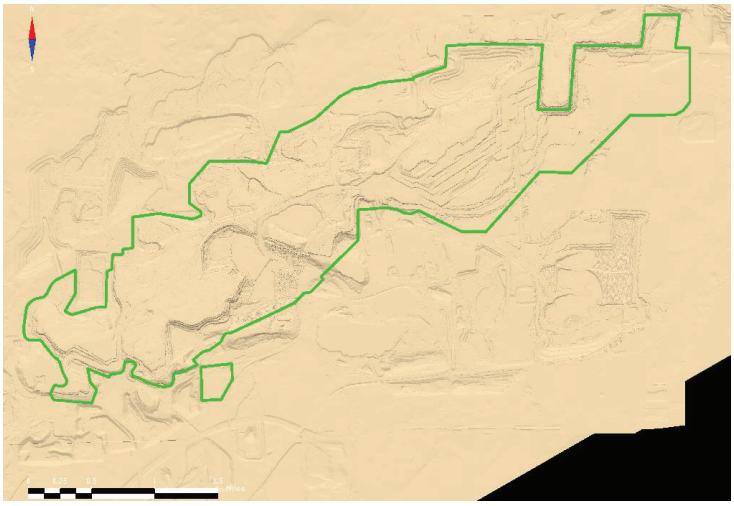
The following table provides details of our iron ore resources and reserves at Minntac as of December 31, 2024, and December 31, 2023. Resources below are stated exclusive of reserves.

Minntac Mine and Pellet Plant

	Amount			Grades/Qualities		Cut-off Grades		Metallurgical Recovery
(Millions of short tons)	Pellets 2024	Pellets 2023	Change (%)	MagFe%	Concentrate Silica %	Min MagFe %	Max Concentrate Silica %	Weight Recovery %
Measured mineral resources	—	—	— %	—	—	—	—	—
Indicated mineral resources	234.2	234.2	— %	18.19	5.82	14.00	9.99	26.98
Measured + indicated mineral resources	**234.2**	**234.2**	**— %**	**18.19**	**5.82**	**14.00**	**9.99**	**26.98**
Inferred mineral resources	96.7	96.7	— %	18.29	6.22	14.00	9.99	27.31
Proven mineral reserves	—	—	— %	—	—	—	—	—
Probable mineral reserves	265.0	280.5	(6)%	19.11	6.20	14.00	9.99	28.55
Total mineral reserves	**265.0**	**280.5**	**(6)%**	**19.11**	**6.20**	**14.00**	**9.99**	**28.55**

Keetac Mine and Pellet Plant

The Keetac Mine and Pellet Plant is located in Keewatin, Minnesota and is wholly owned and operated by U. S. Steel. The Keetac Mine has 18,020 acres of surface rights. The surface mine is in the production stage whereby taconite iron ore is mined using the Truck-Shovel method. The mine is approximately 57 years old and has been operated by U. S. Steel since 2003, when it was acquired as part of the Company's purchase of National Steel Corporation. For discussions regarding encumbrances, violations, fines, etc. related to the Keetac Mine, see Item 3. Legal Proceedings.



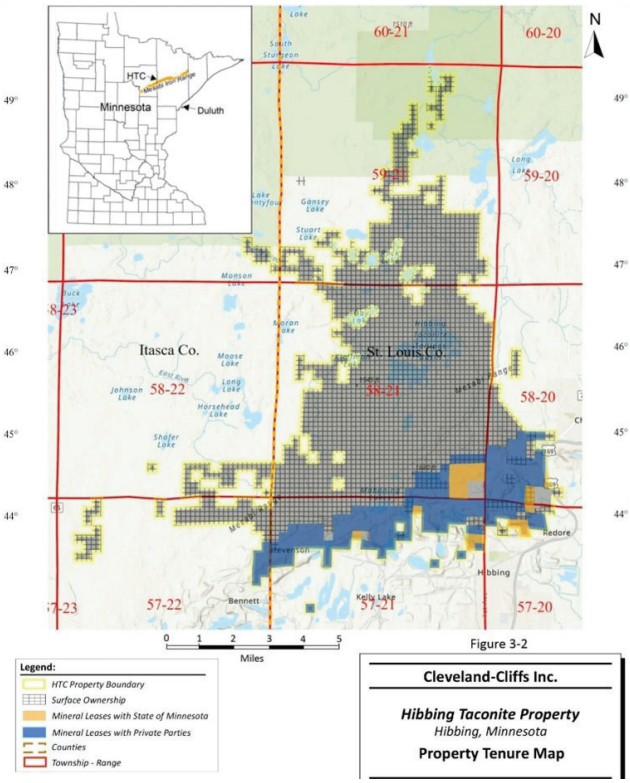
The following table provides details of our iron ore resources and reserves at Keetac as of December 31, 2024, and December 31, 2023. Resources below are stated exclusive of reserves.

Keetac Mine and Pellet Plant

	Amount					Grades/Qualities		Cut-off Grades		Metallurgical Recovery
(Millions of short tons)	DR Grade Pellets 2024	DR Grade Pellets 2023	Pellets 2024	Pellets 2023	Change (%)	MagFe%	Concentrate Silica %	Min MagFe %	Max Concentrate Silica %	Weight Recovery %
Measured mineral resources	—	—	—	—	— %	—	—	—	—	—
Indicated mineral resources	153.3	153.3	214.7	214.7	— %	18.88	3.45	14.00	9.99	27.31
Measured + indicated mineral resources	**153.3**	**153.3**	**214.7**	**214.7**	**— %**	**18.88**	**3.45**	**14.00**	**9.99**	**27.31**
Inferred mineral resources	108.9	108.9	163.2	163.2	— %	18.39	4.37	14.00	9.99	26.83
Proven mineral reserves	—	—	—	—	— %	—	—	—	—	—
Probable mineral reserves	125.9	126.4	175.3	180.6	(2)%	19.36	3.82	14.00	9.99	28.00
Total mineral reserves	**125.9**	**126.4**	**175.3**	**180.6**	**(2)%**	**19.36**	**3.82**	**14.00**	**9.99**	**28.00**

Hibbing Taconite Mine

U. S. Steel maintains a minority interest in the Hibbing Taconite Mine, which is majority-owned by Cleveland-Cliffs Inc. and located in Hibbing, Minnesota. The Hibbing Mine has 30,705 acres of surface rights, of which 1,150 acres are associated with mineral leases. The majority of the mineral rights are leased. The property is comprised of 7,417 acres of mineral leases expiring between 2025 and 2056. The taconite iron ore mine is currently in the production stage.



The following table provides details of our proportionate share of iron ore pellet resources and reserves at Hibbing as of December 31, 2024, and December 31, 2023. Resources below are stated exclusive of reserves.

Hibbing Taconite Company [1]

(Millions of short tons)	Amount			Grades/Qualities
	Pellets 2024	Pellets 2023	Change (%)	MagFe%
Measured mineral resources	0.4	0.4	— %	19.20
Indicated mineral resources	—	—	— %	18.70
Measured + indicated mineral resources	**0.4**	**0.4**	**— %**	**19.20**
Inferred mineral resources	—	—	— %	—
Proven mineral reserves	3.1	2.2	40.91 %	18.96
Probable mineral reserves	0.2	0.4	(50.00)%	18.96
Total mineral reserves	**3.3**	**2.6**	**26.92 %**	**18.96**

[1] Represents U. S. Steel's proportionate share of proven and probable reserves and production as these investments are unconsolidated equity affiliates.

Internal Controls

U. S. Steel estimates its iron ore resources and reserves using exploration drill holes, physical inspections, sampling, laboratory testing, 3-D computer models, economic pit analysis and fully-developed pit designs for its operating mines. Estimates for our share of unconsolidated equity affiliates are based upon information supplied by the joint ventures. Refer to sections 2 and 3 of the technical report summaries filed as Exhibit 96.1 to the 2023 Form 10-K for further details.

Item 3. LEGAL PROCEEDINGS

On January 6, 2025, the Company, NSC and Purchaser jointly filed lawsuits related to the Merger. A Petition for Review was filed in the United States Court of Appeals for the District of Columbia Circuit alleging that the President's order blocking the Merger was issued for purely political reasons, which are irrelevant to U.S. national security, challenging the decision by the President of the United States and the CFIUS process as violating the constitutional due process rights of the Company, NSC and Purchaser, as well as the applicable statute governing CFIUS and the Administrative Procedure Act, and as an exercise of power that exceeded the authority provided to the President of the United States under the applicable statute. The Petition names as respondents Joseph R. Biden, in his official capacity as President of the United States, CFIUS, Janet L. Yellen, in her official capacity as Secretary of the Treasury and Chairperson of CFIUS, and Merrick B. Garland, in his official capacity as United States Attorney General. The Petition asks the Court to set aside the unlawful CFIUS review process and the President's accompanying order, and to instruct CFIUS to conduct a new review of the Merger that is consistent with the petitioners' due process rights and its own statutory obligations. Also on January 6, 2025, the Company, NSC and Purchaser jointly filed a Complaint and motion for a preliminary injunction and for an expedited hearing in the United States District Court for the Western District of Pennsylvania against Cliffs, Cliffs' Chief Executive Officer Lourenco Goncalves, and the President of the USW, David McCall, for engaging in a coordinated series of anticompetitive and racketeering activities illegally designed to prevent any party other than Cliffs from acquiring the Company as part of an illegal campaign to monopolize critical domestic steel markets. This suit asserts antitrust claims under Sections 1 and 2 of the Sherman Act, violations of the Racketeer Influenced and Corrupt Organizations (RICO) Act, and tortious interference claims and seeks an injunction preventing Cliffs, Mr. Goncalves, and Mr. McCall from engaging in further collusive and anticompetitive behavior, and to impose substantial monetary damages for their conduct.

Following announcement of the Merger, the Company received eleven demand letters from putative stockholders (collectively, the "Demand Letters") alleging that the disclosures contained in the preliminary proxy statement, as amended, and/or the definitive proxy statement, in each case filed with the Securities and Exchange Commission in connection with a special meeting of stockholders to consider the Merger, were deficient and demanding that certain corrective disclosures be made. The Company believes that the Demand Letters are without merit; however solely in order to mitigate any risk of the Demand Letters delaying or otherwise adversely affecting the consummation of the Merger and to minimize any costs, risks, and uncertainties inherent in any potential litigation related thereto, and without admitting any liability or wrongdoing, voluntarily made supplemental disclosures on a Form 8-K.

General Litigation

On June 8, 2021, JSW Steel (USA) Inc. and JSW Steel USA Ohio, Inc. (collectively, JSW), U.S. based subsidiaries of Indian steelmaker JSW Steel, filed suit in the United States District Court for the Southern District of Texas against Nucor, U. S. Steel, AK Steel Holding Group and Cleveland-Cliffs Inc. (collectively, the JSW Defendants) alleging that the Defendants operated as a cartel and formed a conspiracy to boycott JSW from obtaining semi-finished steel slabs. JSW alleges that the JSW Defendants acted in violation of Section 1 of the Sherman Act and the Clayton Act (federal antitrust), and violation of the Texas Free Enterprise and Antitrust Act. JSW also alleges that the JSW Defendants formed a civil conspiracy in violation of Texas common law, and that the JSW Defendants tortiously interfered with JSW's business relationships. The basis for JSW's allegations relate to the JSW Defendants' participation in the DOC's Section 232 process, including the JSW Defendants' support of the enactment of the President's Section 232 proclamation, statements made by the JSW Defendants after the enactment of Section 232, and the JSW Defendants' participation in the Section 232 exclusion process. Plaintiffs seek monetary damages including $45 million for payment of Section 232 tariffs and unspecified amounts for financial penalties, termination fees and lost profits as well as other damages. U. S. Steel, along with the other JSW Defendants, filed a Motion to Dismiss the case on August 17, 2021. On February 17, 2022, the Court issued an opinion dismissing JSW's antitrust complaint with prejudice. JSW filed a timely notice of appeal with the United States Court of Appeals for the Fifth Circuit. The Fifth Circuit held oral argument on the appeal on February 6, 2023, and we are awaiting a ruling from the Court. The Company continues to vigorously defend the matter.

On December 24, 2018, U. S. Steel's Clairton Plant experienced a fire, affecting portions of the facility involved in desulfurization of the coke oven gas generated during the coking process. With the desulfurization process out of operation as a result of the fire, U. S. Steel was not able to certify compliance with Clairton Plant's Title V permit levels for sulfur emissions. U. S. Steel promptly notified ACHD (Allegheny County Health Department), which has regulatory jurisdiction for the Title V permit, and updated the ACHD regularly on efforts to mitigate any potential environmental impacts until the desulfurization process was returned to normal operations. Of the approximately 2,400 hours between the date of the fire and April 4, 2019, when the Company resumed desulfurization, there were ten intermittent hours where average SO_2 emissions exceeded the hourly NAAQS for SO_2 at the Allegheny County regional air quality monitors located in Liberty and North Braddock boroughs, which are near U. S. Steel's Mon Valley Works facilities. On April 29, 2019, PennEnvironment and Clean Air Council, both environmental, non-governmental organizations, filed a Complaint in Federal Court in the Western District of Pennsylvania. The ACHD was subsequently granted intervenor status. The parties entered into a Consent Decree that was authorized by the U.S. Department of Justice and U.S. EPA and subsequently approved by the Court on March 26, 2024 that resolved all claims in the suit. The Consent Decree required the Company to undertake operational improvements at Clairton Works, permanently idle Coke Battery #15, pay a $500,000 penalty to the ACHD, fund community-beneficial projects throughout the Mon Valley over a period of five years through contributions which total $4.5 million and pay counsel fees in the amount of $3.0 million. The Consent Decree also established a new H2S Coke Oven Gas standard for Clairton Works. Separately, a class action has been filed in the Court of Common Pleas of Allegheny County on behalf of approximately 123,000 persons who claim that the impacts from the fire created

a nuisance and seek damages for loss of use and enjoyment of properties. That action has been certified as a class action and the Company continues to vigorously defend against it.

Asbestos Litigation

See Note 26 to our Consolidated Financial Statements, Contingencies and Commitments for a description of our asbestos litigation.

ENVIRONMENTAL PROCEEDINGS

No discussion of compliance with sulfate standard. No mention of requirement for compliance with sulfate limit in 2011 permit.

The following is a summary of the proceedings of U. S. Steel that were pending or contemplated as of December 31, 2024, under federal and state environmental laws, and which U. S. Steel reasonably believes may result in monetary sanctions of at least $1 million (the threshold chosen by U. S. Steel as permitted by Item 103 of Regulation S-K). Information about specific sites where U. S. Steel is or has been engaged in significant clean up or remediation activities is also summarized below. Except as described herein, it is not possible to accurately predict the ultimate outcome of these matters.

CERCLA Remediation Sites

Claims under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) have been raised with respect to the cleanup of various waste disposal and other sites. Under CERCLA, potentially responsible parties (each, a PRP) for a site include current owners and operators, past owners and operators at the time of disposal, persons who arranged for disposal of a hazardous substance at a site and persons who transported a hazardous substance to a site. CERCLA imposes strict and joint and several liabilities. Because of various factors, including the ambiguity of the regulations, the difficulty of identifying the responsible parties for any particular site, the complexity of determining the relative liability among them, the uncertainty as to the most desirable remediation techniques, and the amount of damages and cleanup costs and the time period during which such costs may be incurred, we are unable to reasonably estimate U. S. Steel's ultimate liabilities under CERCLA.

As of December 31, 2024, U. S. Steel has received information requests or been identified as a PRP at a total of four CERCLA sites, three of which have liabilities that have not been resolved. Based on currently available information, which is in many cases preliminary and incomplete, management believes that U. S. Steel's liability for CERCLA cleanup and remediation costs at one of the other sites will be over $5 million as described below.

Duluth Works

The former U. S. Steel Duluth Works site was placed on the National Priorities List under CERCLA in 1983 and on the State of Minnesota's Superfund list in 1984. Liability for environmental remediation at the site is governed by a Response Order by Consent executed with the MPCA in 1985 and a Record of Decision signed by MPCA in 1989. U. S. Steel has partnered with the Great Lakes National Program Office (GLNPO) of the U.S. EPA Region 5 to address contaminated sediments in the St. Louis River Estuary and several other operable units that could impact the estuary if not addressed. An amendment to the Project Agreement between U. S. Steel and GLNPO was executed during the second quarter of 2018 to recognize the initial costs associated with implementing the first two phases of the proposed remedial plan at the site.

Remediation contracts were issued by both U. S. Steel and GLNPO for the first phase of the remedial work at the site during the fourth quarter of 2020. U. S. Steel and GLNPO have completed the second phase of work at the site which extended through early 2022. The final phase of the remedial design has been defined and another amendment to the Project Agreement between U. S. Steel and GLNPO was executed in December 2021. Execution of this final phase is in progress and is expected to extend through mid-2025 for habitat restoration. U. S. Steel's portion of additional design, oversight costs, and implementation of all three phases of the preferred remedial alternative on the upland property and Estuary are currently estimated as of December 31, 2024, at approximately $6 million.

Resource Conservation Recovery Act and Other Remediation Sites

U. S. Steel may be liable for remediation costs under other environmental statutes, both federal and state, or where private parties are seeking to impose liability on U. S. Steel for remediation costs through discussions or litigation. There are nine such sites where remediation is being sought involving amounts in excess of $1 million. Based on currently available information, which is in many cases preliminary and incomplete, management believes that liability for cleanup and remediation costs in connection with four sites may involve remediation costs between $1 million and $5 million per site and five sites are estimated to, or could have, costs for remediation, investigation, restoration or compensation in excess of $5 million per site.

For more information on the status of remediation activities at U. S. Steel's significant sites, see the discussions below.

Gary Works

On October 23, 1998, the U.S. EPA issued a final Administrative Order on Consent (Order) addressing Corrective Action for Solid Waste Management Units (SWMU) throughout Gary Works. This Order requires U. S. Steel to perform an RCRA Facility

Investigation, a Corrective Measures Study and Corrective Measure Implementation. Evaluations are underway at six groundwater areas on the east side of the facility. A remedial groundwater treatment system has been operating at one of the six areas since 2021. An Interim Stabilization Measure work plan was approved by the U.S. EPA for a second area where installation and start-up of the remedial system was completed in 2023. Until the remaining Phase I work and Phase II field investigations are completed, it is not possible to assess what additional expenditures will be necessary for Corrective Action projects at Gary Works. In total, the accrued liability for Corrective Action projects is approximately $23 million as of December 31, 2024, based on our current estimate of known remaining costs.

Geneva Works

At U. S. Steel's former Geneva Works, liability for environmental remediation, including the closure of three hazardous waste impoundments and facility-wide corrective action, has been allocated between U. S. Steel and the current property owner pursuant to an agreement and a permit issued by the Utah Department of Environmental Quality (UDEQ). Having completed the investigation on a majority of the remaining areas identified in the permit, U. S. Steel had determined the most effective means to address the remaining impacted material was to manage those materials in a previously approved on-site Corrective Action Management Unit (CAMU). U. S. Steel awarded a contract for the implementation of the CAMU project during the fourth quarter of 2018. Construction, waste stabilization and placement, along with closure of the CAMU, were substantially completed in the fourth quarter of 2020. Work at the site is now focused on addressing groundwater impacts in discrete areas. U. S. Steel has an accrued liability of approximately $17 million as of December 31, 2024 for our estimated share of the remaining costs of remediation at the site.

USS-UPI LLC

In February 2020, U. S. Steel purchased the remaining 50 percent interest in USS-POSCO Industries, a former joint venture that is located in Pittsburg, California between subsidiaries of U. S. Steel and POSCO, now known as USS-UPI, LLC. Prior to formation of the joint venture, UPI's facilities were previously owned and operated solely by U. S. Steel, which assumed responsibility for the existing environmental conditions. U. S. Steel continues to monitor the impacts of the remedial plan implemented in 2016 to address groundwater impacts from trichloroethylene at SWMU 4. Work began in early 2024 to complete removal of hazardous materials and decommission the northwest portion of Building A (SWMU 4.1) and the 54" and 66" Pickle Lines. Additionally, evaluations continue for the SWMUs, known as the Northern Boundary Group, and it is likely that corrective measures will be required, but it is not possible at this time to define a scope or estimate costs for what may be required by the California Department of Toxic Substances Control. As such, there has been no material change in the status of the project during the year ended December 31, 2024. As of December 31, 2024, approximately $6 million has been accrued for ongoing remediation, environmental studies, investigations, and remedial monitoring. Significant additional costs associated with this site are possible and are referenced in Note 26 to the Consolidated Financial Statements "Contingencies and Commitments - Environmental Matters - Remediation Projects - Projects with Ongoing Study and Scope Development."

Fairfield Works

A consent decree was signed by U. S. Steel, the U.S. EPA and the U.S. Department of Justice and filed with the United States District Court for the Northern District of Alabama (United States of America v. USX Corporation) in December 1997. In accordance with the consent decree, U. S. Steel initiated a RCRA corrective action program at the Fairfield Works facility. The Alabama Department of Environmental Management (ADEM), with the approval of the U.S. EPA, assumed primary responsibility for regulation and oversight of the RCRA corrective action program at Fairfield Works. Corrective Measure Implementation Plans (CMIPs) have been submitted to and approved by ADEM for the last two areas on site where impacts to soil and sediments are required to be addressed. Plans are being finalized for contracting the work required under the CMIPs. U. S. Steel has an accrued liability of approximately $8 million at December 31, 2024 for the estimated remaining costs of remediation at the site.

South Works

On August 29, 2017, U. S. Steel was notified by the U.S. Coast Guard of a sheen on the water in the North Vessel Slip at our former South Works in Chicago, Illinois. U. S. Steel has been working with the Illinois Environmental Protection Agency (IEPA) under their voluntary Site Remediation Program since 1993 to evaluate the condition of the property including the North Vessel Slip. The result of this cooperative effort has been the issuance of a series of "No Further Remediation" (NFR) notices to U. S. Steel including one specific to the North Vessel Slip. Following the discovery of the sheen, U. S. Steel notified the IEPA of the changed condition and has since been working closely with the IEPA and the U.S. EPA to determine the source and extent of the sheen as well as options to address the issue. U. S. Steel has also filed a claim against an existing insurance policy at the site and is working closely with the insurers to address the issue. U. S. Steel has an accrued liability of approximately $1 million as of December 31, 2024. Significant additional costs associated with this site are possible and are referenced in Note 26 to the Consolidated Financial Statements "Contingencies and Commitments - Environmental Matters - Remediation Projects - Projects with Ongoing Study and Scope Development."

Air Related Matters

Granite City Works

In October 2015, Granite City Works received a Notice of Violation (NOV) from the IEPA alleging that U. S. Steel violated the emission limits for nitrogen oxides (NOx) and volatile organic compounds from the Basic Oxygen Furnace Electrostatic Precipitator Stack. In addition, the IEPA alleges that U. S. Steel exceeded its natural gas usage limit at its CoGeneration Boiler. U. S. Steel continues to negotiate resolution of the NOV with the IEPA.

Although discussions with the IEPA regarding the foregoing alleged violations are ongoing and the resolution of these matters is uncertain at this time, it is not anticipated that the result of those discussions will be material to U. S. Steel.

Minnesota Ore Operations

On February 6, 2013, the U.S. EPA published a Federal Implementation Plan (FIP) that applies to taconite facilities in Minnesota. The FIP establishes and requires emission limits and the use of low NOx reduction technology on indurating furnaces as Best Available Retrofit Technology (BART). While U. S. Steel installed low NOx burners on three furnaces at Minntac and is currently obligated to install low NOx burners on the two other furnaces at Minntac pursuant to existing agreements and permits, the rule would require the installation of a low NOx burner on the one furnace at Keetac for which U. S. Steel did not have an otherwise existing obligation. U. S. Steel estimates expenditures associated with the installation of low NOx burners of as much as $25 million to $30 million. In 2013, U. S. Steel filed a petition for administrative reconsideration to the U.S. EPA and a petition for judicial review of the 2013 FIP and denial of the Minnesota state implementation plan (SIP) to the Eighth Circuit. In April 2016, the U.S. EPA promulgated a revised FIP with the same substantive requirements for U. S. Steel. In June 2016, U. S. Steel filed a petition for administrative reconsideration of the 2016 FIP to the U.S. EPA and a petition for judicial review of the 2016 FIP before the Eighth Circuit Court of Appeals. While the proceedings regarding the petition for judicial review of the 2013 FIP remained stayed, oral arguments regarding the petition for judicial review of the 2016 FIP were heard by the Eighth Circuit Court of Appeals on November 15, 2017. Thus, both petitions for judicial review remain with the Eighth Circuit. On December 4, 2017, the U.S. EPA published a notification in the Federal Register in which the U.S. EPA denied U. S. Steel's administrative petitions for reconsideration and stay of the 2013 FIP and 2016 FIP. On February 1, 2018, U. S. Steel filed a petition for judicial review of the U.S. EPA's denial of the administrative petitions for reconsideration to the Eighth Circuit Court of Appeals. The U.S. EPA and U. S. Steel reached a settlement regarding the five indurating lines at Minntac. After proposing a revised FIP and responding to public comments, on March 2, 2021, the U.S. EPA promulgated a final revised FIP incorporating the conditions and limits for Minntac to which the parties agreed. U. S. Steel and the U.S. EPA reached a tentative settlement agreement to revise BART limits for Keetac. The proposed settlement agreement was subject to a 30-day public comment period as provided in the April 23, 2024 Federal Register. Per the terms of the proposed settlement agreement, the U.S. EPA will sign a proposed rule to revise the FIP consistent with the settlement agreement by April 23, 2025.

Mon Valley Works

On March 7, 2022, the Company received an enforcement order from the ACHD that includes a civil penalty demand for $1.8 million. In the Order, the ACHD alleges that the Company's Clairton plant is solely and entirely culpable for 153 alleged exceedances of the Pennsylvania hydrogen sulfide ambient air standard that are reported to have occurred during January 1, 2020 through March 1, 2022. On April 5, 2022, the Company appealed the Order and is vigorously defending the matter. On December 29, 2023, the Company received an enforcement order from the ACHD that includes a civil penalty demand for $2.2 million. In the Order, the ACHD alleges that the Company's Clairton plant was the cause for 159 alleged exceedances of the Pennsylvania hydrogen sulfide ambient air standard that are reported to have occurred from March 2, 2022 through November 30, 2023. The Company appealed the Order and consolidated the case with the Order from March 7, 2022. On September 27, 2024, the Company filed a Motion for Summary Judgment which is currently pending. Due to the ACHD not currently having a Hearing Officer, this matter is stayed.

On March 24, 2022, the Company received an enforcement order from the ACHD that includes a civil penalty demand for $4.6 million for alleged air permit violations occurring between January 1, 2020 through March 15, 2022 regarding the Company's Clairton plant's coke oven pushing emission control systems. The Company has appealed the Order. On February 26, 2024, the Company received an enforcement order from the ACHD that includes a civil penalty demand for $2.0 million for alleged air permit violations occurring from March 16, 2022 through December 31, 2023 regarding the Company's Clairton plant's coke oven pushing emission control systems. The Company has appealed the Order. The appeals of the 2022 and 2024 Orders have been consolidated. Due to the ACHD not currently having a Hearing Officer, this matter is stayed.

Item 4. MINE SAFETY DISCLOSURE

The information concerning mine safety violations and other regulatory matters required by Section 1503(a) of the Dodd-Frank Wall Street Reform and Consumer Protection Act and Item 104 of Regulation S-K is included in Exhibit 95 to this Form 10-K.

INFORMATION ABOUT OUR EXECUTIVE OFFICERS

The executive officers of U. S. Steel and their ages as of January 30, 2025, are as follows:

Name	Age	Title	Executive Officer Since
Daniel R. Brown	52	Senior Vice President - Advanced Technology Steelmaking & Chief Operating Officer, Big River Steel	February 1, 2022
James E. Bruno	59	Senior Vice President - Business Development and President, USSE	December 1, 2014
Scott D. Buckiso	57	Senior Vice President and Chief Manufacturing Officer North American Flat-Rolled	May 31, 2015
David B. Burritt	69	President & Chief Executive Officer	September 1, 2013
Jessica T. Graziano	51	Senior Vice President & Chief Financial Officer	August 8, 2022
Manpreet S. Grewal	45	Vice President, Controller & Chief Accounting Officer	March 30, 2020
Duane D. Holloway	52	Senior Vice President, General Counsel and Chief Ethics & Compliance Officer	April 16, 2018

Messrs. Brown, Bruno, Buckiso, Burritt, and Holloway have held responsible management or professional positions with U. S. Steel or its subsidiaries for more than the past five years. Prior to joining U. S. Steel in 2022, Ms. Graziano spent eight years with United Rentals, Inc., culminating in her position as Executive Vice President and Chief Financial Officer. Prior to her work with United Rentals, Ms. Graziano spent five years at Revlon, Inc. where she advanced through positions of increasing responsibility culminating in her being named Senior Vice President, Chief Accounting Officer and Corporate Controller. Prior to joining U. S. Steel in 2020, Mr. Grewal served as vice president, business finance, controller and chief accounting officer at Covanta since February 2017. Prior to Covanta, Mr. Grewal spent fourteen years at Johnson Controls Incorporated (formerly Tyco International) in increasingly responsible roles, including internal audit, accounting, controllership, and financial planning and analysis.

PART II

Item 5. MARKET FOR REGISTRANT'S COMMON EQUITY, RELATED STOCKHOLDER MATTERS AND ISSUER PURCHASES OF EQUITY SECURITIES

Common Stock Information

The principal market on which United States Steel Corporation (U. S. Steel) common stock is traded is the New York Stock Exchange, where the common stock trades under trading symbol "X". U. S. Steel common stock is also traded on the Chicago Stock Exchange under the symbol "X".

As of January 27, 2025, there were 9,505 registered holders of U. S. Steel common stock.

The Board of Directors currently intends to declare and pay dividends on shares of U. S. Steel common stock based on the financial condition and results of operations of U. S. Steel out of legally available funds and in accordance with the requirements set forth by applicable law. Quarterly dividends were declared by U. S. Steel in 2024 and 2023 in the amount of five cents per share.

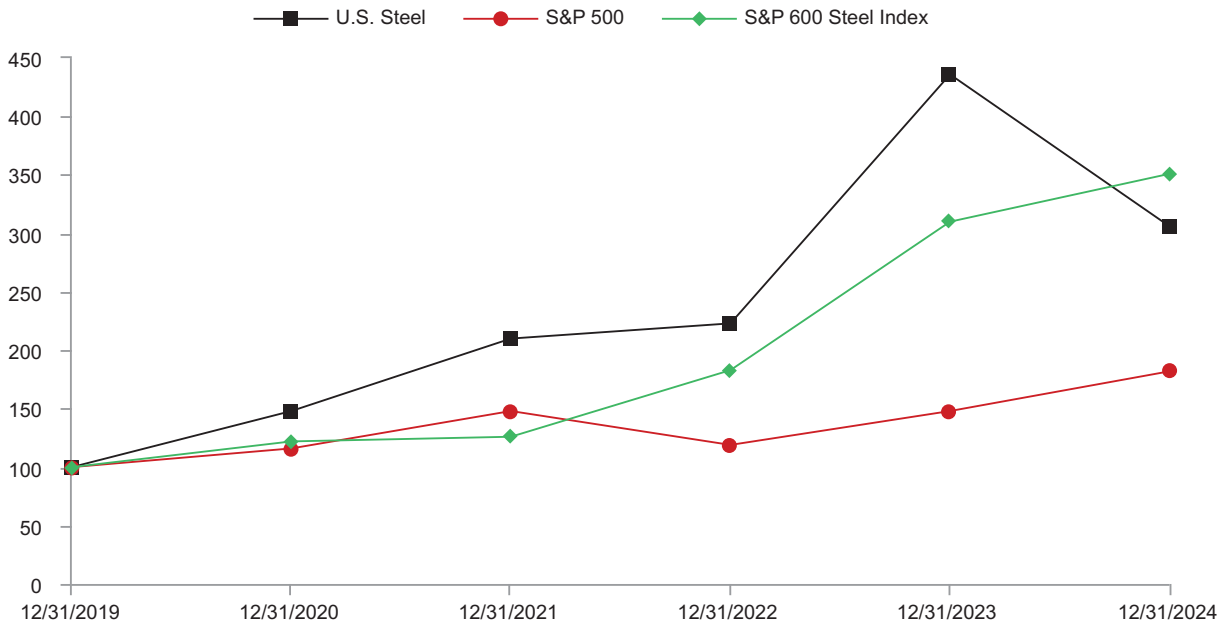
Purchases of Equity Securities by the Issuer and the Affiliated Purchasers

There were no share repurchases in fiscal 2024.

Stockholder Return Performance

The graph below assumes $100 invested on December 31, 2019, and compares the yearly change in cumulative total stockholder return of our common stock with the cumulative total return of the Standard & Poor's (S&P) 500 Stock Index and the S&P 600 Steel Index, and assumes that all dividends were reinvested. U. S. Steel is not included in either the S&P 500 Stock Index or S&P 600 Steel Index as to provide a clearer comparison to the selected indices.

Comparison of Cumulative Total Return
on $100 Invested in U. S. Steel Stock on December 31, 2019
vs
S&P 500 and S&P 600 Steel Index



For information on securities authorized for issuance under our equity compensation plans, see "Item 12. Security Ownership of Certain Beneficial Owners and Management and Related Stockholder Matters."

Unregistered Sales of Equity Securities

U. S. Steel had no sales of unregistered equity securities during the period covered by this report.

Item 6. RESERVED

Item 7. MANAGEMENT'S DISCUSSION AND ANALYSIS OF FINANCIAL CONDITION AND RESULTS OF OPERATIONS

The following discussion should be read in conjunction with the Consolidated Financial Statements and the related Notes that appear elsewhere in this document. Please refer to Item 7 of our 2023 Form 10-K for further discussion and analysis of our 2022 financial condition and results of operations.

Overview

Business Update

As previously disclosed, on December 18, 2023, the Company entered into the Merger Agreement by and among the Company, Purchaser, Merger Sub, and solely as provided in Section 9.13 therein, NSC. Pursuant to the Merger Agreement, and upon the

terms and subject to the conditions described therein, Merger Sub will merge with and into the Company, with the Company continuing as the surviving corporation and a wholly owned subsidiary of Purchaser (the "Merger").

The parties have substantially complied with the Second Requests issued on April 3, 2024 and the statutory waiting period under the Hart-Scott-Rodino Antitrust Improvements Act of 1976 has now expired. In addition, all required regulatory approvals outside of the United States related to the Merger have been received. The closing conditions to the Merger include clearance by the Committee on Foreign Investment in the United States ("CFIUS") under the Defense Production Act of 1950, as amended. Following referral of the transaction by CFIUS to the President of the United States, on January 3, 2025, the President of the United States issued an order prohibiting the Merger and requiring the parties to abandon the Merger Agreement within thirty days. On January 10, 2025, CFIUS granted an extension of that deadline to June 18, 2025.

On January 6, 2025, the Company, NSC and Purchaser jointly filed a lawsuit in the United States Court of Appeals for the District of Columbia Circuit alleging that the President's order blocking the Merger was issued for purely political reasons, which are irrelevant to U.S. national security, challenging the decision by the President of the United States and the CFIUS process as violating the constitutional due process rights of the Company, NSC and Purchaser, as well as the applicable statute governing CFIUS and the Administrative Procedure Act, and as an exercise of power that exceeded the authority provided to the President of the United States under the applicable statute. On the same day, the Company, NSC and Purchaser also filed a lawsuit in the United States District Court for the Western District of Pennsylvania against Cleveland-Cliffs Inc. ("Cliffs"), Cliffs' Chief Executive Officer Lourenco Goncalves, and the President of the United Steel, Paper and Forestry, Rubber, Manufacturing, Energy, Allied Industrial and Service Workers International Union (the "USW"), David McCall, for engaging in a coordinated series of anticompetitive and racketeering activities illegally designed to prevent any party other than Cliffs from acquiring the Company as part of an illegal campaign to monopolize critical domestic steel markets.

Segments Update

In 2024, the Company continued to execute on its strategy by investing in and completing its key strategic projects. U. S. Steel's results in 2024 decreased compared to the previous year for the four reportable segments due to the following factors:

- **North American Flat-Rolled:** Flat-Rolled results decreased primarily due to lower sales volume across most products and lower sales price across most products.

- **Mini Mill:** Mini Mill results decreased primarily due to lower sales volume across most products and lower sales price across all products.

- **U. S. Steel Europe:** USSE results decreased primarily due to lower sales volume across most products and lower sales price across all products.

- **Tubular:** Tubular results decreased primarily due to lower sales price.

Fluctuations in the market price of raw materials and other inflationary impacts have affected the results of each of our reportable segments, and fluctuations going-forward are reasonably likely to have a material impact on future results. We anticipate inflation related headwinds for certain raw materials and other costs, and sales prices and sales volumes may continue to have an adverse effect on our results.

Critical Accounting Estimates

Management's discussion and analysis of U. S. Steel's financial condition and results of operations is based upon U. S. Steel's financial statements, which have been prepared in accordance with accounting standards generally accepted in the United States (U.S. GAAP). The preparation of these financial statements requires management to make estimates and assumptions that affect the reported amounts of assets and liabilities, the disclosure of contingent assets and liabilities at year-end and the reported amount of revenues and expenses during the year. Management regularly evaluates these estimates, including those related to employee benefits liabilities and assets held in trust relating to such liabilities; the carrying value of property, plant and equipment; intangible assets; valuation allowances for receivables, inventories and deferred income tax assets; liabilities for deferred income taxes; potential tax deficiencies; environmental obligations; potential litigation claims and settlements and put and call option assets and liabilities. Management's estimates are based on historical experience, current business and market conditions, and various other assumptions that are believed to be reasonable under the circumstances, the results of which form the basis for making judgments about the carrying values of assets and liabilities that are not readily apparent from other sources. Actual results may differ materially from current expectations under different assumptions or conditions.

Management believes that the following are the more significant judgments and estimates used in the preparation of the financial statements.

Goodwill and intangible assets – Goodwill represents the excess of the cost over the fair value of acquired identifiable tangible and intangible assets and liabilities assumed from businesses acquired. Goodwill and intangible assets deemed to have indefinite lives are not amortized, but are subject to impairment testing annually, or more frequently if events or changes in

circumstances indicate the asset might be impaired. We perform our annual goodwill impairment test as of October 1 and monitor for interim triggering events on an ongoing basis. Application of the goodwill impairment test requires judgment.

We review goodwill for impairment utilizing either a qualitative assessment or a quantitative goodwill impairment test. If we choose to perform a qualitative assessment and we determine that the fair value of the reporting unit more likely than not exceeds the carrying value, no further evaluation is necessary. For reporting units where we perform the quantitative goodwill impairment test, we compare the fair value of each reporting unit, which we primarily determine using an income approach based on the present value of discounted cash flows, to the respective carrying value, which includes goodwill. If the fair value of the reporting unit exceeds its carrying value, we do not consider the goodwill impaired. If the carrying value is higher than the fair value, we recognize the difference as an impairment loss.

A quantitative goodwill impairment testing process requires valuation of the respective reporting unit, which we primarily determine using an income approach based on a discounted cash flow forecast covering discrete periods of time including a terminal value. We compute the terminal value using the constant growth method, which values the forecasted cash flows in perpetuity. The assumptions about future cash flows and growth rates are based on the respective reporting unit's long-term forecast and are subject to review and approval by senior management. A reporting unit's discount rate is a significant assumption and is a risk-adjusted weighted average cost of capital, which we believe approximates the rate from a market participant's perspective. The estimated fair value could be impacted by changes in market conditions, interest rates, growth rates, tax rates, costs, pricing and capital expenditures. We categorize the fair value determination as Level 3 in the fair value hierarchy due to its use of internal projections and unobservable measurement inputs.

Our Mini Mill reporting unit holds the goodwill recognized as a result of the Company's acquisition of Big River Steel and currently is our only reporting unit that has a significant amount of goodwill. This goodwill is primarily attributable to Big River Steel's operational abilities, workforce and the anticipated benefits from their recent expansion. U. S. Steel completed its annual goodwill impairment test using a qualitative assessment during the fourth quarter of 2024 and determined there was no impairment of goodwill.

Intangible assets with indefinite lives are also subject to at least annual impairment testing, which compares the fair value of the intangible assets with their carrying amounts. U. S. Steel has determined that certain of its acquired intangible assets have indefinite useful lives. These assets are also reviewed for impairment annually in the fourth quarter and whenever events or circumstances indicate the carrying value may not be recoverable. U. S. Steel completed its annual evaluation of its indefinite-lived water rights using a qualitative assessment and determined there was no indication of impairment. Key assumptions included in this test relate to the relevant market rate of an acre foot of water.

If business conditions deteriorate or other factors have an adverse effect on our qualitative and quantitative estimates, inclusive of discounted future cash flows or assumed growth rates, or if we experience a sustained decline in our market capitalization, future assessments of goodwill for impairment may result in impairment charges.

Finite-lived intangible assets are amortized on a straight-line basis over their estimated useful lives and are tested for impairment when events occur that indicate that the net book value will not be recovered over future cash flows.

Business combinations – We account for business combinations under the acquisition method of accounting in accordance with Accounting Standards Codification (ASC) Topic 805, *Business Combinations,* which requires an allocation of the consideration we paid to the identifiable assets, intangible assets and liabilities based on the estimated fair values as of the closing date of the acquisition. The excess of the fair value of the purchase price over the fair values of these identifiable assets, intangible assets and liabilities is recorded as goodwill.

Purchased intangibles are initially recognized at fair value and amortized over their useful lives unless those lives are determined to be indefinite. The valuation of acquired assets will impact future operating results. The fair value of identifiable intangible assets is determined using an income approach on an individual asset basis. Specifically, we use the multi-period excess earnings method to estimate the fair value of the customer relationships intangible asset. Determining the fair value of the customer relationships intangible asset involves significant judgments and assumptions, including expected realized price, base year metallic costs, contributory asset charges, and customer attrition rate.

Inventories – Inventories are carried at the lower of cost or market for last-in, first-out (LIFO), moving average and first-in, first-out (FIFO) method inventories. The predominant method of inventory costing for Flat-Rolled and Tubular is LIFO. The Mini Mill segment uses FIFO for raw materials and a moving average costing method to account for semi-finished and finished products. FIFO is the predominant inventory costing method used by the USSE segment. The LIFO method of inventory costing was used on 53 percent of consolidated inventories at both December 31, 2024, and December 31, 2023. Since the LIFO inventory valuation methodology is an annual calculation, interim estimates of the annual LIFO valuation are required. We recognize the effects of the LIFO inventory valuation method on an interim basis by estimating the year-end inventory amounts. The projections of annual LIFO inventory amounts are updated quarterly. Changes in U.S. GAAP rules or tax law, such as the elimination of the LIFO method of accounting for inventories, could negatively affect our profitability and cash flow.

Pensions and Other Benefits – The recording of net periodic benefit costs for defined benefit pensions and Other Benefits is based on, among other things, assumptions of the expected annual return on plan assets, discount rate, mortality, escalation or other changes in retiree health care costs and plan participation levels. Changes in the assumptions or differences between actual and expected changes in the present value of liabilities or assets of U. S. Steel's plans could cause net periodic benefit costs to increase or decrease materially from year to year as discussed below.

U. S. Steel's investment strategy for its U.S. pension and Other Benefits plan assets provides for a diversified mix of high-quality bonds, public equities and selected smaller investments in private equities and private credit. For its U.S. pension, U. S. Steel has a target allocation for plan assets of 68 percent in fixed income investments. The balance is invested in equity securities, private equity and real estate partnerships. U. S. Steel believes that returns on equities over the long term will be higher than returns from fixed-income securities as actual historical returns from U. S. Steel's trusts have shown. Returns on bonds tend to offset some of the short-term volatility of stocks. Both equity and fixed-income investments are made across a broad range of industries and companies (both domestic and foreign) to provide protection against the impact of volatility in any single industry as well as company specific developments. U. S. Steel will use a 6.90 percent assumed rate of return on assets for the development of net periodic cost for the main defined benefit pension plan in 2025. Actual returns since the inception of the plan have exceeded this 6.90 percent rate and while recent annual returns have been volatile, it is U. S. Steel's expectation that rates will achieve this level in future periods.

For its Other Benefits plan, U. S. Steel is employing a liability driven investment strategy. The plan assets are allocated to match the plan cash flows with maturing investments. To achieve this strategy, U. S. Steel has a target allocation for plan assets of 78 percent in fixed income and private credit. The balance is primarily invested in equity securities, private equity and real estate partnerships. U. S. Steel will use a 6.00 percent assumed rate of return on assets for the development of net periodic cost for its Other Benefits plan for 2025.

The expected long-term rate of return on plan assets is applied to the market value of assets as of the beginning of the period less expected benefit payments and considering any planned contributions.

To determine the discount rate used to measure our pension and Other Benefit obligations for U.S. plans we utilize a bond matching approach to select specific bonds that would satisfy our projected benefit payments. At December 31, 2024, the weighted average discount rate used for our pension and Other Benefit obligations was determined to be 5.90 percent and 5.90 percent, respectively, compared to the weighted average discount rate used of 5.49 percent and 5.58 percent, respectively, at December 31, 2023. The discount rate reflects the current rate at which we estimate the pension and Other Benefits liabilities could be effectively settled at the measurement date.

U. S. Steel reviews its actual historical rate experience and expectations of future health care cost trends to determine the escalation of per capita health care costs under U. S. Steel's benefit plans. Approximately 85 percent of our costs for the domestic United Steelworkers participants' retiree health benefits in the Company's main domestic benefit plan are limited to a per capita dollar maximum calculation based on 2006 base year actual costs incurred under the main U. S. Steel benefit plan for USW participants (cost cap). The full effect of the cost cap is expected to be realized around 2031. After 2031, the Company's costs for a majority of USW retirees and their dependents are expected to remain fixed and as a result, the cost impact of health care escalation for the Company is projected to be limited for this group (see Note 18 to the Consolidated Financial Statements). For measurement of its domestic retiree medical plans where health care cost escalation is applicable, U. S. Steel has assumed an initial escalation rate of 5.80 percent for 2025. This rate is assumed to decrease gradually to an ultimate rate of 4.50 percent in 2038 and remain at that level thereafter.

Net periodic pension benefit cost (credit), including multiemployer plans, is expected to total approximately $139 million in 2025 compared to $82 million in 2024. The increase in net periodic pension benefit cost in 2025 is primarily due to a decrease in expected asset return and an increase in amortized actuarial losses. Net periodic other benefit (credit) in 2025 is expected to be approximately $(44) million, compared to $(102) million in 2024. The expected decrease in the 2025 net periodic other benefit (credit) is primarily due to decreases in prior service credits and amortized actuarial gains. See Note 18 to the Consolidated Financial Statements, "Pensions and Other Benefits."

The table below projects the incremental effect of a hypothetical one percentage point change in significant assumptions used in determining the funded status and net periodic benefit cost for pension and other benefits:

	Hypothetical Rate Change Increase (Decrease)	
(In millions)	1%	(1)%
Expected return on plan assets		
Incremental (decrease) increase in:		
Net periodic pension and other benefits costs for 2025	$ (49)	$ 49
Discount rate		
Incremental (decrease) increase in:		
Net periodic pension and other benefits costs for 2025	$ (24)	$ (1)
Pension & other benefits obligations at December 31, 2024	$ (344)	$ 400

Changes in the assumptions for expected annual return on plan assets and the discount rate used for accounting purposes do not impact the funding calculations used to derive minimum funding requirements for the pension plan. However, the discount rate required for minimum funding purposes is also based on corporate bond related indices and as such, the same general sensitivity concepts as above can be applied to increases or decreases to the funding obligations of the plans assuming the same hypothetical rate changes. See Note 18 to the Consolidated Financial Statements for a discussion regarding legislation enacted in March of 2021 that impacts the discount rate used for funding purposes. For further cash flow discussion see “Item 7. Management’s Discussion and Analysis of Financial Condition and Results of Operations, Liquidity and Capital Resources.”

Long-lived assets – U. S. Steel evaluates long-lived assets, primarily property, plant and equipment for impairment whenever changes in circumstances indicate that the carrying amounts of those productive assets exceed their recoverable amount as determined by the asset group’s projected undiscounted cash flows. We evaluate long-lived assets for potential impairment at the asset group level. Our primary asset groups are flat-rolled, mini mill, USSE, welded tubular and seamless tubular.

In 2024, the Company recognized impairment charges of approximately $19 million for certain indefinitely idled assets at Granite City Works and certain permanently idled assets at Clairton Works. In 2023, the Company recognized charges of approximately $123 million for impairment of indefinitely idled iron and steel making assets at Granite City Works. Impairment charges of approximately $151 million were recognized for the write-off of iron making and steel making assets at Great Lakes Works in 2022. The coil finishing processes at Granite City Works and Great Lakes Works continue to operate and remain components of the Company’s operating plans. These impairment charges were the result of actions impacting specific, identifiable assets and were not significantly adverse to the flat-rolled long-lived asset group. No triggering events that required an impairment evaluation of our long-lived asset groups were identified during the years ended December 31, 2024, 2023 and 2022.

Taxes – U. S. Steel records a valuation allowance, related to certain state and foreign net operating losses, state and foreign tax credits and unused capital losses, to reduce deferred tax assets to the amount that is more likely than not to be realized. A valuation allowance is recorded if, based on the weight of all available positive and negative evidence, it is more likely than not that some portion, or all, of a deferred tax asset will not be realized. Each quarter U. S. Steel analyzes the likelihood that our deferred tax assets will be realized. In the future, if we determine that it is more likely than not that we will be able to realize all or a portion of our deferred tax assets, the valuation allowance will be reduced, and we will record a non-cash net benefit to earnings.

At the end of both 2024 and 2023, U. S. Steel did not have any undistributed foreign earnings and profits for which U.S. deferred taxes have not been provided.

For further information on income taxes see Note 11 to the Consolidated Financial Statements.

Environmental remediation – U. S. Steel has been identified as a PRP at four sites under the CERCLA as of December 31, 2024. Of these, there are three sites where information requests have been received or there are other indications that U. S. Steel may be a PRP under CERCLA, but where sufficient information is not presently available to confirm the existence of liability or to make a reasonable estimate with respect to any potential liabilities. There are also nine additional sites where U. S. Steel may be liable for remediation costs in excess of $1 million under other environmental statutes, both federal and state, or where private parties are seeking to impose liability on U. S. Steel for remediation costs through discussions or litigation. At many of these sites, U. S. Steel is one of a number of parties involved and the total cost of remediation, as well as U. S. Steel’s share, is frequently dependent upon the outcome of ongoing investigations and remedial studies. U. S. Steel accrues for environmental remediation activities when the responsibility to remediate is probable and the amount of associated costs is reasonably determinable. As environmental remediation matters proceed toward ultimate resolution or as remediation obligations arise, charges in excess of those previously accrued may be required.

U. S. Steel's accrual for environmental liabilities for U.S. and international facilities as of December 31, 2024, and 2023 was $108 million and $107 million, respectively. These amounts exclude liabilities related to asset retirement obligations, disclosed in Note 19 to the Consolidated Financial Statements.

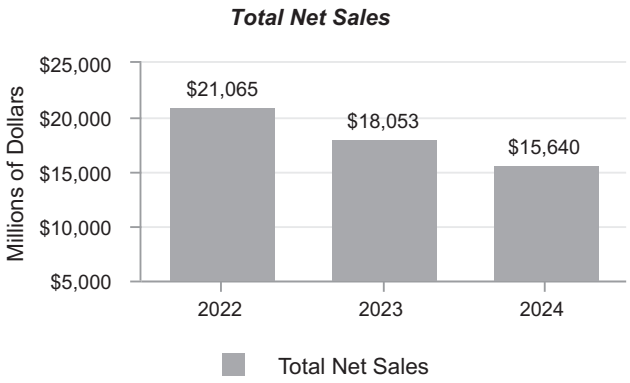
U. S. Steel is the subject of, or a party to, a number of pending or threatened legal actions, contingencies and commitments involving a variety of matters, including laws and regulations relating to the environment. The ultimate resolution of these contingencies could, individually or in the aggregate, be material to the Consolidated Financial Statements.

For discussion of relevant environmental items, see "Part I. Item 3. Legal Proceedings—Environmental Proceedings."

Segments

U. S. Steel has four reportable segments: North American Flat-Rolled, Mini Mill, USSE and Tubular Products. For further description of segment operations and information see Item 1. Business—Segments and Note 4 to the Consolidated Financial Statements, respectively.

Net Sales



Net Sales by Segment

Net sales by segment for the years ended December 31, 2024, and 2023 are set forth in the following table:

(Dollars in millions, excluding intersegment sales)	Years Ended December 31, 2024	2023	% Change
Flat-Rolled	**$ 9,604**	$ 10,744	(11)%
Mini Mill	**2,070**	2,223	(7)%
USSE	**2,983**	3,525	(15)%
Tubular	**970**	1,551	(37)%
Total sales from reportable segments	**15,627**	18,043	(13)%
Other	**13**	10	30 %
Net Sales	**$ 15,640**	$ 18,053	(13)%

Management's analysis of the percentage change in net sales for U. S. Steel's reportable business segments is set forth in the following table:

Year Ended December 31, 2024 versus Year Ended December 31, 2023	Steel Products[a]					
	Volume	Price	Mix	FX[b]	Other[C]	Net Change
Flat-Rolled	(8)%	(2)%	— %	— %	(1)%	(11)%
Mini Mill	(5)%	(3)%	1 %	— %	— %	(7)%
USSE	(8)%	(7)%	— %	— %	— %	(15)%
Tubular	— %	(30)%	(7)%	— %	— %	(37)%

[a] Excludes intersegment sales.

[b] Foreign currency translation effects.

[c] Primarily consists of sales of raw material and coke making by-products.

Net sales for the year ended December 31, 2024, compared to the same period in 2023 were $15,640 million and $18,053 million, respectively.

- For the Flat-Rolled segment, the decrease in sales primarily resulted from decreased shipments (861 thousand tons) across most products and lower average realized prices ($17 per ton) across most products.
- For the Mini Mill segment, the decrease in sales primarily resulted from decreased shipments (117 thousand tons) from lower value products and lower average realized prices ($18 per ton) across all products.
- For the USSE segment, the decrease in sales primarily resulted from decreased shipments (321 thousand tons) across most products and lower average realized prices ($69 per ton) across all products.
- For the Tubular segment, the decrease in sales primarily resulted from lower average realized prices ($1,232 per ton) and mix changes to lower value products.

Operating Expenses

Union profit-sharing costs

(Dollars in millions)	Year Ended December 31, 2024	2023
Allocated to segment results	$ 57	$ 149

The amounts above represent profit-sharing amounts paid to active USW-represented employees and are included in cost of sales on the Consolidated Statements of Operations.

Profit-based amounts are calculated and paid on a quarterly basis as a percentage of consolidated earnings before interest and income taxes based on 7.5 percent of profit between $10 and $50 per ton and 15 percent of profit above $50 per ton.

Net periodic pension and other benefits costs

Pension and other benefit costs (other than service cost) are reflected within net interest and other financial (benefits) costs and the service cost component is reflected within cost of sales in the Consolidated Statements of Operations.

Defined benefit and multiemployer pension plan costs included in cost of sales totaled $108 million in 2024 and $116 million in 2023.

Other benefit service cost included in cost of sales totaled $4 million in 2024 and $6 million in 2023.

Costs related to defined contribution plans totaled $48 million in 2024 and $50 million in 2023.

Selling, general and administrative expenses

Selling, general and administrative expenses were $435 million and $501 million for the years ended December 31, 2024, and December 31, 2023, respectively. The change between periods was primarily due to decreased costs related to pension and OPEB benefits and variable compensation.

Operating configuration adjustments

The Company adjusts its operating configuration in response to changes in market conditions, global overcapacity, import competition arising from unfair trade practices, and changes in customer demand. These operating configuration adjustments can include indefinitely and temporarily idling certain of its facilities as well as restarting production at certain of its facilities.

Idled Operations

In December 2024, the Company temporarily idled Blast Furnace #3 at USSE until demand improves. It is expected that Blast Furnace #3 will be restarted in the first quarter of 2025 based on market conditions.

In 2023, the Company indefinitely idled its subsidiary in Pittsburg, California, USS-UPI, LLC (UPI), resulting in restructuring and other charges of $3 million and $30 million in 2023 and 2022, respectively. Also, in 2023, the Company indefinitely idled the iron and steel making assets at Granite City Works which resulted in a non-cash asset impairment of $123 million and additional charges of $107 million primarily for take-or-pay commitments and employee-related costs. The hot-strip mill, cold mill and coating lines at Granite City Works continue to operate.

In 2023, the Company completed the previously announced permanent shutdown of coke batteries numbers 1 through 3 at the Mon Valley Works.

In 2022, U. S. Steel indefinitely idled the majority of the tin mill operations at Gary Works. This included the Tin Line #5 and the Tin Line #6. As of December 31, 2024, the carrying value of the indefinitely idled tin mill operations assets at Gary Works is $65 million. Tin mill operations continue to operate at the Midwest plant.

At Great Lakes Works, the Company permanently idled the ironmaking operations in 2022, which resulted in non-cash impairment of $151 million. The coil finishing process at Great Lakes Works continues to operate and remains a component of the Company's operating plans.

The Company's Lorain Tubular Operations, Lone Star Tubular Operations and the Wheeling Machine Products coupling production facility at Hughes Springs, Texas were initially idled in 2020 and remained indefinitely idled as of December 31, 2024. The carrying value of the Lorain Tubular Operations assets is $50 million as of December 31, 2024.

Depreciation, depletion and amortization

Depreciation, depletion and amortization expenses were $913 million in 2024 and $916 million in 2023.

Earnings from investees

Earnings from investees were $112 million in 2024 compared to $115 million in 2023. The decrease in 2024 from the prior year is primarily due to decreased earnings from Hibbing Development Company and Patriot Premium Threading Services, partially offset by our PRO-TEC joint venture.

Restructuring and other charges

During 2024, the Company recorded restructuring and other charges of $8 million, which related primarily to idling of certain coke-making facilities. Charges for restructuring and ongoing cost reduction initiatives are recorded in the period U. S. Steel commits to a restructuring or cost reduction plan, or executes specific actions contemplated by the plan and all criteria for liability recognition have been met. Charges related to restructuring and cost reductions are reported in Restructuring and other charges in the Consolidated Statements of Operations.

Earnings (loss) before interest, taxes, depreciation and amortization by segment

Segment performance is measured primarily on the basis of segment level earnings (loss) before interest, taxes, depreciation and amortization (EBITDA). EBITDA for reportable segments and the Other category does not include net interest and other financial costs (income), income taxes, and certain other items that management believes are not indicative of future results.

Earnings (loss) before interest, taxes, depreciation and amortization by segment [a]	Year Ended December 31,	
(Dollars in Millions)	**2024**	**2023**
Flat-Rolled	**$ 934**	$ 1,023
Mini Mill	**233**	383
USSE	**71**	98
Tubular	**135**	638
Other loss before interest, taxes, depreciation and amortization	**(7)**	(3)
Depreciation, depletion and amortization	**(913)**	(916)
Segment earnings before interest and income taxes	**453**	1,223
Other items not allocated to segments:		
Restructuring and other charges [b]	**(8)**	(36)
Stock-based compensation expense	**(51)**	(51)
Asset impairment charges [c]	**(19)**	(127)
Environmental remediation charges	**(18)**	(11)
Strategic alternatives review process costs	**(90)**	(79)
Granite City idling costs	**(11)**	(121)
Other charges, net	**(16)**	1
Total earnings before interest and income taxes	**$ 240**	$ 799

[a] See Note 4 to the Consolidated Financial Statements for reconciliations and other details.

[b] Included in restructuring and other charges on the Consolidated Statements of Operations. See Note 25 to the Consolidated Financial Statements for further details.

[c] See Note 1 to the Consolidated Financial Statements for further details.

Segment results for Flat-Rolled

	Year Ended December 31,		%
(Dollars in Millions)	**2024**	**2023**	**Change**
Earnings before interest, taxes, depreciation and amortization (EBITDA)	**$ 934**	$ 1,023	(9)%
Depreciation, depletion and amortization	**535**	605	(12)%
Earnings before interest and income taxes	**$ 399**	$ 418	(5)%
Gross margin	**11 %**	11 %	— %
Raw steel production (mnt)	**8,389**	9,399	(11)%
Capability utilization	**63 %**	71 %	(8)%
Steel shipments (mnt)	**7,845**	8,706	(10)%
Average realized steel price per ton	**1,013**	1,030	(2)%

The decrease in Flat-Rolled EBITDA for 2024 compared to 2023 was primarily due to:

- lower average realized prices, including mix (approximately $50 million)
- decreased shipments (approximately $220 million)
- lower other sales (approximately $70 million)
- higher operating costs (approximately $110 million),

these changes were partially offset by:

- lower raw material costs, including inventory revaluations (approximately $90 million)
- lower energy costs (approximately $105 million)
- favorable equity affiliate income (approximately $70 million)
- lower other costs, primarily profit-based payments (approximately $95 million).

Gross margin for 2024 as compared to 2023 was unchanged.

Segment results for Mini Mill

(Dollars in Millions)	Year Ended December 31, 2024	2023	% Change
Earnings before interest, taxes, depreciation and amortization (EBITDA)	**$ 233**	$ 383	(39)%
Depreciation, depletion and amortization	**203**	168	21 %
Earnings before interest and income taxes	**$ 30**	$ 215	(86)%
Gross margin	**17 %**	19 %	(2)%
Raw steel production (mnt)	**2,838**	2,953	(4)%
Capability utilization	**80 %**	89 %	(9)%
Steel shipments (mnt)	**2,307**	2,424	(5)%
Average realized steel price per ton	**857**	875	(2)%

The decrease in Mini Mill EBITDA results for 2024 compared to 2023 was primarily due to:

- lower average realized prices, including mix (approximately $55 million)
- decreased shipments (approximately $140 million)
- higher other costs, primarily strategic projects startup costs (approximately $105 million),

these changes were partially offset by:

- lower raw material costs (approximately $150 million).

Gross margin for 2024 as compared to 2023 decreased primarily due to lower sales volume and price.

Segment results for USSE

(Dollars in Millions)	Year Ended December 31, 2024	2023	% Change
Earnings before interest, taxes, depreciation and amortization (EBITDA)	**$ 71**	$ 98	(28)%
Depreciation, depletion and amortization	**125**	94	33 %
(Loss) earnings before interest and income taxes	**$ (54)**	$ 4	(1,450)%
Gross margin	**4 %**	4 %	— %
Raw steel production (mnt)	**3,832**	4,395	(13)%
Capability utilization	**77 %**	88 %	(11)%
Steel shipments (mnt)	**3,578**	3,899	(8)%
Average realized steel price per ton	**805**	873	(8)%

The decrease in USSE EBITDA results for 2024 compared to 2023 was primarily due to:

- lower average realized prices, including mix (approximately $220 million)
- decreased shipments, including volume inefficiencies (approximately $70 million)
- lower other sales (approximately $5 million)
- higher operating costs (approximately $40 million),

these changes were partially offset by:

- lower raw material costs, including inventory revaluations and CO_2 accrual adjustments (approximately $215 million)
- lower energy costs (approximately $85 million)
- lower other costs (approximately $5 million)
- strengthening of the U.S. dollar versus the Euro (approximately $5 million).

Gross margin for 2024 as compared to 2023 was unchanged.

Segment results for Tubular

(Dollars in Millions)	Year ended December 31, 2024	2023	% Change
Earnings before interest, taxes, depreciation and amortization (EBITDA)	**$ 135**	$ 638	(79)%
Depreciation, depletion and amortization	**50**	49	2 %
Earnings before interest and income taxes	**$ 85**	$ 589	(86)%
Gross margin	**14 %**	40 %	(26)%
Raw steel production (mnt)	**575**	568	1 %
Capability utilization	**64 %**	63 %	1 %
Steel shipments (mnt)	**476**	478	— %
Average realized steel price per ton	**1,905**	3,137	(39)%

The decrease in Tubular EBITDA results for 2024 compared to 2023 was primarily due to:

- lower average realized prices (approximately $520 million)
- decreased shipments (approximately $35 million),

these changes were partially offset by:

- lower raw material costs (approximately $20 million)
- lower other costs, primarily variable compensation (approximately $30 million).

Gross margin for 2024 as compared to 2023 decreased primarily as a result of lower average realized prices.

Results for Other

The Other category had a loss before interest, taxes, depreciation and amortization of $7 million for the year ended December 31, 2024, compared to a loss before interest, taxes, depreciation and amortization of $3 million for the year ended December 31, 2023.

Net Interest and Other Financial Costs

(Dollars in millions)	Year Ended December 31, 2024	2023
Interest expense	**24**	72
Interest income	**(96)**	(141)
Loss on debt extinguishment	**2**	—
Other financial costs	**29**	18
Net periodic benefit income	**(132)**	(154)
Net gain from investments related to active employee benefits	**(25)**	(43)
Net interest and other financial benefits	**$ (198)**	$ (248)

Net interest and other financial benefits declined in 2024 compared to 2023 primarily due to reduced net periodic benefit income from increases in actuarial losses, reduced investment gains related to active employee benefits from lower 2024 asset performance, and decreased interest income from a lower cash balance. These were partially offset by lower interest expense as a result of increased capitalized interest. For additional information on U. S. Steel indebtedness see Note 17 to the Consolidated Financial Statements.

For additional information on U. S. Steel's foreign currency exchange activity see Note 16 to the Consolidated Financial Statements and Item 7A. "Quantitative and Qualitative Disclosures about Market Risk – Foreign Currency Exchange Rate Risk."

Income Tax

The income tax expense for the year ended December 31, 2024, was $54 million compared to an income tax expense of $152 million in 2023. The change from the prior year period was primarily due to a decrease in earnings before taxes. In addition, the current year period includes a benefit of $59 million related to the 2023 federal and state income tax returns.

For further information on income taxes see Note 11 to the Consolidated Financial Statements.

Net earnings attributable to U. S. Steel

Net earnings attributable to U. S. Steel in 2024 were $384 million compared to net earnings of $895 million in 2023. The decrease was primarily attributable to the factors discussed above.

Liquidity and Capital Resources

Cash Flows and Capital Requirements

Net Cash Provided by Operating Activities

Net cash provided by operating activities was $919 million in 2024 compared to $2,100 million in 2023. The decrease in 2024 compared to 2023 was primarily due to lower net earnings and changes in working capital. Changes in working capital can vary significantly depending on factors such as the timing of inventory production and purchases, which is affected by the length of our business cycles as well as our captive raw materials position, customer payments of accounts receivable and payments to vendors in the regular course of business.

Our cash conversion cycle increased by 7 days in the fourth quarter of 2024 from the fourth quarter of 2023 as shown below:

Cash Conversion Cycle	2024		2023	
	$ millions	Days	$ millions	Days
Accounts receivable, net [a]	$ 1,398	40	$ 1,549	34
+ Inventories [b]	$ 2,168	58	$ 2,128	53
- Accounts Payable and Other Accrued Liabilities [c]	$ 2,601	72	$ 2,867	68
= Cash Conversion Cycle [d]		26		19

[a] Calculated as Average Accounts Receivable, net divided by total Net Sales multiplied by the number of days in the period.
[b] Calculated as Average Inventory divided by total Cost of Sales multiplied by the number of days in the period.
[c] Calculated as Average Accounts Payable and Other Accrued Liabilities less bank checks outstanding and other current liabilities divided by total Cost of Sales multiplied by the number of days in the period.
[d] Calculated as Accounts Receivable Days plus Inventory Days less Accounts Payable Days.

The cash conversion cycle is a non-GAAP financial measure. We believe the cash conversion cycle is a useful measure in providing investors with information regarding our cash management performance and is a widely accepted measure of working capital management efficiency. The cash conversion cycle should not be considered in isolation or as an alternative to other GAAP metrics as an indicator of performance.

The days in our cash conversion cycle in comparison to the prior year increased primarily from higher inventory levels in our Mini Mill segment due to the BR2 start-up and lower net sales in the fourth quarter of the current year compared to the same period in the prior year.

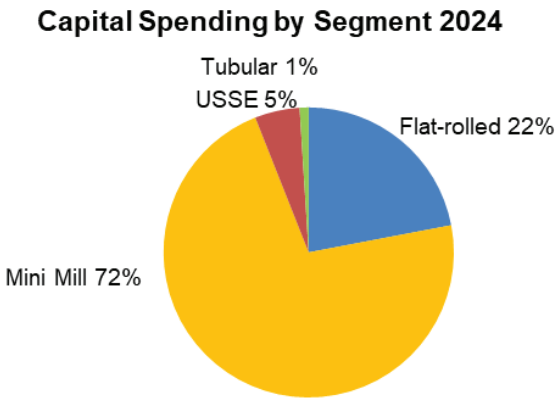
The LIFO inventory method is the predominant method of inventory costing for our Flat-Rolled and Tubular segments. The FIFO and moving average methods are the predominant inventory costing methods for our Mini Mill segment and the FIFO method is the predominant inventory costing method for our USSE segment. In the U.S., management monitors the inventory realizability by comparing the LIFO cost of inventory with the replacement cost of inventory. To the extent the replacement cost (i.e., market value) of inventory is lower than the LIFO cost of inventory, management will write the inventory down. As of December 31, 2024, and 2023, the replacement cost of the LIFO inventory was higher by approximately $1,143 million and $1,248 million, respectively.

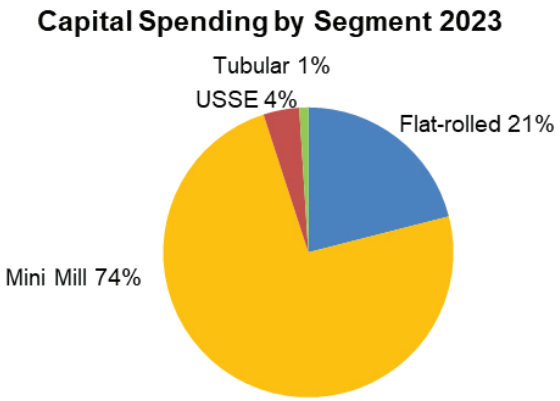
Net cash provided by operating activities for 2024 and 2023 reflects employee benefits payments as shown in the following table.

Benefits Payments for Employees	Year Ended December 31,	
(Dollars in millions)	**2024**	2023
Other employee benefits payments not funded by trusts	**32**	40
Payments to a multiemployer pension plan	**79**	84
Pension related payments not funded by trusts	**1**	3
Reductions in cash flows from operating activities	**$ 112**	$ 127

Net Cash Used in Investing Activities

Net cash used in investing activities was $2,276 million in 2024 compared to $2,568 million in 2023. The decrease in net cash used in investing activities was primarily due to decreased capital expenditures (discussed in more detail below).





Capital expenditures in 2024 were $2,287 million compared to $2,576 million in 2023. Mini Mill capital expenditures were $1,641 million and included $1,368 million for BR2, exclusive of the air separation unit, which began operations during the fourth quarter, as well as spending for the dual Galvalume®/galvanized coating and color coating lines at the existing Big River Steel facility. Flat-Rolled capital expenditures were $495 million which includes spending for the DR grade pellet facility at Keetac, as well as for mining equipment, blast furnace repairs and a stove rebuild at Gary Works, and other infrastructure and environmental projects across the Flat-Rolled footprint. USSE capital expenditures were $118 million and included spending for the blast furnace stove repairs and upgrades, enterprise resource planning (ERP) project, 5-stand control system upgrades, and various other projects. Tubular capital expenditures were $33 million and included spending to support steelmaking, infrastructure, and environmental projects within the Tubular footprint. Other businesses had no capital expenditures.

Net Cash used in Financing Activities

Net cash used in financing activities was $199 million for the twelve months ended December 31, 2024, compared to net cash used in financing activities of $98 million for the same period in 2023. The period over period change in financing activities was primarily due to the absence of proceeds received from the issuance of long-term debt in the current year period, partially offset by the absence of repurchases of common stock in the current year period.

In 2023, U. S. Steel received net proceeds of approximately $238 million after closing on an aggregate principal amount of $240 million unsecured Arkansas Development Finance Authority environmental improvement revenue bonds with a coupon rate of 5.700% and a final maturity of 2053.

Certain of our credit facilities, including the Credit Facility Agreement, the Big River Steel ABL Facility, the USSK Credit Agreement and the Export Credit Agreement, contain standard terms and conditions including customary material adverse change clauses. If a material adverse change was to occur, our ability to fund future operating and capital requirements could be negatively impacted. The €150 million (approximately $156 million) USSK Credit Agreement contains certain USSK specific financial covenants. The USSK Credit Agreement requires USSK to maintain a net debt to EBITDA ratio of less than 3.50:1 (the "EBITDA Ratio Covenant"), as measured on a rolling twelve month basis on June 30th and December 31st of each year. As of December 31, 2024 USSK was in compliance with the EBITDA Ratio Covenant. There are currently no amounts outstanding under the facility.

We use surety bonds, trusts and letters of credit to provide financial assurance for certain transactions and business activities. The use of some forms of financial assurance and cash collateral have a negative impact on liquidity. U. S. Steel has committed $192 million of liquidity sources for financial assurance purposes as of December 31, 2024. Increases in certain of these commitments which use collateral are reflected within cash, cash equivalents and restricted cash on the Consolidated Statement of Cash Flows.

The maximum guarantees of the indebtedness of unconsolidated entities of U. S. Steel totaled $7 million at December 31, 2024. If any default related to the guaranteed indebtedness occurs, U. S. Steel has access to its interest in the assets of the investees to reduce its potential losses under the guarantees.

We may from time to time seek to retire or repurchase our outstanding long-term debt through open market purchases, privately negotiated transactions, exchange transactions, redemptions or otherwise. Such purchases or exchanges, if any, will depend on prevailing market conditions, our liquidity requirements and other factors and may be commenced or suspended at any time. The amounts involved may be material. See Note 17 to the Consolidated Financial Statements for further details regarding U. S. Steel's debt.

Share Repurchases

In the third quarter of 2022, the Board of Directors authorized a share repurchase program that allows for the repurchase of up to $500 million of our outstanding common stock from time to time in the open market or privately negotiated transactions at the discretion of management. The Company's share repurchase program does not obligate it to acquire any specific number of shares. In the twelve months ended December 31, 2024 no common stock was repurchased under our share repurchase program. We do not expect to utilize the remainder of this authorization as the Merger Agreement contains a customary prohibition on our ability to engage in additional share repurchases without the consent of Purchaser. See Note 27 to the Consolidated Financial Statements, "Common Stock Repurchased" for further details.

Capital Requirements

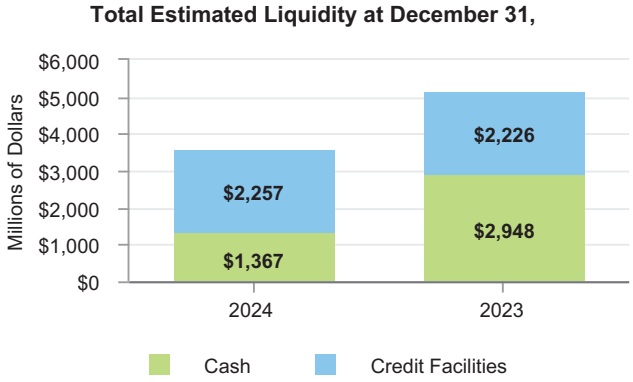
Capital expenditures for 2025 are expected to total approximately $1.0 billion and are significantly lower than 2024 due to the on time completion of several strategic projects as well as the nearing completion and commissioning of the remaining strategic projects to expand our competitive advantages in low-cost iron ore, mini mill steelmaking and best-in-class finishing capabilities.

U. S. Steel's contractual commitments to acquire property, plant and equipment at December 31, 2024, totaled $683 million.

Liquidity

The following table summarizes U. S. Steel's liquidity as of December 31, 2024:

(Dollars in millions)		
Cash and cash equivalents	$	1,367
Amount available under Credit Facility Agreement		1,746
Amount available under Big River Steel - Revolving Line of Credit		340
Amounts available under USSK Credit Agreement and USSK Credit Facility		171
Total estimated liquidity	$	3,624



As of December 31, 2024, $171 million of the total cash and cash equivalents was held by our foreign subsidiaries. Substantially all of the liquidity attributable to our foreign subsidiaries can be accessed without the imposition of income taxes as a result of the election effective December 31, 2013, to liquidate for U.S. income tax purposes a foreign subsidiary that holds most of our international operations.

We expect that our estimated liquidity requirements will consist primarily of our 2025 planned strategic capital expenditures, working capital requirements, debt service, and operating costs and employee benefits for our operations. Our available liquidity at December 31, 2024, consists principally of our cash and cash equivalents and available borrowings under the Credit Facility Agreement, Big River Steel ABL Facility, USSK Credit Agreement and the USSK Credit Facility.

Management continues to evaluate market conditions in our industry and our global liquidity position, and may consider additional actions to further strengthen our balance sheet and optimize liquidity, including but not limited to the repayment or refinancing of outstanding debt and the incurrence of additional debt to opportunistically finance strategic projects.

U. S. Steel management believes that U. S. Steel's liquidity will be adequate to satisfy our cash requirements and obligations for the next twelve months and for the foreseeable future, including obligations to complete currently authorized capital spending programs. Future requirements for U. S. Steel's business needs, including the funding of capital expenditures, scheduled debt maturities, repurchase of debt, dividends, contributions to employee benefit plans and any amounts that may ultimately be paid

in connection with contingencies, are expected to be funded by a combination of internally generated funds (including asset sales), borrowings, refinancings and other external financing sources.

The Company has a supply chain finance (SCF) arrangement with a third-party administrator which allows participating suppliers, at their sole discretion, to make offers to sell payment obligations of the Company prior to their scheduled due dates at a discounted price to a participating financial institution. The third-party administrator entered into a separate agreement with the Export Import Bank of the United States to guarantee 90 percent of supplier obligations sold for up to $95 million. No guarantees or collateral are provided by the Company or any of its subsidiaries under the SCF program.

The Company's goal is to capture overall supplier savings and improve working capital efficiency. The agreements facilitate the suppliers' ability to sell payment obligations, while providing them with greater working capital flexibility. The Company has no economic interest in the sale of the suppliers' receivables and no direct financial relationship with the financial institution concerning these services. The Company's obligations to its suppliers, including amounts due and scheduled payment dates, are not impacted by suppliers' decisions to sell amounts under the arrangements. The SCF program requires the Company to pay the third-party administrator the stated amount of the confirmed participating supplier invoices. The payment terms for confirmed invoices range from 45 to 90 days after the end of the month in which the invoice was issued.

The underlying costs from suppliers that elected to participate in the SCF program are generally recorded in cost of sales in the Company's Consolidated Statements of Operations. Amounts due to suppliers who participate in the SCF program are reflected in accounts payable and accrued expenses on the Company's Consolidated Balance Sheets and payments on the obligations by our suppliers are included in cash used in operating activities in the Consolidated Statements of Cash Flows. As of December 31, 2024, accounts payable and accrued expenses included $62 million of outstanding payment obligations which suppliers elected to sell to participating financial institutions.

The following table summarizes the Company's contractual obligations at December 31, 2024, and the effect such obligations are expected to have on our liquidity and cash flows in future periods.

(Dollars in millions)

		Payments Due by Period			
Contractual Obligations	**Total**	**2025**	**2026 through 2027**	**2028 through 2029**	**Beyond 2029**
Debt (including interest) and finance leases[a]	$ 7,077	$ 338	$ 1,133	$ 1,573	$ 4,033
Operating leases[b]	88	39	39	8	2
Contractual purchase commitments[c]	7,720	5,386	1,286	386	662
Capital commitments[d]	683	518	165	—	—
Environmental commitments[d]	108	26	—	—	82
Steelworkers Pension Trust[e]	411	79	163	169	—
Employee related benefits[f]	138	31	55	52	—
Total contractual obligations	$ 16,225	$ 6,417	$ 2,841	$ 2,188	$ 4,779

(a) See Note 17 to the Consolidated Financial Statements.
(b) See Note 24 to the Consolidated Financial Statements.
(c) Reflects estimated contractual purchase commitments under purchase orders and "take or pay" arrangements. "Take or pay" arrangements are primarily for purchases of gases and certain energy and utility services.
(d) See Note 26 to the Consolidated Financial Statements.
(e) While it is difficult to make a prediction of cash requirements beyond the term of the 2022 Labor Agreements with the USW, which expire on September 1, 2026, projected amounts shown through 2028 assume the contribution rate per hour included in the 2022 Labor Agreements of $4.00 per hour worked.
(f) The amounts reflect corporate cash outlays for expected benefit payments to be paid by the Company. (See Note 18 to the Consolidated Financial Statements.) The accuracy of this forecast of future cash flows depends on future medical health care escalation rates and restrictions related to our trusts for retiree healthcare and life insurance (VEBA) that impact the timing of the use of trust assets. Projected amounts have been reduced to reflect withdrawals from the USW VEBA trust available under its agreements with the USW. Due to these factors, it is not possible to reliably estimate cash requirements beyond five years and actual amounts experienced may differ significantly from those shown.

Other Commercial Commitments

The following table summarizes U. S. Steel's commercial commitments at December 31, 2024, and the effect such commitments could have on our liquidity and cash flows in future periods.

(Dollars in millions)

Commercial Commitments	Total	Scheduled Reductions by Period				
		2025	2026 through 2027	2028 through 2029	Beyond 2029	
Standby letters of credit[a]	$ 60	$ 43	$ —	$ —	$ 17	[b]
Surety bonds[a]	98	—	—	—	98	[b]
Funded Trusts[a]	35	—	—	—	35	[b]
Total commercial commitments	$ 193	$ 43	$ —	$ —	$ 150	

(a) Reflects a commitment or guarantee for which future cash outflow is not considered likely.
(b) Timing of potential cash outflows is not determinable.

Off-Balance Sheet Arrangements

U. S. Steel has invested in several joint ventures that are reported as equity investments. Several of these investments involved a transfer of assets in exchange for an equity interest. U. S. Steel has supply arrangements with several of these joint ventures.

U. S. Steel's other off-balance sheet arrangements include guarantees, indemnifications, unconditional purchase obligations, surety bonds, trusts and letters of credit disclosed in Note 26 to the Consolidated Financial Statements.

Derivative Instruments

See "Item 7A. Quantitative and Qualitative Disclosures About Market Risk" for discussion of derivative instruments and associated market risk for U. S. Steel.

Environmental Matters

U. S. Steel's environmental expenditures were as follows:

(Dollars in millions)

	2024	2023	2022
North America:			
Capital	**$ 80**	$ 60	$ 38
Compliance			
Operating & maintenance	**247**	243	255
Remediation[a]	**10**	15	18
Total North America	**$ 337**	$ 318	$ 311
USSE:			
Capital	**$ 3**	$ 10	$ 6
Compliance			
Operating & maintenance	**10**	12	11
Remediation[a]	**4**	5	6
Total USSE	**$ 17**	$ 27	$ 23
Total U. S. Steel	**$ 354**	$ 345	$ 334

[a] These amounts include spending charged against remediation reserves, net of recoveries where permissible, but do not include non-cash provisions recorded for environmental remediation.

U. S. Steel's environmental capital expenditures accounted for 4 percent of total capital expenditures in 2024, 3 percent in 2023 and 2 percent in 2022.

Environmental compliance expenditures represented 2 percent of U. S. Steel's total costs and expenses in 2024, 2023, and 2022. Remediation spending during 2022 through 2024 was mainly related to remediation activities at former and present operating locations.

For discussion of other relevant environmental items see "Part I, Item 3. Legal Proceedings – Environmental Proceedings."

The following table shows activity with respect to environmental remediation liabilities for the years ended December 31, 2024, and December 31, 2023. These amounts exclude liabilities related to asset retirement obligations accounted for in accordance with ASC Topic 410. See Note 19 to the Consolidated Financial Statements.

(Dollars in millions)	2024	2023
Beginning Balance	**$ 107**	$ 126
Plus: Additions	**18**	15
Adjustments for changes in estimates	**—**	(11)
Less: Obligations settled	**(17)**	(23)
Ending Balance	**$ 108**	$ 107

New or expanded environmental requirements, which could increase U. S. Steel's environmental costs, may arise in the future. U. S. Steel intends to comply with all legal requirements regarding the environment, but since many of them are not fixed or presently determinable (even under existing legislation) and may be affected by future legislation, it is not possible to predict accurately the ultimate cost of compliance, including remediation costs which may be incurred and penalties which may be imposed. U. S. Steel's environmental capital expenditures are expected to be approximately $156 million in 2025, $6 million of which is related to projects at USSE. U. S. Steel's environmental expenditures for 2025 for operating and maintenance and for remediation projects are expected to be approximately $273 million and $65 million, respectively, of which approximately $14 million and $5 million for operating and maintenance and remediation, respectively, is related to USSE. Although the outcome of pending environmental matters is not estimable at this time, it is reasonably possible that U. S. Steel's environmental capital and operating and maintenance expenditures could materially increase as a result of the future resolution of these matters. Predictions of future environmental expenditures beyond 2025 can only be broad-based estimates, which have varied, and will continue to vary, due to the ongoing evolution of specific regulatory requirements, the possible imposition of more stringent requirements and the availability of new technologies to remediate sites, among other factors.

Accounting Standards

See Notes 2 and 3 to the Consolidated Financial Statements in Part II Item 8 of this Form 10-K.

Item 7A. QUANTITATIVE AND QUALITATIVE DISCLOSURES ABOUT MARKET RISK

U. S. Steel is exposed to certain risks related to its ongoing business operations, including financial, market, political, and economic risks. The following discussion provides information regarding U. S. Steel's exposure to the risks of changing foreign currency exchange rates, commodity prices and interest rates.

U. S. Steel may enter into derivative financial instrument transactions in order to manage or reduce these market risks. The use of derivative instruments is subject to our corporate governance policies. These instruments are used solely to mitigate market exposure and are not used for trading or speculative purposes.

U. S. Steel may elect to use hedge accounting for certain commodity or currency transactions. For those transactions, the impact of the hedging instrument will be recognized in other comprehensive income until the transaction is settled. Once the transaction is settled, the effect of the hedged item will be recognized in income. For further information regarding derivative instruments see Notes 1 and 16 to the Consolidated Financial Statements.

Foreign Currency Exchange Rate Risk

U. S. Steel is subject to the risk of price fluctuations due to the effects of exchange rates on revenues and operating costs, firm commitments for capital expenditures and existing assets or liabilities denominated in currencies other than the U.S. dollar, particularly the euro. U. S. Steel historically has made limited use of forward currency contracts to manage exposure to certain currency price fluctuations. U. S. Steel elected cash flow hedge accounting for foreign exchange forwards and had no material open forward exchange contracts that were subject to mark-to-market accounting as of December 31, 2024.

The fair value of our derivatives is determined using Level 2 inputs, which are defined as "significant other observable" inputs. The inputs used include quotes from counterparties that are corroborated with market sources.

Volatility in the foreign currency markets could have significant implications for U. S. Steel as a result of foreign currency transaction effects. Future foreign currency impacts will depend upon changes in currencies and the extent to which we engage in derivatives transactions. For additional information on U. S. Steel's foreign currency exchange activity, see Note 16 to the Consolidated Financial Statements.

Commodity Price Risk and Related Risks

In the normal course of our business, U. S. Steel is exposed to market risk or price fluctuations related to the purchase, production or sale of steel products. U. S. Steel is also exposed to price risk related to the purchase, production or sale of coal, coke, natural gas, steel scrap, iron ore and pellets, and zinc, tin and other nonferrous metals used as raw materials. U. S. Steel is also subject to market price risk for the purchase of a portion of its electricity at certain facilities. See Note 16 to the Consolidated Financial Statements for further details on U. S. Steel's derivatives.

U. S. Steel's market risk strategy has generally been to obtain competitive prices for our products and services and allow operating results to reflect market price movements dictated by supply and demand; however, from time to time U. S. Steel has made forward physical purchases to manage exposure to price risk related to the purchases of natural gas and certain non-ferrous metals used in the production process. As of December 31, 2024, U. S. Steel did not have forward buy contracts for natural gas or any of the other significant raw materials used in the domestic production process.

Interest Rate Risk

U. S. Steel is subject to the effects of interest rate fluctuations on the fair value of certain of our non-derivative financial instruments. A sensitivity analysis of the projected incremental effect of a hypothetical 10 percent increase/decrease in year-end 2024 and 2023 interest rates on the fair value of U. S. Steel's non-derivative financial instruments is provided in the following table:

(Dollars in millions)	**2024**		**2023**	
Non-Derivative Financial Instruments[a]	**Fair Value[b]**	**Change in Fair Value[c]**	Fair Value[b]	Change in Fair Value[c]
Financial liabilities:				
Debt[d][e]	**$ 4,243**	**$ 256**	$ 4,797	$ 325

(a) Fair values of cash and cash equivalents, current accounts and notes receivable, accounts payable, bank checks outstanding and accrued interest approximate carrying value and are relatively insensitive to changes in interest rates due to the short-term maturity of the instruments. Accordingly, these instruments are excluded from the table.

(b) See Note 20 to the Consolidated Financial Statements for carrying value of instruments.

(c) Reflects, by class of financial instrument, the estimated incremental effect of a hypothetical 10 percent change in interest rates at December 31, 2024, and 2023, on the fair value of U. S. Steel's non-derivative financial instruments. For financial liabilities, this assumes a 10 percent decrease in the weighted average yield to maturity of U. S. Steel's long-term debt at December 31, 2024, and December 31, 2023.

(d) Excludes finance lease obligations.

(e) Fair value was determined using Level 2 inputs which were derived from quoted market prices and is based on the yield on public debt where available or current borrowing rates available for financings with similar terms and maturities.

U. S. Steel's sensitivity to interest rate declines and corresponding increases in the fair value of our debt portfolio would unfavorably affect our results and cash flows only to the extent that we elected to repurchase or otherwise retire all or a portion of our fixed-rate debt portfolio at prices above carrying value.

Item 8. CONSOLIDATED FINANCIAL STATEMENTS AND SUPPLEMENTARY DATA

The information required by this item is set forth in our Consolidated Financial Statement contained in this Annual Report on Form 10-K. Specific financial statements can be found at the page listed below:

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MANAGEMENT'S REPORT TO STOCKHOLDERS

January 31, 2025

To the Stockholders of United States Steel Corporation:

Financial Statements and Practices

The accompanying consolidated financial statements of United States Steel Corporation are the responsibility of and have been prepared by United States Steel Corporation in conformity with accounting principles generally accepted in the United States of America. They necessarily include some amounts that are based on our best judgments and estimates. United States Steel Corporation's financial information displayed in other sections of this report is consistent with these financial statements.

United States Steel Corporation seeks to assure the objectivity and integrity of its financial records by careful selection of its managers, by organizational arrangements that provide an appropriate division of responsibility and by communication programs aimed at assuring that its policies, procedures and methods are understood throughout the organization.

United States Steel Corporation has a comprehensive, formalized system of internal controls designed to provide reasonable assurance that assets are safeguarded, that financial records are reliable and that information required to be disclosed in reports filed with or submitted to the Securities and Exchange Commission is recorded, processed, summarized and reported within the required time limits. Appropriate management monitors the system for compliance and evaluates it for effectiveness, and the independent registered public accounting firm measures its effectiveness and recommends possible improvements thereto.

The Board of Directors exercises its oversight role in the area of financial reporting and internal control over financial reporting through its Audit Committee. This committee, composed solely of independent directors, regularly meets (jointly and separately) with the independent registered public accounting firm, management, internal audit and other executives to monitor the proper discharge by each of their responsibilities relative to internal control over financial reporting and United States Steel Corporation's financial statements.

Internal Control Over Financial Reporting

United States Steel Corporation's management is responsible for establishing and maintaining adequate internal control over financial reporting, as defined in Exchange Act Rule 13a-15(f). Under the supervision and with the participation of United States Steel Corporation's management, including the Chief Executive Officer and Chief Financial Officer, United States Steel Corporation conducted an evaluation of the effectiveness of its internal control over financial reporting based on the framework in *Internal Control – Integrated Framework (2013)* issued by the Committee of Sponsoring Organizations of the Treadway Commission.

Based on this evaluation, United States Steel Corporation's management concluded that United States Steel Corporation's internal control over financial reporting was effective as of December 31, 2024.

The effectiveness of United States Steel Corporation's internal control over financial reporting as of December 31, 2024, has been audited by PricewaterhouseCoopers LLP, an independent registered public accounting firm, as stated in their report which is included herein.

/s/ David B. Burritt	/s/ Jessica T. Graziano
David B. Burritt	Jessica T. Graziano
President and Chief Executive Officer	Senior Vice President and Chief Financial Officer

/s/ Manpreet S. Grewal
Manpreet S. Grewal
Vice President, Controller & Chief Accounting Officer

Report of Independent Registered Public Accounting Firm

To the Board of Directors and Stockholders of United States Steel Corporation

Opinions on the Financial Statements and Internal Control over Financial Reporting

We have audited the accompanying consolidated balance sheets of United States Steel Corporation and its subsidiaries (the "Company") as of December 31, 2024 and 2023, and the related consolidated statements of operations, of comprehensive income (loss), of stockholders' equity and of cash flows for each of the three years in the period ended December 31, 2024, including the related notes and financial statement schedule listed in the index appearing under Item 15(a)(2) (collectively referred to as the "consolidated financial statements"). We also have audited the Company's internal control over financial reporting as of December 31, 2024, based on criteria established in *Internal Control - Integrated Framework* (2013) issued by the Committee of Sponsoring Organizations of the Treadway Commission (COSO).

In our opinion, the consolidated financial statements referred to above present fairly, in all material respects, the financial position of the Company as of December 31, 2024 and 2023, and the results of its operations and its cash flows for each of the three years in the period ended December 31, 2024 in conformity with accounting principles generally accepted in the United States of America. Also in our opinion, the Company maintained, in all material respects, effective internal control over financial reporting as of December 31, 2024, based on criteria established in *Internal Control - Integrated Framework* (2013) issued by the COSO.

Basis for Opinions

The Company's management is responsible for these consolidated financial statements, for maintaining effective internal control over financial reporting, and for its assessment of the effectiveness of internal control over financial reporting, included in the accompanying Management's Report to Stockholders on Internal Control Over Financial Reporting. Our responsibility is to express opinions on the Company's consolidated financial statements and on the Company's internal control over financial reporting based on our audits. We are a public accounting firm registered with the Public Company Accounting Oversight Board (United States) (PCAOB) and are required to be independent with respect to the Company in accordance with the U.S. federal securities laws and the applicable rules and regulations of the Securities and Exchange Commission and the PCAOB.

We conducted our audits in accordance with the standards of the PCAOB. Those standards require that we plan and perform the audits to obtain reasonable assurance about whether the consolidated financial statements are free of material misstatement, whether due to error or fraud, and whether effective internal control over financial reporting was maintained in all material respects.

Our audits of the consolidated financial statements included performing procedures to assess the risks of material misstatement of the consolidated financial statements, whether due to error or fraud, and performing procedures that respond to those risks. Such procedures included examining, on a test basis, evidence regarding the amounts and disclosures in the consolidated financial statements. Our audits also included evaluating the accounting principles used and significant estimates made by management, as well as evaluating the overall presentation of the consolidated financial statements. Our audit of internal control over financial reporting included obtaining an understanding of internal control over financial reporting, assessing the risk that a material weakness exists, and testing and evaluating the design and operating effectiveness of internal control based on the assessed risk. Our audits also included performing such other procedures as we considered necessary in the circumstances. We believe that our audits provide a reasonable basis for our opinions.

Definition and Limitations of Internal Control over Financial Reporting

A company's internal control over financial reporting is a process designed to provide reasonable assurance regarding the reliability of financial reporting and the preparation of financial statements for external purposes in accordance with generally accepted accounting principles. A company's internal control over financial reporting includes those policies and procedures that (i) pertain to the maintenance of records that, in reasonable detail, accurately and fairly reflect the transactions and dispositions of the assets of the company; (ii) provide reasonable assurance that transactions are recorded as necessary to permit preparation of financial statements in accordance with generally accepted accounting principles, and that receipts and expenditures of the company are being made only in accordance with authorizations of management and directors of the company; and (iii) provide reasonable assurance regarding prevention or timely detection of unauthorized acquisition, use, or disposition of the company's assets that could have a material effect on the financial statements.

Because of its inherent limitations, internal control over financial reporting may not prevent or detect misstatements. Also, projections of any evaluation of effectiveness to future periods are subject to the risk that controls may become inadequate because of changes in conditions, or that the degree of compliance with the policies or procedures may deteriorate.

Critical Audit Matters

The critical audit matter communicated below is a matter arising from the current period audit of the consolidated financial statements that was communicated or required to be communicated to the audit committee and that (i) relates to accounts or

disclosures that are material to the consolidated financial statements and (ii) involved our especially challenging, subjective, or complex judgments. The communication of critical audit matters does not alter in any way our opinion on the consolidated financial statements, taken as a whole, and we are not, by communicating the critical audit matter below, providing a separate opinion on the critical audit matter or on the accounts or disclosures to which it relates.

Sales Recognition

As described in Notes 1 and 6 to the consolidated financial statements, revenue is generated primarily from contracts to produce, ship and deliver steel products, and to a lesser extent, raw materials such as iron ore pellets and coke by-products. Sales are recognized when the Company's performance obligations are satisfied. Generally, the Company's performance obligations are satisfied, control of products is transferred, and revenue is recognized at a single point in time, when title transfers to customers for products shipped. For the year ended December 31, 2024, the Company's net sales were $15.6 billion.

The principal consideration for our determination that performing procedures relating to sales recognition is a critical audit matter is a high degree of auditor effort in performing procedures related to the Company's sales recognition.

Addressing the matter involved performing procedures and evaluating audit evidence in connection with forming our overall opinion on the consolidated financial statements. These procedures included testing the effectiveness of controls relating to the sales recognition process. These procedures also included, among others, for certain components (i) evaluating revenue transactions by testing the issuance and settlement of invoices and credit memos (ii) tracing transactions not settled to a detailed listing of accounts receivable, (iii) testing the completeness and accuracy of data provided by management, and (iv) confirming a sample of outstanding customer invoice balances as of December 31, 2024 and, for confirmations not returned, obtaining and inspecting source documents, such as invoices, delivery documents, and subsequent cash receipts. For other components, these procedures included testing the revenue recognized for a sample of revenue transactions by obtaining and inspecting source documents, such as evidence of customer arrangement, invoices, proof of shipment, and subsequent cash receipts.

/s/ PricewaterhouseCoopers LLP
Pittsburgh, Pennsylvania
January 31, 2025

We have served as the Company's auditor since 1903.

UNITED STATES STEEL CORPORATION
CONSOLIDATED STATEMENTS OF OPERATIONS

(Dollars in millions, except per share amounts)	Year Ended December 31, 2024	2023	2022
Net sales:			
Net sales	**$ 13,135**	$ 16,038	$ 19,123
Net sales to related parties (Note 23)	**2,505**	2,015	1,942
Total (Note 6)	**15,640**	18,053	21,065
Operating expenses (income):			
Cost of sales (excludes items shown below)	**14,060**	15,803	16,777
Selling, general and administrative expenses	**435**	501	422
Depreciation, depletion and amortization (Notes 13 and 14)	**913**	916	791
Earnings from investees (Note 12)	**(112)**	(115)	(243)
Asset impairment charges (Note 1)	**19**	129	163
Restructuring and other charges (Note 25)	**8**	36	48
Gain on equity investee transactions (Note 12)	**—**	—	(6)
Other losses (gains), net	**77**	(16)	(47)
Total	**15,400**	17,254	17,905
Earnings before interest and income taxes	**240**	799	3,160
Interest expense	**24**	72	159
Interest income	**(96)**	(141)	(44)
Loss on debt extinguishment (Note 7)	**2**	—	—
Other financial costs	**29**	18	32
Net periodic benefit income	**(132)**	(154)	(246)
Net gain from investments related to active employee benefits (Note 20)	**(25)**	(43)	—
Net interest and other financial (benefits) costs (Note 7)	**(198)**	(248)	(99)
Earnings before income taxes	**438**	1,047	3,259
Income tax expense (Note 11)	**54**	152	735
Net earnings	**384**	895	2,524
Less: Net earnings attributable to noncontrolling interests	**—**	—	—
Net earnings attributable to United States Steel Corporation	**$ 384**	$ 895	$ 2,524
Earnings per common share (Note 8)			
Earnings per share attributable to United States Steel Corporation stockholders:			
— Basic	**$ 1.71**	$ 3.98	$ 10.22
— Diluted	**$ 1.57**	$ 3.56	$ 9.16

The accompanying notes are an integral part of these Consolidated Financial Statements.

UNITED STATES STEEL CORPORATION
CONSOLIDATED STATEMENTS OF COMPREHENSIVE INCOME (LOSS)

(Dollars in millions)	Year Ended December 31, 2024	2023	2022
Net earnings	**$ 384**	$ 895	$ 2,524
Other comprehensive income (loss), net of tax:			
Changes in foreign currency translation adjustments [(a)]	**(70)**	54	(91)
Changes in pension and other employee benefit accounts [(a)]	**(77)**	81	(297)
Changes in derivative financial instruments [(a)]	**80**	(9)	(28)
Changes in fair value of active employee benefit investments [(a)]	**—**	5	—
Total other comprehensive income (loss), net of tax	**(67)**	131	(416)
Comprehensive income including noncontrolling interest	**317**	1,026	2,108
Comprehensive income attributable to noncontrolling interest	**—**	—	—
Comprehensive income attributable to United States Steel Corporation	**$ 317**	$ 1,026	$ 2,108

[(a)] Related income tax (provision) benefit:

Foreign currency translation adjustments	**$ 28**	$ (3)	$ 24
Pension and other benefits adjustments	**23**	(24)	95
Derivative adjustments	**(26)**	3	9
Active employee benefit investments	**—**	(2)	—

The accompanying notes are an integral part of these Consolidated Financial Statements.

UNITED STATES STEEL CORPORATION
CONSOLIDATED BALANCE SHEETS

(Dollars in millions)	December 31, 2024	2023
Assets		
Current assets:		
Cash and cash equivalents (Note 9)	**$ 1,367**	$ 2,948
Receivables, less allowance of $26 and $38	**1,236**	1,390
Receivables from related parties (Note 23)	**162**	158
Inventories (Note 10)	**2,168**	2,128
Other current assets	**299**	319
Total current assets	**5,232**	6,943
Long-term restricted cash (Note 9)	**35**	32
Investments and long-term receivables, less allowance of $3 in both periods (Note 12)	**757**	761
Operating lease assets (Note 24)	**72**	109
Property, plant and equipment, net (Note 13)	**11,973**	10,393
Intangibles, net (Note 14)	**416**	436
Deferred income tax benefits (Note 11)	**—**	19
Goodwill (Note 14)	**920**	920
Other noncurrent assets	**830**	838
Total assets	**$ 20,235**	$ 20,451
Liabilities		
Current liabilities:		
Accounts payable and other accrued liabilities	**$ 2,601**	$ 2,889
Accounts payable to related parties (Note 23)	**146**	139
Payroll and benefits payable	**295**	442
Accrued taxes	**131**	222
Accrued interest	**70**	70
Current operating lease liabilities (Note 24)	**35**	44
Short-term debt and current maturities of long-term debt (Note 17)	**95**	142
Total current liabilities	**3,373**	3,948
Noncurrent operating lease liabilities (Note 24)	**44**	73
Long-term debt, less unamortized discount and debt issuance costs (Note 17)	**4,078**	4,080
Employee benefits (Note 18)	**117**	126
Deferred income tax liabilities (Note 11)	**657**	587
Deferred credits and other noncurrent liabilities	**526**	497
Total liabilities	**8,795**	9,311
Contingencies and commitments (Note 26)		
Stockholders' Equity		
Common stock issued — 288,088,141 and 285,959,739 shares issued (par value $1 per share, authorized 400,000,000 shares) (Note 8)	**288**	286
Treasury stock, at cost (62,907,930 shares and 62,288,523 shares)	**(1,446)**	(1,418)
Additional paid-in capital	**5,307**	5,253
Retained earnings	**7,219**	6,880
Accumulated other comprehensive (loss) income (Note 21)	**(21)**	46
Total United States Steel Corporation stockholders' equity	**11,347**	11,047
Noncontrolling interests	**93**	93
Total liabilities and stockholders' equity	**$ 20,235**	$ 20,451

The accompanying notes are an integral part of these Consolidated Financial Statements.

UNITED STATES STEEL CORPORATION
CONSOLIDATED STATEMENTS OF CASH FLOWS

(Dollars in millions)	Year Ended December 31, 2024	2023	2022
Increase (decrease) in cash and cash equivalents			
Operating activities:			
Net earnings	**$ 384**	$ 895	$ 2,524
Adjustments to reconcile net cash provided by operating activities:			
Depreciation, depletion and amortization (Notes 13 and 14)	**913**	916	791
Asset impairment charges (Note 1)	**19**	129	163
Gain on equity investee transactions (Note 12)	**—**	—	(6)
Restructuring and other charges (Note 25)	**8**	36	48
Loss on debt extinguishment (Note 7)	**2**	—	—
Pensions and other post-employment benefits	**(133)**	(157)	(213)
Active employee benefit investments	**65**	32	—
Deferred income taxes (Note 11)	**113**	97	501
Net gain on sale of assets	**(5)**	(6)	(12)
Equity investees earnings, net of distributions received	**(3)**	(1)	(215)
Changes in:			
Current receivables	**174**	103	370
Inventories	**(71)**	257	(222)
Current accounts payable and accrued expenses	**(285)**	25	(180)
Income taxes receivable/payable	**(126)**	(27)	(15)
All other, net	**(136)**	(199)	(29)
Net cash provided by operating activities	**919**	2,100	3,505
Investing activities:			
Capital expenditures	**(2,287)**	(2,576)	(1,769)
Proceeds from cost reimbursement government grants (Note 26)	**—**	—	54
Proceeds from sale of assets	**5**	8	32
Proceeds from sale of ownership interests in equity investees (Note 12)	**—**	—	12
Other investing activities	**6**	—	(8)
Net cash used in investing activities	**(2,276)**	(2,568)	(1,679)
Financing activities:			
Issuance of long-term debt, net of financing costs (Note 17)	**—**	241	343
Repayment of long-term debt (Note 17)	**(128)**	(89)	(382)
Common stock repurchased (Note 27)	**—**	(175)	(849)
Proceeds from government incentives (Note 26)	**—**	—	82
Other financing activities	**(71)**	(75)	(62)
Net cash used in financing activities	**(199)**	(98)	(868)
Effect of exchange rate changes on cash	**(19)**	15	(19)
Net (decrease) increase in cash, cash equivalents and restricted cash	**(1,575)**	(551)	939
Cash, cash equivalents and restricted cash at beginning of year (Note 9)	**2,988**	3,539	2,600
Cash, cash equivalents and restricted cash at end of year (Note 9)	**$ 1,413**	$ 2,988	$ 3,539

See Note 22 for supplemental cash flow information.

The accompanying notes are an integral part of these Consolidated Financial Statements.

UNITED STATES STEEL CORPORATION
CONSOLIDATED STATEMENTS OF STOCKHOLDERS' EQUITY

	Dollars in Millions			Shares in Thousands		
	2024	**2023**	**2022**	**2024**	**2023**	**2022**
Common stock:						
Balance at beginning of year	**$ 286**	$ 283	$ 280	**285,960**	282,487	279,522
Common stock issued	**2**	3	3	**2,128**	3,473	2,965
Balance at end of year	**$ 288**	$ 286	$ 283	**288,088**	285,960	282,487
Treasury stock:						
Balance at beginning of year	**$ (1,418)**	$ (1,204)	$ (334)	**(62,289)**	(54,090)	(15,709)
Common stock repurchased	**—**	(175)	(849)	**—**	(7,092)	(37,559)
Excise tax on common stock repurchased	**—**	(1)	—	**—**	—	—
Common stock repurchased for employee/ non-employee director stock plans	**(28)**	(38)	(21)	**(619)**	(1,107)	(822)
Balance at end of year	**$ (1,446)**	$ (1,418)	$ (1,204)	**(62,908)**	(62,289)	(54,090)
Additional paid-in capital:						
Balance at beginning of year	**$ 5,253**	$ 5,194	$ 5,199			
Employee stock plans	**54**	59	72			
Cumulative effect upon adoption of Accounting Standards Update 2020-06	**—**	—	(77)			
Balance at end of year	**$ 5,307**	$ 5,253	$ 5,194			

The accompanying notes are an integral part of these Consolidated Financial Statements.

UNITED STATES STEEL CORPORATION
CONSOLIDATED STATEMENTS OF STOCKHOLDERS' EQUITY

(Continued)

(Dollars in millions)	2024	2023	2022	Comprehensive Income (Loss) 2024	2023	2022
Retained earnings:						
Balance at beginning of year	**$ 6,880**	$ 6,030	$ 3,534			
Net earnings attributable to United States Steel Corporation	**384**	895	2,524	**$ 384**	$ 895	$ 2,524
Dividends on common stock	**(46)**	(45)	(50)			
Cumulative effect upon adoption of Accounting Standards Update 2020-06	**—**	—	22			
Other	**1**	—	—			
Balance at end of year	**$ 7,219**	$ 6,880	$ 6,030			
Accumulated other comprehensive (loss) income:						
Pension and other benefit adjustments (Note 18):						
Balance at beginning of year	**$ (241)**	$ (322)	$ (25)			
Changes during year, net of taxes [(a)]	**(77)**	79	(304)	**(77)**	79	(304)
Changes during year, equity investee net of taxes [(a)]	**—**	2	7	**—**	2	7
Balance at end of year	**$ (318)**	$ (241)	$ (322)			
Foreign currency translation adjustments:						
Balance at beginning of year	**$ 334**	$ 280	$ 371			
Changes during year, net of taxes [(a)]	**(70)**	54	(91)	**(70)**	54	(91)
Balance at end of year	**$ 264**	$ 334	$ 280			
Derivative financial instruments:						
Balance at beginning of year	**$ (52)**	$ (43)	$ (15)			
Changes during year, net of taxes [(a)]	**80**	(9)	(28)	**80**	(9)	(28)
Balance at end of year	**$ 28**	$ (52)	$ (43)			
Fair value of active employee benefit investments						
Balance at beginning of year	**$ 5**	$ —	$ —			
Changes during year, net of taxes [(a)]	**—**	5	—	**—**	5	—
Balance at end of year	**$ 5**	$ 5	$ —			
Total balances at end of year	**$ (21)**	$ 46	$ (85)			
Total stockholders' equity	**$ 11,347**	$ 11,047	$ 10,218			
Noncontrolling interests:						
Balance at beginning of year	**$ 93**	$ 93	$ 93			
Net loss	**—**	—	—	**—**	—	—
Balance at end of year	**$ 93**	$ 93	$ 93			
Total comprehensive income				**$ 317**	$ 1,026	$ 2,108

[(a)] Related income tax (provision) benefit:

Foreign currency translation adjustments	**$ 28**	$ (3)	$ 24
Pension and other benefits adjustments	**23**	(24)	95
Derivative adjustments	**(26)**	3	9
Active employee benefit investments	**—**	(2)	—

The accompanying notes are an integral part of these Consolidated Financial Statements.

1. Nature of Business and Significant Accounting Policies

Nature of Business

U. S. Steel produces and sells steel products, including flat-rolled and tubular products, in North America and Europe. Operations in the United States also include iron ore and coke production facilities and real estate operations. Operations in Europe also include coke production facilities.

Agreement and Plan of Merger with Nippon Steel Corporation

On December 18, 2023, the Company entered into an Agreement and Plan of Merger (such agreement, as it may be amended, modified or supplemented from time to time, the "Merger Agreement") by and among the Company, Nippon Steel North America, Inc., a New York corporation ("Purchaser"), 2023 Merger Subsidiary, Inc., a Delaware corporation and a wholly owned subsidiary of Purchaser ("Merger Sub"), and solely as provided in Section 9.13 therein, Nippon Steel Corporation, a Japanese corporation ("NSC"). Pursuant to the Merger Agreement, and upon the terms and subject to the conditions described therein, Merger Sub will merge with and into the Company, with the Company continuing as the surviving corporation and a wholly owned subsidiary of Purchaser (the "Merger"). On April 12, 2024, the Company obtained the approval of its stockholders required to adopt the Merger Agreement. U. S. Steel stockholders approved the Merger with 98.8% approval of shares voted, satisfying a condition to closing. Subject to the terms and conditions set forth in the Merger Agreement, each share of the Company's common stock, par value $1.00 per share, outstanding immediately prior to the effective time of the Merger (the "Effective Time") will, at the Effective Time, automatically be converted into the right to receive $55.00 per share in cash, without interest, subject to any required tax withholding. The parties have substantially complied with the Second Requests issued on April 3, 2024 and the statutory waiting period under the Hart-Scott-Rodino Antitrust Improvements Act of 1976 has now expired. In addition, all required regulatory approvals outside of the United States related to the Merger have been received. The closing conditions to the Merger include clearance by the Committee on Foreign Investment in the United States ("CFIUS") under the Defense Production Act of 1950, as amended. Following referral of the transaction by CFIUS to the President of the United States, on January 3, 2025, the President of the United States issued an order prohibiting the Merger and requiring the parties to abandon the Merger Agreement within thirty days. On January 10, 2025, CFIUS granted an extension of that deadline to June 18, 2025.

On January 6, 2025, the Company, NSC and Purchaser jointly filed a lawsuit in the United States Court of Appeals for the District of Columbia Circuit alleging that the President's order blocking the Merger was issued for purely political reasons, which are irrelevant to U.S. national security, challenging the decision by the President of the United States and the CFIUS process as violating the constitutional due process rights of the Company, NSC and Purchaser, as well as the applicable statute governing CFIUS and the Administrative Procedure Act, and as an exercise of power that exceeded the authority provided to the President of the United States under the applicable statute. The Company, NSC and Purchaser also filed a lawsuit in the United States District Court for the Western District of Pennsylvania against Cleveland-Cliffs Inc. ("Cliffs"), Cliffs' Chief Executive Officer Lourenco Goncalves, and the President of the United Steel, Paper and Forestry, Rubber, Manufacturing, Energy, Allied Industrial and Service Workers International Union (the "USW"), David McCall, for engaging in a coordinated series of anticompetitive and racketeering activities illegally designed to prevent any party other than Cliffs from acquiring the Company as part of an illegal campaign to monopolize critical domestic steel markets.

The Merger Agreement requires us to operate in the ordinary course of business and restricts us, without the consent of Purchaser, from taking certain specified actions agreed by the parties to be outside the ordinary course of business until the pending Merger occurs or the Merger Agreement terminates.

Significant Accounting Policies

Principles applied in consolidation

These financial statements include the accounts of U. S. Steel and its majority-owned subsidiaries. Additionally, variable interest entities for which U. S. Steel is the primary beneficiary are included in the Consolidated Financial Statements, and their impacts are either partially or completely offset by noncontrolling interests. Intercompany accounts, transactions and profits have been eliminated in consolidation.

Investments in entities over which U. S. Steel has significant influence are accounted for using the equity method of accounting and are carried at U. S. Steel's share of net assets plus loans, advances and our share of earnings less distributions.

Earnings or loss from investees includes U. S. Steel's share of earnings or loss from equity method investments (and any amortization of basis differences), which are generally recorded a month in arrears.

Use of estimates

Generally accepted accounting principles require management to make estimates and assumptions that affect the reported amounts of assets and liabilities, the disclosure of contingent assets and liabilities at year-end and the reported amounts of revenues and expenses during the year. Significant items subject to such estimates and assumptions include the carrying value of property, plant and equipment; intangible assets; the fair value of assets or liabilities acquired in a business combination; valuation allowances for receivables, inventories and deferred income tax assets and liabilities;

environmental liabilities; liabilities for potential tax deficiencies; potential litigation claims and settlements; assets and obligations related to employee benefits; and restructuring and other charges. Actual results could differ materially from the estimates and assumptions used.

The preparation of the financial statements includes an assessment of certain accounting matters using all available information including consideration of forecasted financial information in context with other information reasonably available to us. However, our future assessment of current expectations could result in material impacts to our consolidated financial statements in future reporting periods. All such adjustments are of a normal recurring nature unless disclosed otherwise.

Sales recognition
Sales are recognized when U. S. Steel's performance obligations are satisfied. Generally, U. S. Steel's performance obligations are satisfied, control of our products is transferred, and revenue is recognized at a single point in time, when title transfers to our customer for product shipped or when services are provided. Revenues are recorded net of any sales incentives. Shipping and other transportation costs charged to customers are treated as fulfillment activities and are recorded as additional net sales and cost of sales at the time control is transferred to the customer. See Note 6 for further details on U. S. Steel's revenue.

Inventories
Inventories are carried at the lower of cost or net realizable value. Fixed costs related to abnormal production capacity are expensed in the period incurred rather than capitalized into inventory.

LIFO is the predominant method of inventory costing for inventories held by the Flat-Rolled and Tubular segments. The Mini Mill segment uses a moving average costing method to account for semi-finished and finished products and FIFO to account for raw materials. FIFO is the predominant method used by the USSE segment. The LIFO method of inventory costing was used on 53 percent of consolidated inventories at both December 31, 2024, and December 31, 2023.

Derivative instruments
From time to time, U. S. Steel may use fixed price forward physical purchase contracts to partially manage our exposure to price risk. Generally, forward physical purchase contracts qualify for the normal purchase normal sales exclusion in Accounting Standards Codification (ASC) 815, *Derivatives and Hedging*, and are not subject to mark-to-market accounting. U. S. Steel also uses derivatives such as commodity-based financial swaps and foreign currency exchange forward contracts to manage its exposure to purchase and sale price fluctuations and foreign currency exchange rate risk. The USSE and Flat-Rolled segments elect hedge accounting for some of their derivatives. Under hedge accounting, fluctuations in the value of the derivative are recognized in Accumulated other comprehensive (loss) income (AOCI) until the associated underlying is recognized in earnings. When the associated underlying is recognized in earnings, the value of the derivative is reclassified to earnings from AOCI. The Mini Mill segment has not elected hedge accounting. Therefore, the changes in fair value of the Mini Mill segment's foreign exchange forwards, as well as fair value changes for other derivatives where hedge accounting has not been elected, are recognized immediately in earnings. In accordance with the guidance in ASC Topic 820 on fair value measurements and disclosures, the fair value of our foreign exchange forwards, commodity purchase swaps and sales swaps was determined using Level 2 inputs, which are defined as "significant other observable" inputs. The inputs used are from market sources that aggregate data based upon market transactions. See Note 16 for further details on U. S. Steel's derivatives.

Property, plant and equipment
Property, plant and equipment is carried at cost less accumulated depreciation and is depreciated on a straight-line basis over the estimated useful lives of the assets.

Depletion of mineral properties is based on rates which are expected to amortize cost over the estimated tonnage of minerals to be removed.

When property, plant and equipment is sold or otherwise disposed of, any gains or losses are reflected in income. If a loss on disposal is expected, such losses are recognized when the assets are reclassified as assets held for sale or when impaired as part of an asset group's impairment.

Asset Impairment
U. S. Steel evaluates impairment of its property, plant and equipment whenever circumstances indicate that the carrying value may not be recoverable. We evaluate the impairment of long-lived assets at the asset group level. Our asset groups are flat-rolled, mini mill, USSE, welded tubular and seamless tubular. Asset impairments are recognized when the carrying value of an asset group exceeds its recoverable amount as determined by the asset group's aggregate projected undiscounted cash flows.

In 2024, the Company recognized impairment charges of approximately $19 million for certain indefinitely idled assets at Granite City Works and certain permanently idled assets at Clairton Works. In 2023, the Company recognized charges of approximately $123 million for impairment of indefinitely idled iron and steel making assets at Granite City Works. In 2022,

impairment charges of approximately $151 million were recognized for the write-off of iron making and steel making assets at Great Lakes Works. The coil finishing processes at Granite City Works and Great Lakes Works continue to operate and remain components of the Company's operating plans. These impairment charges were the result of actions impacting specific, identifiable assets and were not significantly adverse to the flat-rolled long-lived asset group.

No triggering events that required an impairment evaluation of our long-lived asset groups were identified during the years ended December 31, 2024, 2023, and 2022.

Goodwill and identifiable intangible assets

Goodwill represents the excess of the cost over the fair value of acquired identifiable tangible and intangible assets and liabilities assumed from businesses acquired. Goodwill and intangible assets deemed to have indefinite lives are not amortized, but are subject to impairment testing annually, or more frequently if events or changes in circumstances indicate the asset might be impaired. We perform our annual goodwill impairment test as of October 1 and monitor for interim triggering events on an ongoing basis.

We review goodwill for impairment utilizing either a qualitative assessment or a quantitative goodwill impairment test. If we choose to perform a qualitative assessment and we determine that the fair value of the reporting unit more likely than not exceeds the carrying value, no further evaluation is necessary. For reporting units where we perform the quantitative goodwill impairment test, we compare the fair value of each reporting unit, which we primarily determine using an income approach based on the present value of discounted cash flows, to the respective carrying value, which includes goodwill. If the fair value of the reporting unit exceeds its carrying value, we do not consider the goodwill impaired. If the carrying value is higher than the fair value, we recognize the difference as an impairment loss.

A quantitative goodwill impairment testing process requires valuation of the respective reporting unit, which we primarily determine using an income approach based on a discounted cash flow forecast covering discrete periods of time including a terminal value. We compute the terminal value using the constant growth method, which values the forecasted cash flows in perpetuity. The assumptions about future cash flows and growth rates are based on the respective reporting unit's long-term forecast and are subject to review and approval by senior management. A reporting unit's discount rate is a significant assumption and is a risk-adjusted weighted average cost of capital, which we believe approximates the rate from a market participant's perspective. The estimated fair value could be impacted by changes in market conditions, interest rates, growth rates, tax rates, costs, pricing and capital expenditures. We categorize the fair value determination as Level 3 in the fair value hierarchy due to its use of internal projections and unobservable measurement inputs.

Our Mini Mill reporting unit holds the goodwill recognized as a result of the Company's acquisition of Big River Steel and currently is our only reporting unit that has a significant amount of goodwill. This goodwill is primarily attributable to Big River Steel's operational abilities, workforce and the anticipated benefits from their recent expansion. U. S. Steel completed its annual goodwill impairment test using a qualitative assessment during the fourth quarter of 2024 and determined there was no impairment of goodwill.

Intangible assets with indefinite lives are also subject to at least annual impairment testing, which compares the fair value of the intangible assets with their carrying amounts. U. S. Steel has determined that certain of its acquired intangible assets have indefinite useful lives. These assets are also reviewed for impairment annually in the fourth quarter and whenever events or circumstances indicate the carrying value may not be recoverable. U. S. Steel completed its annual evaluation of its indefinite-lived water rights using a qualitative assessment and determined there was no indication of impairment.

Finite-lived intangible assets are amortized on a straight-line basis over their estimated useful lives and are tested for impairment when events occur that indicate that the net book value will not be recovered over future cash flows.

Environmental remediation

Environmental expenditures are capitalized if the costs mitigate or prevent future contamination or if the costs improve existing assets' environmental safety or efficiency. U. S. Steel provides for remediation costs and penalties when the responsibility to remediate is probable and the amount of associated costs is reasonably estimable. The timing of remediation accruals typically coincides with completion of studies defining the scope of work to be undertaken or when it is probable that a formal plan of action will be approved by the oversight agency. Remediation liabilities are accrued based on estimates of believed environmental exposure and are discounted if the amount and timing of the cash disbursements are readily determinable.

Asset retirement obligations

Asset retirement obligations (AROs) are initially recorded at fair value and are capitalized as part of the cost of the related long-lived asset and depreciated in accordance with U. S. Steel's depreciation policies for property, plant and equipment. The fair value of the obligation is determined as the discounted value of expected future cash flows. Accretion expense is recorded each month to increase this discounted obligation over time. Certain AROs related to disposal costs of the majority of assets at our integrated steel facilities are not recorded because they have an indeterminate settlement date.

These AROs will be initially recognized in the period in which sufficient information exists to estimate their fair value. See Note 19 for further details on U. S. Steel's AROs.

Pensions and other post-employment benefits
U. S. Steel has defined contribution plans or multiemployer arrangements for pension benefits for more than 85 percent of its employees in the United States and defined benefit pension plans covering the remaining employees. For hires before January 1, 2016, U. S. Steel has defined benefit retiree health care and life insurance plans (Other Benefits) that cover its represented employees in North America upon their retirement. Government-sponsored programs into which U. S. Steel makes required contributions cover U. S. Steel's European employees. For more details regarding pension and other post-employment benefits see Note 18 of the Consolidated Financial Statements.

The pension and Other Benefits obligations and the related net periodic benefit costs are based on, among other things, assumptions regarding the discount rate, estimated return on plan assets, salary increases, the projected mortality of participants and the current level and future escalation of health care costs. Additionally, U. S. Steel recognizes an obligation to provide post-employment benefits for disability-related claims covering indemnity and medical payments for certain employees in North America. The obligation for these claims and the related periodic costs are measured using actuarial techniques and assumptions. Actuarial gains and losses occur when actual experience differs from any of the many assumptions used to value the benefit plans, or when assumptions change. For pension and Other Benefits, the Company recognizes into income on an annual basis a portion of unrecognized actuarial net gains or losses that exceed 10 percent of the larger of projected benefit obligations or plan assets (the corridor). These unrecognized amounts in excess of the corridor are amortized over the plan participants' average life expectancy or average future service, depending on the demographics of the plan. Unrecognized actuarial net gains and losses for disability-related claims are immediately recognized into income.

Deferred taxes
Deferred tax assets and liabilities are recognized for the estimated future tax consequences attributable to differences between the financial statement carrying amounts of existing assets and liabilities and their respective tax basis. The realization of deferred tax assets is assessed quarterly based on several interrelated factors. These factors include U. S. Steel's expectation to generate sufficient future taxable income and the projected time period over which these deferred tax assets will be realized. U. S. Steel records a valuation allowance when necessary to reduce deferred tax assets to the amount that will more likely than not be realized. See Note 11 for further details of deferred taxes.

2. New Accounting Standards

During the year ended December 31, 2024, there were no new accounting standards and interpretations issued which are expected to have a material impact on the Company's financial position, operations or cash flows.

In November 2024, the Financial Accounting Standards Board (FASB) issued Accounting Standards Update 2024-03, *Disaggregation of Income Statement Expenses* (ASU 2024-03). ASU 2024-03 includes requirements that an entity disclose in the notes to the financial statements specified information about certain costs and expenses, including the amounts of (a) purchases of inventory, (b) employee compensation, (c) depreciation, (d) intangible asset amortization, and (e) other amounts of depletion expense included in each relevant expense caption presented on the statement of operations. The standard also requires disclosure of a qualitative description of the amounts remaining in relevant expense captions that are not separately disaggregated quantitatively, as well as the total amount of selling expenses and an entity's definition of selling expenses. ASU 2024-03 is effective for annual periods beginning after December 15, 2026, and interim periods beginning after December 15, 2027. U. S. Steel is currently assessing the impact of ASU 2024-03 on its disclosures.

In March 2024, the SEC adopted final rules that would require certain climate related disclosures. Certain disclosures would be required in the notes to the financial statements beginning in fiscal year 2025. The audited financial statement disclosures include capitalized costs and expenses related to severe weather events and other natural conditions subject to certain materiality thresholds. Beginning in annual disclosures for fiscal year 2026, certain greenhouse gas emission disclosures would also be required. In April 2024, the SEC issued a stay on the rules until legal challenges to the rule are addressed. U. S. Steel is monitoring the legal challenges and assessing the impact of the rules on its disclosures.

In December 2023, the FASB issued Accounting Standards Update 2023-09, *Improvements to Income Tax Disclosures* (ASU 2023-09). ASU 2023-09 includes requirements that an entity disclose specific categories in the rate reconciliation and provide additional information for reconciling items that are greater than five percent of the amount computed by multiplying pretax income (or loss) by the applicable statutory income tax rate. The standard also requires that entities disclose income (or loss) from continuing operations before income tax expense (or benefit) and income tax expense (or benefit) each disaggregated between domestic and foreign. In addition, the standard also requires disclosure of payment amounts disaggregated by federal, state, and foreign, as well as by major jurisdiction. ASU 2023-09 is effective for annual periods beginning after December 15, 2024. U. S. Steel is currently assessing the impact of ASU 2023-09 on its disclosures.

3. Recently Adopted Accounting Standards

In November 2023, the FASB issued Accounting Standards Update 2023-07, *Improvements to Reportable Segment Disclosures* (ASU 2023-07). ASU 2023-07 includes requirements that an entity disclose the title of the chief operating decision maker (CODM) and on an interim and annual basis, significant segment expenses and the composition of other segment items for each segment's reported profit. The standard also permits disclosure of additional measures of segment profit. ASU 2023-07 is effective for fiscal years beginning after December 15, 2023, and interim periods within fiscal years beginning after December 15, 2024. U. S. Steel adopted this guidance effective January 1, 2024 for annual reporting and applied the amendments retrospectively to all prior periods presented in the Consolidated Financial Statements. The amendments for interim periods will be adopted in our fiscal year beginning on January 1, 2025. See Note 4 for further details on segment information.

In September 2022, the FASB issued Accounting Standards Update 2022-04, *Disclosure of Supplier Finance Program Obligations* (ASU 2022-04). ASU 2022-04 requires that an entity disclose certain information about supplier finance programs used in connection with the purchase of goods and services. ASU 2022-04 is effective for all entities with fiscal years beginning after December 15, 2022, and interim periods within those fiscal years, except for the amendment on roll-forward information, which is effective for fiscal years beginning after December 15, 2023. U. S. Steel adopted this guidance effective January 1, 2023, with the exception of the amendment on roll-forward information, which was adopted in our fiscal year beginning on January 1, 2024 and applied prospectively.

The Company has a SCF arrangement with a third-party administrator which allows participating suppliers, at their sole discretion, to make offers to sell payment obligations of the Company prior to their scheduled due dates at a discounted price to a participating financial institution. The third-party administrator entered into a separate agreement with the Export Import Bank of the United States to guarantee 90 percent of supplier obligations sold for up to $95 million. No guarantees or collateral are provided by the Company or any of its subsidiaries under the SCF program, and the Company does not benefit from any preferential payment terms or discounts as a result of supplier participation.

The Company's goal is to capture overall supplier savings and improve working capital efficiency. The agreements facilitate the suppliers' ability to sell payment obligations, while providing them with greater working capital flexibility. The Company has no economic interest in the sale of the suppliers' receivables and no direct financial relationship with the financial institution concerning these services. The Company's obligations to its suppliers, including amounts due and scheduled payment dates, are not impacted by suppliers' decisions to sell amounts under the arrangements. The SCF program requires the Company to pay the third-party administrator the stated amount of the confirmed participating supplier invoices. The payment terms for confirmed invoices range from 45 to 90 days after the end of the month in which the invoice was issued.

The underlying costs from suppliers that elected to participate in the SCF program are generally recorded in cost of sales in the Company's Consolidated Statements of Operations. Amounts due to suppliers who participate in the SCF program are reflected in accounts payable and accrued expenses on the Company's Consolidated Balance Sheets and payments on the obligations by our suppliers are included in cash used in operating activities in the Consolidated Statements of Cash Flows. As of December 31, 2024 and 2023, accounts payable and accrued expenses included $62 million and $84 million, respectively, of outstanding payment obligations which suppliers elected to sell to participating financial institutions.

The roll-forward of the Company's outstanding obligations confirmed as valid under its SCF program for the year ended December 31, 2024 is as follows:

(in millions)	2024
Confirmed obligations outstanding at beginning of year	$197
Invoices confirmed	936
Invoices paid	(935)
Confirmed obligations outstanding at end of year	$198

4. Segment Information

U. S. Steel has four reportable segments: North American Flat-Rolled, Mini Mill, USSE and Tubular Products. The results of our real estate business are disclosed in the Other category. The majority of U. S. Steel's customers are located in North America and Europe. During 2024, sales to one customer of the Flat-Rolled segment were approximately $2.0 billion which exceeded 10 percent of U. S. Steel's total consolidated net sales. During 2023 and 2022, no single customer accounted for more than 10 percent of U. S. Steel's total consolidated net sales.

The Flat-Rolled segment includes the operating results of U. S. Steel's integrated steel plants and equity investees in the United States involved in the production of slabs, strip mill plates, sheets and tin mill products, as well as all pig iron, iron

ore and coke production facilities in the United States. These operations primarily serve North American customers in the service center, conversion, transportation (including automotive), construction, container and appliance and electrical markets.

The Mini Mill segment includes the operating results of Big River Steel and BR2 steel plants in Osceola, Arkansas involved in the production of sheets and electrical products. These operations primarily serve North American customers in the service center, conversion, transportation (including automotive), construction, container and appliance and electrical markets.

The USSE segment includes the operating results of USSK, U. S. Steel's integrated steel plant and coke production facilities in Slovakia, and its subsidiaries. USSE conducts its business mainly in Central and Western Europe and primarily serves customers in the European transportation (including automotive), construction, container, appliance, electrical, service center, conversion and oil, gas and petrochemical markets. USSE produces and sells slabs, hot-rolled, cold-rolled, hot-dip galvanized, and color-coated coils, electrical steel, strip mill plate, sheet, tin mill products and spiral welded pipe.

The Tubular segment includes the operating results of U. S. Steel's tubular production facilities and an equity investee in the United States. These operations produce and sell rounds, seamless and electric resistance welded (welded) steel casing and tubing (commonly known as oil country tubular goods or OCTG), standard and line pipe and mechanical tubing and primarily serve customers in the oil, gas and petrochemical markets. The Tubular segment includes the electric arc furnace at our Fairfield Tubular Operations in Fairfield, Alabama.

The chief operating decision maker (CODM), which is the chief executive officer, evaluates segment performance and determines resource allocations based on a number of factors, the primary measure being earnings (loss) before interest, taxes, depreciation and amortization (EBITDA). EBITDA for reportable segments and the Other category does not include net interest and other financial costs (income), income taxes, and certain other items that management believes are not indicative of future results. For all of the segments, the CODM uses segment EBITDA in the Annual Operating Plan (AOP) and monthly performance review processes. The CODM considers AOP-to-actual EBITDA variances on a monthly, quarter-to-date, and year-to-date basis for evaluating performance of each segment and making decisions about resource allocations to each segment.

The accounting principles applied at the operating segment level in determining earnings (loss) before interest, taxes, depreciation and amortization are generally the same as those applied at the consolidated financial statement level. Intersegment sales and transfers are accounted for at market-based prices and are eliminated at the corporate consolidation level. Corporate-level selling, general and administrative expenses and costs related to certain former businesses are allocated to the reportable segments and Other based on measures of activity that management believes are reasonable.

The results of segment operations are as follows:

(In millions)	Flat-Rolled	Mini Mill	USSE	Tubular	Total
2024					
Customer sales	$ 9,604	$ 2,070	$ 2,983	$ 970	$ 15,627
Intersegment sales	211	347	24	7	589
Net sales	9,815	2,417	3,007	977	16,216
Other net sales [a]					13
Elimination of intersegment sales					(589)
Total net sales					$ 15,640
Less: [b]					
Cost of sales	8,775	2,063	2,898	843	14,579
Earnings from investees	(90)	—	—	(22)	(112)
Other segment items [c]	196	121	38	21	376
Segment earnings before interest, taxes, depreciation and amortization	934	233	71	135	$ 1,373
Other loss before interest, taxes, depreciation and amortization [a]					(7)
Depreciation, depletion and amortization					(913)
Items not allocated to segments:					
Restructuring and other charges (Note 25)					(8)
Stock-based compensation expense (Note 15)					(51)
Asset impairment charges					(19)
Environmental remediation charges					(18)
Strategic alternatives review process costs					(90)
Granite City idling costs					(11)
Other charges, net					(16)
Earnings before interest and income taxes					$ 240
Net interest and other financial benefits					(198)
Earnings before income taxes					$ 438
Capital expenditures [d]	495	1,641	118	33	2,287
Segment depreciation, depletion and amortization	(535)	(203)	(125)	(50)	(913)
Other depreciation, depletion and amortization [a]					—
Total depreciation, depletion and amortization					$ (913)
Assets [d]	7,266	9,339	1,904	975	19,484
Other assets [a]					127
Corporate, reconciling items, and eliminations [e]					624
Total assets					$ 20,235

[a] The results of our real estate business are disclosed in the Other category.

[b] The significant expense (income) categories and amounts align with the segment-level information that is regularly provided to the CODM. Intersegment expenses are included within the amounts shown.

[c] Other segment items primarily include selling, general and administrative expenses, BRS recycling tax credit revenue and BR2 startup costs.

[d] Mini Mill includes capital expenditures and assets related to BR2 of $1.4 billion and $4.7 billion, respectively.

[e] The majority of Corporate, reconciling items, and eliminations total assets is comprised of cash and the elimination of intersegment amounts.

(In millions)	Flat-Rolled	Mini Mill	USSE	Tubular	Total
2023					
Customer sales	$ 10,744	$ 2,223	$ 3,525	$ 1,551	$ 18,043
Intersegment sales	365	455	25	1	846
Net sales	11,109	2,678	3,550	1,552	18,889
Other net sales [a]					10
Elimination of intersegment sales					(846)
Total net sales					$ 18,053
Less: [b]					
Cost of sales	9,899	2,259	3,413	931	16,502
Earnings from investees	(72)	—	—	(43)	(115)
Other segment items [c]	259	36	39	26	360
Segment earnings before interest, taxes, depreciation and amortization	1,023	383	98	638	$ 2,142
Other loss before interest, taxes, depreciation and amortization [a]					(3)
Depreciation, depletion and amortization					(916)
Items not allocated to segments:					
Restructuring and other charges (Note 25)					(36)
Stock-based compensation expense (Note 15)					(51)
Asset impairment charges					(127)
Environmental remediation charges					(11)
Strategic alternatives review process costs					(79)
Granite City idling costs					(121)
Other charges, net					1
Earnings before interest and income taxes					$ 799
Net interest and other financial benefits					(248)
Earnings before income taxes					$ 1,047
Capital expenditures [d]	536	1,899	109	32	2,576
Segment depreciation, depletion and amortization	(605)	(168)	(94)	(49)	(916)
Other depreciation, depletion and amortization [a]					—
Total depreciation, depletion and amortization					$ (916)
Assets [d]	7,546	7,569	2,229	1,002	18,346
Other assets [a]					140
Corporate, reconciling items, and eliminations [e]					1,965
Total assets					$ 20,451

[a] The results of our real estate business are disclosed in the Other category.

[b] The significant expense (income) categories and amounts align with the segment-level information that is regularly provided to the CODM. Intersegment expenses are included within the amounts shown.

[c] Other segment items primarily include selling, general and administrative expenses, BRS recycling tax credit revenue and BR2 startup costs.

[d] Mini Mill includes capital expenditures and assets related to BR2 of $1.5 billion and $3.0 billion, respectively.

[e] The majority of Corporate, reconciling items, and eliminations total assets is comprised of cash and the elimination of intersegment amounts.

(In millions)	Flat-Rolled	Mini Mill	USSE	Tubular	Total
2022					
Customer sales	$ 12,522	$ 2,681	$ 4,243	$ 1,611	$ 21,057
Intersegment sales	350	366	13	5	734
Net sales	12,872	3,047	4,256	1,616	21,791
Other net sales [a]					9
Elimination of intersegment sales					(735)
Total net sales					$ 21,065
Less: [b]					
Cost of sales	10,357	2,381	3,696	1,024	17,458
Earnings from investees	(220)	—	—	(23)	(243)
Other segment items [c]	228	27	31	23	309
Segment earnings before interest, taxes, depreciation and amortization	2,507	639	529	592	$ 4,267
Other earnings before interest, taxes, depreciation and amortization [a]					23
Depreciation, depletion and amortization					(791)
Items not allocated to segments:					
Restructuring and other charges (Note 25)					(48)
Stock-based compensation expense (Note 15)					(57)
Asset impairment charges					(163)
Environmental remediation charges					(13)
United Steelworkers labor agreement signing bonus and related costs					(64)
Gains on assets sold & previously held investments					6
Earnings before interest and income taxes					$ 3,160
Net interest and other financial benefits					(99)
Earnings before income taxes					$ 3,259
Capital expenditures [d]	503	1,159	90	17	1,769
Segment depreciation, depletion and amortization	(499)	(158)	(85)	(48)	(790)
Other depreciation, depletion and amortization [a]					(1)
Total depreciation, depletion and amortization					$ (791)
Assets [d]	7,936	5,787	2,175	1,140	17,038
Other assets [a]					141
Corporate, reconciling items, and eliminations [e]					2,279
Total assets					$ 19,458

[a] The results of our real estate business are disclosed in the Other category.

[b] The significant expense (income) categories and amounts align with the segment-level information that is regularly provided to the CODM. Intersegment expenses are included within the amounts shown.

[c] Other segment items primarily include selling, general and administrative expenses, BRS recycling tax credit revenue and adjustments in electricity purchase agreements.

[d] Mini Mill includes capital expenditures and assets related to BR2 of $0.8 billion and $1.4 billion, respectively.

[e] The majority of Corporate, reconciling items, and eliminations total assets is comprised of cash and the elimination of intersegment amounts.

Geographic Area:
The information below summarizes external sales, property, plant and equipment and equity method investments based on the location of the operating segment to which they relate.

(In millions)	Year	External Sales	Assets
North America	**2024**	**$ 12,657**	**$ 11,853** [(a)]
	2023	14,528	10,223 [(a)]
	2022	16,822	8,459 [(a)]
Europe	**2024**	**2,983**	**854**
	2023	3,525	910
	2022	4,243	843
Total	**2024**	**15,640**	**12,707**
	2023	18,053	11,133
	2022	21,065	9,302

[(a)] Assets with a book value of $11,853 million, $10,223 million, and $8,459 million were located in the United States at December 31, 2024, 2023, and 2022, respectively.

5. Dispositions

Real Property, South Works Disposition
In July 2024, the Company entered into a purchase and sale agreement with a third party for the sale of substantially all of the Company's former South Works real property. The Company will convey title of the property to the buyer for expected proceeds of approximately $70 million. This transaction is expected to close within the next 12 months.

USS-UPI, LLC Disposition
In December 2023, production at USS-UPI, LLC (UPI) was indefinitely idled. The Company has accrued a total of $44 million and $108 million for severance, exit costs and employee benefits as of December 31, 2024 and 2023, respectively. Payments of $59 million for these items were made during the year ended December 31, 2024. The Company has previously committed to, and continues to intend to, pursue the disposition of certain assets related to the UPI facility.

As of December 31, 2024, the South Works property and the UPI facility are recorded as assets held-for-sale which are recorded in Other current assets on the Consolidated Balance Sheet.

6. Revenue

Revenue is generated primarily from contracts to produce, ship and deliver steel products, and to a lesser extent, raw materials sales such as iron ore pellets and coke by-products and real estate sales. Generally, U. S. Steel's performance obligations are satisfied and revenue is recognized when title transfers to our customer for product shipped or services are provided. Revenues are recorded net of any sales incentives. Shipping and other transportation costs charged to customers are treated as fulfillment activities and are recorded in both revenue and cost of sales at the time control to the related good being shipped is transferred to the customer. Costs related to obtaining sales contracts are incidental and are expensed when incurred. Because customers are invoiced at the time title transfers and U. S. Steel's right to consideration is unconditional at that time, U. S. Steel does not maintain contract asset balances. Additionally, U. S. Steel does not maintain contract liability balances, as performance obligations are satisfied prior to customer payment for product. U. S. Steel offers industry standard payment terms.

The following tables disaggregate our revenue by product for each of our reportable business segments for the years ended December 31, 2024, 2023, and 2022, respectively (Net Sales by Product, excluding intersegment sales, in millions):

Year Ended December 31, 2024	Flat-Rolled	Mini Mill	USSE	Tubular	Other	Total
Semi-finished	$ 84	$ —	$ 78	$ 35	$ —	$ 197
Hot-rolled sheets	1,566	1,039	1,468	—	—	4,073
Cold-rolled sheets	3,665	325	229	—	—	4,219
Coated sheets	2,899	701	1,052	—	—	4,652
Tubular products	—	—	51	913	—	964
All other [(a)]	1,390	5	105	22	13	1,535
Total	$ 9,604	$ 2,070	$ 2,983	$ 970	$ 13	$ 15,640

[(a)] Consists primarily of sales of raw materials and coke making by-products

Year Ended December 31, 2023	Flat-Rolled	Mini Mill	USSE	Tubular	Other	Total
Semi-finished	$ 242	$ —	$ 168	$ —	$ —	$ 410
Hot-rolled sheets	1,926	1,215	1,637	—	—	4,778
Cold-rolled sheets	3,568	365	269	—	—	4,202
Coated sheets	3,484	639	1,278	—	—	5,401
Tubular products	—	—	52	1,528	—	1,580
All other [(a)]	1,524	4	121	23	10	1,682
Total	$ 10,744	$ 2,223	$ 3,525	$ 1,551	$ 10	$ 18,053

[(a)] Consists primarily of sales of raw materials and coke making by-products.

Year Ended December 31, 2022	Flat-Rolled	Mini Mill	USSE	Tubular	Other	Total
Semi-finished	$ 193	$ —	$ 103	$ —	$ —	$ 296
Hot-rolled sheets	2,338	1,570	1,919	—	—	5,827
Cold-rolled sheets	3,898	356	385	—	—	4,639
Coated sheets	4,461	745	1,622	—	—	6,828
Tubular products	—	—	68	1,594	—	1,662
All other [(a)]	1,632	10	146	17	8	1,813
Total	$ 12,522	$ 2,681	$ 4,243	$ 1,611	$ 8	$ 21,065

[(a)] Consists primarily of sales of raw materials and coke making by-products.

7. Net Interest and Other Financial Costs

(In millions)	2024	2023	2022
Interest income:			
Interest income	**$ (96)**	$ (141)	$ (44)
Interest expense and other financial costs:			
Interest incurred	**237**	234	218
Less interest capitalized	**213**	162	59
Total interest expense	**24**	72	159
Loss on debt extinguishment [(a)]	**2**	—	—
Net periodic benefit income	**(132)**	(154)	(246)
Net gain from investments related to active employee benefits	**(25)**	(43)	—
Foreign currency net loss (gain) [(b)]	**6**	(5)	11
Financial costs on:			
Amended Credit Agreement	**5**	5	5
USSK Credit Facility	**2**	4	3
Other	**7**	5	4
Amortization of discounts and deferred financing costs	**9**	9	9
Total other financial costs	**29**	18	32
Net interest and other financial (benefits) costs	**$ (198)**	$ (248)	$ (99)

[(a)] Represents a net pretax charge of $2 million during 2024 related to the repayments of the 2026 Senior Convertible Notes.

[(b)] The functional currency for USSE is the euro. Foreign currency net loss (gain) is a result of transactions denominated in currencies other than the euro.

8. Earnings and Dividends Per Common Share

Earnings per Share Attributable to United States Steel Corporation Stockholders

Basic earnings per common share is based on the weighted average number of common shares outstanding during the period.

Diluted earnings per common share assumes the exercise of stock options, the vesting of restricted stock units and performance awards, provided in each case the effect is dilutive. The “if-converted” method is used to calculate the dilutive effect of the Senior Convertible Notes due in 2026 as a result of ASU 2020-06.

The computations for basic and diluted earnings per common share from continuing operations are as follows:

(Dollars in millions, except per share amounts)	2024	2023	2022
Net earnings attributable to United States Steel Corporation stockholders			
Basic	**$ 384**	$ 895	$ 2,524
Interest expense on Senior Convertible Notes, net of tax	**13**	13	13
Diluted	**$ 397**	$ 908	$ 2,537
Weighted-average shares outstanding (in thousands):			
Basic	**224,817**	224,761	246,986
Effect of Senior Convertible notes	**26,159**	26,194	26,194
Effect of stock options, restricted stock units and performance awards	**3,028**	4,405	3,783
Diluted	**254,004**	255,360	276,963
Net earnings per share attributable to United States Steel Corporation stockholders:			
Basic	**$ 1.71**	$ 3.98	$ 10.22
Diluted	**$ 1.57**	$ 3.56	$ 9.16

The following table summarizes the securities that were antidilutive, and therefore, were not included in the computation of diluted earnings per common share:

(In thousands)	2024	2023	2022
Securities granted under the 2016 Omnibus Incentive Compensation Plan, as amended and restated	**125**	475	960

Dividends Paid per Share
Quarterly dividends on common stock were five cents per share for each quarter in 2024, 2023, and 2022.

9. Cash, Cash Equivalents and Restricted Cash

The following table provides a reconciliation of cash, cash equivalents and restricted cash reported within U. S. Steel's Consolidated Balance Sheets that sum to the total of the same amounts shown in the Consolidated Statements of Cash Flows:

	December 31,		
(In millions)	2024	2023	2022
Cash and cash equivalents	**$ 1,367**	$ 2,948	$ 3,504
Restricted cash in other current assets	**11**	8	4
Long-term restricted cash	**35**	32	31
Total cash, cash equivalents and restricted cash	**$ 1,413**	$ 2,988	$ 3,539

Amounts included in restricted cash represent cash balances which are legally or contractually restricted, primarily for insurance purposes, environmental liabilities and other capital projects.

10. Inventories

(In millions)	December 31, 2024	December 31, 2023
Raw materials	**$ 959**	$ 773
Semi-finished products	**828**	877
Finished products	**331**	428
Supplies and sundry items	**50**	50
Total	**$ 2,168**	$ 2,128

Current acquisition costs for LIFO inventories were estimated to exceed the above inventory values at December 31 by $1.1 billion in 2024 and $1.2 billion in 2023. As a result of the liquidation of LIFO inventories, cost of sales increased and earnings before interest and income taxes decreased by $3 million in 2024. Cost of sales decreased and earnings before interest and income taxes increased by $43 million and $44 million in 2023 and 2022, respectively.

11. Income Taxes

Components of earnings before income taxes:

(In millions)	2024	2023	2022
United States	**$ 499**	$ 1,041	$ 2,847
Foreign	**(61)**	6	412
Earnings before income taxes	**$ 438**	$ 1,047	$ 3,259

At the end of 2024, 2023, and 2022, U. S. Steel does not have any undistributed foreign earnings and profits for which U.S. deferred taxes have not been provided.

Income tax provision (benefit):

	2024			2023			2022		
(In millions)	Current	Deferred	Total	Current	Deferred	Total	Current	Deferred	Total
Federal	**$ (53)**	**$ 60**	**$ 7**	$ 56	$ 116	$ 172	$ 101	$ 451	$ 552
State and local	**(6)**	**24**	**18**	(3)	(14)	(17)	54	43	97
Foreign	**—**	**29**	**29**	2	(5)	(3)	79	7	86
Total	**$ (59)**	**$ 113**	**$ 54**	$ 55	$ 97	$ 152	$ 234	$ 501	$ 735

A reconciliation of the federal statutory tax rate of 21 percent to total provision follows:

(In millions)	2024	2023	2022
Statutory rate applied to earnings before income taxes	**$ 92**	$ 220	$ 684
Valuation allowance	**47**	(14)	(38)
Excess percentage depletion	**(23)**	(18)	(48)
Capitalized transaction costs	**13**	10	—
State and local income taxes after federal income tax effects	**8**	(1)	97
Effects of foreign operations	**(12)**	(3)	85
U.S. impact of foreign operations	**(29)**	(19)	6
Impact of tax credits	**(11)**	(7)	(83)
Adjustment of prior years' federal income taxes	**(42)**	(34)	40
Unrecognized tax benefits	**12**	2	—
Stock compensation	**(7)**	(5)	(3)
Nondeductible executive compensation	**5**	16	6
Other	**1**	5	(11)
Total provision	**$ 54**	$ 152	$ 735

Included in the 2024 provision is a benefit of $59 million related to the 2023 federal and state income tax returns.

In March 2022, the Company and the Arkansas Economic Development Commission entered into the Recycling Tax Credit Incentive Agreement, whereby the Company may earn state income tax credits in an amount equal to 30 percent of the cost of waste reduction, reuse, or recycling equipment, subject to meeting the requirements of the Arkansas Code Ann. Section 26-51-506, for the Company's BR2 facility near Osceola, Arkansas. Documentation supporting the Company's investment in qualifying equipment must be submitted as part of an application for certification expected to be completed in 2025. In March 2022, the Company received a lump-sum payment of approximately $82 million as proceeds from the sale of a portion of expected future tax credits to be earned by the Company (see Note 26 for additional information). The Company estimates that it could earn tax credits in excess of $700 million, exclusive of the amount sold in March 2022, which the Company will recognize in the year it meets the requirements of Arkansas Code Ann. Section 26-51-506. Any unused tax credit that cannot be claimed in a tax year may be carried forward indefinitely by the Company and applied to its future state tax liability.

On August 16, 2022, H.R. 5376 (commonly called the Inflation Reduction Act of 2022) was signed into law, which, among other things, implemented a corporate alternative minimum tax (CAMT) of 15 percent on net book income of certain large corporations adjusted for certain items prescribed by the legislation.

The Organization for Economic Co-operation and Development (the "OECD"), an international association of 38 countries including the U.S., has proposed changes to numerous long-standing tax principles, including a global minimum tax initiative. On December 12, 2022, the European Union member states agreed to implement the OECD's Pillar 2 global

corporate minimum tax rate of 15 percent on companies with revenues of at least €750 million, which went into effect in 2024. The law on minimum top-up tax for multinational enterprise groups and large-scale domestic groups in Slovakia was approved by the parliament on December 8, 2023 and signed by the President on December 21, 2023, with an effective date of December 31, 2023.

The Company analyzed the 2024 tax effects of CAMT and Pillar 2, which were not material to the Consolidated Financial Statements.

Included in the 2023 provision is a benefit of $43 million related to the 2022 federal and state income tax returns, as well as an additional benefit of $12 million related to the adjustment of prior years' federal income taxes. Also included in the 2023 provision is a benefit of $23 million related to the recognition of a deferred tax asset on unrecognized currency losses in branch operations pursuant to proposed regulations under Internal Revenue Code Section 987.

Deferred taxes
Deferred tax assets and liabilities resulted from the following:

	December 31,	
(In millions)	**2024**	**2023**
Deferred tax assets:		
Federal tax loss carryforwards (no expiration)	**$ 213**	$ 1
Federal capital loss carryforwards (expiring in 2026)	**69**	69
State tax credit carryforwards (expiring in 2027 through 2038)	**17**	14
State tax loss carryforwards (expiring in 2026 through 2044)	**91**	80
State capital loss carryforwards (expiring in 2026 through 2036)	**21**	23
General business credit carryforwards (expiring in 2044)	**9**	—
Foreign tax loss and credit carryforwards (expiring in 2027 through 2034)	**26**	8
Operating lease liabilities	**19**	28
Capitalized research and development	**76**	69
Receivables, payables and debt	**149**	140
Inventory	**48**	57
Other temporary differences	**71**	41
Valuation allowance	**(149)**	(101)
Total deferred tax assets	**$ 660**	$ 429
Deferred tax liabilities:		
Property, plant and equipment	**$ 589**	$ 642
Operating right-of-use assets	**17**	26
Investments in subsidiaries and equity investees	**557**	184
Employee benefits	**109**	72
Contingencies and accrued liabilities	**45**	73
Total deferred tax liabilities	**$ 1,317**	$ 997
Net deferred tax liability	**$ (657)**	$ (568)

U. S. Steel recognizes deferred tax assets and liabilities for the estimated future tax consequences attributable to differences between the financial statement carrying amounts of existing assets and liabilities and their respective tax basis. The realization of deferred tax assets is assessed quarterly based on several interrelated factors. These factors include U. S. Steel's expectation to generate sufficient future taxable income and the projected time period over which these deferred tax assets will be realized.

Each quarter U. S. Steel analyzes the likelihood that our deferred tax assets will be realized. A valuation allowance is recorded if, based on the weight of all available positive and negative evidence, it is more likely than not that some portion, or all, of a deferred tax asset will not be realized.

At December 31, 2024, the net domestic deferred tax liability was $619 million, net of an established valuation allowance of $103 million. At December 31, 2023, the net domestic deferred tax liability was $566 million, net of an established valuation allowance of $98 million.

At December 31, 2024, the net foreign deferred tax liability was $38 million, net of an established valuation allowance of $46 million. At December 31, 2023, the net foreign deferred tax liability was $2 million, net of an established valuation allowance of $3 million. The net foreign deferred tax asset will fluctuate as the value of the U.S. dollar changes with respect to the euro.

U. S. Steel will continue to monitor the realizability of its deferred tax assets on a quarterly basis. In the future, if we determine that realization is more likely than not for a deferred tax asset with a valuation allowance, the related valuation allowance will be reduced, and we will record a non-cash benefit to earnings.

Unrecognized tax benefits
Unrecognized tax benefits are the differences between a tax position taken, or expected to be taken, in a tax return and the benefit recognized for accounting purposes pursuant to the guidance in ASC Topic 740 on income taxes. The total amount of gross unrecognized tax benefits was $18 million, $4 million, and $2 million as of December 31, 2024, 2023, and 2022, respectively.

The total amount of net unrecognized tax benefits that, if recognized, would affect the effective tax rate was $18 million and $4 million as of December 31, 2024, and 2023, respectively.

U. S. Steel records interest related to uncertain tax positions as a part of net interest and other financial costs in the Consolidated Statements of Operations. Any penalties are recognized as part of selling, general and administrative expenses. U. S. Steel had accrued liabilities for interest and penalties related to uncertain tax positions of less than $1 million and $2 million as of December 31, 2024, and 2023, respectively.

A tabular reconciliation of unrecognized tax benefits follows:

(In millions)	2024	2023	2022
Unrecognized tax benefits, beginning of year	**$ 4**	$ 2	$ 3
Increases – tax positions taken in prior years	**16**	—	—
Increases – current tax positions	**—**	2	—
Lapse of statute of limitations	**(2)**	—	(1)
Unrecognized tax benefits, end of year	**$ 18**	$ 4	$ 2

Tax years subject to examination
Below is a summary of the tax years open to examination by major tax jurisdiction:

U.S. Federal – 2021 and forward
U.S. States – 2017 and forward
Slovakia – 2013 and forward

Status of Internal Revenue Service (IRS) examinations
The IRS completed its audit of the Company's 2017-2018 federal consolidated tax returns in 2023.

12. Investments, Long-Term Receivables and Equity Investee Transactions

	December 31,	
(In millions)	2024	2023
Equity method investments	**$ 734**	$ 740
Receivables due after one year, less allowance of $3 in both periods	**21**	19
Other	**2**	2
Total	**$ 757**	$ 761

Summarized financial information of all investees accounted for by the equity method of accounting is as follows (amounts represent 100% of investee financial information):

(In millions)	2024	2023	2022
Income data – year ended December 31:			
Net Sales	**$ 3,184**	$ 3,335	$ 3,222
Operating income	**245**	342	492
Net earnings	**220**	319	462
Balance sheet date – December 31:			
Current Assets	**$ 847**	$ 876	$ 1,085
Noncurrent Assets	**788**	844	974
Current liabilities	**226**	255	314
Noncurrent Liabilities	**392**	398	513

U. S. Steel's portion of the income (loss) from investees reflected on the Consolidated Statements of Operations was $112 million, $115 million, and $243 million for the years ended December 31, 2024, 2023, and 2022, respectively.

All of our significant investees are located in the U.S. Investees accounted for using the equity method include:

Investee	December 31, 2024 Interest
Chrome Deposit Corporation	50 %
Double G Coatings Company, Inc.	50 %
Hibbing Development Company	24.1 %
Hibbing Taconite Company[(a)]	14.7 %
Patriot Premium Threading Services, LLC	50 %
PRO-TEC Coating Company, LLC	50 %

[(a)] Hibbing Taconite Company (Hibbing) is an unincorporated joint venture that is owned, in part, by Hibbing Development Company (HDC), which is accounted for using the equity method. Through HDC we are able to influence the activities of Hibbing, and as such, its activities are accounted for using the equity method.

In 2022, the Company recognized pre-tax gains on equity investee transactions of approximately $6 million from Worthington Specialty Processing pertaining to the sale of the former joint venture's facilities and subsequent distribution to the investors. The joint venture is expected to be dissolved in the future and is expected to result in an immaterial distribution to the Company.

Dividends or partnership distributions received from equity investees were $109 million, $114 million and $28 million in 2024, 2023 and 2022, respectively.

U. S. Steel evaluates impairment of its equity method investments whenever circumstances indicate that a decline in value below carrying value is other than temporary. Under these circumstances, we would adjust the investment down to its estimated fair value, which then becomes its new carrying value.

We supply substrate to certain of our equity method investees and from time to time will extend the payment terms for their trade receivables. For discussion of transactions and related receivable and payable balances between U. S. Steel and its investees, see Note 23.

13. Property, Plant and Equipment

(In millions)	Useful Lives	December 31, 2024	2023
Land and depletable property	—	**$ 257**	$ 216
Buildings	35-40 years	**2,883**	1,854
Machinery and equipment			
Steel producing	2-30 years	**19,790**	16,806
Other	5-30 years	**102**	97
Information technology	5-15 years	**857**	829
Assets under finance lease	5-15 years	**379**	302
Construction in process	—	**1,971**	3,871
Total		**26,239**	23,975
Less accumulated depreciation and depletion		**14,266**	13,582
Net		**$ 11,973**	$ 10,393

Amounts in accumulated depreciation and depletion for assets acquired under finance leases were $182 million and $126 million at December 31, 2024, and 2023, respectively.

14. Goodwill and Intangible Assets

Intangible assets are being amortized on a straight-line basis over their estimated useful lives and are detailed below:

(In millions)	Useful Lives	As of December 31, 2024 Gross Carrying Amount	Accumulated Amortization	Net Amount	As of December 31, 2023 Gross Carrying Amount	Accumulated Amortization	Net Amount
Customer relationships	22 Years	**$ 413**	**$ 75**	**$ 338**	$ 413	$ 56	$ 357
Patents	5-15 Years	**17**	**14**	**3**	17	13	4
Energy Contract	2 Years	**—**	**—**	**—**	54	54	—
Total amortizable intangible assets		**$ 430**	**$ 89**	**$ 341**	$ 484	$ 123	$ 361

Amortization expense was $20 million and $42 million for years ended December 31, 2024, and December 31, 2023, respectively. We expect approximately $97 million in total amortization expense from 2025 through 2029 and approximately $244 million in remaining amortization expense thereafter.

The carrying amount of acquired water rights with indefinite lives as of December 31, 2024, and December 31, 2023, totaled $75 million.

Below is a summary of goodwill by segment for the twelve months ended December 31, 2024:

(In millions)	Flat-Rolled	Mini Mill	USSE	Tubular	Total
Balance at December 31, 2023	$ —	$ 916	$ 4	$ —	$ 920
Additions	—	—	—	—	—
Balance at December 31, 2024	**$ —**	**$ 916**	**$ 4**	**$ —**	**$ 920**

15. Stock-Based Compensation Plans

U. S. Steel has outstanding stock-based compensation awards that were granted by the Compensation & Organization Committee of the Board of Directors (the Committee), or its designee, under the 2005 Stock Incentive Plan (the 2005 Plan) and the 2016 Omnibus Incentive Compensation Plan, as amended and restated (the Omnibus Plan) (collectively the Plans). On April 26, 2016, the Company's stockholders approved the Omnibus Plan and authorized the Company to issue up to 7,200,000 shares of U. S. Steel common stock under the Omnibus Plan. The Company's stockholders authorized the issuance of an additional 6,300,000 shares under the Omnibus Plan on April 25, 2017, an additional 4,700,000 shares under the Omnibus Plan on April 28, 2020, and an additional 14,500,000 shares under the Omnibus Plan on April 27, 2021. While awards that were previously granted under the 2005 Plan remain outstanding, all future awards will be granted under the Omnibus Plan. As of December 31, 2024, there were 4,084,130 shares available for future grants under the Omnibus Plan.

Generally, a share issued under the Omnibus Plan pursuant to an award other than a stock option will reduce the number of shares available under the Stock Plan by 1.78 shares. Shares related to awards under either plan (i) that are forfeited, (ii) that terminate without shares having been issued or (iii) for which payment is made in cash or property other than shares, are again available for awards under the Omnibus Plan. Shares delivered to U. S. Steel or withheld for purposes of satisfying the exercise price or tax withholding obligations are not available for future awards. The purpose of the Plans is to attract, reward and retain employees and non-employee directors who create long-term value for our stockholders by delivering on objectives that support our long-term strategy. The Committee administers the Plans, and under the Omnibus Plan may make grants of stock options, restricted stock units (RSUs), performance awards, and other stock-based awards.

The following table summarizes the total stock-based compensation awards granted during the years 2024, 2023, and 2022:

	Restricted Stock Units	TSR Performance Awards	ROCE Performance Awards	Performance-Based Restricted Stock Units
2024	**801,540**	**—**	**230,350**	**—**
2023	2,126,020	185,120	357,020	—
2022	1,249,830	236,520	408,870	83,951

Stock-based compensation expense
The following table summarizes the total compensation expense recognized for stock-based compensation awards:

(In millions, except per share amounts)	Year Ended December 31, 2024	Year Ended December 31, 2023	Year Ended December 31, 2022
Stock-based compensation expense recognized:			
Cost of sales	**$ 20**	$ 16	$ 15
Selling, general and administrative expenses	**32**	35	42
Total	**52**	51	57
Related deferred income tax benefit	**12**	12	14
Decrease in net income	**40**	39	43
Decrease in basic earnings per share	**0.18**	0.17	0.18
Decrease in diluted earnings per share	**0.16**	0.15	0.16

As of December 31, 2024, total future compensation cost related to nonvested stock-based compensation arrangements was $43 million and the average period over which this cost is expected to be recognized is approximately 21 months.

Stock options
Compensation expense for stock options is recorded over the vesting period based on the fair value on the date of grant, as calculated by U. S. Steel using the Black-Scholes model and the assumptions listed below. Awards generally vest ratably over a three-year service period and have a term of ten years. Stock options are generally issued at the average market price of the underlying stock on the date of the grant. Upon exercise of stock options, shares of U. S. Steel stock are issued from treasury stock or from authorized, but unissued common stock. There were 171,000 performance-based stock options granted in 2021. There were no stock options granted in 2024, 2023, and 2022.

The 171,000 performance-based stock options granted in December 2021 do not become vested and exercisable until the Company's 20-trading day average closing stock price meets or exceeds the following stock price hurdles during the seven-year period beginning on the grant date, as follows:

20-trading day Average Closing Stock Price Achievement During 7-Year Period Beginning on Grant Date [(a)]	Percentage of Performance-Based Stock Options Exercisable
$ 35.00	33.33 %
$ 45.00	33.33 %
$ 55.00	33.34 %

[(a)] The $35.00 tranche vested in April 2022 and the $45.00 tranche vested in January 2024.

The following table shows a summary of the status and activity of stock options for the year ended December 31, 2024:

	Shares	Weighted-Average Exercise Price (per share)	Weighted-Average Remaining Contractual Term (in years)	Aggregate Intrinsic Value (in millions)
Outstanding at January 1, 2024	760,238	$ 27.98		
Granted	—	$ —		
Exercised	(158,908)	$ 24.90		
Forfeited or expired	(13,057)	$ 37.33		
Outstanding at December 31, 2024	588,273	$ 28.60	2.11	$ 4
Exercisable at December 31, 2024	531,273	$ 29.15	1.91	$ 4
Exercisable and expected to vest at December 31, 2024	588,273	$ 28.60	2.11	$ 4

The aggregate intrinsic value in the table above represents the total pretax intrinsic value (difference between our closing stock price on the last trading day of 2024 and the exercise price, multiplied by the number of in-the-money options). Intrinsic value changes are a function of the fair market value of our stock.

The total intrinsic value of stock options exercised (i.e., the difference between the market price at exercise and the price paid by the employee to exercise the option) was $3 million, $4 million, and $5 million during the years ended December 31, 2024, December 31, 2023, and December 31, 2022, respectively. The total amount of cash received by U. S. Steel from the exercise of options was $4 million and $10 million during the years ended December 31, 2024, and

December 31, 2023, respectively. The related net tax benefit realized from the exercise of these options was immaterial in 2024 and 2023.

Stock awards
Compensation expense for nonvested stock awards is recorded over the vesting period based on the fair value at the date of grant.

RSUs awarded as part of annual grants generally vest ratably over three years. Their fair value is the average market price of the underlying common stock on the date of grant. RSUs granted in connection with new-hire or retention awards generally cliff vest three years from the date of the grant.

Total shareholder return (TSR) performance awards may vest at varying levels at the end of a three-year performance period if U. S. Steel's total shareholder return compared to the total shareholder return of a peer group of companies meets performance criteria during the three-year performance period. TSR is calculated as follows: 20 percent for each year in the three-year performance period and 40 percent for the full three-year period. TSR performance awards may vest and pay out 50 percent at the threshold level, 100 percent at the target level and 200 percent at the maximum level. Payment for performance in between the threshold percentages will be interpolated. The fair value of the performance awards is calculated using a Monte-Carlo simulation.

Performance awards based on the return on capital employed (ROCE) metric will be measured based on the Company's consolidated worldwide earnings (loss) before interest and income taxes, as adjusted, divided by consolidated worldwide capital employed, as adjusted, over a three year period.

ROCE is calculated based on the ROCE achieved in the first, second and third years of the performance period, weighted at 20 percent for each year in the three-year performance period and 40 percent for the full three-year period. The ROCE awards will pay out 50 percent at the threshold level, 100 percent at the target level and 200 percent at the maximum level. Payment for performance in between the threshold percentages will be interpolated.

Compensation expense associated with the ROCE awards will be contingent based upon the achievement of the specified ROCE performance goals and will be adjusted on a quarterly basis to reflect the probability of achieving the ROCE metric.

ROCE performance awards may vest at the end of a three-year performance period contingent upon meeting ROCE performance goals approved by the Committee. The fair value of the ROCE performance awards is the average market price of the underlying common stock on the date of grant.

In December 2021 and August 2022, special performance-based restricted stock unit awards (PSUs) were granted to members of the Company's executive leadership team. Shares are earned based on the achievement of certain pre-set quantitative performance criteria during the four-year performance period, January 1, 2022, through December 31, 2025. Shares may vest following the expiration of the Performance Period if the Company satisfies the performance criteria.

The Chief Executive Officer was granted PSUs that vest with the following, equally weighted, performance metrics: (i) EBITDA margin expansion, (ii) greenhouse gas emissions intensity reduction, (iii) asset portfolio optimization, (iv) leverage metrics and (v) corporate relative valuation. Other members of the executive leadership team were granted PSUs that vest with performance criteria related to: (i) on time and on budget completion of the second mini mill (30% of the grant), (ii) EBITDA margin expansion (40% of the grant) and (iii) greenhouse gas emissions intensity reduction (30% of the grant).

For the PSU awards, a payout is achievable at threshold (50% of target), target (100% of target) or maximum (200% of target) performance achievement. Pay out amounts will be interpolated between the threshold, target and maximum amounts.

The following table shows a summary of the performance awards outstanding as of December 31, 2024, and their fair market value on the respective grant date:

Performance Period	Fair Value (in millions)	Minimum Shares	Target Shares	Maximum Shares
2024 - 2026	$ 10	—	219,770	439,540
2023 - 2025	$ 16	—	495,852	991,704
2022 - 2024	$ 15	—	595,662	1,191,324
2021 - 2025	$ 13	—	574,168	1,148,336

The following table shows a summary of the status and activity of nonvested stock awards for the year ended December 31, 2024:

	Restricted Stock Units	TSR Performance Awards [a]	ROCE Performance Awards [a]	Performance-Based Restricted Stock Units [a]	Total	Weighted-Average Grant-Date Fair Value
Nonvested at January 1, 2024	3,432,338	728,570	952,969	697,485	5,811,362	$ 26.95
Granted	801,540	—	230,350	—	1,031,890	47.22
Vested	(1,332,529)	(192,040)	(404,952)	—	(1,929,521)	23.00
Performance adjustment factor [b]	—	(114,890)	202,476	—	87,586	15.89
Forfeited or expired	(139,826)	(32,612)	(58,587)	(123,317)	(354,342)	29.60
Nonvested at December 31, 2024	2,761,523	389,028	922,256	574,168	4,646,975	$ 32.68

[a] The number of shares shown for the performance awards is based on the target number of share awards.
[b] Consists of adjustments to vested performance awards to reflect actual performance.

The following table presents information on RSUs and performance awards granted:

	2024	2023	2022
Number of awards granted	**1,031,890**	2,668,160	1,979,171
Weighted-average grant-date fair value per share	**$ 47.22**	$ 31.68	$ 24.52

During the years ended December 31, 2024, 2023, and 2022, the total fair value of shares vested was $44 million, $45 million, and $45 million, respectively.

16. Derivative Instruments

U. S. Steel is exposed to foreign currency exchange rate risks in our European operations. USSE's revenues are primarily in euros and costs are primarily in euros and U.S. dollars. U. S. Steel uses foreign exchange forward sales contracts (foreign exchange forwards) with maturities up to 12 months to manage our currency requirements and exposure to foreign currency exchange rate fluctuations. Derivative instruments are required to be recognized at fair value in the Consolidated Balance Sheet. The USSE and Flat-Rolled segments use hedge accounting for their foreign exchange forwards.

U. S. Steel also uses financial swaps to mitigate commodity price risks related to the procurement of natural gas, zinc, tin, electricity, and iron ore (commodity purchase swaps). We have elected cash flow hedge accounting for these commodity purchase swaps, which have maturities of up to 15 months.

U. S. Steel has entered into financial swaps that are used to partially manage the sales price risk of certain hot-rolled coil sales and iron ore sales (sales swaps). The sales swaps are accounted for using hedge accounting and have maturities of up to 12 months.

Generally, forward physical purchase contracts qualify for the normal purchase and normal sales exceptions described in ASC Topic 815 and are not subject to mark-to-market accounting. In accordance with the guidance in ASC Topic 820 on fair value measurements and disclosures, the fair value of our foreign exchange forwards, commodity purchase swaps and sales swaps was determined using Level 2 inputs, which are defined as "significant other observable" inputs. The inputs used are from market sources that aggregate data based upon market transactions.

The table below shows the outstanding swap quantities used to hedge forecasted purchases and sales as of December 31, 2024, and December 31, 2023:

Hedge Contracts	Classification	December 31, 2024	December 31, 2023
Natural gas (in mmbtus)	Commodity purchase swaps	**20,706,000**	20,625,000
Tin (in metric tons)	Commodity purchase swaps	**—**	5
Zinc (in metric tons)	Commodity purchase swaps	**9,300**	26,148
Electricity (in megawatt hours)	Commodity purchase swaps	**16,400**	183,288
Iron ore pellets (in metric tons)	Commodity purchase swaps	**2,880,000**	0
Iron ore pellets (in metric tons)	Sales swaps	**300,900**	120,834
Hot-rolled coils (in tons)	Sales swaps	**236,000**	261,000
Foreign currency (in millions of euros)	Foreign exchange forwards	**€ 368**	€ 452
Foreign currency (in millions of dollars)	Foreign exchange forwards	**$ —**	$ 37

The following summarizes the fair value amounts included in our Consolidated Balance Sheets as of December 31, 2024, and December 31, 2023:

Balance Sheet Location (in millions)	December 31, 2024	December 31, 2023
Designated as Hedging Instruments		
Accounts receivable	**$ 41**	$ 4
Accounts payable	**8**	81
Investments and long-term receivables	**1**	—
Other long-term liabilities	**1**	2

The table below summarizes the effect of hedge accounting on AOCI and amounts reclassified from AOCI into earnings for 2024, 2023, and 2022:

	Gain (Loss) on Derivatives in AOCI				Amount of Gain (Loss) Recognized in Income		
(In millions)	2024	2023	2022	Location of Reclassification from AOCI	2024	2023	2022
Sales swaps	**$ 58**	$ (41)	$ 14	Net sales	**$ 8**	$ (15)	$ (18)
Commodity purchase swaps	**20**	34	(35)	Cost of sales [(a)]	**(40)**	(105)	103
Foreign exchange forwards	**28**	(5)	(16)	Cost of sales	**7**	(1)	44

[(a)] Costs for commodity purchase swaps are recognized in cost of sales as products are sold.

The table below summarizes the impact of derivative activity where hedge accounting has not been elected on our Consolidated Statements of Operations for 2024, 2023, and 2022:

		Amount of Gain (Loss) Recognized in Income		
(In millions)	Consolidated Statements of Operations Location	2024	2023	2022
Commodity purchase swaps	Cost of sales	**$ —**	$ (13)	$ 18
Foreign exchange forwards	Other financial costs	**(1)**	1	(7)

At current contract values, $22 million in AOCI as of December 31, 2024, will be recognized as a decrease in cost of sales over the next year, and $12 million in AOCI as of December 31, 2024, will be recognized as an increase in net sales over the next year.

17. Debt

(In millions)	Issuer/Borrower	Interest Rates %	Maturity	December 31, 2024	December 31, 2023
2037 Senior Notes	U. S. Steel	6.650	2037	**$ 274**	$ 274
2026 Senior Convertible Notes	U. S. Steel	5.000	2026	**349**	350
2029 Senior Notes	U. S. Steel	6.875	2029	**475**	475
2029 Senior Secured Notes	Big River Steel	6.625	2029	**720**	720
Environmental Revenue Bonds	U. S. Steel	4.125 - 6.750	2026 - 2053	**1,104**	1,164
Environmental Revenue Bonds	Big River Steel	4.500 - 4.750	2049	**752**	752
Finance leases and all other obligations	U. S. Steel	Various	2025 - 2029	**189**	157
Finance leases and all other obligations	Big River Steel	Various	2025 - 2027	**162**	167
ECA Credit Agreement	U. S. Steel	Variable	2031	**84**	97
Credit Facility Agreement	U. S. Steel	Variable	2027	**—**	—
Big River Steel ABL Facility	Big River Steel	Variable	2026	**—**	—
USSK Credit Agreement	U. S. Steel Kosice	Variable	2026	**—**	—
USSK Credit Facility	U. S. Steel Kosice	Variable	2027	**—**	—
Total debt				**4,109**	4,156
Less unamortized discount, premium and debt issuance costs				**(64)**	(66)
Less short-term debt, long-term debt due within one year, and short-term issuance costs				**95**	142
Long-term debt				**$ 4,078**	$ 4,080

Arkansas Development Finance Authority Environmental Improvement Revenue Bonds, Series 2023 (United States Steel Corporation Project) (Green Bonds)

On May 18, 2023, U. S. Steel closed on an offering consisting of an aggregate principal amount of $240 million unsecured Arkansas Development Finance Authority environmental improvement revenue bonds, which carry a green bond designation. The bonds, issued through Arkansas Development Finance Authority, have a coupon rate of 5.700% and a final maturity of 2053 (2053 ADFA Green Bonds). U. S. Steel received net proceeds of approximately $238 million after fees of approximately $2 million related to the underwriting and third-party expenses, and will pay semiannual interest. The net proceeds from the issuance of the 2053 ADFA Green Bonds were used to partially fund work related to the BR2 facility near Osceola, Arkansas.

On and after May 1, 2026, the Company may redeem the 2053 ADFA Green Bonds at its option, at any time in whole or from time to time in part at the redemption prices (expressed in percentages of principal amount) listed below, plus accrued and unpaid interest on the 2053 ADFA Green Bonds, if any, to, but excluding, the applicable redemption date, if redeemed during the twelve-month period beginning on May 1 of each of the years indicated below.

Year	Redemption Price
2026	105.000 %
2027	104.000 %
2028	103.000 %
2029	102.000 %
2030	101.000 %
2031 and thereafter	100.000 %

At any time prior to May 1, 2026, U. S. Steel may also redeem the 2053 ADFA Green Bonds, at our option, in whole or in part, or from time to time, at a price equal to the greater of 100 percent of the principal amount of the 2053 ADFA Green Bonds plus accrued and unpaid interest, if any, or the sum of the present value of the redemption price of the 2053 ADFA Green Bonds if they were redeemed on May 1, 2026, plus interest payments due through May 1, 2026, discounted to the date of redemption on a semi-annual basis at the applicable tax-exempt municipal bond rate, plus accrued and unpaid interest, if any.

2026 Senior Convertible Notes
In October 2019, U. S. Steel issued $350 million of 5.00% Senior Convertible Notes due November 1, 2026 (2026 Senior Convertible Notes). Interest on the 2026 Senior Convertible Notes is payable semi-annually on May 1 and November 1 of each year. The initial conversion rate for the 2026 Senior Convertible Notes is 74.8391 shares of U. S. Steel common stock per $1,000 principal amount, equivalent to an initial conversion price of approximately $13.36 per share of common stock, subject to adjustment pursuant to the 2026 Senior Convertible Notes indenture. Based on the initial conversion rate, the 2026 Senior Convertible Notes are convertible into 26,155,592 shares of U. S. Steel common stock and we reserved for the possible issuance of 33,348,361 shares, which is the maximum amount that could be issued upon conversion. Prior to August 1, 2026, holders of notes may convert all or a portion of their notes at their option only upon the satisfaction of specified conditions and during certain periods. On or after August 1, 2026, holders may convert all or a portion of their notes prior to the maturity date. Upon conversion, we will satisfy the obligation with cash, common stock, or a combination thereof, at our election. Anytime prior to August 1, 2026, if the price per share of U. S. Steel's common stock has been at least 130% of the conversion price for specified periods, U. S. Steel may redeem all or a portion of the 2026 Senior Convertible Notes at a cash redemption price of 100% of the principal amount, plus accrued and unpaid interest.

If U. S. Steel undergoes a fundamental change, as defined in the 2026 Senior Convertible Notes, holders may require us to repurchase the 2026 Senior Convertible Notes in whole or in part for cash at a price equal to 100% of the principal amount of the 2026 Senior Convertible Notes to be purchased plus any accrued and unpaid interest up to, but excluding the repurchase date.

Big River Steel — Sustainability Linked ABL Facility
Big River Steel's amended senior secured asset-based revolving credit facility (Big River Steel ABL Facility) matures on July 23, 2026. The facility is secured by first-priority liens on accounts receivable and inventory and certain other assets and second priority liens on most tangible and intangible assets of Big River Steel in each case subject to permitted liens. Additionally, the amendment includes sustainability targets related to greenhouse gas emissions intensity reduction, safety performance and facility certification by ResponsibleSteel™.

The Big River Steel ABL Facility provides for borrowings for working capital and general corporate purposes in an amount equal up to the lesser of (a) $350 million and (b) a borrowing base calculated based on specified percentages of eligible accounts receivables and inventory, subject to certain adjustments and reserves.

Big River Steel LLC must maintain a fixed charge coverage ratio of at least 1.00 to 1.00 for the most recent twelve consecutive months when availability under the Big River Steel ABL Facility is less than the greater of ten percent of the borrowing base availability and $13 million. Based on the most recent four quarters as of December 31, 2024, Big River Steel would have met the fixed charge coverage ratio test. The facility includes affirmative and negative covenants and events of default that are customary for facilities of this type.

There were no loans outstanding under the Big River Steel ABL Facility at December 31, 2024. Availability under the Big River Steel ABL Facility, pursuant to the available borrowing base, was $340 million at December 31, 2024.

U. S. Steel — Sustainability Linked Credit Facility Agreement
On May 27, 2022, U. S. Steel entered into the Sixth Amended and Restated Credit Facility Agreement (Credit Facility Agreement) to replace the existing Fifth Amended and Restated Credit Facility Agreement (Fifth Credit Facility Agreement). The Credit Facility Agreement has substantially the same terms as the Fifth Credit Facility Agreement, except the Credit Facility Agreement references the Secured Overnight Financing Rate instead of the London Interbank Offered Rate, adjusts the individual lenders' commitments, and renews the five-year maturity to May 27, 2027. The Credit Facility Agreement also adjusts the threshold for the fixed charge coverage ratio. The total availability under the facility remained the same at $1,750 million, and the financial impact from replacing the Fifth Credit Facility Agreement was immaterial. Consistent with the Fifth Credit Facility Agreement, the Credit Facility Agreement is secured by first-priority liens on certain accounts receivable and inventory and includes targets related to greenhouse gas emissions intensity reduction, safety performance and facility certification by ResponsibleSteel™.

The Credit Facility Agreement provides for borrowings for working capital and general corporate purchases in an amount equal to the lesser of (a) $1,750 million or (b) a borrowing base calculated based on specified percentages of eligible accounts receivable and inventory, subject to certain adjustments and reserves. As of December 31, 2024, there were approximately $4 million of letters of credit issued and no loans drawn under the Credit Facility Agreement. U. S. Steel must maintain a fixed charge coverage ratio of at least 1.00 to 1.00 for the most recent four consecutive quarters when availability under the Credit Facility Agreement is less than the greater of ten percent of the maximum facility availability and $140 million. Based on the most recent four quarters as of December 31, 2024, the Company would have met the fixed charge coverage ratio test.

USSK Credit Facilities
On September 28, 2023, the Company elected to reduce the size of the USSK Credit Agreement from €300 million to €150 million (approximately $156 million). The reduced credit facility size supports USSK's liquidity needs and is consistent with efforts to optimize costs and the global liquidity position. The USSK Credit Agreement matures in 2026 and contains sustainability targets related to greenhouse gas emissions intensity reduction, safety performance and facility certification by ResponsibleSteel™.

Under the USSK Credit Agreement, USSK is required to maintain a net debt to EBITDA ratio of less than 3.50:1.00 (the "EBITDA Ratio Covenant"), as measured on a rolling twelve month basis on June 30th and December 31st of each year. As of December 31, 2024, USSK met the EBITDA Ratio Covenant, and the USSK Credit Agreement was undrawn and fully available.

During the first quarter of 2023, USSK increased the size of its €20 million credit facility to €30 million (approximately $31 million) (the USSK Credit Facility). On September 13th, 2024, the Company amended the USSK Credit Facility to extend the term by three years, maturing in 2027. At December 31, 2024, USSK had no borrowings under the USSK Credit Facility, and the availability was approximately $15 million due to approximately $16 million of customs and other guarantees outstanding.

Debt Maturities – Aggregate maturities of debt are as follows (in millions):

2025	2026	2027	2028	2029	Later Years	Total
$ 99	$ 562	$ 130	$ 41	$ 1,216	$ 2,061	$ 4,109

18. Pensions and Other Benefits

U. S. Steel has defined contribution plans or multiemployer retirement benefits for more than 85 percent of its employees in the United States and non-contributory defined benefit pension plans covering the remaining employees. Benefits under the defined benefit pension plans are based upon years of service and final average pensionable earnings, with a minimum benefit based upon years of service. In addition, pension benefits for most non-represented employees under these plans are based upon a percent of total career pensionable earnings. Effective December 31, 2015, non-represented participants in the defined benefit plan no longer accrue additional benefits under the plan. For most non-represented employees without defined benefit coverage (the defined benefit pension plan was closed to new participants in 2003) and those for which the defined benefit plan was frozen, the Company also provides in the defined contribution plans (401(k) plans) a retirement account benefit based on salary and attained age. Most non-represented employees also participate in the 401(k) plans whereby the Company matches a certain percentage of salary based on the amount contributed by the participant. As of December 31, 2024, approximately 80 percent of U. S. Steel's represented employees in the United States are covered by the SPT, a multiemployer pension plan, to which U. S. Steel contributes on the basis of a fixed dollar amount for each hour worked.

Certain hourly employees of U. S. Steel's flat-rolled, tubular, cokemaking and iron ore operations in the United States are covered by collective bargaining agreements with the USW entered into effective September 1, 2022 (the 2022 Labor Agreements) that expire on September 1, 2026. The 2022 Labor Agreements include a signing bonus for each eligible USW-represented employee and annual 5% wage increases effective September 1, 2022, 2023, 2024, and 2025. Additionally, the 2022 Labor Agreements provide for increases to our pension and retirement benefits, including increases to our defined benefit pension plan, and to the contribution rate to the SPT from $3.50 to $4.00 per hour effective January 1, 2023. During the fourth quarter of 2022, U. S. Steel recorded a charge of approximately $67 million for the 2022 Labor Agreements signing bonus and related costs which was recognized primarily as a component of Cost of sales on the Consolidated Statements of Operations.

In addition, as part of the collective bargaining process, U. S. Steel and the USW agreed to leverage the overfunded OPEB plans to support the benefits provided to active represented employees. Beginning January 1, 2023, this agreement allows the Company to use a certain amount of surplus VEBA assets (the surplus amount) to pay for legally permissible benefits under Section 501(c)(9) of the Internal Revenue Code for active employees and retirees of the USW. The surplus amount represented a one-time transfer of VEBA assets to a subaccount to be used for these employees and retirees, which took place in the first quarter of 2023. The surplus amount of $595 million was determined as of December 31, 2022, (after merger of the UPI VEBA) and is the balance of VEBA assets in excess of 135% of the retiree obligation. The Company is permitted to withdraw a target of $75 million annually, with a guaranteed annual minimum of $50 million, and which may be in excess of $75 million in certain circumstances, on a quarterly pro rata basis, from the subaccount to cover the cost of the permissible benefits for active USW employees and USW retirees. As a result of its designation for this purpose, the surplus amount is presented as $75 million in Other current assets and $430 million in Other noncurrent assets on the Consolidated Balance Sheet as of December 31, 2024. Pursuant to the agreement with the USW, the Company merged the United States Steel Corporation Plan for Active Employee Insurance Benefits with and into the United States Steel Corporation Plan for Retiree Insurance Benefits effective January 1, 2023. Upon the one-time transfer from the VEBA to the subaccount, the surplus assets were no longer accounted for under ASC 715, *Compensation-Retirement Benefits*, and are instead accounted for primarily under the provisions of ASC 825 *Financial Instruments,* resulting in the portfolio being accounted for on a fair value basis, with gains and losses recognized in earnings, and reported as activity in Other financial costs (benefits) on the Company's Consolidated Statements of Operations.

On November 8, 2021, U. S. Steel entered into a commitment agreement with Banner Life Insurance Company and William Penn Life Insurance Company of New York (the "Insurers") and State Street Global Advisors Trust Company, as

independent fiduciary to the United States Steel Corporation Plan for Employee Pension Benefits (Revision of 2003), for the plan to purchase group annuity contracts and transfer approximately $284 million of its pension plan obligations to the Insurers. The purchase of the group annuity contracts was funded directly by the assets of the pension plan. The purchase resulted in the transfer of administrative and benefit-paying responsibilities for approximately 17,800 U.S. retirees and beneficiaries to the Insurers. The Insurers began paying benefits for certain retirees and beneficiaries in the Plan on January 1, 2022. There was no change to the pension benefits for any retirees and beneficiaries as a result of the transaction.

On October 26, 2022, U. S. Steel entered into an agreement with Pacific Life Insurance Company (the Insurer) and State Street Global Advisors Trust Company, as independent fiduciary to the UPI Pension Plan, for the plan to purchase group annuity contracts and transfer approximately $115 million of its pension plan obligations to the Insurer. The purchase of the group annuity contracts was completed on November 1, 2022, and funded directly by the assets of the pension plan. The purchase resulted in the transfer of the administrative and benefit-paying responsibilities for approximately 850 U.S. retirees and beneficiaries to the Insurer. The Insurer began paying benefits for certain retirees and beneficiaries in the Plan on January 1, 2023. There was no change to the pension benefits for any retirees and beneficiaries as a result of the transaction. As a result of the transaction, the Corporation recognized a non-cash pension settlement credit of approximately $3 million. This amount was reclassified to earnings through Net interest and other financial costs from accumulated other comprehensive loss.

Effective as of December 15, 2022, the UPI Pension Plan was merged into the U. S. Steel pension plan.

U. S. Steel's defined benefit retiree health care and life insurance plans (Other Benefits) cover the majority of its represented employees in the United States upon their retirement. Health care benefits are provided for Medicare and pre-Medicare retirees, with Medicare retirees largely enrolled in Medicare Advantage Plans. Both are subject to various cost sharing features, and in most cases domestically, an employer cap on total costs. The Other Benefits plan was closed to represented employees hired or rehired under certain conditions on or after January 1, 2016.

The retiree medical and retiree life insurance plans, for non-represented employees were amended to eliminate retiree medical benefits effective December 31, 2017, and to eliminate retiree life insurance benefits for employees who retired after December 31, 2017.

The majority of U. S. Steel's European employees are covered by government-sponsored programs into which U. S. Steel makes required contributions. Also, U. S. Steel sponsors defined benefit plans for most European employees covering benefit payments due to employees upon their retirement, some of which are government mandated. These same employees receive service awards throughout their careers based on stipulated service and, in some cases, age and service.

U. S. Steel uses a December 31 measurement date for its plans and may have an interim measurement date if significant events occur. Details relating to pension benefits and Other Benefits are below.

	Pension Benefits		Other Benefits	
(In millions)	**2024**	**2023**	**2024**	**2023**
Change in benefit obligations				
Benefit obligations at January 1	**$ 4,071**	$ 4,186	**$ 1,119**	$ 1,236
Service cost	**29**	33	**4**	6
Interest cost	**212**	220	**60**	67
Actuarial losses (gains)	**(118)**	24	**(18)**	(77)
Exchange rate gain	**(1)**	(1)	**—**	—
Settlements, curtailments and termination benefits	**—**	12	**—**	—
Benefits paid	**(411)**	(403)	**(103)**	(113)
Benefit obligations at December 31	**$ 3,782**	$ 4,071	**$ 1,062**	$ 1,119
Change in plan assets				
Fair value of plan at January 1	**$ 4,174**	$ 4,170	**$ 1,172**	$ 1,151
Actual return on plan assets	**148**	406	**18**	98
Asset reversion	**—**	—	**(10)**	(2)
Benefits paid from plan assets	**(410)**	(402)	**(71)**	(75)
Fair value of plan assets at December 31	**$ 3,912**	$ 4,174	**$ 1,109**	$ 1,172
Funded status of plans at December 31	**130**	103	**47**	53

For pension benefits, the largest contributor to the actuarial gain in 2024 was the increase in the discount rate from 5.49% at December 31, 2023, to 5.90% at December 31, 2024. In 2023, the largest contributor of actuarial loss was the decrease in the discount rate from 5.55% at December 31, 2022, to 5.49% at December 31, 2023.

For Other Benefits, the largest contributor to the actuarial gain in 2024 was the increase in the discount rate from 5.58% at December 31, 2023, to 5.90% at December 31, 2024. In 2023, the largest contributor to the actuarial gain was attributable to reductions in future health care costs.

Amounts recognized in accumulated other comprehensive loss:

		2024		
(In millions)	12/31/2023	Amortization	Activity	12/31/2024
Pensions				
Prior Service Cost	$ 113	$ (17)	$ —	**$ 96**
Actuarial Losses	1,409	(43)	31	**1,397**
Other Benefits				
Prior Service Credit	(18)	27	—	**9**
Actuarial Gains	(664)	65	38	**(561)**

As of December 31, 2024, and 2023, the following amounts were recognized in the Consolidated Balance Sheets:

	Pension Benefits		Other Benefits	
(In millions)	2024	2023	2024	2023
Noncurrent assets	**$ 154**	$ 131	**$ 51**	$ 57
Current liabilities	**(2)**	(2)	**(1)**	(1)
Noncurrent liabilities	**(22)**	(26)	**(3)**	(3)
Accumulated other comprehensive loss [(a)]	**1,493**	1,522	**(552)**	(682)
Net amount recognized	**$ 1,623**	$ 1,625	**$ (505)**	$ (629)

[(a)] Accumulated other comprehensive loss effects associated with accounting for pensions and other benefits in accordance with ASC Topic 715 at December 31, 2024, and December 31, 2023, respectively, are reflected net of tax of $626 million and $602 million respectively, on the Consolidated Statements of Stockholders' Equity.

The Accumulated Benefit Obligation (ABO) for all defined benefit pension plans was $3,679 million and $3,956 million at December 31, 2024, and 2023, respectively.

	December 31,	
(In millions)	2024	2023
Information for pension plans with an accumulated benefit obligation in excess of plan assets:		
Aggregate accumulated benefit obligations (ABO)	**$ (18)**	$ (21)
Aggregate projected benefit obligations (PBO)	**(24)**	(28)
Aggregate fair value of plan assets	**—**	—

The aggregate PBO in excess of plan assets reflected above is included in the payroll and benefits payable and employee benefits lines on the Consolidated Balance Sheets.

Following are the details of net periodic benefit costs (credits) related to Pension and Other Benefits:

	Pension Benefits			Other Benefits		
(In millions)	**2024**	**2023**	**2022**	**2024**	**2023**	**2022**
Components of net periodic benefit cost (credits):						
Service cost	**$ 29**	$ 33	$ 45	**$ 4**	$ 6	$ 9
Interest cost	**212**	220	157	**60**	67	49
Expected return on plan assets	**(298)**	(328)	(357)	**(74)**	(61)	(91)
Amortization - prior service costs (credits)	**17**	17	2	**(27)**	(23)	(24)
- actuarial losses (gains)	**43**	13	73	**(65)**	(72)	(53)
Net periodic benefit cost (credits), excluding	**3**	(45)	(80)	**(102)**	(83)	(110)
Multiemployer plans [(a)]	**79**	83	74	**—**	—	—
Settlement, termination and curtailment losses	**—**	13	12	**—**	—	2
Net periodic benefit cost (credits)	**$ 82**	$ 51	$ 6	**$ (102)**	$ (83)	$ (108)

(a) Primarily represents pension expense for the SPT covering USW employees hired from National Steel Corporation and new USW employees hired after May 21, 2003.

Net periodic benefit expense (credits) for pensions and Other Benefits is projected to be approximately $139 million and approximately $(44) million, respectively, in 2025. The pension cost projection includes approximately $79 million of contributions to the SPT.

Weighted average assumptions used to determine the benefit obligation at December 31 and net periodic benefit cost for the year ended December 31 are detailed below.

	Pension Benefits		Other Benefits	
	2024	**2023**	**2024**	**2023**
	U.S. and Europe	**U.S. and Europe**	**U.S.**	**U.S.**
Actuarial assumptions used to determine benefit obligations at December 31:				
Discount rate	**5.90 %**	5.49 %	**5.90 %**	5.58 %
Increase in compensation rate	**3.15 %**	3.15 %	**N/A**	N/A

	Pension Benefits			Other Benefits		
	2024	**2023**	**2022**	**2024**	**2023**	**2022**
	U.S. and Europe	**U.S. and Europe**	**U.S. and Europe**	**U.S.**	**U.S.**	**U.S.**
Actuarial assumptions used to determine net periodic benefit cost for the year ended December 31:						
Discount rate	**5.49 %**	5.55 %	3.01 %	**5.58 %**	5.66 %	3.11 %
Expected annual return on plan assets	**6.90 %**	6.90 %	6.82 %	**6.00 %**	4.50 %	4.50 %
Increase in compensation rate	**3.15 %**	3.15 %	2.60 %	**N/A**	N/A	N/A

The discount rate reflects the current rate at which the pension and Other Benefit liabilities could be effectively settled at the measurement date. In 2017, we refined our discount rate determination process for our U.S. plans by using a bond matching approach to select specific bonds that would satisfy our projected benefit payments. We believe the bond matching approach more closely reflects the process we would employ to settle our pension and other benefits obligations. For our European pension plan, the discount rate is determined using data published by European Central Bank and underlying data provided by EuroMTS Ltd. The discount rate assumptions are updated annually.

	2024	**2023**
Assumed health care cost trend rates at December 31:	**U.S.**	**U.S.**
Health care cost trend rate assumed for next year	**5.80%**	5.90%
Rate to which the cost trend rate is assumed to decline (the ultimate trend rate)	**4.50%**	4.50%
Year that the rate reaches the ultimate trend rate	**2038**	2038

U. S. Steel reviews its actual historical rate experience and expectations of future health care cost trends to determine the escalation of per capita health care costs under U. S. Steel's benefit plans. About 85 percent of our costs for the domestic USW participants' retiree health benefits in the Company's main domestic benefit plan are limited to a per capita dollar maximum calculation based on 2006 base year actual costs incurred under the main U. S. Steel benefit plan for USW participants (cost cap). The full effect of the cost cap is expected to be realized around 2031. After 2031, the Company's costs for a majority of USW retirees and their dependents are expected to remain fixed and as a result, the cost impact of health care escalation for the Company is projected to be limited for this group.

Plan Assets

ASC Topic 820 establishes a single definition of fair value, creates a three-tier hierarchy as a framework for measuring fair value based on inputs used to value the Plan's investments, and requires additional disclosure about fair value. The hierarchy gives the highest priority to unadjusted quoted prices in active markets for identical assets or liabilities (level 1) and the lowest priority to unobservable inputs (level 3). The three levels of the fair value hierarchy are summarized below:

- Level 1 – Inputs to the valuation methodology are unadjusted quoted prices for identical assets or liabilities in active markets that the Plan has the ability to access.
- Level 2 – Inputs to the valuation methodology include:
  - Quoted prices for similar assets or liabilities in active markets;
  - Quoted prices for identical or similar assets or liabilities in inactive markets;
  - Inputs other than quoted prices that are observable for the asset or liability;
  - Inputs that are derived principally from or corroborated by observable market data by correlation or other means

  If the asset or liability has a specified (contractual) term, the level 2 input must be observable for substantially the full term of the asset or liability.

- Level 3 – Inputs to the valuation methodology are unobservable and significant to the fair value measurement.

An instrument's level is based on the lowest level of any input that is significant to the fair value measurement. Valuation techniques maximize the use of relevant observable inputs and minimize the use of unobservable inputs. The following is a description of the valuation methodologies used for assets measured at fair value. There have been no changes in the methodologies used at December 31, 2024, and 2023.

Short-term investments are valued at amortized cost which approximates fair value due to the short-term maturity of the instruments. Equity securities - U.S. & International are valued at the closing price reported on the active exchange on which the individual securities are traded. U.S. and Non U.S. government bonds are valued using pricing models maximizing the use of observable inputs for similar securities. Corporate U.S. & Non U.S. bonds are also valued using pricing models maximizing the use of observable inputs for similar securities, which includes basing value on yields currently available on comparable securities of issuers with similar credit ratings. When quoted prices are not available for identical or similar bonds, the bond is valued under a discounted cash flow approach that maximizes observable inputs, such as current yields of similar instruments, but includes adjustments for certain risks that may not be observable, such as credit and liquidity risks. Mortgage and asset-backed securities are valued using quotes from a broker dealer. Private equities and real estate are valued using information provided by external managers for each individual investment held in the fund or using NAV (net asset value) as a practical expedient. Timberland investments are valued at their appraised value. Mineral Interests and other alternatives are valued at the present value of estimated future cash flows discounted at estimated market rates for assets of similar quality and duration.

The fair value of U. S. Steel's pension plan assets by asset category at December 31 was as follows (in millions):

	2024					2023				
	Level 1	Level 2	Level 3	measured at NAV [a]	Total	Level 1	Level 2	Level 3	measured at NAV [a]	Total
Asset Category										
Equity										
U. S. companies	$ 295	$ —	$ —	$ —	$ 295	$ 332	$ —	$ —	$ —	$ 332
International companies	419	—	—	—	419	433	—	—	—	433
Derivative financial instruments	38	—	—	—	38	—	7	—	—	7
Total equity	752	—	—	—	752	765	7	—	—	772
Fixed Income										
Corporate Bonds - U.S.	—	706	—	—	706	—	654	—	—	654
Corporate Bonds - Non-U.S.	—	116	—	—	116	—	129	—	—	129
U.S. government and agencies	—	178	—	—	178	—	197	—	—	197
Non-U.S. government	—	66	—	—	66	—	43	—	—	43
Mortgage and asset-backed securities	—	6	—	—	6	—	9	—	—	9
Derivative financial instruments	2	2	—	—	4	—	6	—	—	6
Total fixed income	2	1,074	—	—	1,076	—	1,038	—	—	1,038
Alternatives										
Timberlands	—	—	—	1	1	—	—	—	21	21
Mineral Interests and other alternatives	—	—	—	348	348	—	—	—	239	239
Private equity	—	—		210	210	—	—	—	235	235
Real estate	—	—	—	251	251	—	—	—	245	245
Total alternatives	—	—	—	810	810	—	—	—	740	740
Commingled Funds	—	—	—	1,154	1,154	—	—	—	1,499	1,499
Short-Term Investments	55	36	—	—	91	106	5	—	—	111
Other [b]	29	—	—	—	29	14	—	—	—	14
Total assets at fair value	$ 838	$ 1,110	$ —	$ 1,964	$ 3,912	$ 885	$ 1,050	$ —	$ 2,239	$ 4,174

[a] In accordance with ASC Topic 820, certain investments that were measured at net asset value per share (or its equivalent) have not been classified in the fair value hierarchy.

[b] Includes cash, accrued income, and miscellaneous payables.

The following table sets forth a summary of changes in the fair value of U. S. Steel's Pension plan Level 3 assets for the years ended December 31, 2024, and 2023:

	Level 3 assets only	
(In millions)	2024	2023
Balance at beginning of period	$ —	$ 89
Transfers in and/or out of Level 3	—	(89)
Actual return on plan assets:		
Realized gain	—	—
Net unrealized gain	—	—
Purchases, sales, issuances and settlements:		
Purchases	—	—
Sales	—	—
Balance at end of period	$ —	$ —

The fair value of U. S. Steel's Other Benefits plan assets by asset category at December 31 was as follows (in millions):

	2024					2023				
	Level 1	Level 2	Level 3	measured at NAV [a]	Total	Level 1	Level 2	Level 3	measured at NAV [a]	Total
Asset Category										
Equity										
U. S. companies	$ 64	$ —	$ —	$ —	$ 64	$ 65	$ —	$ —	$ —	$ 65
International companies	24	—	—	—	24	31	—	—	—	31
Total equity	88	—	—	—	88	96	—	—	—	96
Fixed Income										
Corporate Bonds - U.S.	—	435	—	—	435	—	476	—	—	476
Corporate Bonds - Non-U.S.	—	78	—	—	78	—	108	—	—	108
U.S. government and agencies	—	89	—	—	89	—	105	—	—	105
Non-U.S. government	—	15	—	—	15	—	14	—	—	14
Mortgage and asset-backed securities	—	11	—	—	11	—	11	—	—	11
Total fixed income	—	628	—	—	628	—	714	—	—	714
Alternatives										
Timberlands	—	—	—	—	—	—	—	—	3	3
Other alternatives	—	—	29	70	99	—	—	—	41	41
Private equity	—	—	—	50	50	—	—	—	54	54
Real estate	—	—	—	20	20	—	—	—	30	30
Total alternatives	—	—	29	140	169	—	—	—	128	128
Commingled Funds	—	—	—	177	177	—	—	—	190	190
Short-Term Investments	19	10	—	—	29	13	1	—	—	14
Other [b]	18	—	—	—	18	30	—	—	—	30
Total assets at fair value	$ 125	$ 638	$ 29	$ 317	$ 1,109	$ 139	$ 715	$ —	$ 318	$ 1,172

[a] In accordance with ASC Topic 820, certain investments that were measured at net asset value per share (or its equivalent) have not been classified in the fair value hierarchy.
[b] Includes cash, accrued income, and miscellaneous payables.

The following table sets forth a summary of changes in the fair value of U. S. Steel's Other Benefits plan Level 3 assets for the years ended December 31, 2024, and 2023:

	Level 3 assets only	
(In millions)	2024	2023
Balance at beginning of period	$ —	$ 47
Transfers in and/or out of Level 3	—	(47)
Actual return on plan assets:		
Realized gain	—	—
Net unrealized loss	—	—
Purchases, sales, issuances and settlements:		
Purchases	29	—
Sales	—	—
Balance at end of period	$ 29	$ —

U. S. Steel's investment strategy for its U.S. pension and Other Benefits plan assets provides for a diversified mix of high quality bonds, public equities and selected smaller investments in private equities and private credit. For its U.S. pension, U. S. Steel has a target allocation for plan assets of 68 percent in corporate bonds, government bonds and mortgage, private credit, and asset-backed securities. The balance is invested in equity securities, timber, private equity and real estate partnerships. U. S. Steel believes that returns on equities over the long term will be higher than returns from fixed-income securities as actual historical returns from U. S. Steel's trusts have shown. Returns on bonds tend to offset some of the short-term volatility of stocks. Both equity and fixed-income investments are made across a broad range of industries and companies (both domestic and foreign) to provide protection against the impact of volatility in any single industry as well as company specific developments. U. S. Steel will use a 6.90 percent assumed rate of return on assets for the development of net periodic cost for the main defined benefit pension plan in 2025. Actual returns since the inception of the plan have exceeded this 6.90 percent rate and while recent annual returns have been volatile, it is U. S. Steel's expectation that rates will achieve this level in future periods.

For its Other Benefits plan, U. S. Steel is employing a liability driven investment strategy. The plan assets are allocated to match the plan cash flows with maturing investments. To achieve this strategy, U. S. Steel has a target allocation for plan assets of 78 percent in fixed income and private credit. The balance is primarily invested in equity securities, private equity and real estate partnerships. U. S. Steel will use a 6.00 percent assumed rate of return on assets for the development of net periodic cost for its Other Benefit plans for 2025.

Steelworkers Pension Trust

For most bargaining unit employees participating in the SPT, U. S. Steel contributed to the SPT a fixed dollar amount for each hour worked of $4.00 through December 31, 2024. SPT contributions increased from $3.50 to $4.00 per hour effective January 1, 2023. U. S. Steel's contributions to the SPT represented greater than 30% of the total combined contributions of all employers participating in the plan for the years ended December 31, 2024, 2023, and 2022.

Participation in a multiemployer pension plan agreed to under the terms of a collective bargaining agreement differ from a traditional qualified single employer defined benefit pension plan. The SPT shares risks associated with the plan in the following respects:

a. Contributions to the SPT by U. S. Steel may be used to provide benefits to employees of other participating employers;

b. If a participating employer stops contributing to the SPT, the unfunded obligations of the plan may be borne by the remaining participating employers; and

c. If U. S. Steel chooses to stop participating in the SPT, U. S. Steel may be required to pay an amount based on the underfunded status of the plan, referred to as a withdrawal liability.

On March 21, 2011, the Board of Trustees of the SPT elected funding relief which has the effect of decreasing the amount of required minimum contributions in near-term years, but will increase the minimum funding requirements during later plan years. As a result of the election of funding relief, the SPT's zone funding under the Pension Protection Act may be impacted.

In addition to the funding relief election, the Board of Trustees also elected a special amortization rule, which allows the SPT to separately amortize investment losses incurred during the SPT's December 31, 2008 plan year-end over a 29 year period, whereas they were previously required to be amortized over a 15 year period.

U. S. Steel's participation in the SPT for the annual periods ended December 31, 2024, 2023, and 2022 is outlined in the table below.

Pension Fund	Employer Identification Number/ Pension Plan Number	Pension Protection Act Zone Status as of December 31 [a]		FIP/RP Status Pending/ Implemented[b]	U. S. Steel Contributions (in millions)			Surcharge Imposed[c]		Expiration Date of Collective Bargaining Agreement
		2024	2023		2024	2023	2022	2024	2023	
Steelworkers Pension Trust	23-6648508/499	Green	Green	No	$ 83	$ 82	$ 74	No	No	September 1, 2026

[a] The zone status is based on information that U. S. Steel received from the plan and is certified by the plan's actuary. Among other factors, plans in the green zone are at least 80 percent funded, while plans in the yellow zone are less than 80 percent funded and plans in the red zone are less than 65 percent funded.

[b] Indicates if a financial improvement plan (FIP) or a rehabilitation plan (RP) is either pending or has been implemented.

[c] Indicates whether there were charges to U. S. Steel from the plan.

Cash Flows

The following information is in addition to the contributions to the SPT noted in the table above.

Employer Contributions **–** U. S. Steel did not make any voluntary or mandatory contributions to the U. S. Steel Retirement Plan Trust in 2024 or 2023. The U. S. Steel Retirement Plan Trust is the funding vehicle for the Company's main defined benefit pension plan.

For pension plans not funded by trusts, U. S. Steel made $1 million, $3 million, and $2 million of pension payments in 2024, 2023, and 2022, respectively.

Cash payments totaling $32 million, $40 million, and $32 million were made for other post-employment benefit payments not funded by trusts in 2024, 2023, and 2022, respectively. In 2024, 2023, and 2022, U. S. Steel continued to use assets from our VEBA trust for represented retiree health care and life insurance benefits to pay USW post-employment benefit claims.

Estimated Future Benefit Payments – The following benefit payments, which reflect expected future service as appropriate, are expected to be paid from U. S. Steel's defined benefit plans:

(In millions)	Pension Benefits	Other Benefits
2025	$ 1,043	$ 102
2026	1,043	109
2027	1,154	111
2028	1,182	114
2029	1,451	117
Years 2030 - 2034	9,904	437

Defined contribution plans
U. S. Steel also contributes to two defined contribution plans for its salaried employees. Most non-represented salaried employees in North America receive pension benefits in the form of a separate retirement account through a defined contribution plan with contribution percentages based upon age, for which company contributions totaled $19 million, $21 million, and $21 million in 2024, 2023, and 2022, respectively. U. S. Steel's matching contributions to the salaried employees' defined contribution plans, which for most non-represented salaried employees are 100 percent of the employees' contributions up to six percent of their eligible salary, totaled $20 million, $20 million, and $19 million in 2024, 2023, and 2022, respectively. U. S. Steel also maintains non-qualified defined contribution plans to provide benefits which are otherwise limited by the terms of the qualified plan or the Internal Revenue Code. U. S. Steel's contributions under these defined contribution plans were less than $1 million in 2024, $2 million in 2023, and less than $1 million in 2022.

Most represented employees are eligible to participate in a defined contribution plan where there is no company match on savings or separate retirement account except for certain Tubular hourly employees. Effective with the 2015 Labor Agreement, represented employees hired on or after January 1, 2016, are eligible for a $0.50 per hour savings account contribution. As a result of the 2018 Labor Agreements, the retiree healthcare account contribution for each hour worked increased to $0.55 effective January 1, 2019, $0.60 effective January 1, 2020, and $0.65 effective January 1, 2021. As a result of the 2022 Labor Agreements, the retiree healthcare account contribution for each hour worked increased to $0.75 effective January 1, 2023. These Company contributions for represented employees totaled $7 million, $7 million, and $5 million in 2024, 2023, and 2022, respectively.

Other post-employment benefits
The Company provides benefits to former or inactive employees after employment but before retirement. Certain benefits including workers' compensation and black lung benefits represent material obligations to the Company and under the guidance for nonretirement post-employment benefits, have historically been treated as accrued benefit obligations. Liabilities for these benefits recorded at December 31, 2024, totaled $81 million as compared to $91 million at December 31, 2023. Liability amounts were developed assuming a discount rate of 5.76% and 5.66% at December 31, 2024, and 2023. Net periodic benefit cost for these benefits is projected to be $15 million in 2025 compared to $9 million in 2024 and $24 million in 2023.

Pension Funding

In March 2021, the American Rescue Plan Act (ARPA - H.R. 1319) further extended the pension relief interest rate corridor used to measure defined benefit pension obligations for calculating minimum annual contributions. The new interest rate formula results in higher interest rates for minimum funding calculations as compared to prior law over the next few years, which will improve the funded status of our main defined benefit pension plan and reduce minimum required contributions.

U. S. Steel will monitor the funded status of the plan to determine when voluntary contributions may be prudent in order to mitigate potentially larger mandatory contributions in later years.

19. Asset Retirement Obligations

U. S. Steel's asset retirement obligations (AROs) primarily relate to mine, landfill closure and post-closure costs. The following table reflects changes in the carrying values of AROs for the years ended December 31, 2024, and 2023:

	December 31,	
(In millions)	**2024**	**2023**
Balance at beginning of year	**$ 79**	$ 66
Additional obligations incurred	**—**	12
Obligations settled	**(4)**	(1)
Change in estimate of obligations	**(1)**	—
Foreign currency translation effects	**(1)**	—
Accretion expense	**4**	2
Balance at end of period	**$ 77**	$ 79

Certain AROs related to disposal costs of the majority of fixed assets at our integrated steel facilities have not been recorded because they have an indeterminate settlement date. These AROs will be initially recognized in the period in which sufficient information exists to estimate their fair value.

20. Fair Value of Financial Instruments

The carrying value of cash and cash equivalents, current accounts and notes receivable, accounts payable and accrued interest included in the Consolidated Balance Sheets approximate fair value. See Note 16 for disclosure of U. S. Steel's derivative instruments, which are accounted for at fair value on a recurring basis.

Stelco Option for Minntac Mine Interest
On April 30, 2020, the Company entered into an Option Agreement with Stelco, a subsidiary of Cleveland-Cliffs Inc., that grants Stelco the option to purchase a 25 percent interest (the Option Interest) in a to-be-formed entity (the Joint Venture) that will own the Company's current iron ore mine located in Mt. Iron, Minnesota (the Minntac Mine). As consideration for the option, Stelco paid the Company an aggregate amount of $100 million in five $20 million installments during the year ended December 31, 2020, which are recorded net of transaction costs in the Consolidated Balance Sheet. The option can be exercised any time before January 31, 2027, and in the event Stelco exercises the option, Stelco will contribute an additional $500 million to the Joint Venture, which amount shall be remitted solely to U. S. Steel in the form of a one-time special distribution, and the parties will engage in good faith negotiations to finalize the master agreement (pursuant to which Stelco will acquire the Option Interest) and the limited liability company agreement of the Joint Venture.

Surplus VEBA assets
During the fourth quarter 2022, U. S. Steel and the USW agreed to utilize the overfunded OPEB plans to support the benefits provided to active represented employees. Beginning January 1, 2023, this agreement allows the Company to use a certain amount of surplus VEBA assets (the surplus amount) to pay for legally permissible benefits under Section 501(c)(9) of the Internal Revenue Code for active employees and retirees of the USW. The surplus amount of $595 million was determined as of December 31, 2022, and was the balance of VEBA assets in excess of 135% of the retiree obligation at that time. On January 1, 2023, a subaccount was created and consisted of a pro-rata share of the existing trust. On February 1, 2023, using January 31, 2023 asset values, a new investment strategy was implemented and comprised of existing investments from the VEBA trust and cash. On February 1, 2023, certain assets were transferred from the VEBA to the subaccount. The Company is permitted to withdraw a target of $75 million annually, with a guaranteed annual minimum of $50 million, on a quarterly pro rata basis, from the subaccount to cover the cost of the permissible benefits for active USW employees and USW retirees. If after the annual withdrawal of $75 million, the subaccount value decreases by less than $40 million annually (defined as the "Threshold Surplus"), the Company may withdraw the excess above the Threshold Surplus. Based on the value of the subaccount as of December 31, 2023, approximately $15 million was withdrawn from the subaccount in January 2024 to pay for permissible benefits. The surplus VEBA assets subaccount portfolio consists of fixed income securities including corporate bonds, U.S. government bonds, and U.S. Treasury notes, in addition to alternatives including investments in private credit partnerships and real estate funds. A portion of the corporate bonds are classified as available-for-sale debt securities, with unrealized gains and losses reported in Accumulated other comprehensive (loss) income. Upon sale, realized gains and losses are reported in earnings. All other investments in the subaccount are financial instruments measured at fair value or net asset value, with gains and losses recognized through net earnings and are reported as Net gain from investments related to active employee benefits on the Company's Consolidated Statements of Operations

As of December 31, 2024, the fair value of the surplus VEBA assets subaccount portfolio was $505 million, with $75 million in Other current assets and $430 million in Other noncurrent assets on the Consolidated Balance Sheet.

As of December 31, 2023, the fair value of the surplus VEBA assets subaccount portfolio was $570 million, with $89 million in Other current assets and $481 million in Other noncurrent assets on the Consolidated Balance Sheet.

The value of corporate bonds classified as available-for-sale debt securities was $131 million and $208 million as of December 31, 2024, and 2023, respectively. A total pretax net gain related to available-for-sale securities of $7 million was included in Accumulated other comprehensive (loss) income as of both December 31, 2024, and 2023.

During 2024, pretax net gains of $25 million were recognized in Net gain from investments related to active employee benefits. During 2024, immaterial pretax net gains were recognized in Accumulated other comprehensive (loss) income.

During 2023, pretax net gains of $43 million and $7 million were recognized in Net gain from investments related to active employee benefits and in Accumulated other comprehensive (loss) income, respectively.

The fair value of the subaccount portfolio by asset category as of December 31, 2024 and 2023 was as follows (in millions):

	December 31, 2024					December 31, 2023				
	Level 1	Level 2	Level 3	measured at NAV [a]	Total	Level 1	Level 2	Level 3	measured at NAV [a]	Total
Asset Category										
Fixed Income										
Corporate bonds - U.S.	—	129	—	—	129	—	191	—	—	191
Corporate bonds - Non-U.S.	—	46	—	—	46	—	56	—	—	56
U.S. government bonds	—	43	—	—	43	—	86	—	—	86
Mortgage and asset-backed securities	—	14	—	—	14	—	12	—	—	12
Total fixed income	$ —	$ 232	$ —	$ —	$ 232	$ —	$ 345	$ —	$ —	$ 345
Alternatives										
Private credit partnerships	—	—	29	157	186	—	—	58	64	122
Other alternatives	—	—	—	19	19	—	—	—	18	18
Total alternatives	$ —	$ —	$ 29	$ 176	$ 205	$ —	$ —	$ 58	$ 82	$ 140
Commingled Funds	—	—	—	5	5	—	—	—	61	61
Other [b]	63	—	—	—	63	25	—	(1)	—	24
Total assets at fair value	$ 63	$ 232	$ 29	$ 181	$ 505	$ 25	$ 345	$ 57	$ 143	$ 570

[a] In accordance with ASC Topic 820, certain investments that were measured at net asset value per share (or its equivalent) have not been classified in the fair value hierarchy.

[b] Includes cash, accrued income, and miscellaneous payables.

The following table summarizes U. S. Steel's financial assets and liabilities that were not carried at fair value at December 31, 2024, and 2023.

	December 31, 2024		December 31, 2023	
(In millions)	Fair Value	Carrying Amount	Fair Value	Carrying Amount
Financial liabilities:				
Long-term debt [a]	**$ 4,243**	**$ 3,822**	$ 4,797	$ 3,899

[a] Excludes finance lease obligations.

The fair value of long-term debt was determined using Level 2 inputs which were derived from quoted market prices.

Fair value of the financial assets and liabilities disclosed herein is not necessarily representative of the amount that could be realized or settled, nor does the fair value amount consider the tax consequences of realization or settlement.

Financial guarantees are U. S. Steel's only unrecognized financial instrument. For details relating to financial guarantees see Note 26.

21. Reclassifications from Accumulated Other Comprehensive Income (AOCI)

(In millions)	Pension and Other Benefit Items	Foreign Currency Items	Unrealized (Loss) Gain on Derivatives	Active Employee Benefit Investments	Total
Balance at December 31, 2022	$ (322)	$ 280	$ (43)	$ —	$ (85)
Other comprehensive income (loss) before reclassifications	131	54	(77)	6	114
Amounts reclassified from AOCI [(a)]	(50)	—	68	(1)	17
Net current-period other comprehensive income (loss)	81	54	(9)	5	131
Balance at December 31, 2023	$ (241)	$ 334	$ (52)	$ 5	$ 46
Other comprehensive (loss) income before reclassifications	**(52)**	**(70)**	**72**	**3**	**(47)**
Amounts reclassified from AOCI [(a)]	**(25)**	**—**	**8**	**(3)**	**(20)**
Net current-period other comprehensive (loss) income	**(77)**	**(70)**	**80**	**—**	**(67)**
Balance at December 31, 2024	**$ (318)**	**$ 264**	**$ 28**	**$ 5**	**$ (21)**

[(a)] See table below for further details.

Details about AOCI components [(a)]	**Amount reclassified from AOCI**		
(In millions)	**2024**	**2023**	**2022**
Amortization of pension and other benefit items			
Prior service credits [(a)]	**$ (10)**	$ (6)	$ (23)
Actuarial (gains) losses [(a)]	**(22)**	(60)	20
Settlements, termination and curtailment losses (gains) [(a)]	**—**	1	(3)
Total pensions and other benefits items	**(32)**	(65)	(6)
Derivative reclassifications to Consolidated Statements of Operations	**12**	143	(133)
Active employee benefit investment reclassifications to Consolidated Statements of Operations	**(4)**	(1)	—
Total before tax	**(24)**	77	(139)
Tax provision (benefit)	**4**	(60)	32
Net of tax	**$ (20)**	$ 17	$ (107)

[(a)] These AOCI components are included in the computation of net periodic benefit cost (see Note 18 for additional details).

22. Supplemental Cash Flow Information

	Year Ended December 31,		
(In millions)	**2024**	**2023**	**2022**
Net cash used in operating activities included:			
Interest and other financial costs paid (net of amount capitalized)	**$ (35)**	$ (68)	$ (161)
Income taxes paid	**$ (53)**	$ (86)	$ (242)
Non-cash investing and financing activities:			
Change in accrued capital expenditures	**$ (109)**	$ (2)	$ 351
U. S. Steel common stock issued for employee/non-employee director stock plans	**$ 45**	$ 45	$ 46
Capital expenditures funded by finance lease borrowings	**$ 78**	$ 90	$ 52
Export Credit Agreement (ECA) financing	**$ —**	$ 5	$ —

In January 2025, the Company received a refund of approximately $44 million for the 2023 federal income tax return, which included approximately $1 million of interest.

23. Transactions with Related Parties

Related party sales and service transactions are primarily related to equity investees and were $2,505 million, $2,015 million, and $1,942 million in 2024, 2023, and 2022, respectively.

Accounts payable to related parties include balances due to PRO-TEC Coating Company (PRO-TEC) of $144 million and $137 million at December 31, 2024, and 2023, respectively for invoicing and receivables collection services provided by U. S. Steel on PRO-TEC's behalf. U. S. Steel, as PRO-TEC's exclusive sales agent, is responsible for credit risk related to those receivables. U. S. Steel also provides PRO-TEC marketing, selling and customer service functions. Payables to other related parties totaled $2 million for both the period ending December 31, 2024 and the period ending December 31, 2023.

Purchases from related parties for outside processing services provided by equity investees amounted to $19 million, $26 million, and $27 million during 2024, 2023, and 2022, respectively. Purchases of iron ore pellets from related parties amounted to $87 million, $157 million, and $131 million for the years ended December 31, 2024, 2023, and 2022, respectively.

On December 18, 2023, the Company entered into the Merger Agreement by and among the Company, Purchaser, Merger Sub, and solely as provided in Section 9.13 therein, NSC. Pursuant to the Merger Agreement, and upon the terms and subject to the conditions described therein, Merger Sub will merge with and into the Company, with the Company continuing as the surviving corporation and a wholly owned subsidiary of Purchaser.

Wheeling-Nippon Steel, Inc., ("WNS") a wholly owned subsidiary of NSC (party to the Merger Agreement described above), currently is a customer of the Company. Net sales to related parties pertaining to business with WNS amounted to $322 million for the year ended December 31, 2024. Receivables from related parties as of December 31, 2024 include $6 million due from WNS.

24. Leases

Operating lease assets consist primarily of office space, heavy mobile equipment used in our mining operations and facilities and equipment under operating service agreements for electricity generation and scrap processing. We also have operating lease assets for light mobile equipment and information technology assets. The Company also has short-term leases related to transportation services for which we apply the short-term lease exception. Significant finance leases primarily consist of heavy mobile equipment used in our mining operations. Variable lease payments are primarily related to operating service agreements where payment is solely dependent on consumption of certain services, such as raw material and by-product processing. Most long-term leases include renewal options and, in certain leases, purchase options. Generally, we are not reasonably certain that these options will be exercised. We have residual value guarantees under certain light mobile equipment leases. There is no impact to our leased assets for residual value guarantees as the potential loss is not probable (see "Other Contingencies" in Note 26 for further details). We do not have material restrictive covenants associated with our leases or material amounts of sublease income. From time to time, U. S. Steel may enter into arrangements for the construction or purchase of an asset and then enter into a financing arrangement to lease the asset. U. S. Steel recognizes leased assets and liabilities under these arrangements when it obtains control of the asset.

The following table summarizes the lease amounts included in our Consolidated Balance Sheets as of December 31, 2024 and 2023.

(In millions)	Balance Sheet Location	December 31, 2024	December 31, 2023
Assets			
Operating	Operating lease assets [a]	**$ 72**	$ 109
Finance	Property, plant and equipment [b]	**198**	176
Total Lease Assets		**$ 270**	$ 285
Liabilities			
Current			
Operating	Current operating lease liabilities	**$ 35**	$ 44
Finance	Current portion of long-term debt	**58**	43
Non-Current			
Operating	Noncurrent operating lease liabilities	**44**	73
Finance	Long-term debt less unamortized discount and issue costs	**151**	137
Total Lease Liabilities		**$ 288**	$ 297

[a] Operating lease assets are recorded net of accumulated amortization of $129 million and $138 million as of December 31, 2024, and December 31, 2023, respectively.
[b] Finance lease assets are recorded net of accumulated depreciation of $182 million and $126 million as of December 31, 2024, and December 31, 2023, respectively.

The following table summarizes lease costs included in our Consolidated Statements of Operations for the years ended December 31, 2024, December 31, 2023, and December 31, 2022.

(In millions)	Classification	Year Ended December 31, 2024	Year Ended December 31, 2023	Year Ended December 31, 2022
Operating Lease Cost [(a)]	Cost of sales	**$ 48**	$ 54	$ 65
Operating Lease Cost	Selling, general and administrative expenses	**14**	15	14
Finance Lease Cost				
Amortization	Depreciation, depletion and amortization	**56**	42	26
Interest	Interest expense	**14**	11	9
Total Lease Cost		**$ 132**	$ 122	$ 114

[(a)] Operating lease cost recorded in cost of sales includes $13 million of variable lease cost for the year ended December 31, 2024. Operating lease cost recorded in cost of sales includes $12 million of variable lease cost for both the years ended December 31, 2023, and December 31, 2022. Operating lease cost recorded in selling, general and administrative expenses includes $1 million of variable lease cost for the years ended December 31, 2024, December 31, 2023, and December 31, 2022. $1 million of short-term lease cost is included in cost of sales and selling, general and administrative expenses for the years ended December 31, 2024, December 31, 2023, and December 31, 2022.

Lease liability maturities as of December 31, 2024, are shown below.

(In millions)	Operating	Finance	Total
2025	$ 39	$ 78	$ 117
2026	24	70	94
2027	15	50	65
2028	7	29	36
2029	1	7	8
After 2029	2	—	2
Total Lease Payments	**$ 88**	**$ 234**	**$ 322**
Less: Interest	9	25	34
Present value of lease liabilities	**$ 79**	**$ 209**	**$ 288**

Lease terms and discount rates are shown below.

	December 31, 2024
Weighted average lease term	
Finance	3 years
Operating	3 years
Weighted average discount rate	
Finance	6.05 %
Operating	7.14 %

Supplemental cash flow information related to leases is as follows:

(In millions)	Year Ended December 31, 2024	Year Ended December 31, 2023	Year Ended December 31, 2022
Cash paid for amounts included in the measurement of lease liabilities:			
Operating cash flows from operating leases	**$ 47**	$ 55	$ 66
Operating cash flows from finance leases	**14**	11	9
Financing cash flows from finance leases	**49**	34	20
Right-of-use assets exchanged for lease liabilities:			
Operating leases	**4**	14	20
Finance leases	**78**	90	52

25. Restructuring and Other Charges

During 2024, the Company recorded restructuring and other charges of $8 million, which related primarily to the idling of certain coke-making facilities. Cash payments related to previously accrued restructuring programs made during the year ended December 31, 2024 were approximately $89 million.

During 2023, the Company recorded restructuring and other charges of $36 million, which relate primarily to Company-wide headcount reductions. Cash payments were made related to severance and exit costs of approximately $75 million.

During 2022, the Company recorded restructuring and other charges of $48 million, which consists of charges of $30 million related to the planned disposition of a component within the Flat-Rolled segment, $23 million related to headcount reductions under a voluntary early retirement program (VERP) offered at USSK, a $9 million favorable adjustment to the expected exit costs of indefinitely idled facilities, and $4 million of severance-related charges at other facilities. Cash payments were made related to severance and exit costs of approximately $95 million.

Charges for restructuring and ongoing cost reduction initiatives are recorded in the period U. S. Steel commits to a restructuring or cost reduction plan, or executes specific actions contemplated by the plan and all criteria for liability recognition have been met. Charges related to the restructuring and cost reductions are reported in restructuring and other charges in the Consolidated Statements of Operations.

The activity in the accrued balances incurred in relation to restructuring during the years ended December 31, 2023, and December 31, 2024, were as follows:

(in millions)	Employee Related Costs	Exit Costs	Non-cash Charges	Total
Balance at December 31, 2022	$ 132	$ 50	$ —	$ 182
Additional charges	25	11	—	$ 36
Release of prior accruals and other adjustments [(a)]	(2)	(1)	—	$ (3)
Cash payments/utilization [(b)]	(45)	(30)	—	(75)
Balance at December 31, 2023	**$ 110**	**$ 30**	**$ —**	**$ 140**
Additional charges	**(3)**	**11**	**—**	**8**
Cash payments/utilization [(b)]	**(62)**	**(27)**	**—**	**(89)**
Balance at December 31, 2024	**$ 45**	**$ 14**	**$ —**	**$ 59**

[(a)] Includes releases of accruals to reflect the current estimate of costs to complete approved restructuring programs.

(b) $4 million and $10 million of payments were made from the pension fund trust assets in the Employee Related Cost column during the years ended December 31, 2023, and 2024, respectively.

26. Contingencies and Commitments

U. S. Steel is the subject of, or party to, a number of pending or threatened legal actions, contingencies and commitments involving a variety of matters, including laws and regulations relating to the environment. Certain of these matters are discussed below. The ultimate resolution of these contingencies could, individually or in the aggregate, be material to the Consolidated Financial Statements. However, management believes that U. S. Steel will remain a viable and competitive enterprise even though it is possible that these contingencies could be resolved unfavorably.

U. S. Steel accrues for estimated costs related to existing lawsuits, claims and proceedings when it is probable that it will incur these costs in the future and the costs are reasonably estimable.

Asbestos matters – As of December 31, 2024, U. S. Steel was a defendant in approximately 990 active cases involving approximately 2,575 plaintiffs. The vast majority of these cases involve multiple defendants. About 1,600, or approximately 62 percent, of these plaintiff claims are currently pending in jurisdictions which permit filings with massive numbers of plaintiffs. At December 31, 2023, U. S. Steel was a defendant in approximately 915 cases involving approximately 2,505 plaintiffs. Based upon U. S. Steel's experience in such cases, it believes that the actual number of plaintiffs who ultimately assert claims against U. S. Steel will likely be a small fraction of the total number of plaintiffs.

The following table shows the number of asbestos claims in the current year and the prior two years:

Period ended	Opening Number of Claims	Claims Dismissed, Settled and Resolved	New Claims	Closing Number of Claims
December 31, 2022	2,505	230	235	2,510
December 31, 2023	2,510	235	230	2,505
December 31, 2024	2,505	240	310	2,575

The amount U. S. Steel accrues for pending asbestos claims is not material to U. S. Steel's financial condition. However, U. S. Steel is unable to estimate the ultimate outcome of asbestos-related claims due to a number of uncertainties, including: (1) the rates at which new claims are filed, (2) the number of and effect of bankruptcies of other companies traditionally defending asbestos claims, (3) uncertainties associated with the variations in the litigation process from jurisdiction to jurisdiction, (4) uncertainties regarding the facts, circumstances and disease process with each claim, and (5) any new legislation enacted to address asbestos-related claims.

Further, U. S. Steel does not believe that an accrual for unasserted claims is required. At any given reporting date, it is probable that there are unasserted claims that will be filed against the Company in the future. The Company engages an outside valuation consultant to assist in assessing its ability to estimate an accrual for unasserted claims. This assessment is based on the Company's settlement experience, including recent claims trends. This analysis focuses on settlements made over the last several years as these claims are likely to best represent future claim characteristics. After review by the valuation consultant and U. S. Steel management, it was determined that the Company could not estimate an accrual for unasserted claims.

Despite these uncertainties, management believes that the ultimate resolution of these matters will not have a material adverse effect on U. S. Steel's financial condition.

Environmental Matters – U. S. Steel is subject to federal, state, local and foreign laws and regulations relating to the environment. These laws generally provide for control of pollutants released into the environment and require responsible parties to undertake remediation of hazardous waste disposal sites. Penalties may be imposed for noncompliance. Changes in accrued liabilities for remediation activities where U. S. Steel is identified as a named party are summarized in the following table:

	Year Ended December 31,	
(In millions)	2024	2023
Beginning of period	$ 107	$ 126
Accruals for environmental remediation deemed probable and reasonably estimable	18	15
Adjustments for changes in estimates	—	(11)
Obligations settled	(17)	(23)
End of period	$ 108	$ 107

Accrued liabilities for remediation activities are included in the following Consolidated Balance Sheet lines:

(In millions)	December 31, 2024	December 31, 2023
Accounts payable	$ 26	$ 27
Deferred credits and other noncurrent liabilities	82	80
Total	$ 108	$ 107

Expenses related to remediation are recorded in cost of sales and were $18 million and $13 million for the years ended December 31, 2024, and December 31, 2023, respectively. It is not currently possible to estimate the ultimate amount of all remediation costs that might be incurred or the penalties that may be imposed. Due to uncertainties inherent in remediation projects and the associated liabilities, it is reasonably possible that total remediation costs for active matters may exceed the accrued liabilities by as much as 30 to 50 percent.

Remediation Projects

U. S. Steel is involved in environmental remediation projects at or adjacent to several current and former U. S. Steel facilities and other locations that are in various stages of completion ranging from initial characterization through post-closure monitoring. Based on the anticipated scope and degree of uncertainty of projects, we categorize projects as follows:

(1) *Projects with Ongoing Study and Scope Development* – Projects which are still in the development phase. For these projects, the extent of remediation that may be required is not yet known, the remediation methods and plans are not yet developed, and/or cost estimates cannot be determined. Therefore, significant costs, in addition to the accrued liabilities for these projects, are reasonably possible. There are three environmental remediation projects where additional costs for completion are not currently estimable, but could be material. These projects are at South Works, UPI and the former steelmaking plant at Joliet, Illinois. As of December 31, 2024, accrued liabilities for these projects totaled $7 million for the costs of studies, investigations, interim measures, design and/or remediation. It is reasonably possible that additional liabilities associated with future requirements regarding studies, investigations, design and remediation for these projects could be as much as $33 million to $46 million.

(2) *Significant Projects with Defined Scope* – Projects with significant accrued liabilities with a defined scope. As of December 31, 2024, there are four significant projects with defined scope greater than or equal to $5 million each, with a total accrued liability of $54 million. These projects are: Gary Resource Conservation and Recovery Act (RCRA) (accrued liability of $23 million), Duluth Works (accrued liability of $6 million), Fairfield Works (accrued liability of $8 million) and the former Geneva facility (accrued liability of $17 million).

(3) *Other Projects with a Defined Scope* – Projects with relatively small accrued liabilities for which we believe that, while additional costs are possible, they are not likely to be significant, and also include those projects for which we do not yet possess sufficient information to estimate potential costs to U. S. Steel. There are three other environmental remediation projects which each had an accrued liability of between $1 million and $5 million. The total accrued liability for these projects at December 31, 2024, was $5 million. These projects have progressed through a significant portion of the design phase and material additional costs are not expected.

The remaining environmental remediation projects each have an accrued liability of less than $1 million each. The total accrued liability for these projects at December 31, 2024, was approximately $4 million. The Company does not foresee material additional liabilities for any of these sites.

Post-Closure Costs – Accrued liabilities for post-closure site monitoring and other costs at various closed landfills totaled $27 million at December 31, 2024, and were based on known scopes of work.

Administrative and Legal Costs – As of December 31, 2024, U. S. Steel had an accrued liability of $11 million for administrative and legal costs related to environmental remediation projects. These accrued liabilities were based on projected administrative and legal costs for the next three years and do not change significantly from year to year.

Capital Expenditures – For a number of years, U. S. Steel has made substantial capital expenditures to comply with various regulations, laws, and other requirements relating to the environment. Such capital expenditures totaled $83 million and $70 million in 2024 and 2023, respectively. U. S. Steel anticipates making additional such expenditures in the future, which may be material; however, the exact amounts and timing of such expenditures are uncertain because of the continuing evolution of specific regulatory requirements.

European Union (the EU) Environmental Requirements - Phase IV of the EU Emissions Trading System (the EU ETS) commenced on January 1, 2021, and will finish on December 31, 2030. The European Commission issued final approval of the updated 2021-2025 Slovak National Allocation table in February 2022. The Slovak Ministry of Environment allocated 6.2 million metric tons of European Union Emission Allowances (EUA) at no charge (free allowances or free allocation) to USSE in March 2024. As of December 31, 2024, we have pre-purchased approximately 2.22 million EUA totaling €161 million (approximately $167 million) via spot purchases or settled forwards to cover the 2024 shortfall of emission allowances and expected shortfall in subsequent years.

The EU's Industrial Emissions Directive requires implementation of EU-determined best available techniques (BAT) for Iron and Steel production to reduce environmental impacts as well as compliance with BAT associated emission levels. Total capital expenditures for projects to go beyond BAT requirements were €138 million (approximately $143 million). These costs were partially offset by the EU funding received and may be mitigated over the next measurement periods if USSK complies with certain financial covenants, which are assessed annually. If we are unable to meet these covenants in the future, USSK might be required to provide additional collateral (e.g., bank guarantee) to secure 50 percent of the EU funding received. USSK complied with these covenants as of December 31, 2024, and no additional collateral will be required by the end of June 30, 2025. By this next assessment date, we expect that nine of the total fifteen projects will pass the sustainability monitoring and will be excluded from further assessment to provide additional collateral if the covenants are not met. The last assessment of financial covenants will be performed as of June 30, 2026.

Environmental indemnifications – Throughout its history, U. S. Steel has sold numerous properties and businesses and many of these sales included indemnifications and cost sharing agreements related to the assets that were divested. The amount of potential environmental liability associated with these transactions and properties is not estimable due to the nature and extent of the unknown conditions related to the properties divested and deconsolidated. Aside from the environmental liabilities already recorded as a result of these transactions due to specific environmental remediation activities and cases (included in the $108 million of accrued liabilities for remediation discussed above), there are no other known probable and estimable environmental liabilities related to these transactions.

Guarantees – The maximum guarantees of the indebtedness of unconsolidated entities of U. S. Steel totaled $7 million at December 31, 2024.

Other contingencies – Under certain finance lease agreements covering various equipment, U. S. Steel has the option to renew the lease or to purchase the equipment at the end of the lease term. If U. S. Steel does not exercise the purchase option by the end of the lease term, U. S. Steel guarantees a residual value of the equipment as determined at the lease inception date (totaling approximately $14 million at December 31, 2024). No liability has been recorded for these guarantees as the potential loss is not probable.

The Company's BR2 facility in Osceola, Arkansas qualifies for financing and related economic incentives associated with the acquisition, development, construction and operation of the facility. These incentives consist of advance lump-sum payments which are included in deferred credits and other noncurrent liabilities on the Consolidated Balance Sheets. In March 2022, the Company received a lump-sum payment of approximately $82 million as proceeds from the sale of a portion of expected future tax credits to be earned by the Company under the State of Arkansas's Recycling Tax Credit program. These funds are to be used primarily for the acquisition of project related equipment, however they may also be used for the training and development of new employees hired for the facility. The Company is contingently liable for certain repayment penalties if the Company fails to meet certain employment requirements in any given period. In April 2022, the Company received a $3 million grant from Mississippi County, Arkansas, and in May 2022, the Company received a $50 million grant from the State of Arkansas Quick Action Closing Fund. Both grants pertain to the reimbursement of qualifying project costs. For each of these incentives and grants, the balance of deferred income will be recognized into other gains, net in the accompanying Consolidated Statements of Operations on a systematic basis over the periods in which the Company earns the granted funds by complying with the investment and employment requirements of the grant programs.

In July 2024, the Company also received a lump-sum payment of approximately $75 million as proceeds from the sale of a portion of future tax credits to be earned by the Company under the State of Arkansas's Recycling Tax Credit program for the Phase II portion of the Big River Steel facilities. The Company is contingently liable for certain repayment penalties if the Company fails to meet certain employment requirements, and as such a deferred liability was recognized for this grant and will be amortized into other gains, net in the statement of operations on a systematic basis over the periods in which the Company earns the granted funds by complying with the employment requirements of the grant program.

We have incurred and expect to continue to incur significant expenses in connection with the pending Merger, including legal and investment banking fees.

Insurance – U. S. Steel maintains insurance for certain property damage, equipment, business interruption and general liability exposures; however, insurance is applicable only after certain deductibles and retainages. U. S. Steel is self-insured for certain other exposures including workers' compensation (where permitted by law) and auto liability. Liabilities are recorded for workers' compensation and personal injury obligations. Other costs resulting from losses under deductible or retainage amounts or not otherwise covered by insurance are charged against income upon occurrence.

U. S. Steel uses surety bonds, trusts and letters of credit to provide whole or partial financial assurance for certain obligations such as workers' compensation. The total amount of active surety bonds, trusts and letters of credit being used for financial assurance purposes was approximately $192 million as of December 31, 2024, which reflects U. S. Steel's maximum exposure under these financial guarantees, but not its total exposure for the underlying obligations. A significant portion of our trust arrangements and letters of credit are collateralized by the Credit Facility Agreement. The remaining trust arrangements and letters of credit are collateralized by restricted cash. Restricted cash, which is recorded in other current and noncurrent assets, totaled $46 million and $40 million at December 31, 2024, and December 31, 2023, respectively.

Capital Commitments – At December 31, 2024, U. S. Steel's contractual commitments to acquire property, plant and equipment totaled $683 million.

Contractual Purchase Commitments – U. S. Steel is obligated to make payments under contractual purchase commitments, including unconditional purchase obligations. Payments for contracts with remaining terms in excess of one year are summarized below (in millions):

2025	2026	2027	2028	2029	Later years	Total
$1,260	$1,033	$253	$218	$167	$661	$3,592

The majority of U. S. Steel's unconditional purchase obligations relate to the supply of industrial gases, and certain energy and utility services with terms ranging from 13 months to 19 years. Unconditional purchase obligations also include coke and steam purchase commitments related to a coke supply agreement with Gateway Energy & Coke Company LLC (Gateway) under which Gateway is obligated to supply a minimum volume of the expected targeted annual production of the heat recovery coke plant, and U. S. Steel is obligated to purchase the coke from Gateway at the contract price. As of December 31, 2024, if U. S. Steel were to terminate the agreement, it may be obligated to pay in excess of $26 million.

As a result of the indefinite idling of the iron and steel making processes at Granite City Works, there were $60 million and $86 million of liabilities for unconditional purchase obligations as of December 31, 2024, and December 31, 2023, respectively.

Total payments relating to unconditional purchase obligations were approximately $815 million in 2024, $787 million in 2023, and $850 million in 2022.

27. Common Stock Repurchased

On October 25, 2021, the Board of Directors authorized a share repurchase program that allowed for the repurchase of up to $300 million of its outstanding common stock from time to time in the open market or privately negotiated transactions. On January 24, 2022, the Board of Directors authorized an additional $500 million under the share repurchase program.

On July 25, 2022, following the completion of the previously authorized $800 million share repurchase programs, the Board of Directors authorized a share repurchase program that allows for the repurchase of up to $500 million of its outstanding common stock from time to time in the open market or privately negotiated transactions at the discretion of management. The Company's share repurchase program does not obligate it to acquire any specific number of shares.

U. S. Steel repurchased 7.1 million and 37.6 million shares of common stock for approximately $175 million and $849 million under these programs during 2023 and 2022, respectively. We do not expect to utilize the remainder of this authorization. No share repurchases were completed during 2024 as the Merger Agreement prohibits us from engaging in additional share repurchases without the consent of Purchaser.

FIVE-YEAR OPERATING SUMMARY (Unaudited)

(Thousands of tons, unless otherwise noted)	2024	2023	2022	2021	2020
Raw Steel Production					
Gary, IN	**5,665**	5,587	5,326	5,664	4,675
Great Lakes, MI	**—**	—	—	—	328
Mon Valley, PA	**2,724**	2,650	2,161	2,668	2,552
Granite City, IL	**—**	1,162	1,359	1,549	1,758
Total Flat-Rolled facilities	**8,389**	9,399	8,846	9,881	9,313
Mini Mill facility	**2,838**	2,953	2,650	2,688	—
U. S. Steel Košice	**3,832**	4,395	3,839	4,931	3,366
Tubular facility	**575**	568	634	464	16
Total	**15,634**	17,315	15,969	17,964	12,695
Raw Steel Capability					
Flat-Rolled	**13,200**	13,200	13,200	13,200	17,000
Mini Mill [(c)]	**6,300**	3,300	3,300	3,300	—
USSE	**5,000**	5,000	5,000	5,000	5,000
Tubular [(d)]	**900**	900	900	900	900
Total	**25,400**	22,400	22,400	22,400	22,900
Production as % of total capability:					
Flat-Rolled	**63 %**	71 %	67 %	58 %	55 %
Mini Mill [(c)]	**80 %**	89 %	80 %	81 %	— %
USSE	**77 %**	88 %	77 %	99 %	67 %
Tubular [(d)]	**64 %**	63 %	70 %	52 %	7 %
Coke Production					
Flat-Rolled	**3,164**	3,295	3,627	3,848	2,557
USSE	**1,336**	1,485	1,407	1,548	1,116
Total	**4,500**	4,780	5,034	5,396	3,673
Iron Ore Pellet Production [(a)]					
Total	**22,247**	22,134	22,059	23,369	16,981
Steel Shipments by Segment [(b)]					
Flat-Rolled	**7,845**	8,706	8,373	9,018	8,711
Mini Mill	**2,307**	2,424	2,287	2,230	—
USSE	**3,578**	3,899	3,759	4,302	3,041
Tubular	**476**	478	523	444	464
Total steel shipments	**14,206**	15,507	14,942	15,994	12,216
Average Realized Price (dollars per net ton)					
Flat-Rolled	**$ 1,013**	$ 1,030	$ 1,261	$ 1,172	$ 718
Mini Mill	**$ 857**	$ 875	$ 1,134	$ 1,314	$ —
USSE	**$ 805**	$ 873	$ 1,090	$ 966	$ 626
Tubular	**$ 1,905**	$ 3,137	$ 2,978	$ 1,696	$ 1,271

(a) Includes our share of production from Hibbing.

(b) Does not include intersegment shipments or shipments by joint ventures and other equity investees of U. S. Steel. Includes shipments from U. S. Steel to joint ventures and equity investees of substrate materials, primarily hot-rolled and cold-rolled sheets.

(c) The 2024 production as a % of total capability amount is based on Mini Mill's aggregate annual raw steel production capability of 3.3 million tons through the third quarter of 2024 and 6.3 million tons during the fourth quarter of 2024.

(d) The Fairfield Electric Arc Furnace commenced operation in October 2020. The 2020 production as a % of total capability amount is based on an October 1, 2020, start date.

FIVE-YEAR OPERATING SUMMARY (Unaudited) (Continued)

(Thousands of net tons)	2024	2023	2022	2021	2020
Steel Shipments by Market - North American Facilities [a]					
Steel service centers	**1,980**	2,622	2,208	2,660	1,450
Further conversion:					
Trade customers	**2,382**	2,669	2,935	2,385	2,063
Joint ventures [b]	**193**	211	256	490	415
Transportation and automotive [b]	**3,403**	2,893	2,631	2,372	2,012
Construction and construction products	**1,186**	1,422	1,261	1,524	1,295
Containers and packaging	**364**	571	706	959	913
Appliances and electrical equipment	**523**	507	509	679	497
Oil, gas and petrochemicals	**465**	447	494	426	430
All other	**132**	266	183	197	100
Total	**10,628**	11,608	11,183	11,692	9,175
Steel Shipments by Market - USSE					
Steel service centers	**877**	848	839	995	690
Further conversion:					
Trade customers	**245**	293	289	314	202
Transportation and automotive	**559**	636	619	590	517
Construction and construction products	**1,224**	1,319	1,052	1,346	775
Containers and packaging	**223**	312	423	449	435
Appliances and electrical equipment	**176**	172	225	266	194
Oil, gas and petrochemicals	**1**	—	3	8	5
All other	**273**	319	309	334	223
Total	**3,578**	3,899	3,759	4,302	3,041

[a] Does not include shipments by joint ventures and other equity investees of U. S. Steel, but instead reflects the shipments of substrate materials, primarily hot-rolled and cold-rolled sheets, to those entities.

[b] PRO-TEC automotive substrate shipments are included in the Transportation and Automotive category.

Item 9. CHANGES IN AND DISAGREEMENTS WITH ACCOUNTANTS ON ACCOUNTING AND FINANCIAL DISCLOSURE

None.

Item 9A. CONTROLS AND PROCEDURES

Conclusions Regarding the Effectiveness of Disclosure Controls and Procedures

Under the supervision and with the participation of U. S. Steel's management, including the chief executive officer and chief financial officer, U. S. Steel conducted an evaluation of our disclosure controls and procedures, as such term is defined under Rule 13a-15(e) promulgated under the Securities Exchange Act of 1934, as amended (the Exchange Act). Based on this evaluation, U. S. Steel's chief executive officer and chief financial officer concluded that U. S. Steel's disclosure controls and procedures were effective as of the end of the period covered by this annual report.

Management's Report on Internal Control Over Financial Reporting

See "Item 8. Financial Statements and Supplementary Data – Management's Report to Stockholders – Internal Control Over Financial Reporting."

Attestation Report of Independent Registered Public Accounting Firm

See "Item 8. Financial Statements and Supplementary Data – Report of Independent Registered Public Accounting Firm."

Changes in Internal Control Over Financial Reporting

There have not been any changes in U. S. Steel's internal control over financial reporting that occurred during the fourth quarter of 2024 which have materially affected, or are reasonably likely to materially affect, U. S. Steel's internal control over financial reporting.

Item 9B. OTHER INFORMATION

During the quarter ended December 31, 2024, none of our directors or officers adopted or terminated a "Rule 10b5-1 trading arrangement" or a "non-Rule 10b5-1 trading arrangement," as those terms are defined in Item 408 of Regulation S-K.

Item 9C. DISCLOSURE REGARDING FOREIGN JURISDICTIONS THAT PREVENT INSPECTIONS

Not Applicable.

PART III

Item 10. DIRECTORS, EXECUTIVE OFFICERS AND CORPORATE GOVERNANCE

Information concerning the directors of U. S. Steel required by this item is incorporated and made part hereof by reference to the material appearing under the heading "Election of Directors" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders, which will be filed with the Securities and Exchange Commission, pursuant to Regulation 14A, no later than 120 days after the end of the fiscal year. Information concerning the Audit Committee, and its financial expert required by this item is incorporated and made part hereof by reference to the material appearing under the heading "Corporate Governance – Board Committees – Audit" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders. Information regarding the Nominating Committee required by this item is incorporated and made part hereof by reference to the material appearing under the heading "Corporate Governance – Board Committees – Corporate Governance & Sustainability" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders. Information regarding the ability of stockholders to communicate with the Board of Directors is incorporated and made part hereof by reference to the material appearing under the heading "Corporate Governance – Commitment to Stockholder Engagement – Communications from Stockholders and Interested Parties" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders. Information regarding compliance with Section 16(a) of the Exchange Act required by this item is incorporated and made part hereof by reference to the material appearing under the heading "Delinquent Section 16(a) Reports" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders. Information concerning the executive officers of U. S. Steel is contained in Part I of this Form 10-K under the caption "Information about our Executive Officers."

U. S. Steel has adopted a Code of Ethical Business Conduct that applies to all of our directors and officers, including our principal executive officer, principal financial officer, principal accounting officer or controller, or persons performing similar functions. U. S. Steel will provide a copy of this code free of charge upon request. To obtain a copy, contact the Office of the Corporate Secretary, United States Steel Corporation, 600 Grant Street, Pittsburgh, Pennsylvania, 15219-2800 (telephone: 412-433-1121). The Code of Ethical Business Conduct is also available through the Company's website at ***www.ussteel.com***.

We intend to post all required disclosures concerning any amendments to or waivers from, our Code of Ethical Business Conduct on our website. U. S. Steel does not intend to incorporate the contents of our website into this Annual Report on Form 10-K.

U. S. Steel has an Insider Trading Policy governing the purchase, sale and other dispositions of its securities by directors, officers and employees that is reasonably designed to promote compliance with insider trading laws, rules and regulations and NYSE listing standards. The Insider Trading Policy is filed with this Form 10-K as Exhibit 19.

Item 11. EXECUTIVE COMPENSATION

Information required by this item is incorporated and made part hereof by reference to the material appearing under the headings "Director Compensation," "Compensation & Organization Committee Report," "Compensation Discussion and Analysis," "Executive Compensation Tables," "Potential Payments Upon Termination or Change in Control" and "CEO Pay Ratio" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders.

Item 12. SECURITY OWNERSHIP OF CERTAIN BENEFICIAL OWNERS AND MANAGEMENT AND RELATED STOCKHOLDER MATTERS

Equity Compensation Plan Information

Plan Category	(1) Number of securities to be issued upon exercise of outstanding options, warrants and rights	(2) Weighted-average exercise price of outstanding options, warrants and rights	(3) Number of securities remaining available for future issuance under equity compensation plans excluding securities reflected in Column (1) [(b)]
Equity compensation plans approved by security holders [(a)]	7,872,563	$28.60	4,084,130
Equity compensation plans not approved by security holders[(c)]	—	(one for one)	—
Total	7,872,563	—	4,084,130

(a) The numbers in columns (1) and (2) of this row contemplate all shares that could potentially be issued as a result of outstanding grants under the 2005 Stock Incentive Plan and the 2016 Omnibus Incentive Compensation Plan, as amended and restated as of December 31, 2024. (For more information, see Note 15 to the Consolidated Financial Statements). Column (1) includes (i) 751,863 shares of common stock that could be issued for the Common Stock Units outstanding under the Deferred Compensation Program for Non-Employee Directors and (ii) 3,770,904 shares that could be issued for the 1,885,452 performance awards outstanding under the Long-Term Incentive Compensation Program (a program under the 2016 Omnibus Incentive Compensation Plan, as amended and restated). The calculation in column (2) does not include the Common Stock Units since the weighted average exercise price for Common Stock Units is one for one; that is, one share of common stock will be given in exchange for each unit of such phantom stock accumulated through the date of the director's retirement. Also, the calculation in column (2) does not include the performance awards since the shares issued for performance awards can range from zero for one to two for one; that is, performance awards may result in up to 3,770,904 of common stock being issued (two for one), or some lesser number of shares (including zero shares of common stock issued), depending upon the Corporation's common stock performance versus that of a peer group of companies or the Corporation's return on capital employed performance over a performance period.

(b) Represents shares available under the 2016 Omnibus Incentive Compensation Plan, as amended and restated.

(c) At December 31, 2024, U. S. Steel had no securities remaining for future issuance under equity compensation plans that had not been approved by security holders. Column (1) represents Common Stock Units that were issued pursuant to the Deferred Compensation Plan for Non-Employee Directors prior to its being amended to make it a program under the 2005 Stock Incentive Plan and 2016 Omnibus Incentive Compensation Plan, as amended and restated. The weighted average exercise price for Common Stock Units in column (2) is one for one; that is, one share of common stock will be given in exchange for each unit of phantom stock upon the director's retirement from the Board of Directors. All future grants under this amended plan/program will count as shares issued under the 2016 Omnibus Incentive Compensation Plan, as amended and restated, a stockholder approved plan.

Other information required by this item is incorporated and made part hereof by reference to the material appearing under the headings "Stock Ownership of Directors and Executive Officers" and "Stock Ownership of Certain Beneficial Owners" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders.

Item 13. CERTAIN RELATIONSHIPS AND RELATED TRANSACTIONS, AND DIRECTOR INDEPENDENCE

Information required by this item is incorporated and made part hereof by reference to the material appearing under the headings "Corporate Governance – Related Person Transactions Policy" and "Corporate Governance – Director Independence" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders.

Item 14. PRINCIPAL ACCOUNTANT FEES AND SERVICES

Information required by this item is incorporated and made part hereof by reference to the material appearing under the heading "Audit Fees" in U. S. Steel's Proxy Statement for the 2025 Annual Meeting of Stockholders.

PART IV

Item 15. EXHIBITS AND FINANCIAL STATEMENT SCHEDULE

A. Documents Filed as Part of the Report

1. **Financial Statements and Supplementary Data**
   Financial Statements filed as part of this report are included in "Item 8 – Financial Statements and Supplementary Data".

2. **Financial Statement Schedules**
   See "Schedule II – Valuation and Qualifying Accounts and Reserves" for years ended December 31, 2024, 2023, and 2022. All other schedules are omitted because they are not applicable or the required information is contained in the applicable financial statements or notes thereto.

B. Exhibits

Exhibit No.

2. Plan of acquisition, reorganization, arrangement, liquidation or succession

(a)	Agreement and Plan of Merger, dated as of December 18, 2023, by and among the Company, Parent, Merger Sub and NSC (solely as provided in Section 9.13 therein).**	Incorporated by reference to Exhibit 2.1 to United States Steel Corporation's Form 8-K filed on December 18, 2023, Commission File Number 1-16811.

3. Articles of Incorporation and By-Laws

(a)	Amended and Restated Certificate of Incorporation of United States Steel Corporation, dated April 25, 2017	Incorporated by reference to Exhibit 3.1 to United States Steel Corporation's Form 8-K filed on April 28, 2017, Commission File Number 1-16811.
(b)	Amended and Restated By-Laws of United States Steel Corporation, dated as of January 31, 2023.	Incorporated by reference to Exhibit 3.1 to United States Steel Corporation's Form 8-K filed on February 2, 2023, Commission File Number 1-16811.

4. Instruments Defining the Rights of Security Holders, Including Indentures

(a)	Indenture between United States Steel Corporation and The Bank of New York Mellon (f/k/a The Bank of New York), dated as of May 21, 2007.	Incorporated by reference to Exhibit 4.1 to United States Steel Corporation's Form 8-K filed on May 22, 2007, Commission File Number 1-16811.
(b)	First Supplemental Indenture between United States Steel Corporation and The Bank of New York Mellon (f/k/a The Bank of New York), dated as of May 21, 2007.	Incorporated by reference to Exhibit 4.2 to United States Steel Corporation's Form 8-K filed on May 22, 2007, Commission File Number 1-16811.
(c)	Tenth Supplemental Indenture between United States Steel Corporation and The Bank of New York Mellon, dated as of February 11, 2021.	Incorporated by reference to Exhibit 4.1 to United States Steel Corporation's Form 8-K filed on February 11, 2021, Commission File Number 1-16811.
(d)	Indenture by and between United States Steel Corporation and The Bank of New York Mellon, dated as of October 21, 2019.	Incorporated by reference to Exhibit 4.1 to United States Steel Corporation's Form 8-K filed on October 21, 2019, Commission File Number 1-16811.
(e)	Indenture among Big River Steel LLC, BRS Finance Corp., BRS Intermediate Holdings LLC, each Guarantor that may become party thereto and U.S. Bank National Association, dated as of September 18, 2020.	Incorporated by reference to Exhibit 4.1 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(f)	Description of Securities.	Incorporated by reference to Exhibit 4.1 to United States Steel Corporation's Form 10-K filed on February 14, 2020, Commission File Number 1-16811.

Certain long-term debt instruments are omitted pursuant to Item 601(b)(4)(iii) of Regulation S-K. U. S. Steel agrees to furnish to the Commission on request a copy of any instrument defining the rights of holders of long-term debt of U. S. Steel and of any subsidiary for which consolidated or unconsolidated financial statements are required to be filed.

10. Material Contracts

(a)	Option Agreement by and between United States Steel Corporation and Stelco Inc., dated as of April 30, 2020.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on April 30, 2020, Commission File Number 1-16811.
(b)	Commitment Agreement by and among United States Steel Corporation, Banner Life Insurance Company, William Penn Life Insurance Company of New York and State Street Global Advisors Trust Company, dated November 8, 2021.**	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(c)	Sixth Amended and Restated Credit Agreement, dated as of May 27, 2022, among United States Steel Corporation, the Subsidiary Guarantors from time to time party thereto, the Lenders party thereto, the LC Issuing Banks party thereto, and JPMorgan Chase Bank, N.A., as Administrative and Collateral Agent.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on June 3, 2022, Commission File Number 1-16811.
(d)	Amendment No. 1 to Sixth Amended and Restated Credit Agreement, dated as of December 19, 2022, among United States Steel Corporation, the Subsidiary Guarantors from time to time party thereto, the Lenders party thereto, the LC Issuing Banks party thereto, and JPMorgan Chase Bank, N.A., as Administrative and Collateral Agent.**	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-K filed on February 3, 2023, Commission File Number 1-16811.
(e)	Third Amended and Restated Borrower Security Agreement, dated as of May 27, 2022, between United States Steel Corporation and JPMorgan Chase Bank, N.A., as Collateral Agent.	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 8-K filed on June 3, 2022, Commission File Number 1-16811.
(f)	Third Amended and Restated Subsidiary Security Agreement, dated as of May 27, 2022, between the Subsidiary Guarantors and JPMorgan Chase Bank, N.A., as Collateral Agent.	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 8-K filed on June 3, 2022, Commission File Number 1-16811.
(g)	Second Amended and Restated Borrower Canadian Security Agreement, dated as of May 27, 2022, between United States Steel Corporation and JPMorgan Chase Bank, N.A., as Collateral Agent.	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 8-K filed on June 3, 2022, Commission File Number 1-16811.
(h)	Agreement of Sale between United States Steel Corporation and The Industrial Development Board of the City of Hoover, dated as of October 1, 2019.	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 8-K filed on October 28, 2019, Commission File Number 1-16811.
(i)	Loan Agreement between United States Steel Corporation and the Allegheny County Industrial Development Authority, dated as of October 1, 2019.	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 8-K filed on October 28, 2019, Commission File Number 1-16811.
(j)	Amended and Restated Credit Agreement, dated December 22, 2022, among Exploratory Ventures, LLC, as Borrower, United States Steel Corporation, as Parent Guarantor, KFW IPEX-Bank GMBH, as Mandated Lead Arranger and ECA Structuring Bank, KFW IPEX-Bank GMBH, as Facility Agent and ECA Agent, and lenders party thereto from time to time.**	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on October 27, 2023, Commission File Number 1-16811.
(k)	Agreement of Sale between United States Steel Corporation and The Industrial Development Board of the City of Hoover regarding $63,400,000 million 6.375% Environmental Improvement Revenue Bonds, Series 2020 (United States Steel Corporation Project), dated as of November 1, 2020.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on November 24, 2020, Commission File Number 1-16811.
(l)	Loan Agreement dated between United States Steel Corporation and the Indiana Finance Authority regarding $33,300,000 Indiana Finance Authority Environmental Improvement Revenue Bonds, Series 2020A (United States Steel Corporation Project) and Indiana Finance Authority Environmental Improvement Revenue Refunding Bonds, Series 2020B (United States Steel Corporation Project), dated as of November 1, 2020.	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 8-K filed on November 24, 2020, Commission File Number 1-16811.
(m)	ABL Credit Agreement by and among Big River Steel LLC, BRS Intermediate Holdings LLC, Goldman Sachs Bank USA and each lender party thereto, dated as of August 23, 2017.**	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.

(n)	First Amendment to ABL Credit Agreement by and among Big River Steel LLC, BRS Intermediate Holdings LLC, Goldman Sachs Bank USA and each lender party thereto, dated as of September 10, 2020.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(o)	Second Amendment to ABL Credit Agreement by and among Big River Steel LLC, BRS Intermediate Holdings LLC, Goldman Sachs Bank USA and each lender party thereto, dated as of July 23, 2021.	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-Q filed on July 30, 2021, Commission File Number 1-16811.
(p)	Third Amendment to ABL Credit Agreement by and among Big River Steel LLC, BRS Intermediate Holdings LLC, Goldman Sachs Bank USA and each lender party thereto, dated as of December 19, 2022.**	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-K filed on February 3, 2023, Commission File Number 1-16811.
(q)	Fourth Amendment to ABL Credit Agreement by and among BRS Intermediate Holdings LLC, Big River Steel LLC, BRS Finance Corp. and Goldman Sachs Bank USA and each lender party thereto, dated as of June 16, 2023.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on July 28, 2023, Commission File Number 1-16811.
(r)	Bond Financing Agreement between Arkansas Development Finance Authority and each of Big River Steel LLC, BRS Finance Corp. and BRS Intermediate Holdings LLC relating to $265 million Arkansas Development Finance Authority Industrial Development Revenue Bonds (Big River Steel Project), Tax-Exempt Series 2020 (Green Bonds), dated as of September 10, 2020.	Incorporated by reference to Exhibit 10.2.1 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(s)	Definitions Annex relating to Arkansas Development Finance Authority Industrial Development Revenue Bonds (Big River Steel Project), Tax-Exempt Series 2020 (Green Bonds), dated as of September 10, 2020.	Incorporated by reference to Exhibit 10.2.2 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(t)	Bond Financing Agreement between Arkansas Development Finance Authority and each of Big River Steel LLC, BRS Finance Corp. and BRS Intermediate Holdings LLC relating to $487 million Arkansas Development Finance Authority Industrial Development Revenue Bonds (Big River Steel Project), Series 2019, dated as of May 31, 2019.	Incorporated by reference to Exhibit 10.3.1 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(u)	Definitions Annex relating to Arkansas Development Finance Authority Industrial Development Revenue Bonds (Big River Steel Project), Series 2019, dated as of May 31, 2019.	Incorporated by reference to Exhibit 10.3.2 to United States Steel Corporation's Form 8-K filed on January 19, 2021, Commission File Number 1-16811.
(v)	Loan Agreement between Arkansas Development Finance Authority and each of United States Steel Corporation and Exploratory Ventures, LLC relating to $290,000,000 Arkansas Development Finance Authority Environmental Improvement Revenue Bonds, Series 2022 (United States Steel Corporation Project) (Green Bonds), dated as of September 1, 2022.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on October 28, 2022, Commission File Number 1-16811.
(w)	EUR 300,000,000 Unsecured Sustainability Linked Revolving Credit Facility Agreement among U. S. Steel Košice, s.r.o., and ING Bank N.V., Komerèní banka, a.s., Slovenská sporiteľňa, a.s., UniCredit Bank Czech Republic and Slovakia , a.s., Československá Obchodná banka, a.s., and Citibank Europe plc, dated September 29, 2021.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on October 1, 2021, Commission File Number 1-16811.
(x)	Supplemental Agreement No. 9 between U. S. Steel Košice, s.r.o. and ING Bank N.V., dated December 3, 2021.	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(y)	First Supplemental Agreement, dated December 15, 2022, between U. S. Steel Košice, s.r.o., and ING Bank N.V., as Facility Agent, relating to an up to EUR 300,000,000 credit agreement dated September 29, 2021.**	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-K filed on February 3, 2023, Commission File Number 1-16811.
(z)	United States Steel Corporation 2005 Stock Incentive Plan, Amended and Restated Effective April 29, 2014.*	Incorporated by reference to Appendix A to United States Steel Corporation's Definitive Proxy Statement on Schedule 14A filed on March 14, 2014, Commission File Number 1-16811.

(aa)	Form of Stock Option Grant under the Long-Term Incentive Compensation Program, a program under the United States Steel Corporation 2005 Stock Incentive Plan.*	Incorporated by reference to Exhibit 10(x) to United States Steel Corporation's Form 10-K filed on February 27, 2007, Commission File Number 1-16811.
(bb)	Form of Stock Option Grant Agreement under the Long-Term Incentive Compensation Program, a program under the United States Steel Corporation 2005 Stock Incentive Plan.*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on April 26, 2011, Commission File Number 1-16811.
(cc)	Form of Non-Qualified Stock Option Grant Agreement under the Long-Term Incentive Compensation Program, a program under the United States Steel Corporation 2005 Stock Incentive Plan.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on April 29, 2015, Commission File Number 1-16811.
(dd)	Administrative Procedures for the Long-Term Incentive Compensation Program under the United States Steel Corporation 2005 Stock Incentive Plan, as Amended and Restated, and under the United States Steel Corporation Annual Incentive Compensation Plan, Effective February 22, 2016.*	Incorporated by reference to Exhibit 10(kk) to United States Steel Corporation's Form 10-K filed on February 29, 2016, Commission File Number 1-16811.
(ee)	United States Steel Corporation Deferred Compensation Program for Non-Employee Directors, a program under the 2005 Stock Incentive Plan, effective as of November 29, 2005.*	Incorporated by reference to Exhibit 10(d) to United States Steel Corporation's Form 10-K filed on February 28, 2012, Commission File Number 1-16811.
(ff)	United States Steel Corporation 2010 Annual Incentive Compensation Plan, Effective April 27, 2010.*	Incorporated by reference to Appendix B to United States Steel Corporation's Definitive Proxy Statement on Schedule 14A filed on March 12, 2010, Commission File Number 1-16811.
(gg)	Administrative Procedures for the Executive Management Annual Incentive Compensation Program under the United States Steel Corporation 2010 Annual Incentive Compensation Plan, as amended November 1, 2016.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on November 2, 2016, Commission File Number 1-16811.
(hh)	United States Steel Corporation Supplemental Thrift Program, Amended and Restated Effective July 31, 2013.*	Incorporated by reference to Exhibit 10.7 to United States Steel Corporation's Form 10-Q filed on October 29, 2013, Commission File Number 1-16811.
(ii)	United States Steel Corporation Supplemental Thrift Program, Amended and Restated Effective November 1, 2016.*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on November 2, 2016, Commission File Number 1-16811.
(jj)	United States Steel Corporation Supplemental Thrift Program, Amended and Restated Effective January 1, 2019.*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-Q filed on May 3, 2019, Commission File Number 1-16811.
(kk)	United States Steel Corporation Non Tax-Qualified Retirement Account Program, Amended and Restated Effective November 13, 2013.	Incorporated by reference to Exhibit 10(dd) to United States Steel Corporation's Form 10-K filed on February 25, 2014, Commission File Number 1-16811.
(ll)	United States Steel Corporation Non Tax-Qualified Retirement Account Program, Amended and Restated Effective January 1, 2016.*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on November 2, 2016, Commission File Number 1-16811.
(mm)	United States Steel Corporation Non Tax-Qualified Retirement Account Program, Amended and Restated Effective January 1, 2019.*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on May 3, 2019, Commission File Number 1-16811.
(nn)	United States Steel Corporation Non Tax-Qualified Pension Plan, Amended and Restated Effective December 31, 2015.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on August 21, 2015, Commission File Number 1-16811.
(oo)	United States Steel Corporation Executive Management Supplemental Pension Program, Amended and Restated Effective December 31, 2015.*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 8-K filed on August 21, 2015, Commission File Number 1-16811.
(pp)	United States Steel Corporation Supplemental Retirement Account Program, Amended and Restated Effective January 1, 2016.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on November 4, 2015, Commission File Number 1-16811.
(qq)	United States Steel Corporation Supplemental Retirement Account Program, Amended and Restated Effective January 1, 2019.*	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 10-Q filed on May 3, 2019, Commission File Number 1-16811.
(rr)	United States Steel Corporation Change in Control Severance Plan, Amended and Restated Effective July 28, 2020*.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on July 30, 2020, Commission File Number 1-16811.

(ss)	United States Steel Corporation Executive Severance Plan, Effective December 14, 2021.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on December 20, 2021, Commission File Number 1-16811.
(tt)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Effective April 26, 2016.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on April 27, 2016, Commission File Number 1-16811.
(uu)	First Amendment to United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Effective February 28, 2017.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on April 26, 2017, Commission File Number 1-16811.
(vv)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Amended and Restated Effective April 28, 2020*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 8-K filed on May 1, 2020, Commission File Number 1-16811.
(ww)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Amended and Restated Effective April 27, 2021.*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(xx)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan (Stock Option Grant).*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on July 27, 2016, Commission File Number 1-16811.
(yy)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2021 Grants).*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(zz)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Cash Award Grant Agreement (2021 Grants).*	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(aaa)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (2021 Grants).*	Incorporated by reference to Exhibit 10.6 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(bbb)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Retention Restricted Stock Unit Grant Agreement (2021 Grants).*	Incorporated by reference to Exhibit 10.7 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(ccc)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan TSR Performance Share Award Grant Agreement (2021 Grants).*	Incorporated by reference to Exhibit 10.8 to United States Steel Corporation's Form 10-Q filed on April 30, 2021, Commission File Number 1-16811.
(ddd)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2021 SVP Performance Awards)* **	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(eee)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2021 CEO Performance Award)* **	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(fff)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Non-Qualified Stock Option Grant Agreement (2021 CEO PSO)* **	Incorporated by reference to Exhibit 10.6 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(ggg)	Form of United States Steel Corporation 2016 Omnibus Incentive Plan Performance Share Award Grant Agreement (ROCE Grant)*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on April 29, 2022, Commission File Number 1-16811.
(hhh)	Form of United States Steel Corporation 2016 Omnibus Incentive Plan Performance Share Award Grant Agreement (TSR Grant)*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on April 29, 2022, Commission File Number 1-16811.
(iii)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2023 ROCE Grants).*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.
(jjj)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2023 TSR Grants).*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.
(kkk)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (2023 Annual Grants).*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.
(lll)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (2023 Retention Grants).*	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.

(mmm)	Administrative Procedures for the Executive Management Annual Incentive Compensation Plan under the United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Effective February 28, 2023.*	Incorporated by reference to Exhibit 10.6 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.
(nnn)	United States Steel Corporation Deferred Compensation Program for Non-Employee Directors, a program under the United States Steel Corporation 2016 Omnibus Incentive Plan, effective as of July 26, 2016, as amended on October 30, 2018.*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-K filed on February 15, 2019, Commission File Number 1-16811.
(ooo)	United States Steel Corporation Deferred Compensation Program for Non-Employee Directors, a program under the United States Steel Corporation 2016 Omnibus Incentive Plan, effective as of July 26, 2016, as amended on October 28, 2019.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-K filed on February 14, 2020, Commission File Number 1-16811.
(ppp)	United States Steel Corporation Deferred Compensation Program for Non-Employee Directors, a program under the United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Effective January 1, 2022.*	Incorporated by reference to Exhibit 10.7 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(qqq)	United States Steel Corporation Non-Employee Director Stock Program of the 2016 Omnibus Incentive Compensation Plan, Effective November 1, 2019.*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-K filed on February 14, 2020, Commission File Number 1-16811.
(rrr)	United States Steel Corporation Non-Employee Director Compensation Policy, Effective as of January 1, 2022.*	Incorporated by reference to Exhibit 10.8 to United States Steel Corporation's Form 10-K filed on February 11, 2022, Commission File Number 1-16811.
(sss)	United States Steel Corporation Non-Employee Director Compensation Policy, Adopted as of December 14, 2021, Amended as of December 15, 2022.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on April 28, 2023, Commission File Number 1-16811.
(ttt)	Letter Agreement between Scott D. Buckiso and United States Steel Corporation, dated July 30, 2020.*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-K filed on February 12, 2021, Commission File Number 1-16811.
(uuu)	Separation Agreement and Release by and between Douglas R. Matthews and United States Steel Corporation, dated December 24, 2020.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-K filed on February 12, 2021, Commission File Number 1-16811.
(vvv)	Special Transition Agreement and Release by and between Christine S. Breves and United States Steel Corporation, dated February 15, 2022.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on April 29, 2022, Commission File Number 1-16811.
(www)	Form of Offer Letter to Jessica T. Graziano, dated June 6, 2022.**	Incorporated by reference to Exhibit 10.5 to United States Steel Corporation's Form 10-Q filed on July 29, 2022, Commission File Number 1-16811.
(xxx)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (Non-Employee Directors – Base Retainer)*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-K filed on February 2, 2024, Commission File Number 1-16811.
(yyy)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (Non-Employee Directors – Chair Retainer)*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-K filed on February 2, 2024, Commission File Number 1-16811.
(zzz)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Deferred Stock Unit Grant Agreement (Non-Employee Directors – Base Retainer)*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-K filed on February 2, 2024, Commission File Number 1-16811.
(aaaa)	United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Deferred Stock Unit Grant Agreement (Non-Employee Directors – Chair Retainer)*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-K filed on February 2, 2024, Commission File Number 1-16811.
(bbbb)	Administrative Procedures for the Executive Management Annual Incentive Compensation Plan under the United States Steel Corporation 2016 Omnibus Incentive Compensation Plan, Effective February 27, 2024.*	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on May 3, 2024, Commission File Number 1-16811.
(cccc)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Performance Share Award Grant Agreement (2024 ROCE Grants).*	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on May 3, 2024, Commission File Number 1-16811.

(dddd)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (2024 Retention Grants).*	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on May 3, 2024, Commission File Number 1-16811.
(eeee)	Form of United States Steel Corporation 2016 Omnibus Incentive Compensation Plan Restricted Stock Unit Grant Agreement (2024 Annual Grants).*	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-Q filed on May 3, 2024, Commission File Number 1-16811.
(ffff)	Amendment No. 2 to the Sixth Amended and Restated Credit Agreement, dated May 3, 2024, by and among United States Steel Corporation, JPMorgan Chase Bank, N.A. and the other lenders party thereto.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on August 2, 2024, Commission File Number 1-16811.
(gggg)	Second Supplement Agreement between U. S. Steel Kosice, S.R.O. and ING Bank N.V., dated May 3, 2024.	Incorporated by reference to Exhibit 10.2 to United States Steel Corporation's Form 10-Q filed on August 2, 2024, Commission File Number 1-16811.
(hhhh)	Supplemental Agreement No. 11 between U. S. Steel Kosice, S.R.O. and ING Bank N.V., dated May 3, 2024.	Incorporated by reference to Exhibit 10.3 to United States Steel Corporation's Form 10-Q filed on August 2, 2024, Commission File Number 1-16811.
(iiii)	First Amendment and Consent to Amended and Restated Credit Agreement, dated June 7, 2024, by and among Exploratory Ventures, LLC, United States Steel Corporation, KFW IPEX-Bank GMBH, and other lenders and parties thereto.	Incorporated by reference to Exhibit 10.4 to United States Steel Corporation's Form 10-Q filed on August 2, 2024, Commission File Number 1-16811.
(jjjj)	Supplemental Agreement No. 12 between U. S. Steel Košice, s.r.o. and ING Bank N.V., dated September 13, 2024.	Incorporated by reference to Exhibit 10.1 to United States Steel Corporation's Form 10-Q filed on November 11, 2024, Commission File Number 1-16811.

19	**United States Steel Corporation Insider Trading Policy, effective February 27, 2023.**
21	**List of Subsidiaries**
23	**Consent of PricewaterhouseCoopers LLP**
24	**Powers of Attorney**
31.1	**Certification of Chief Executive Officer required by Rules 13a-14(a) or 15d-14(a) of the Securities Exchange Act of 1934, as promulgated by the Securities and Exchange Commission pursuant to Section 302 of the Sarbanes-Oxley Act of 2002**
31.2	**Certification of Chief Financial Officer required by Rules 13a-14(a) or 15d-14(a) of the Securities Exchange Act of 1934, as promulgated by the Securities and Exchange Commission pursuant to Section 302 of the Sarbanes-Oxley Act of 2002**
32.1	**Certification of Chief Executive Officer Pursuant to 18 U.S.C. Section 1350, as Adopted Pursuant to Section 906 of the Sarbanes-Oxley Act of 2002**
32.2	**Certification of Chief Financial Officer Pursuant to 18 U.S.C. Section 1350, as Adopted Pursuant to Section 906 of the Sarbanes-Oxley Act of 2002**
95	**Mine Safety Disclosure required under Section 1503 of the Dodd-Frank Wall Street Reform and Consumer Protection Act**
97	**United States Steel Corporation Executive Officer Recoupment (Clawback) Policy, effective July 25, 2023.**
101	**The following financial information from United States Steel Corporation's Annual Report on Form 10-K for the year ended December 31, 2024, formatted in Inline XBRL (Extensible Business Reporting Language) includes: (i) the Consolidated Statements of Operations, (ii) the Consolidated Statements of Comprehensive Income (Loss), (iii) the Consolidated Balance Sheets, (iv) the Consolidated Statements of Cash Flows, and (v) Notes to the Consolidated Financial Statements.**
104	**Cover Page Interactive Data File (formatted as Inline XBRL and contained in Exhibit 101)**

* Indicates management contract or compensatory plan or arrangement.
** Schedules and exhibits have been omitted pursuant to Item 601(b)(2) of Regulation S-K. The Corporation hereby undertakes to furnish supplemental copies of the omitted schedules and exhibits upon request by the U.S. Securities and Exchange Commission; provided, however, that the parties may request confidential treatment pursuant to Rule 24b-2 of the Exchange Act for any document so furnished.

SCHEDULE II – VALUATION AND QUALIFYING ACCOUNTS AND RESERVES

YEARS ENDED DECEMBER 31, 2024, 2023, AND 2022

(millions of dollars)

Description	Balance at Beginning of Period	Additions		Deductions		Balance at End of Period
		Charged to Costs and Expenses	Charged to Other Accounts	Charged to Costs and Expenses	Charged to Other Accounts	
Year ended December 31, 2024:						
Reserves deducted in the balance sheet from the assets to which they apply:						
Allowance for doubtful accounts	**$ 38**	**$ 8**	**$ —**	**$ —**	**$ 20**	**$ 26**
Investments and long-term receivables reserve	**3**	**—**	**—**	**—**	**—**	**3**
Deferred tax valuation allowance:						
Domestic	**98**	**5**	**—**	**—**	**—**	**103**
Foreign	**3**	**43**	**—**	**—**	**—**	**46**
Year ended December 31, 2023:						
Reserves deducted in the balance sheet from the assets to which they apply:						
Allowance for doubtful accounts	$ 38	$ 14	$ —	$ —	$ 14	$ 38
Investments and long-term receivables reserve	4	—	—	—	1	3
Deferred tax valuation allowance:						
Domestic	116	—	—	18	—	98
Foreign	3	—	—	—	—	3
Year ended December 31, 2022:						
Reserves deducted in the balance sheet from the assets to which they apply:						
Allowance for doubtful accounts	$ 44	$ 17	$ —	$ —	$ 23	$ 38
Investments and long-term receivables reserve	4	—	—	—	—	4
Deferred tax valuation allowance:						
Domestic	159	18	—	61	—	116
Foreign	3	—	—	—	—	3

Item 16. FORM 10-K SUMMARY

None.

SIGNATURES

Pursuant to the requirements of Section 13 or 15(d) of the Securities Exchange Act of 1934, the Registrant has duly caused this report to be signed on its behalf by the undersigned, thereunto duly authorized.

Date: January 31, 2025

UNITED STATES STEEL CORPORATION

By: */s/ Manpreet S. Grewal*
Manpreet S. Grewal
Vice President, Controller & Chief Accounting Officer

Signature	Title
/s/ David B. Burritt **David B. Burritt**	President & Chief Executive Officer & Director (Principal Executive Officer)
/s/ Jessica T. Graziano **Jessica T. Graziano**	Senior Vice President & Chief Financial Officer (Principal Financial Officer)
/s/ Manpreet S. Grewal **Manpreet S. Grewal**	Vice President, Controller & Chief Accounting Officer (Principal Accounting Officer)
* **Tracy A. Atkinson**	Director
* **Andrea J. Ayers**	Director
* **Alicia J. Davis**	Director
* **Terry L. Dunlap**	Director
* **John J. Engel**	Director
* **John V. Faraci**	Director
* **Murry S. Gerber**	Director
* **Paul A. Mascarenas**	Director
* **Michael H. McGarry**	Director
* **David S. Sutherland**	Board Chair
* **Patricia A. Tracey**	Director

* **BY:** */s/ Manpreet S. Grewal*
Manpreet S. Grewal
Attorney-in-Fact

GLOSSARY OF CERTAIN DEFINED TERMS

The following definitions apply to terms used in this document:

2026 Senior Convertible Notes	Senior Convertible Notes due November 1, 2026
2053 ADFA Green Bonds	Arkansas Development Finance Authority Environmental Improvement Revenue Bonds due 2053
401(k) plans	defined contribution plans
ABL	Asset-Based Loan
ACCCI	American Coke and Coal Chemicals Institute
ACHD	Allegheny County Health Department
AD	antidumping
AD/CVD	antidumping and countervailing duty
ADEM	Alabama Department of Environmental Management
AHSS	advanced high-strength steel
AI	Artificial Intelligence
AISI	American Iron and Steel Institute
AOCI	Accumulated Other Comprehensive Income
AOP	Annual Operating Plan
ARO	Asset Retirement Obligation
ARPA	American Rescue Plan Act
ASC	Accounting Standards Codification
ASU	Accounting Standards Update
BART	Best Available Retrofit Technology
BAT	Best Available Technique
Big River Steel ABL Facility	Big River Steel amended senior secured asset-based revolving credit facility
BOF	Basic Oxygen Furnace Steelmaking
Big River 2 or BR2	Big River Steel 2
BRS	Big River Steel
CAA	Clean Air Act
CAFC	U.S. Court of Appeals for the Federal Circuit
CAFE	Corporate Average Fuel Economy
CAMT	corporate alternative minimum tax
CAMU	Corrective Action Management Unit
CBAM	carbon border adjustment mechanism
CDC	Chrome Deposit Corporation
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
CFIUS	the Committee on Foreign Investment in the United States
CIO	Chief Information Officer
CISO	the Chief Information Security Officer
CISSP	Certified Information Systems Security Professional
CIT	U.S. Court of International Trade
Cliffs	Cleveland-Cliffs Inc.
CMIP	Corrective Measure Implementation Plan
CO_2	carbon dioxide
CODM	chief operating decision maker
COETF	Coke Environmental Task Force
commodity purchase swaps	financial swaps associated with purchases of natural gas, zinc, tin and electricity
CORE	corrosion-resistant steel
COSO	Committee of Sponsoring Organizations of the Treadway Commission
cost cap	per capita dollar maximum the company is expected to pay per participant under the main U. S. Steel benefit plan
Credit Facility Agreement	Sixth Amended and Restated Credit Facility Agreement
CSIRP	Cybersecurity Incident Response Plan
CVD	countervailing duties
CWA	Clean Water Act
DAFW	OSHA Days Away From Work
D.C. Circuit	the United States Court of Appeals for the D.C. Circuit
DEI	diversity equity and inclusion
DOC	U.S. Department of Commerce

Double G	Double G Coatings Company LLC
DR	direct reduced
DRA	DRA Global and Barr Engineering Co.
EAF	Electric Arc Furnace
EBITDA	earnings before interest, taxes, depreciation and amortization
EC	European Commission
ECA	Export Credit Agreement
the “Effective Time”	effective time of the Merger
EGU	electric generating unit
EPS	earnings per share
ERGs	employee resource groups
ERISA	Employee Retirement Income Security Act of 1974
ERM	Enterprise Risk Management
ERP	enterprise resource planning
ERW	electric resistance welded
ESG	environmental, social and governance
ETS	Emissions Trading System
EU	European Union
EU ETS	EU Emissions Trading System
EUA	European Union Emission Allowances
EWPM	electric-weld pipe mill
FASB	Financial Accounting Standards Board
FIFO	first in, first out
FIP	Federal Implementation Plan
Fifth Credit Facility Agreement	Fifth Amended and Restated Credit Facility Agreement
Flat-Rolled	North American Flat-Rolled segment
foreign exchange forwards	foreign exchange forward sales contracts
FX	foreign exchange
GAAP	Generally Accepted Accounting Principles
Gateway	Gateway Energy and Coke Company LLC
GEN3	generation 3
GHG	greenhouse gas
GLNPO	Great Lakes National Program Office
Green Steels	steels made with low greenhouse gas emissions intensity
HDC	Hibbing Development Company
Hibbing	Hibbing Taconite Company
IEPA	Illinois Environmental Protection Agency
IRS	Internal Revenue Service
ISO	International Organization for Standardization
ITC	U.S. International Trade Commission
JSW	JSW Steel (USA) Inc. and JSW Steel USA Ohio, Inc.
JSW Defendants	Nucor, U. S. Steel, AK Steel Holding Group and Cleveland-Cliffs Inc.
Keetac	U. S. Steel’s iron ore operations at Keewatin, Minnesota
LIFO	last in, first out
MACT	Maximum Achievable Control Technology
Merger Sub	2023 Merger Subsidiary, Inc., a Delaware corp. and a wholly owned subsidiary of Purchaser
Midwest	Midwest Plant
Minntac	U. S. Steel’s iron ore operations at Mt. Iron, Minnesota
mmbtus	One Million British Thermal Units
mnt	thousand net tons
MPCA	Minnesota Pollution Control Agency
NAAQS	National Ambient Air Quality Standards
NAV	net asset value
NESHAP	National Emission Standards for Hazardous Air Pollutants
NGO	non-grain oriented
NIST	the National Institute of Standards and Technology
non-GAAP	Non-Generally Accepted Accounting Principles

NOV	Notice of Violation
NOx	nitrogen oxide
NPDES	National Pollutant Discharge Elimination System
NSC	Nippon Steel Corporation, a Japanese corporation
OCTG	oil country tubular goods
OECD	The Organization for Economic Co-operation and Development
OEM	original equipment manufacturer
OPEB	Other Post-Employment Benefit
Order	Administrative Order on Consent
Other Benefits	defined benefit retiree health care and life insurance plans
Patriot	Patriot Premium Threading Services, LLC
PBO	Projected Benefit Obligation
PCAOB	Public Company Accounting Oversight Board (United States)
PFAS	Per- and Polyfluoroalkyl Substances
PII	Personally Identifiable Information
PM	Particulate Matter
PPA	Pension Protection Act of 2006
ppb	parts per billion
PRO-TEC	PRO-TEC Coating Company, U. S. Steel and Kobe Steel Ltd. joint venture
PRP	potentially responsible party
PSU	Performance-based restricted Stock Unit
Purchaser	Nippon Steel North America, Inc., a New York corporation
RCRA	Resource Conservation and Recovery Act
RICO	Racketeer Influenced and Corrupt Organizations
ROCE	Return On Capital Employed
RP	Rehabilitation plan
RSU	Restricted Stock Unit
S&P	Standard & Poor's
sales swaps	financial swaps hot-rolled coil and iron ore pellet sales
SCF	supply chain finance
SEC	Securities and Exchange Commission
SIP	State Implementation Plan
SO_2	Sulfur dioxide
SPT	Steelworkers Pension Trust
SSS	site-specific standard
Stelco	Stelco Inc., a subsidiary of Cleveland-Cliffs Inc.
sustainable steels	steels made with low greenhouse gas emissions
SWMU	Solid Waste Management Units
the 2005 Plan	2005 Stock Incentive Plan
the 2022 Labor Agreements	collective bargaining agreements with United Steelworkers effective September 1, 2022
the Committee	the Compensation & Organization Committee of the Board of Directors
the "Company"	United States Steel Corporation and its subsidiaries
the Exchange Act	the Securities Exchange Act of 1934, as amended
the "Insurers"	Banner Life Insurance Company and William Penn Life Insurance Company of New York
the insurer	Pacific Life Insurance Company
the "Merger"	pursuant to which Merger Sub will merge with and into the Company
the "Merger Agreement"	On December 18, 2023, the Company entered into an Agreement and Plan of Merger with Nippon Steel North America, Inc.
the Minntac Mine	iron ore mine located in Mt. Iron, Minnesota
the Omnibus Plan	2016 Omnibus Incentive Compensation Plan, as amended and restated
USSK Credit Agreement	USSK €150 million unsecured sustainability linked credit agreement
TRQ	tariff rate quotas
Transtar	Transtar LLC and its direct and indirect subsidiaries
TSR	Total Shareholder Return
Tubular	Tubular Products segment

U. S. Steel	United States Steel Corporation
U.S. EPA	United States Environmental Protection Agency
U.S. GAAP	accounting standards generally accepted in the United States
U.S. Supreme Court	the Supreme Court of the United States
UDEQ	Utah Department of Environmental Quality
ug/m3	micrograms per cubic meter
UK	United Kingdom
UPI	USS-POSCO Industries
USSE	U. S. Steel Europe segment
USSK	U. S. Steel Košice
USSTP	U. S. Steel Tubular Products LLC
USTR	United States Trade Representative
USW	United Steel, Paper and Forestry, Rubber, Manufacturing, Energy, Allied Industrial and Service Workers International Union or United Steelworkers
USX	United States Steel Corporation
VEBA	trusts for retiree healthcare and life insurance
VERP	voluntary early retirement program
welded	seamless and electric resistance welded
WTO	World Trade Organization
WQS	water quality standard

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