WaterLegacy

WaterLegacy submits the uploaded Comments and their accompanying Attachments A through D.

Sincerely yours,
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Katrina Kessler, Commissioner
Minnesota Pollution Control Agency
520 Lafayette Road N.
St. Paul, MN 55155-4194

RE: Minnesota Wild Rice Sulfate Water Quality Standard NPDES/SDS Wastewater Permit Implementation.

Framework for developing and evaluating site-specific sulfate standards for the protection of wild rice.

Procedures for implementing the Class 4A wild rice sulfate standards in NPDES wastewater permits in Minnesota.

Dear Commissioner Kessler,

The following comments are submitted by WaterLegacy regarding the Minnesota Pollution Control Agency's (MPCA) proposed implementation for the wild rice sulfate standard in the NPDES/SDS process, which includes both procedures for developing and evaluating site-specific standards[1] and procedures for implementing wild rice sulfate standards in NPDES permits.[2] WaterLegacy appreciates the efforts made by the MPCA to describe the value of wild rice and its ecological cyclicity. However, WaterLegacy is deeply disappointed in the proposed implementation concepts and procedures for enforcing the wild rice sulfate standard.

MPCA has resisted enforcement of the 1973 duly enacted and federally-approved wild rice sulfate standard for decades. WaterLegacy had hoped that decisions in the past five years by the Administrative Law Judge (ALJ) and Chief ALJ, the Minnesota courts, and the United States Environmental Protection Agency (EPA) determining that wild rice water quality standard (WQS) must be enforced to comply with the Clean Water Act in permitting and in listing and restoring impaired waters would change MPCA's modus operandi. However, both MPCA's site-specific standards and implementation procedures appear to allow the Agency and permittees to

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[1] MPCA, Framework for developing and evaluating site-specific sulfate standards for the protection of wild rice (June 2023), https://www.pca.state.mn.us/sites/default/files/wq-s6-66.pdf (Wild Rice Sulfate SSS Framework).

[2] MPCA, Procedures for implementing the Class 4A wild rice sulfate standards in NPDES wastewater permits in Minnesota, https://www.pca.state.mn.us/sites/default/files/wq-wwprm2-109.pdf, (Wild Rice Sulfate Permit Procedures).

avoid application of Minnesota's wild rice standard or to allow sulfate far discharge far in excess of the 10 milligrams per liter (mg/L) wild rice sulfate WQS.

MPCA's proposals are contrary to law and/or inimical to science and must be altered before procedures for implementing the wild rice sulfate standard are finalized.

1. The 10 mg/L Wild Rice Sulfate Water Quality Standard is the Effect Criterion.

MPCA's concept that a site-specific standard may be chosen based on "sediment-based equation" rejected in the 2017-2018 contested case rulemaking or a "likely sulfate effect threshold" based on the review of literature or sulfide concentrations in sediment porewater,[3] is contrary to scientific evidence, contrary to law, and contrary to EPA's recent decisions overruling MPCA failure to list wild rice waters where sulfate exceeds 10 mg/L as impaired due to excessive sulfate.

MPCA's proposal to allow more sulfate pollution where there is also a high level of iron in sediments was rejected by the Administrative Law Judge (ALJ) in the 2018 rulemaking, rejected by the Chief ALJ, and then withdrawn by the MPCA. There is no scientific justification for resuscitating this unprotective approach. MPCA's 2017 "novel approach" that a model for sediment iron, organic carbon and surface water sulfate should be used to determine a sulfate effect threshold to replace Minnesota's 10 mg/L numeric wild rice sulfate standard was debunked in the contested case rulemaking process.

There is robust scientific evidence that the mechanism of sulfate impairment of wild rice is not ameliorated by iron in sediments. Iron sulfide plaques form on roots and impair nutrient uptake and seed production. It was further demonstrated in the 2017-2018 administrative process that, from a mathematical perspective, the "equation" MPCA proposed in 2017 to replace the 10 mg/L wild rice water quality standard would have the effect of allowing more sulfate pollution, not protecting wild rice. Although the modeling equation approach is favored by both taconite and sulfide ore mining interests to avoid or minimize the need for sulfate treatment, it is not scientifically supported.[4]

MPCA states that "tailoring" the wild rice sulfate WQS is consistent with the Clean Water Act, suggesting, in effect, that Minnesota's 10 mg/L adopted and federally approved WQS is merely an advisory starting point, not a standard. This approach is contrary to the Clean Water Act, where water quality standards set criteria to protect the beneficial use. 33 U.S.C. § 1313(c)(2)(A); 40 C.F.R. § 131.3(i). Numeric standards, like the 10 mg/L wild rice WQS provide criteria establishing quantifiable concentrations of pollutants that can't be exceeded in order to support a particular beneficial use. 40 C.F.R. § 131.3(b). Consideration of "guidance" under

[3] Wild Rice Sulfate SSS Framework at 3.

[4] Expert reports from the 2017-2018 contested case hearing and subsequent peer-reviewed literature are provided in Attachment A (John Pastor opinion and supporting documents, 2017); Attachment B (Joel Roberts opinion 2017); and Attachment C (Sophia LaFond Hudson articles, 2018, 2020, 2022).

Clean Water Act regulations, 40 C.F.R. § 131.11(b)(1)(i) is used to adopt a numeric standard, not to deviate from that federally-approved standard.[5]

MPCA's proposal that permittees return to the open-ended and unprotective equation proposed and rejected in rulemaking is also inconsistent with the EPA's April 27, 2021 Decision Document Regarding the Sulfate Impaired Waters EPA is Adding to the Minnesota 2020 Clean Water Act Section 303(d) List (EPA Sulfate Impaired Waters Decision). In that Decision, EPA overruled MPCA's failure to identify *any* Minnesota wild rice waters impaired due to sulfate exceeding the wild rice sulfate standard of 10 mg/L.

The EPA emphasized, "Since 2012, EPA has also strongly encouraged MPCA to develop an assessment methodology and to engage in a substantive effort to assess and list waters *against its current wild rice criterion*."[6] EPA summarized the history of the rule, noting that after "the 2018 Chief ALJ Order disapproving [MPCA's] proposed standards revision . . . MPCA withdrew its effort to clarify the wild rice beneficial use and associated criterion." *Id.* at 9. EPA listed sulfate impaired Minnesota wild rice waters under Section 303(d) of the Clean Water Act based on data showing the "exceedance of the numeric 10 mg/L sulfate criterion." *Id*. at 14.

MPCA's Wild Rice Sulfate SSS Framework further demonstrates that MPCA is not intending to use site-specific standards in a manner that would be protective of wild rice beneficial uses. MPCA's Mississippi River Pool 8 example presumes that a less stringent standard should apply to this waterbody since the number of wild rice locations has not declined since 1989. Wild Rice Sulfate SSS Framework at 8. However, beginning the analysis of decline at 1989 is insufficient under the Clean Water Act. In addition, MPCA's own criteria for protection of the wild rice beneficial use requires more than counting locations. *Id*. at 3.

It is clear that any implementation of site-specific wild rice sulfate standards needs clear guardrails not provided in MPCA's draft:

- The Wild Rice Sulfate SSS Framework must state that the 10 mg/L wild rice sulfate standard is the applicable water quality criterion that will be incorporated into all NPDES permits pending attempts by any party to conduct the research and devise a less stringent site-specific sulfate standard.
- The Wild Rice Sulfate SSS Framework must further state that the 10 mg/L WQS represents the "threshold effect" on wild rice.

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[5] MPCA's discussion of this regulation in the Wild Rice Sulfate SSS Framework at 1, is simply incorrect.

[6] EPA, Sulfate Impaired Waters Decision, https://www.epa.gov/sites/default/files/2021-04/documents/2021.4.27_2020_mn_303d_dd_phase_2_.pdf, Attachment D at 8 (emphasis added)

- The Wild Rice Sulfate SSS Framework must state that any person seeking a less stringent site-specific standard must demonstrate that the wild rice beneficial use has been fully protected at all historical times for tribal uses and since November 28, 1975 for other uses, not merely that wild rice plants have survived despite elevated sulfate.

2. Permitting Cannot Allow Degradation or Fail to Consider High Quality Waters,

The decision tree in MPCA's Wild Rice Sulfate Permit Procedures has several important flaws that will result in inadequate protection of wild rice beneficial use. They will not ensure that sufficient effluent controls are imposed to prevent sulfate discharge from causing or contributing to exceedance of numeric standards or from degrading wild rice.

First, MPCA's failure to address sulfate loading as well as sulfate concentration in waters that are impaired or lack assimilative capacity is scientifically untenable and inconsistent with applicable law. Sulfate is toxic to wild rice due to its effects on sediment chemistry and biochemical reactions that result in sulfide formation. Sulfate may form chemoclines in lakes, where sulfate concentrations are higher near the lake bottom than surface sampling data would suggest. Wild rice waters where water is shallow, slow-moving, or backwater may not sluice away the sulfate in the water flow. Limiting additional loading of sulfate to wild rice waters is necessary to avoid sulfide toxicity and excessive release of nitrogen and phosphorus nutrients that can adversely impact wild rice.

Applicable federal and state laws explicitly require that limits be placed on loading of new discharge of pollutants to impaired waters to comply with wasteload allocations and that water quality-based effluent limits control pollutants by weight or mass, not only by concentration. 40 C.F.R. §§ 122.4(i), 122.44(d)(1)(vii); Minn. Stat. §115.03, subd. 10; Minn. R. 7001.1080, subps. 1, 2(A); *see also In re Cities of Annandale & Maple Lake NPDES/SDS Permit Issuance for Discharge of Treated Wastewater*, 731 N.W.2d 502 (Minn. 2007).

Second, MPCA's proposed "boundary condition" between wasteload discharge and wild rice waters is not sufficient to control discharge that has "the reasonable potential to cause ,or contribute" to an exceedance of the wild rice sulfate standard in a downstream water. 40 C.F.R. §122.44(d)(1)(i); Minn. R. 7001.0180, subp. 1. If there are sulfate dischargers above and below a low-sulfate tributary that both contribute to an exceedance in a downstream waterbody, both dischargers of sulfate require water quality-based effluent limits. For example, in an otherwise 1.5 mg/L low sulfate stream, if upstream (NPDES 1) discharge of 100 mg/L sulfate is diluted to 9.5 mg/L by a clean tributary and then contributes to sulfate discharged by a second (NPDES 2) discharger, resulting in a sulfate level of 12 mg/L in a wild rice water, both the NPDES 1 and NPDES 2 entities should have effluent limits, since both have the reasonable potential to cause or contribute to the exceedance of the 10 mg/L wild rice sulfate standard in a wild rice water.

Third, neither MPCA's Wild Rice Sulfate Permit Procedures nor MPCA's Wild Rice Sulfate SSS Framework address the need to prevent degradation of the productivity, ecological health, and/or genetic diversity of wild rice. *See* Minn. R. 7050.0224, subp. 1; 7050.0250; 7050.0265; 7050.0280. In the areas of wild rice abundance, sulfate concentrations are generally much lower than 10 mg/L. For example, average sulfate concentrations in Big Sandy Lake are 1.2 mg/L sulfate. Wild rice waters on which wildlife and harvesters rely for food and where tribal members exercise treaty-reserved usufructuary rights, are often low-sulfate waters. MPCA has cited no research and WaterLegacy knows of none demonstrating that increasing sulfate loading to low-sulfate wild rice waters or increasing sulfate concentrations until they approach 10 mg/L will not degrade the quality or quantity of wild rice.

MPCA's discussion of site-specific standards, similarly, contains no guidance for development of *more* stringent site-specific standards to preserve low-sulfate wild rice waters or wild rice waters with outstanding value for wildlife, human harvest, exercise of treaty-reserved rights, or preservation of genetic diversity. MPCA does not appear to have evaluated conditions under which a more stringent site-specific sulfate standard would be imposed. Given the devastation of wild rice caused by anthropogenic land use, pollution, and climate change across the nation as well as across Minnesota, MPCA must adopt a more more proactive approach.

MPCA's Wild Rice Sulfate Permit Procedures and Wild Rice Sulfate SSS Framework must be revised to provide protection of wild rice consistent with ecosystems knowledge and applicable law:

- MPCA's Wild Rice Sulfate Permit Procedures must limit sulfate loading to comply with wasteload allocations, restore impaired waters, and prevent degradation.
- MPCA's Wild Rice Sulfate Permit Procedures must require water quality-based effluent limits if discharge causes <u>or contributes</u> to an exceedance, whether or nor there is an intervening waterbody with less than 10 mg/L of sulfate.
- MPCA's Wild Rice Sulfate Permit Procedures must impose effluent limits to prevent degradation of low-sulfate and high value wild rice waters and must explicitly set forth a process of setting more stringent site-specific standards to protect these waters.

3. <u>Regional Data Shows Gaps in Monitoring and Analysis</u>.

MPCA's discussions of regional waters and historical sulfate data are not well developed, and their rationale is dubious. The example of monitoring high sulfate in the main channel of the Mississippi River upstream of back channels with wild rice is ambiguous. It is not clear whether MPCA is asserting that the wild rice in back channels should be identified as impaired with or without more proximate sampling or whether MPCA is asserting that factors other than sulfate influence the growth of wild rice in these locations.

Similarly, MPCA's documentation of ambient sulfate in regional waters is poorly connected to the topic of site-specific standards or protection of wild rice beneficial use. The MPCA has not distinguished "baseline" conditions that may be due to anthropogenic land use and pollution from "natural background" conditions that occurred before European settlement. The map of high and low sulfate conditions is uninformative for the wild rice sulfate standard implementation since it does not illustrate the relationship between wild rice and sulfate levels.

The previous map on this topic prepared by the MPCA in 2014 in the wild rice sulfate standard rulemaking[7] (below) is a more useful indicator of the relationships between sulfate and wild rice.

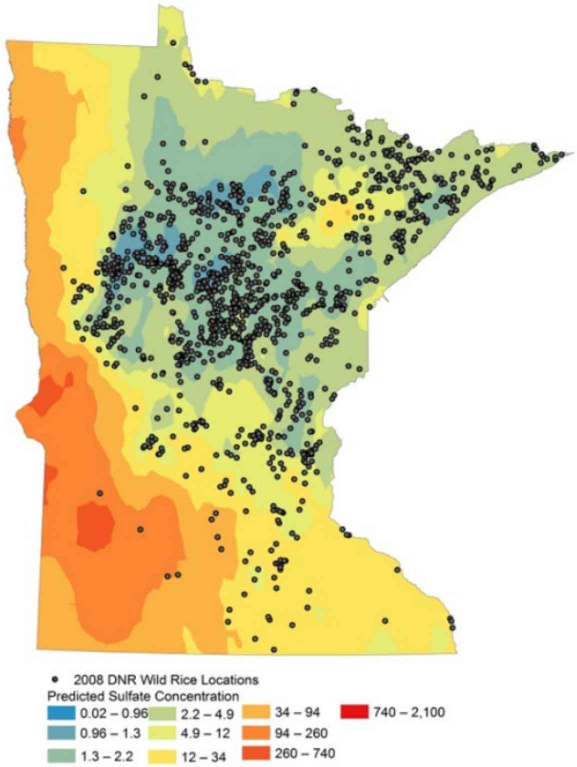

Figure 1. Locations of reported lakes with wild rice (black symbols; from DNR 2008) as compared to surface water sulfate concentrations (in mg/L). The sulfate contours were generated from 3,230 surface water sulfate values in DNR and MPCA databases (see Table 6 for summary statistics of these data).

WaterLegacy requests a more rigorous analysis of the relationships between sulfate and wild rice prevalence as well as the specific policy changes in the Wild Rice Sulfate Permit Procedures and Wild Rice Sulfate SSS Framework detailed above. MPCA proposed procedures and framework must be substantially revised to protect the beneficial use of waters for the growth of wild rice.

Respectfully submitted,

Janet R. Keough
Janet Keough, Water Legacy Board President

Paula G. Maccabee
Paula Maccabee, WaterLegacy Advocacy Director and Counsel

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[7] MPCA, Analysis of the Wild Rice Sulfate Standard Study, June 2014 at 9, Figure 1 https://www.leg.mn.gov/docs/2014/other/140594.pdf

Technical Review Comments on MPCA's Proposed Flexible Standard for Sulfate in Wild Rice Beds

Proposed Minnesota Pollution Control Agency Rulemaking

John Pastor, PhD (November 2017)

Background and Research

I am a Professor of Biology at the University of Minnesota Duluth, past Co-Chair of the Natural History Section of the Ecological Society of America, and an Honorary Member of the Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

I received my B.S. in Geology from the University of Pennsylvania in 1974, and my Ph.D. in Forestry and Soil Science in 1980 from the University of Wisconsin-Madison. I've also done post-doctoral research in the Environmental Sciences Division at Oak Ridge National Laboratory. I've authored two books on ecology, over 100 peer-reviewed papers, and over 20 book chapters. My papers have been cited over 17,000 times by other scientists. My *curriculum vitae* is provided (attachment A) with these comments.

For the past ten years, my research has focused on the ecology of wild rice, including the effects of sulfate pollution and iron on wild rice. This work has been funded by the National Science Foundation, Minnesota Pollution Control Agency, Fond du Lac and Grand Portage Bands of Lake Superior Chippewa, and Minnesota Sea Grant. I was the lead researcher for the hydroponic experiments and tank mesocosm studies of sulfate and wild rice coordinated by the Minnesota Pollution Control Agency (MPCA) in the Wild Rice Sulfate Standard Study funded by the Minnesota Legislature. However, our mesocosm studies of wild rice and sulfates began several years before the MPCA study and have continued through 2017.

Results of the first several years of my research regarding effects of sulfate and sulfide on the life cycle of wild rice in hydroponic and mesocosm experiments were published in a peer-reviewed journal article (Pastor *et al*. 2017) provided (attachment B) with these comments.

For the past several years, I have continued mesocosm research designed to test the MPCA's hypothesis that sediment iron would protect wild rice from the effects of high surface water concentrations of sulfate. The results of this research are reflected in a Minnesota Sea Grant Progress 2016 report (attachment C) and a Minnesota Sea Grant Progress 2017 report (attachment D) provided with these comments. One of my graduate students, Sophia LaFond-Hudson, studied iron and sulfur cycling in the root zones of wild rice in an experimental growing wild rice in buckets. Her 2016 Master's thesis on this research (LaFond-Hudson, 2016) is also provided with my comments (attachment E). The 2016 Sea Grant Progress Report and Ms. LaFond-Hudson's thesis were provided to the MPCA in the summer of 2016. I also presented a slide presentation on the experimental effects of iron and sulfate on wild rice to the MPCA and Wild Rice Sulfate Standard Study Advisory Committee in August 2016. That slide presentation is also provided with my comments (attachment F).

I was contacted by WaterLegacy to review the MPCA's proposal to replace Minnesota's existing fixed standard of 10 milligrams per liter (mg/L) sulfate applicable to water used for the production of wild rice (Minn. R. 7050.0224, subp. 2) with a flexible standard derived through the use of an equation. Throughout the past six years, I have read numerous MPCA draft proposals, internal memos, peer review materials, submitted and published articles and comments of various entities and experts. In preparing these comments, I also reviewed the MPCA's draft rule, Statement of Need and Reasonableness and Exhibit 1 Technical Support Document.

Summary

1) Our recent research at the University of Minnesota Duluth demonstrates that sulfide, not sulfate, is toxic to seedlings of wild rice. The MPCA proposes that iron can protect wild rice by precipitating with the sulfide. However, the addition of iron to mesocosms with high sulfate concentrations did not entirely mitigate the toxic effects of sulfide to seedlings. Our research also demonstrates that precipitation of iron sulfide on wild rice roots can inhibit nutrient uptake needed to ripen seeds, so iron sulfide can have negative effects on wild rice sustainability. Setting sulfate limits based on the level of sediment iron is premature and is not reasonable.

2) In addition, the MPCA's model assumes that concentrations of sulfide, sulfate, reactive iron and organic matter are in a steady state. This is not a reasonable assumption, especially once sulfate loading increases from various sources of pollution.

3) Both historic field data and the recent field surveys performed by the University of Minnesota as part of the Wild Rice Sulfate Standards Study demonstrate that concentrations of sulfate in surface water above 10 mg/L proposed in the MPCA's flexible standard may not adequately protect wild rice.

Statement of the problem

The State of Minnesota now has a fixed standard of "10 mg/L sulfate applicable to 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, subp. 2). This standard, developed during the 1970s, is based on research by DNR botanist John Moyle, who found that "No large stands of rice occur in water having sulfate content greater than 10 ppm [parts per million, or mg/L], and rice generally is absent from water with more than 50 ppm" (Moyle 1944).

Application of Minnesota's sulfate standard has been rare and controversial. To put this in perspective, EPA drinking water standards for sulfate are 250 mg/L, while EPA standards for sulfide in surface waters to protect aquatic life are very low; 2 parts per billion (2ug/L). Although ecologists, including John Moyle, have long believed that wild rice toxicity resulted from conversion of sulfate to sulfide in sediments with low concentrations of oxygen, little experimental data confirmed that hypothesis. Research was designed to evaluate what factors resulted in wild rice toxicity and whether limiting sulfate was necessary to prevent sulfide-induced toxicity.

Sulfate, Sulfide and Iron Research

Sulfate is released to surface waters by several industrial processes, but *sulfate* is transformed into *sulfide* in waterlogged sediments with low concentrations of oxygen. Our initial investigations of the effects of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris* L.) in hydroponic solutions and in outdoor mesocosm tanks demonstrated that sulfide, not sulfate, is toxic to seedlings of wild rice. 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.

When we added sulfate to experimental mesocosm tanks where wild rice was grown in sediments from a wild rice lake under low oxygen conditions similar to those in a natural environment, sulfate additions to overlying water increased sulfide production in sediments. Seedling emergence, seedling survival, vegetative growth and seed production all declined in proportion to the amount of sulfate added and the amount of sulfide produced.

In each spring after the initial planting in 2011, the number of seedlings that emerged from the sediment declined significantly with increased sulfate concentrations ($p < 0.001$). The rate of seedling survival also declined significantly with increased sulfate concentrations ($p < 0.001$) and became worse in each subsequent year ($p < 0.001$). The rate of decline in seedling survival with amended sulfate was twice as high in 2014 and 2015 as it was in 2012 and 2013 (Pastor *et al*. 2017).

Elevated sulfate and presumably sulfide concentrations decreased vegetative growth, measured as plant biomass ($p < 0.001$), and the rate of decline increased significantly during the course of the experiment. Although the overall number of seeds produced per plant did not change across sulfate concentrations, the proportion of seeds produced that were filled and thus able to propagate declined significantly with increasing sulfate concentrations ($p < 0.001$). The proportion of filled seeds declined more steeply with each successive year ($p < 0.001$) (Pastor *et al.* 2017).

These declines in seed production and seedling survival lead to the extinction of wild rice populations after 5 years at sulfate concentrations comparable to drinking water standards (Pastor *et al*. 2017). Populations of wild rice exposed to sulfate concentrations of 150 mg/L have continued to decline over the course of the mesocosm experiments, nearing the point of extinction (Progress Report 2017). In addition, we have noticed a parallel decline in other species in the tanks with enhanced sulfate concentrations. These species include the larvae of dragonflies and caddisflies, which are important foods for fish such as walleye that typically inhabit wild rice lakes. Therefore, the decline in population densities with enhanced sulfate concentrations may not be limited to wild rice but in fact may happen to other important species of the food web.

The MPCA also coordinated a parallel field study of over 100 wild rice lakes. The MPCA's preliminary findings seemed to support retaining the existing 10 mg/L sulfate limit to protect wild rice from sulfide-induced toxicity. However, the MPCA is currently proposing to replace its 10 mg/L fixed sulfate standard with a flexible standard based on a model which attempts to predict sulfide concentrations in sediment of each individual lake from the concentration of sulfate in surface waters and the concentrations of reactive iron and organic matter in sediments from these lakes.

Geochemistry supports the MPCA's basic premise that iron may reduce sulfide concentrations in sediments. Sulfate is converted to sulfide by microorganisms that also obtain energy by decomposing organic matter. Iron is present in many forms in wild rice beds but the more important form for the purpose of this model is ferrous iron, a form that can reduce the reactivity of sulfide in sediment.

However, MPCA's proposed model relies on a critical assumption that is tenuous and has not been experimentally verified. The MPCA assumes that any precipitation of sulfide by iron helps to protect wild rice. Our experimental mesocosm research has substantially undermined this assumption. During the course of our initial mesocosm (tank) experiments, we noticed that wild rice roots in tanks with more than 50 mg/L sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle. Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques, whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides. (Pastor *et al*. 2017; Sea Grant Report 2017).

Figure 1. Orange healthy roots (left) of wild rice grown under low sulfate concentrations near the current standard and black iron sulfide coatings on roots of plants grown with high sulfate concentrations.

We learned that iron sulfide precipitates rapidly on wild rice roots in midsummer at the time when the plants are beginning to flower and take up additional nutrients for the ripening seeds. The iron sulfide precipitates gave the roots a black appearance, compared to amber or rust colored roots on healthy plants exposed to sulfate concentrations near the current fixed standard of 10 mg/L. Seed nitrogen, seed count and seed weight were all markedly reduced in plants with back root surfaces exposed to high sulfate surface water concentrations (300 mg/L) because these black iron sulfide precipitates inhibit the uptake of nutrients necessary for the filling and ripening of seeds necessary for propagation of wild rice. This happened even though the amount of iron remaining in the sediment was sufficient to remove sulfide from sediment porewater. These experiments are detailed in Progress Report (2017) and LaFond-Hudson (2016). Plants grown at lower concentrations of sulfate had black iron sulfide coatings in proportionally lower amounts, as well as proportionally reduced seed production (Pastor et al. 2017).

Our experimental mesocosms contained sediment iron near the median of that observed in field conditions. Our more recent experiments, in which we tripled the amount of sediment iron in the first growing season and removed litter to reduce carbon supply for microbes under sulfate conditions of 300 mg/L, began in 2015. During the three years of this experiment, sulfate amendments had the greatest effect on outcomes, reducing seedling survival, plant growth, and seed production. Litter removal had no effect on seedlings, vegetative growth, or seed production. Adding iron without sulfate had no effect on seedling survival, plant growth, or seed production. Iron amendments in the presence of sulfate increased seedling survival compared with seedlings grown under sulfate amendments alone, but seedling survival in the tanks with both iron and sulfate additions was still less than in control tanks. (Progress Report 2017). Our experiments found that precipitation of iron sulfide in the sediment may temporarily ameliorate the effects of sulfate on seedling survival, but by the spring of year three, iron amendment no longer had an effect on seedling survival, possibly because almost all the added iron had been precipitated. (Progress Report 2017).

Our experiments demonstrate that precipitation of sulfide in the presence of high levels of iron has both ameliorative and negative effects on wild rice growth. Iron additions may partly ameliorate sulfide toxicity to seedlings in spring. However, precipitation of iron sulfide plaques on roots during the flowering and seed production period of wild rice's life cycle appears to block uptake of nitrogen, leading to fewer and smaller seeds with reduced nitrogen content. The net effect of sulfate additions to wild rice populations is to drive the populations to extinction within 4 or 5 years at high concentrations of sulfate (300 mg/l), even when iron was added to the sediments. Sulfate loading greatly reduce population viability at lower concentrations.

How and whether iron mitigates sulfide toxicity to wild rice is not fully understood and appears not to be related to the amount of reactive iron in sediments in the simple way assumed by MPCA's model. Therefore, setting sulfate standards based on the amount of reactive iron in sediments is premature at best. Based on current scientific evidence, an equation determining "protective" sulfate levels based on iron in sediments and available carbon is not a defensible strategy to protect wild rice.

Finally, MPCA claims, on p. 82 in their Statement of Need and Reasonableness, that concentrations of sulfate above the allowable standard in one year out of ten would not have a significant impact on wild rice populations in the long run. They cite our experiments in support of this conclusion. While I agree that it is important to determine the allowable frequency and degree of excursions to avoid impacts on wild rice, I must also point out that our experiments were not designed to determine what these might be. At present, a one-in-ten year allowable excursion is premature and requires further experiments designed specifically to determine what level of excursions does not harm the long term sustainability of wild rice populations.

Steady State Concentrations

In addition to assuming a simple relationship between iron in sediments and survival of wild rice, MPCA's model assumes that the concentrations of sulfide, sulfate, reactive iron, and organic matter in the sites from which the equation was developed are in steady state, which means that their concentrations do not change over long periods of time.

MPCA claims that the assumption of steady state is verified by data that concentrations of these elements of the model did not change during one growing season. But one growing season is insufficient to test the assumption of steady state. The steady state assumption must be tested against data across years, particularly in systems subject to transient changes to sulfate from industrial discharges. Until longer-term information is obtained, we do not know if these ecosystems are in a steady state from one year to the next. If the ecosystems are not in steady state, then the calculation that a certain sulfate concentration in surface water creates lower-than-toxic levels of sulfide during one year may not apply to subsequent years. A sulfate concentration deemed "protective" in year one could become toxic in subsequent years.

Once sulfate inputs to a wild rice bed increase as a result from discharge of wastewater, ecosystems will no longer be in steady state. Microbes in the sediments will convert some of this sulfate to additional sulfide and the sulfide will precipitate with some of the reactive iron and convert it to iron sulfide precipitates. But the iron in these precipitates will no longer be available to precipitate any additional sulfide. The reactive iron removed by precipitation with sulfide must be replenished by inputs of additional iron for the initial calculation to remain valid. In an ecosystem, it cannot be assumed that natural inputs of reactive iron from streams and groundwater or from weathering of sediments will keep pace with sulfate pollution.

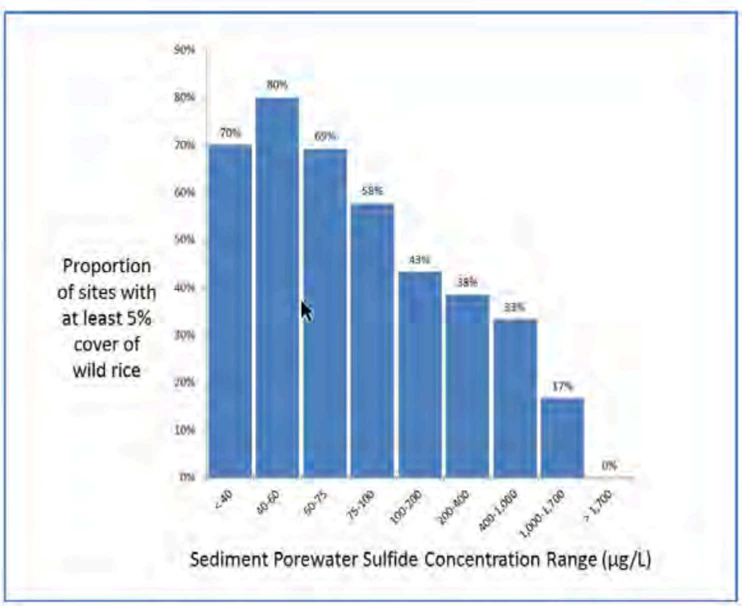
The amount of reactive iron in a localized area will decline with increased sulfate loading, just as a checkbook balance declines when withdrawals increase without a matching increase in deposits. MPCA's model does not demonstrate that natural inputs of iron would replenish the reactive iron in the sediment commensurate with sulfate discharge. The model assumes, without evidence, that iron input will remain at a rate sufficient to ameliorate sulfide toxicity from the additional sulfate without creating additional adverse consequences for wild rice survival.

As also pointed out by Prof. David Schimpf (Schimpf, 2015), a decision to allow sulfate concentrations in surface waters above their current levels in certain sites could look reasonable for a while, but become inadvisable and fail to protect wild rice over time.

Concentrations of Sulfate Greater than 10 mg/L May Not Adequately Protect Wild Rice

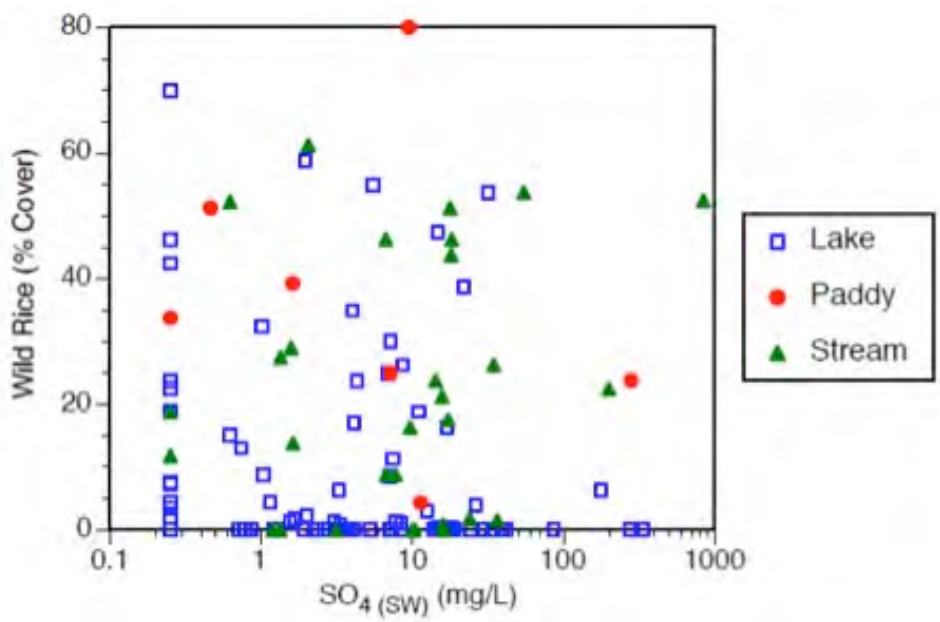
Professor Shimpf has also raised the concern that the MPCA's proposal, by focusing on the presence of wild rice may redefine "protect wild rice" in a weaker sense than that of the existing standard, which was based on John Moyle's field research finding no large stands of wild rice in Minnesota where sulfate exceeded 10 mg/L and that wild rice was "generally absent" where sulfate exceeded 50 mg/L. (Schimpf, 2015)

Data from MPCA's survey lakes demonstrate a decline in wild rice abundance at sulfide concentrations above 75 µg/L, which is below MPCA's proposed EC10 of 120 µg/L. (MPCA, 2014). In addition, a standard that is based on 5% wild rice cover may not protect wild rice sustainability.



MPCA's flexible standard, based on calculating a "protective sulfate concentration" to attain a sulfide level of 120 ug/L, would allow sulfate concentrations more than an order of magnitude above the current sulfate limit of 10 mg/L in many cases and could sometimes result in allowing sulfate concentrations two orders of magnitude higher than the current standard. For example, the MPCA has calculated that a "protective sulfate concentration" for the St. Louis Estuary would range from 99.5 mg/L to 241.1 mg/L, while a "protective" concentration of sulfate for the Embarrass River would be 1248.9 mg/L. (See MPCA spreadsheet, attachment G).

Current data collected by MPCA demonstrate that allowing sulfate concentrations much greater than 10 mg/L (the current standard) may not protect wild rice. This chart prepared by an MPCA staff scientist from the 119 field study sites [1] shows that over 70% of wild rice ecosystems are found in sulfate concentrations of 10 mg/L or less and 94 % are found in lakes or streams with sulfate concentrations below 50 mg/L. Even though the MPCA field survey was designed to study sites with wild rice present despite high sulfate levels (MPCA, 2014), field survey findings strongly corroborate Moyle's (1944) conclusions.



This figure illustrates the infrequency of wild rice presence and density in waters with sulfate concentrations above the current standard of 10 mg/L. Based on its model and equation, MPCA's proposed flexible standard would allow for much higher concentrations of sulfate to be defined as "protective" if high levels of iron were present. Sulfate limits set for individual water bodies above the current standard of 10 mg/L incur increased risk to the sustainability of wild rice populations.

Sandy Lake provides an example of the decline of wild rice populations in the presence of sulfate exceeding the existing 10 mg/L standard despite high sediment iron concentrations. Sandy Lake (MN DNR ID 69-0730-00, in St. Louis County) had extensive and productive wild rice populations in the past. Sandy Lake has received discharge from a nearby tailings pond of an iron mine since the

[1] Edward Swain, MPCA, "The world's 4 species of wild rice," slide presentation to Minnesota Native Plant Society, Feb. 4, 2016.

mid-1960s. The MPCA sampled water and sediment and counted wild rice stem density in Sandy Lake 10 times from June through September in 2013 (Appendix G). The sulfate concentration in Sandy Lake during 2013 averaged 95 mg/L, which is not significantly different from the calculated average allowable sulfate concentration using MPCA's flexible standard model of 79 mg/L, although it is significantly higher than the existing wild rice sulfate limit of 10 mg/L. The sediment of Sandy Lake has high iron content, 23,540 ug/g, which is nearly three times the statewide average (8800 μg/mg) for all non-paddy wild rice water bodies sampled by MPCA. Despite this high iron content, wild rice was largely absent at all times and sampling locations in Sandy Lake, except for two locations with very low population densities (0.6 stems per m^2 at one location on Sept. 17 and 3.8 stems per m^2 at another location on Sept. 21). These low densities are highly unlikely to be viable in the long run.

If MPCA's model is correct, then wild rice should be present and abundant in Sandy Lake because of the high sediment iron content and the similarity of the concentration of sulfate in the water compared to the allowable sulfate concentrations. And yet, despite the high iron content of the sediment, MPCA could barely find any wild rice in Sandy Lake. Although wild rice is present in Sandy Lake and thus appears in MPCA's modeling as a lake with wild rice despite high sulfate concentrations the populations of wild rice in Sandy Lake are clearly not healthy, especially compared to what is known to have been present in the past.

Conclusion

The Wild Rice Sulfate Standard Study wild rice research funded by the Minnesota Legislature and coordinated by the MPCA has made important contributions to our understanding of the process of sulfide-induced toxicity resulting from sulfate concentrations in surface waters in the presence of iron and other factors. However, based on my training and experience, it is my opinion that the weight of the scientific evidence supports retaining Minnesota's existing sulfate standard of 10 mg/L to protect wild rice. As sulfate concentrations rise above the current standard, the risk to sustainable wild rice populations increases because of increased sulfide production.

Although the MPCA's conceptual framework pertaining to sulfate reduction to sulfide and iron sulfide precipitation has substantial merit, making the leap from this conceptual understanding to the MPCA's proposed flexible standard equation makes important assumptions about the ameliorative effects of iron and the continuation of a steady state over time despite sulfate addition to the ecosystems. These assumptions cannot be defended based on scientific evidence. Both experimental research and field data suggest that sulfate concentrations above 10 mg/L may not protect wild rice and that sulfate concentrations an order of magnitude or more above 10 mg/L, as would be allowed in some water bodies by MPCA's proposed flexible standard, are likely to result in decline and extinction of wild rice over time.

Attachments

A. John Pastor *curriculum vitae*.

B. John Pastor *et al*., Effects of sulfate and sulfide on the life cycle of Zizania palustris in hydroponic and mesocosm experiments, Ecological Applications, 27(1), 2017, pp. 321-336.

C. John Pastor, Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*), August 18, 2016 slide presentation.

D. John Pastor, The biogeochemical Habitat of Wild Rice, Minnesota Sea Grant Report May 5, 2016.

E. John Pastor, Progress Report on Experiments on Effects of Sulfate and Sulfide on Wild Rice, June 28, 2017.

F. Sophia LaFondn Hudson, Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*) May 2016, Masters dissertation.

G. MPCA, Field Data with CPSC (All MN Data), Aug. 17, 2016.

Additional References

John Moyle, Wild Rice in Minnesota, Journal of Wildlife Management, Vol. 8, No. 3 (1944)

MPCA, Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, June 9, 2014.

David Schimpf, Comments on the Minnesota Pollution Control Agency's draft proposed approach for Minnesota's sulfate standard to protect wild rice (March 24, 2015), Dec. 14, 2015.

Ed Swain, MPCA, Plant-of-the-month: The world's 4 species of wild rice (Zizania Linnaeus) slide presentation at Minnesota Native Plan Society, Feb. 4, 2016.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment A

(27 pages)

JOHN PASTOR
Department of Biology
University of Minnesota Duluth
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Education

Ph.D., Forestry and Soil Science, University of Wisconsin, Madison, June 1980
M.S., Soil Science, University of Wisconsin, Madison, December 1977
B.S., Geology, University of Pennsylvania, May 1974

Present Positions

Professor, Dept. of Biology, University of Minnesota Duluth (July 1996 – present)

Director, Natural History Minor, University of Minnesota Duluth (March 2009 – present)

Previous Positions

Associate Director of Graduate Studies, Ecological, Organismal, and Population Biology Track, Integrated Biosciences Graduate Program, University of Minnesota Duluth (March 2006 – May 2009)

Director of Graduate Studies, Biology Graduate Program, University of Minnesota Duluth (July 2000 – August 2009)

Visiting Scientist, Dept. of Animal Ecology, Swedish University of Agricultural Sciences, Umeå, Sweden (June – July 1998, and annually thereafter)

Visiting Scientist, Macaulay Land Use Research Institute, Aberdeen, Scotland (May 1997)

Distinguished Visiting Professor, College of Forestry, University of Washington, Seattle, Washington (March 1991)

Visiting Scientist, Institute of Applied Ecology, Shenyang, People's Republic of China (July – August 1988)

Senior Research Associate, Natural Resources Research Institute, University of Minnesota Duluth (July 1985 – 2006)

Postdoctoral Fellow, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 (August 1983 – June 1985)

Postdoctoral Research Associate, Department of Forestry, University of Wisconsin, Madison, WI 53706 (June 1980 – July 1983)

Graduate Student, Departments of Soil Science and Forestry, University of Wisconsin, Madison, WI 53706 (September 1975 – May 1980)

Staff Geologist, Ralph Stone Engineers, Los Angeles, CA 97821 (September 1974 – August 1975)

Research Interests

Species effects on nutrient cycling, plant-herbivore interactions, northern ecosystems, mathematical ecology

Awards and Honors

Honorary Life Member, Finnish Society of Forest Science, elected May 1999

First Recipient, Chancellor's Distinguished Research Award, University of Minnesota Duluth, November 1999

Institute of Scientific Information, Highly Cited List, Ecology and Environment, 2002 – 2012

Sabra and Dennis Anderson Scholar/Teacher Award, College of Science and Engineering, University of Minnesota Duluth, May 2007

University of Minnesota Council of Graduate Students Outstanding Faculty Award, April 2010

Doctores honoris causa, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, October 2010

Distinguished Ecologist Lecture, Colorado State University, April 2012

Teaching

Courses

Dept. of Biology, University of Minnesota Duluth: Biology 5776, "Ecosystem Ecology" (Spring 1990, Fall 1993, Fall 1998 and alternate years to present)

Dept. of Fisheries and Wildlife, University of Minnesota, St. Paul: Fisheries and Wildlife 8579, "Ecosystem Analysis and Simulations" (Winter 1993)

Province of Ontario and Lakehead University: "Ontario Advanced Forestry Program", Lecturer, 1992 and 1993

Dept. of Biology, University of Minnesota Duluth: Biology 5774, "Forest Ecology" (Summer 1994), with George Host

Dept. of Biology, University of Minnesota Duluth: Biology 5155, "Evolutionary Biology" (Fall 1994), with Carl Richards

Dept. of Biology, University of Minnesota Duluth: Biology 8871, "Graduate Seminar: Soil Genesis" (Winter 1994)

Dept. of Biology, University of Minnesota Duluth: Biology 8871, "Graduate Seminar: Measurement of Ecological Diversity" (Winter 1995 and Winter 1998)

Dept. of Biology, University of Minnesota Duluth: Biology 3871, "Issues in Global Change" (Winter 1996)

Dept. of Biology, University of Minnesota Duluth: Biology 5821, "Mathematical Ecology" (Fall 1997 and alternate years to present)

Dept. of Biology, University of Minnesota Duluth: Biology, "Graduate Seminar: Species Diversity in Time and Space" (Winter 1997)

Dept. of Biology, University of Minnesota Duluth: Biology 1102, "Biology & Society" (Spring 1998)

Dept. of Biology, University of Minnesota Duluth: Biology, "Graduate Seminar: Ecological Stoichiometry" (Spring 2005)

Dept. of Biology, University of Minnesota Duluth: Biology 5583, "Animal Behavior" (Spring 1999 – present)

Dept. of Biology, University of Minnesota Duluth: Biology 1097, "Biological Illustration" (Fall 1999 – present)

Dept. of Biology, University of Minnesota Duluth: Biology 8099, "The Biological Practitioner" (Fall 1997 – 2005)

Dept. of Biology, University of Minnesota Duluth: Integrated BioSciences 8011, "Integrated Biological Systems" (Fall 2006 – present)

Dept. of Biology, University of Minnesota Duluth: Integrated BioSciences 8201, "Ecological Processes" (Spring 2007 – present)

Graduate Students and Postdoctoral Fellows

Pamela McInnes, M.S. Wildlife Conservation, 1989 (co-advised with Y. Cohen)
Thesis title: Moose browsing and boreal forest dynamics, Isle Royale, Michigan, USA

Carmen Chapin, M.S. Biology, 1994
Thesis title: Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*.

Ron Moen, Ph.D. Wildlife Conservation, 1995 (co-advised with Y. Cohen)
Thesis title: Evaluating foraging strategies with linked spatially explicit models of moose energetics, plant growth, and moose population dynamics

Cindy Hale, M.S. Biology, 1996
Thesis title: Comparison of structural and compositional characteristics and coarse woody debris dynamics in old-growth versus mature hardwood forests of Minnesota, USA

John Terwilliger, M.S. Biology, 1997
Thesis title: Small mammals, ectomycorrhizae, and conifer succession in beaver meadows

Jean Fujikawa, M.S. Wildlife Conservation, 1997 (co-advised with Y. Cohen)
Thesis title: Interfacing songbird habitats with simulation processes

Scott McGovern, M.S. Biology, 1999
Thesis title: The effects of nitrogen, bacteria, and tachinid parasitoids on the nutrition of the spruce budworm (*Choristoneura fumiferana* Clem.)

Bingbing Li, M.S. Applied and Computational Mathematics, 2001
Thesis title: Mapping and modelling change in a boreal forest landscape

David VanderMeulen, M.S. Water Resources Science, 2001
Thesis title: Decay and nutrient dynamics of litter from peatland plant species

Nathan DeJager, M.S. Biology, 2004
Thesis title: Interactions between moose and the fractal geometries of birch (*Betula pubescens* and *B. pendula*) and Scots Pine (*Pinus sylvestris*)

Wendy Graves, M.S. Applied and Computational Mathematics, 2004 (co-advised with B. Peckham)
Thesis title: A Bifurcation Analysis of a Differential Equations Model for Mutualism

Laura Zimmerman, M. S., Applied and Computational Mathematics, 2006 (co-advised with B. Peckham)
Thesis title: A producer-consumer model with stoichiometry

Rachel Durkee Walker, Ph.D. Water Resources Science, 2008
Thesis title: Wild rice: the dynamics of its population cycles and the debate over its control at the Minnesota Legislature

Laurence Lin, M.S. Applied and Computational Mathematics, 2008 (co-advised with B. Peckham and H. Stech)
Thesis title: A stoichiometric model of two producers and one consumer

Nathan DeJager, Ph.D. Ecology, Evolution, and Behavior, 2008
Thesis title: Multiple scale spatial dynamics of the moose-forest-soil ecosystem of Isle Royale National Park, MI, USA

Rachel MaKarrall, M.S. Biology, 2009 (co-advised with T. Craig)
Thesis title: Creating useful tools for learning insect anatomy

Diana Ostrowski, M.S. Integrated BioSciences, 2009
Thesis title: White-tailed deer browsing and the conservation of forest songbirds and understory vegetation: A natural experiment within the Apostle Islands National Lakeshore

Angela Hodgson, Ph.D. Ecology, Evolution, and Behavior, 2010
Thesis title: Temporal changes in spatial patterns in a boreal ecosystem, causes and consequences

Lauren Hildebrandt, M.S., Integrated BioSciences, 2011
Thesis title: Decay and nutrient dynamics of wild rice litter in response to N and P availability and litter quality

Lee Sims, M.S. Integrated BioSciences, 2011
Thesis title: Light, nitrogen, and phosphorus effects on growth, allocation of biomass and nutrients, reproduction, and fitness in wild rice (*Zizania palustris* L.)

Angelique Edgerton, M.S. Integrated BioSciences, 2013
Thesis title: Structure of relict arctic plant communities along the north shore of Lake Superior

David Wedin, Postdoctoral Fellow, 1990 – 1992

Scott Bridgham, Postdoctoral Fellow, 1993 – 1995 (co-advised with C. Johnston)

Ron Moen, Postdoctoral Fellow, 1995 – 1998 (co-advised with Y. Cohen)

Terry Brown, Postdoctoral Fellow, 1997 – 2000 (co-advised with C. Johnston)

Thesis Opponent for the Following Ph.D. students

Otso Suominen, Ph.D. Biology, Turku University, Turku, Finland, 1999
Thesis title: Mammalian herbivores, vegetation, and invertebrate assemblages in boreal forests: feeding selectivity, ecosystem engineering and trophic effects

Johan Olofsson, Ph.D. Ecology and Environmental Science, Umeå University, Umeå, Sweden, 2001
Thesis title: Long term effects of herbivory on tundra ecosystems

Sari Stark, Ph.D. Biology, University of Oulu, Oulu, Finland, 2002
Thesis title: Reindeer grazing and soil nutrient cycling in boreal and tundra ecosystems

Caroline Lundmark, Ph.D. Wildlife, Fish, and Conservation, Swedish University of Agricultural Sciences, 2008
Thesis title: Morphological and behavioural adaptations of moose to climate, snow, and forage

Professional Service

National Science Foundation

Ad Hoc Reviewer for Ecosystems, Ecology, Long-Term Research in Environmental Biology, Computational Biology, Mathematics, Geography, Hydrology, and Polar Programs

Review Team, Louisiana State University's application to National Science Foundation's EPSCOR Program (January 1986)

Ecosystems Studies Panel (March 1989 – October 1991; reappointed October 2004 – October 2008)

Review Team, Central Plains Long-Term Ecological Research Site (June 1990)

Review Team, Jornada Long-Term Ecological Research Site (May 1991)

Terrestrial Ecology and Global Change (TECO) Research Panel (June 1995)

Research Training Centers Panel (April 1996)

Board, National Center for Ecological Analysis and Synthesis (September 1998 – September 1999)

Long Term Ecological Research Panel (April 2000; reappointed April 2010)

Biocomplexity Panel (June 2000)

Frontiers in Integrated Biological Research Panel (December 2002; reappointed November 2004)

Long-Term Research in Environmental Biology (LTREB) Workshop (September 2003)

Review Team, Coweeta Long-Term Ecological Research Site (June 2005)

Review Team, Bonanza Creek and Toolik Lake Long-Term Ecological Research Sites (June 2007)

Review Team, Virginia Coast Reserve Long-Term Ecological Research Site (September 2009)

National Academy of Sciences / National Research Council

Committee on Scholarly Communications with the People's Republic of China (March 1991 – December 1991)

Committee to Review the Environmental Protection Agency's Environmental Monitoring and Assessment Program (July 1991 – March 1995)

Committee to Review the U.S. Navy's Extremely Low Frequency Submarine Communication Ecological Monitoring Program (March 1995 – June 1997)

Committee to Evaluate Indicators for Monitoring Aquatic and Terrestrial Environments (January 1997 – July 2000)

Review Coordinator for Progress Towards Adaptive Monitoring and Assessment for the Comprehensive Everglades Restoration Plan (September 2002 – February 2003)

Department of Interior

Review Team, Value of Downed Logs in Second Growth Douglas-Fir, Bureau of Land Management (August 1986)

Technical Advisor, U.S. Fish and Wildlife Service and Bell Museum, Endangered Species Exhibition (October 1993 – October 1994)

Department of Agriculture

Committee to Review U.S. Dept. of Agriculture's Research Initiative Program on Water Quality and Ecosystems (August 1993)

U.S. Dept. of Agriculture, National Research Initiative Program, Ecosystems Panel (March 1994)

Environmental Protection Agency

Review Team, Environmental Protection Agency's Research Initiative on Forest Ecosystems (March 1988)

Chair, Review Team, Corvallis Laboratory (August 2001)

NASA

Panel Member, Earth Observing System satellite (September 1988)

U.S. Congress

Testimony on Voyageurs National Park and Boundary Waters Wilderness, U.S. House of Representatives, Subcommittee on National Parks, Forests, and Lands (October 28, 1995 and July 16, 1996)

Testimony on Voyageurs National Park and Boundary Waters Wilderness, U.S. Senate, Committee on Energy and Natural Resources (July 18, 1996)

The White House

National Environmental Monitoring and Research Workshop, National Science and Technology Council (September 1996)

National Sciences and Engineering Research Council, Canada

Grant Selection Committee for Evolution and Ecology (August 1996 – June 1998)

State of Minnesota

Expert Witness on the Effects of Global Climate Change on Minnesota's Ecosystems, Attorney General's Office (1994)

Testimony on the Effects of Global Climate Change on Minnesota's Ecosystems, House Environmental Policy Committee (April 1998)

Local Governments

Co-Founder, City of Duluth Tree Commission (October 1994); Board Member (October 1994 – October 1999); Chair (October 1998 – October 1999)

City of Duluth Secondary Education Mathematics Curriculum Committee (October 1995 – October 1996)

City of Duluth Cities for Climate Protection Program, Steering Committee (November 2001 – October 2002)

University of Minnesota

Chair, Search Committee, Director of the Center for Water and the Environment, Natural Resources Research Institute (1990)

University of Minnesota Duluth Campus Planning Committee (1994)

College of Science and Engineering Executive Committee (May 1998-June 1999; reappointed September 2004 – June 2005)

Chair, Search Committee, Vertebrate Physiologist, Dept. of Biology (September 1998 – June 1999)

Research Ethics Advocates Committee (November 2000 – November 2001)

College of Science and Engineering Academic Standards Committee (September 2001 – 2002)

College of Science and Engineering Integrated Biosciences Program Executive Committee (June 2000 – May 2009)

College of Science and Engineering Single Semester Leave Committee (October 2003)

Chair, University of Minnesota Duluth Graduate Council (September 2004 – May 2005)

College of Science and Engineering Curriculum Committee (September 2007 – June 2009)

Office of Vice-President for Research, Research and Scholarship Advisory Panel (September 2010 – present).

Office of Vice-President for Research, Minnesota Futures Proposal Review Committee (June 2012).

Professional Journals and Societies

Member, Society of American Naturalists, American Mathematical Society, Ecological Society of America

Ad Hoc Reviewer for Science, Nature, Ecology, Forest Science, Canadian Journal of Forest Research, Canadian Journal of Botany, Biogeochemistry, Climatic Change, and other journals

Chair, Committee on Ecosystems and Macroscale Phenomenon, Society of Conservation Biology (April 1988).

Secretary, Association of Ecosystem Research Centers (November 1993 – November 1994)

Associate Editor, The American Naturalist (September 1990 – June 1994)

Associate Editor, Silva Fennica (December 1993 – December 1998)

Ad Hoc Associate Editor, Ecology (May 1994 – August 1996)

Associate Editor, Vegetatio (now Plant Ecology) (March 1995 – March 1998)

Associate Editor, Conservation Ecology (October 1995 – June 2004)

Associate Editor, Ecosystems (January 2001 – present)

R.H. MacArthur Award Committee, Ecological Society of America (2012)

Private Organizations

Joint Coordinating Committee, Climate Systems Modeling Initiative, University Corporation for Atmospheric Research (January 1989 – January 1990)

Technical Advisor, North Central Caribou Corporation (January 1992 – October 1995)

Board of Directors, Voyageurs Region National Park Association (January 1993 – January 2003)

Board of Directors, Sigurd Olson Environmental Institute, Northland College (May 1995 – September 1998)

Board of Directors, Biodiversity Fund, Duluth-Superior Area Community Foundation (October 2010-present)

Board of Trustees, Minnesota, South Dakota, and North Dakota Chapter of The Nature Conservancy (July 2013-present)

Symposia and Workshops, Co-Organizer

"Geomorphology and Ecosystem Processes," Ecological Society of America Annual Meeting, Syracuse, New York, August 1986 (co-organizer with D. Schimel)

"Sustainability of Boreal Regions: Sources and Consequences of Variability," MacArthur Foundation and the Beijer Institute, Itasca State Park, Minnesota, October 1997 (co-organizer with C.S. Holling and S. Light). The papers from this symposium were published in a special issue of *Conservation Ecology*.

"The Role of Large Herbivores in Ecosystem Processes", World Wildlife Fund, Hällnäs, Sweden, May 2002 (co-organizer with K. Danell). The papers from this symposium were published in Danell, K., R. Bergström, P. Duncan, and J. Pastor, (editors). 2006. *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*. Cambridge University Press, Cambridge, Great Britain.

"Mathematical Problems of Global Climate Change", Mathematical Biosciences Institute, Columbus, Ohio, June 2006. (co-organizer with D. Schimel and J. Harte).

"Modeling Nutrient Constraints: Stoichiometry of Cells, Populations, and Ecosystems", Society of Industrial and Applied Mathematics Conference on Applications of Dynamical Systems, Snowbird, Utah, May 2007 (co-organizer with B. Peckham).

Symposia and Workshops, Invited Speaker

"Predicting the Consequences of Intensive Forest Harvesting on Long-Term Productivity," Swedish University of Agricultural Sciences, Jaadrås, Sweden, May 1986

"Positive Feedbacks and the Global Carbon Cycle," Oak Ridge National Laboratory, Tennessee, May 1987

"Influence of Large Mammals on Ecosystem Processes," Symposium at the Ecological Society of America Annual Meeting, Columbus, Ohio, August 1987

"Ecology and Forest Policy for the Lake States," Society of American Foresters Annual Meeting, Minneapolis, Minnesota, October 1987

"Problems in Conservation Biology," Society of Conservation Biology, Hawk's Kay, Florida, June 1988

"Modeling Forest Response to Climatic Change," Scientific Committee on Problems of the Environment, Oxford, England, September 1988

"Ecology for a Changing Earth," National Science Foundation, Santa Fe, New Mexico, December 1988

"Climate Systems Modeling Initiative - First Workshop," University Corporation for Atmospheric Research, Boulder, Colorado, January 1989

"Production-decomposition linkages in northern forests and grasslands and response to climate change," Scientific Committee on Problems of the Environment, Woods Hole, Massachusetts, April 1989

"Explaining Records of Past Global Changes," Global Change Institute, Aspen, Colorado, July 1989

"New Perspectives for Watershed Management: Balancing Long-Term Sustainability with Cumulative Environmental Change," University of Washington and Oregon State University, Seattle, Washington, November 1990

"Hydrological-Geochemical-Biological Interactions in Forested Catchments," Gordon Conference, Holderness School, New Hampshire, July 1991

"Workshop on Northern Herbivory," National Science Foundation, LTER Program, Ecosystems Center, Woods Hole, Massachusetts, November 1992

"Biodiversity of Arctic and Alpine Tundra," Scientific Committee on Problems of the Environment, Kongsvold Biological Station, Oppdal, Norway, August 1993

“Functional Roles of Biodiversity: A Global Perspective,” Scientific Committee on Problems of the Environment, Asilomar, California, March 1994

“Ungulates in Temperate Forest Ecosystems,” Netherlands Institute for Forestry and Nature Research, Wageningen, The Netherlands, April 1995

“Control and Chaos,” National Science Foundation, Hawaii, June 1995

“Managing Ungulates as Components of Ecosystems,” The Wildlife Society Annual Conference, Portland, Oregon, September 1995

“Synthesis, Science, and Ecosystem Management,” National Center for Ecological Analysis and Synthesis, Santa Barbara, California, November 1996

“Hydrobiogeochemistry of Forested Catchments,” Gordon Conference, Colby-Sawyer College, New London, New Hampshire, August 1997

“Herbivore-Plant Interactions,” Third European Congress of Mammalogy, Jyväskylä, Finland, June 1999

“How Nutrient Cycles Constrain Carbon Balances in Boreal Forests and Arctic Tundra,” GCTE-IGBP, Abisko, Sweden, June 1999

“Understanding Ecosystems: The Role of Quantitative Models in Observation, Synthesis, and Prediction,” Cary Conference IX, Institute of Ecosystem Studies, Millbrook, New York, May 2001

“Third North American Forest Ecology Conference,” Duluth, Minnesota, June 2001

“Biogeochemistry of Wetlands,” Duke University Wetland Center, Durham, North Carolina, June 2001

“Twenty-fifth National Indian Timber Symposium” Intertribal Timber Council, Fond du Lac Reservation, Minnesota, June 2001

“Fifth International Moose Symposium”, Lillehammer, Norway, August 2002

“The Importance of Spatial Heterogeneity on Ecosystem Ecology”, Cary Conference X, Institute of Ecosystem Studies, Millbrook, New York, May 2003

“Third ManOMin Watershed Conference: Rainy River Basin”, International Falls, Minnesota, November 2003

“New Directions in Research in Grazing Ecology”, The Macaulay Institute, Aberdeen, Scotland, December 2003

“Novel Approaches to Climate Change”, Aspen Institute of Physics, Aspen, Colorado, June 2005

“Wild Rice Roundtable”, Ecological Society of America Annual Meeting, Milwaukee, Wisconsin, Aug. 4, 2008

"Understanding the Vegetation and Hydrology of Upper Midwest Wetlands", Fond du Lac Band of Lake Superior Ojibway, Carlton, MN, Sept. 22, 2010.

Research Grant Support

Dept. of Energy, "Changes in forest carbon storage with intensive management and climatic change," $93,567 (1985 – 1987). To Pastor

Environmental Protection Agency, "Factors controlling the recovery of aquatic systems from disturbance," $221,032 (1986 – 1987). To Niemi, Naiman, and Pastor

National Science Foundation, "The effects of large mammal browsing on the dynamics of northern ecosystems," $258,645 (1987-1989) to Pastor and Naiman; $419,170 (1989 – 1992) to Pastor and Mladenoff

National Science Foundation, "Reconstructing forest stand histories and soil development from paleoecological evidence," $405,000 (1987 – 1989). To Davis and Pastor

National Science Foundation, "A cooperative facility for research on the ecology of spatial heterogeneity," $403,066 (1988 – 1990). To Johnston and Pastor

Dept. of Energy, "Response of northern ecosystems to global change," $45,150 (1989). To Pastor, Gorham, and Shaver

National Science Foundation, "Animal influences on the aquatic landscape: vegetative patterns, successional transitions, and nutrient dynamics," $430,974 (1989 – 1992). To Naiman, Johnston, and Pastor and $660,000 (1992-1995) to Johnston and Pastor

NASA, "Regional modeling of trace gas production in grassland and boreal ecosystems," $240,000 (1989 – 1992). To Johnston and Pastor

Legislative Commission on Minnesota's Resources, "The relationship between heavy metal biogeochemistry and airborne spectral radiometry as an exploration method," $250,000 (1989 – 1991). To Hauck and Pastor

U.S. Forest Service and The Nature Conservancy, "A landscape approach to biological diversity management using geographic information systems and a forest succession model," $32,000 (1989 – 1991). To Mladenoff and Pastor

U.S. Forest Service and The North Central Caribou Corporation, "Woodland caribou assessment of northern Minnesota," $40,000 (1990 – 1991). To Pastor and Mladenoff

National Science Foundation, "The use of fractal and chaos theory to verify, simplify, and extend forest ecosystem models," $220,975 (1991 – 1993). To Cohen and Pastor

National Science Foundation, “Spatial modelling of forest ecosystem landscapes and bird species diversity,” $200,000 (1994 – 1996). To Cohen, Pastor, and Niemi

U.S. Forest Service, "Investigating ecological and economic interactions between soil and forest conditions and harvesting regimes on the Chippewa National Forest," $25,000 (1992 – 1993). To Pastor and Mladenoff

National Science Foundation, "Moose foraging strategy, energetics, and ecosystem processes in boreal landscapes," $90,000 (1993 – 1994). To Pastor, Mladenoff, and Cohen

National Science Foundation, "Long-term dynamics of moose populations, community structure, and ecosystem properties on Isle Royale," $250,000 (1993 – 1998). To Pastor, Mladenoff and Cohen

National Science Foundation, "Direct and indirect effects of climate change on boreal peatlands," $800,000 (1993 – 1997). To Bridgham, Pastor, Malterer, and Janssens

National Science Foundation, “Landscape control of trophic structure in arctic Alaskan lakes,” $200,000 (1995 – 1997). To Hershey, McDonald, Pastor, and Richards

Legislative Commission on Minnesota's Resources, "Forest management to maintain structural and species diversity," $160,000 (1995 – 1997). To Pastor and Rusterholz

National Science Foundation, "Moose foraging strategy, energetics, and ecosystem processes in boreal landscapes," $765,000 (1995 – 2000). To Pastor and Cohen

National Science Foundation, “Grizzly bear digging in subalpine meadows: Influences on plant distributions and nitrogen availability,” $111,549 (1995 – 1998). To Stanford and Pastor

National Science Foundation, “Control of productivity and plant species segregation by nitrogen fluxes to wetland beaver meadows,” $600,000 (1997 – 2000). To Johnston, Pastor, and Mooers

National Science Foundation, “Carbon and energy flow and plant community response to climate change in peatlands,” $1,200,000 (1997-2001). To Bridgham, Pastor, and Chen

National Science Foundation, “Moose population cycles, ecosystem properties, and landscape patterns on Isle Royale,” $300,000 (1998 – 2003). To Pastor, Cohen, Moen, and Dewey

NASA, “Mapping and modeling forest change in a boreal landscape,” $350,000 (2000 – 2003). To Pastor and Wolter

National Science Foundation, “Wild rice population dynamics and nutrient cycles.” $543,046 (2002 – 2006). To Pastor

National Science Foundation, “LTREB: Spatial dynamics of the moose-forest-soil ecosystem on Isle Royale.” $300,000 (2004 – 2009). To Pastor and Cohen

National Science Foundation, “OPUS: A synthesis of long-term research on moose-boreal forest interactions.” $143,911 (2007 – 2009). To Pastor and Cohen

National Science Foundation, “GK-12: Graduate Fellows in Science and Mathematics Education.” $2,931,828 (2007 – 2011). To Latterell, Hale, Munson, Morton, and Pastor

National Science Foundation, “Wild rice population oscillations, allocation patterns, and nutrient cycling.” $547,000 (2007 – 2012). To Pastor and Lee

Biodiversity Fund, Duluth-Superior Area Community Foundation. “Tundra conservation and monitoring along the North Shore of Lake Superior”, $8,396 (2011-2012). To Pastor

Minnesota Pollution Control Agency, “Wild rice sulfate standards study”, $88,000 (2012-2014). To Pastor

Minnesota Sea Grant, “The biogeochemical habitat of wild rice”. $200,000 (2014-2016). To Pastor, Johnson, and Cotner

Books

Danell, K., R. Bergström, P. Duncan, and J. Pastor, (editors). 2006. *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*. Cambridge University Press, Cambridge, Great Britain.

Pastor, J. 2008. *Mathematical Ecology of Populations and Ecosystems*. Blackwell, Oxford, Great Britain.

Peer-reviewed Journal Articles

Pastor, J., and J.G. Bockheim. 1980. Soil development on moraines of the Taylor Glacier, Lower Taylor Valley, Antarctica. Soil Science Society of America Journal 44: 341-348.

Pastor, J., and J.G. Bockheim. 1981. Biomass and production of an aspen-mixed hardwood-spodosol ecosystem in northern Wisconsin. Canadian Journal of Forest Research 11: 132-138.

Aber, J.D., J. Pastor, and J.M. Melillo. 1982. Changes in forest canopy structure along a site quality gradient in southern Wisconsin. American Midland Naturalist 108: 256-265.

Pastor, J., J.D. Aber, C.A. McClaugherty, and J. Melillo. 1982. Geology, soils, and vegetation of Blackhawk Island, Wisconsin. American Midland Naturalist 198: 266-277.

Pastor, J., J.D. Aber, and J.M. Melillo. 1984. Biomass prediction using generalized allometric regressions for some northeast tree species. Forest Ecology and Management 7: 256-274.

Pastor, J., J.D. Aber, C.A. McClaugherty, and J.M. Melillo. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology 65: 256-268.

Pastor, J., and J.G. Bockheim. 1984. Distribution and cycling of nutrients in an aspen-mixed hardwood-spodosol ecosystem in northern Wisconsin. Ecology 65: 339-353.

Pastor, J., and W.M. Post. 1984. Calculating Thornthwaite's and Mather's actual evapotranspiration using an approximating function. Canadian Journal of Forest Research 13: 466-477.

McClaugherty, C.A., J. Pastor, J.D. Aber, and J.M. Melillo. 1985. Forest litter decomposition in relationship to soil nitrogen dynamics and litter quality. Ecology 66: 266-275.

Post, W.M., J. Pastor, P. Zinke, and A. Stangenberger. 1985. Global patterns of soil nitrogen storage. Nature 317: 613-616.

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, C.A. McClaugherty, and J. Pastor. 1985. Fine root turnover in forest ecosystems in relation to quantity and forms of nitrogen availability: a comparison of two methods. Oecologia 66: 317-321.

Pastor, J., and W.M. Post. 1986. Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2: 3-27.

Binkley, D., J.D. Aber, J. Pastor, and K.J. Nadelhoffer. 1986. Nitrogen availability in some Wisconsin forests: comparisons of resin bags and on-site incubations. Biology and Fertility of Soils 2: 77-82.

Norby, R.J., J. Pastor, and J.M. Melillo. 1986. Carbon-nutrient interactions in response to CO_2 enrichment: physiological and long-term perspectives. Tree Physiology 2: 233-242.

Pastor, J., M.A. Stillwell, and D. Tilman. 1987. Nitrogen mineralization and nitrification in four Minnesota old fields. Oecologia 71: 481-485.

Pastor, J., M. A. Stillwell, and D. Tilman. 1987. Little bluestem litter dynamics in Minnesota old fields. Oecologia 72: 327-330.

Pastor, J., R.H. Gardner, V.H. Dale, and W.M. Post. 1987. Successional changes in soil nitrogen availability as a potential factor contributing to spruce dieback in boreal North America. Canadian Journal of Forest Research 17: 1394-1400.

Pastor, J., R.J. Naiman, and B. Dewey. 1987. A hypothesis of the effects of moose and beaver foraging on soil nitrogen and carbon dynamics, Isle Royale. Alces 23: 107-124.

Pastor, J. and W.M. Post. 1988. Response of northern forests to CO_2-induced climatic change. Nature 334: 55-58.

[*]Pastor, J., R.J. Naiman, B. Dewey, and P. McInnes. 1988. Moose, microbes, and the boreal forest. BioScience 38: 770-777.

[†]Naiman, R.J., H. Décamps, J. Pastor, and C.A. Johnston. 1988. The potential importance of boundaries to fluvial ecosystems. Journal of the North American Benthological Society 7: 289-306.

O'Neill, R.V., D.L. DeAngelis, J. Pastor, B.J. Handley, and W.M. Post. 1989. Multiple nutrient limitations in ecological processes. Ecological Modeling 46: 147-163.

Pastor, J. and M. Broschart. 1990. The spatial pattern of a northern conifer-hardwood landscape. Landscape Ecology 4: 55-68.

Cohen, Y. and J. Pastor. 1991. The responses of a forest ecosystem model to serial correlations of global warming. Ecology 72: 1161-1165.

Ågren, G.I., R.E. McMurtrie, W.J. Parton, J. Pastor, and H.H. Shugart. 1991. State-of-the-art of models of production-decomposition linkages in conifer and grassland ecosystems. Ecological Applications 1: 118-138.

Bryant, J.P., F.D. Provenza, J. Pastor, P.B. Reichardt, T.P. Clausen, and J.T. du Toit. 1991. Interactions between woody plants and browsing mammals mediated by secondary metabolites. Annual Review of Ecology and Systematics 22: 431-446.

[*] Included in the anthology *Readings in Ecology*, S. I. Dodson et al. (editors). Oxford University Press, 1999.

[†] Included in the anthology *Foundation Papers in Landscape Ecology*, J. Wiens et al. (editors). Columbia University Press, 2006.

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, J. Pastor, and R. Boone. 1991. Factors controlling nitrogen cycling and nitrogen saturation in northern temperate forest ecosystems. Ecological Applications 1: 303-315.

Moen, R., J. Pastor, and Y. Cohen. 1991. Effects of moose and beaver on the vegetation of Isle Royale National Park. Alces 26: 51-63.

Pastor, J. and R.J. Naiman. 1992. Selective foraging and ecosystem processes in boreal forests. The American Naturalist 139: 690-705.

Post, W.M., J. Pastor, A.W. King, and W.R. Emanuel. 1992. Aspects of the interaction between vegetation and soil under global change. Water, Air, and Soil Pollution 64:345-363.

McInnes, P.F., R.J. Naiman, J. Pastor, and Y. Cohen. 1992. Effects of moose browsing on vegetation and litterfall of the boreal forest, Isle Royale, Michigan, USA. Ecology 73: 2059-2075.

Pastor, J., B. Dewey, R.J. Naiman, P.F. McInnes, and Y. Cohen. 1993. Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. Ecology 74:467-480.

Pastor, J. and W.M. Post. 1993. Linear regressions do not predict the transient responses of eastern North American forests to CO_2 induced climate change. Climatic Change 23:111-119.

Geng Xiaoyuan, J. Pastor, and B. Dewey. 1993. Studies on leaf decomposition of some tree species on Changbai Mountain. Acta Phytoecologica et Geobotanica Sinica 17:90-96 [in Chinese].

Mladenoff, D.J., M.A. White, J. Pastor and T.R. Crow. 1993. Comparing spatial pattern in unaltered old-growth and disturbed forest landscapes for biodiversity design and management. Ecological Applications 3:294-306.

Hershey, A.E., J. Pastor, B.J. Peterson, and G.W. Kling. 1993. Stable isotopes resolve the drift paradox for *Baetis* mayflies in an arctic river. Ecology 74:2315-2326.

Alban, D.H. and J. Pastor. 1993. Decomposition of aspen, spruce, and pine boles on two sites in Minnesota. Canadian Journal of Forest Research 23: 1744-1749.

Geng Xiaoyuan, J. Pastor, and B. Dewey. 1993. Decay and nitrogen dynamics of litter from disjunct, congeneric tree species in Wisconsin and northeastern China. Canadian Journal of Botany 71: 693-699.

Wedin, D.A. and J. Pastor. 1993. Nitrogen mineralization dynamics in grass monocultures. Oecologia 96: 186-192.

Frelich, L.E., R.R. Calcote, M.B. Davis, and J. Pastor. 1993. Patch formation and maintenance in an old-growth hemlock-hardwood forest. Ecology 74: 513-527.

Mladenoff, D.J., M.A. White, T.R. Crow, and J. Pastor. 1994. Applying principles of landscape design and management to integrate old-growth forest enhancement and commodity use. Conservation Biology 8: 752-762.

Updegraff, K., J. Pastor, S.D. Bridgham, and C.A. Johnston. 1995. Environmental and substrate quality controls over carbon and nitrogen mineralization in a beaver meadow and a bog. Ecological Applications 5: 151-163.

Bridgham, S.D., J. Pastor, C.A. McClaugherty and C.J. Richardson. 1995. Nutrient-use efficiency: a litterfall index, a model, and a test along a nutrient availability gradient in North Carolina peatlands. The American Naturalist 145: 1-21.

Wedin, D.A., L.L. Tieszen, B. Dewey, and J. Pastor. 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. Ecology 76: 1383-1392.

Bridgham, S.D. C.A. Johnston, J. Pastor, and K. Updegraff. 1995. Potential feedbacks of northern wetlands on climate change. Bioscience 45: 262-274.

Chapin, C.T. and J. Pastor. 1995. Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*. Canadian Journal of Botany 73: 728-734.

Pastor, J., B. Dewey, and D. Christian. 1996. Carbon and nutrient mineralization and fungal spore composition of vole fecal pellets in Minnesota. Ecography 19: 52-61.

Post, W.M. and J. Pastor. 1996. Linkages - an individual-based forest ecosystem model. Climatic Change 34: 253-261.

Bridgham, S.D., J. Pastor, J.A. Janssens, C. Chapin, and T. J. Malterer. 1996. Multiple nutrient limitations in peatlands: a call for a new paradigm. Wetlands 16: 45-65.

Moen, R., J. Pastor, Y. Cohen, and C.C. Schwartz. 1996. Effect of moose movement and habitat use on GPS collar performance. Journal of Wildlife Management 60: 659-668.

Cohen, Y., and J. Pastor. 1996. Interactions among nitrogen, carbon, plant shape, and photosynthesis. The American Naturalist 147: 847-865.

Sarkar, S., Y. Cohen, and J. Pastor. 1996. Mathematical formulation and parallel implementation of a spatially explicit ecosystem control model. In: Conference Proceedings, Grand Challenges in Computer Simulations, Society for Computer Simulation, New Orleans.

Pastor, J., A. Downing, and H. E. Erickson. 1996. Species-area curves and diversity-productivity relationships in beaver meadows of Voyageurs National Park, U.S.A. Oikos 77: 399-406.

Keenan, R.J., C.E. Prescott, J.P. Kimmins, J. Pastor, and B. Dewey. 1996. Litter decomposition in western red cedar and western hemlock forests on northern Vancouver Island, British Columbia. Canadian Journal of Botany 74: 1626-1634.

Moen, R., J. Pastor, and Y. Cohen. 1997. A spatially-explicit model of moose foraging and energetics. Ecology 78: 505-521.

Moen, R., J. Pastor, and Y. Cohen. 1997. Accuracy of GPS telemetry collar location with differential correction in theory and practice. Journal of Wildlife Management 61: 530-539.

Moen, R., J. Pastor, and Y. Cohen. 1997. Interpreting behavior from activity counters in GPS collars on moose. Alces 32: 101-108.

Pastor, J. and Y. Cohen. 1997. Herbivores, the functional diversity of plants species, and the cycling of nutrients in ecosystems. Theoretical Population Biology 51: 165 -179.

Pastor, J., R. Moen, and Y. Cohen. 1997. Spatial heterogeneities, carrying capacity, and feedbacks in animal-landscape interactions. Journal of Mammalogy 78: 1040-1052.

Moen, R., Y. Cohen, and J. Pastor. 1998. Evaluating foraging strategies with a moose energetics model. Ecosystems 1: 52-63.

Moen, R. and J. Pastor. 1998. Simulating antler growth and energy, nitrogen, calcium, and phosphorus metabolism in caribou. Rangifer Special Issue No. 10: 85-97.

Bridgham, S. D., K. Updegraff, and J. Pastor. 1998. Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79: 1545-1561.

Jordan, P.A., J.L. Nelson, and J. Pastor. 1998. Progress towards the experimental reintroduction of woodland caribou to Minnesota and adjacent Ontario. Rangifer Special Issue No. 10: 169-181.

Pastor, J. and D. Binkley. 1998. Nitrogen fixation and the mass balances of carbon and nitrogen in ecosystems. Biogeochemistry 43: 63-78.

Pastor, J., B. Dewey, R. Moen, M. White, D. Mladenoff, and Y. Cohen. 1998. Spatial patterns in the moose-forest-soil ecosystem on Isle Royale, Michigan, USA. Ecological Applications 8: 411-424.

Updegraff, K., S.D. Bridgham, J. Pastor, and P. Weishampel. 1998. Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. Biogeochemistry 43: 253-272.

Hale, C.M. and J. Pastor. 1998. Nitrogen content, decay rates, and decompositional dynamics of hollow versus solid hardwood logs in old-growth and mature hardwood forests of Minnesota, U.S.A. Canadian Journal of Forest Research 28: 1276-1285.

Pastor, J., S. Light, and L. Sovell (editors). 1998. Sustainability and Resilience in Boreal Regions: Sources and Consequences of Variability. Conservation Ecology 2 (Special Issue).

Moen, R., J. Pastor, and Y. Cohen. 1999. Antler growth and extinction of the Irish elk. Evolutionary Ecology Research 1: 235-249.

Cohen, Y., J. Pastor, and R. Moen. 1999. Bite, chew, and swallow. Ecological Modelling 116: 1-14.

Pastor, J. and S.D. Bridgham. 1999. Nutrient efficiency along nutrient availability gradients. Oecologia 118: 50-58.

Pastor, J., Y. Cohen, and R. Moen. 1999. The generation of spatial patterns in boreal landscapes. Ecosystems 2: 439-450.

Bridgham, S.D., J. Pastor, K. Updegraff, T.J. Malterer, K. Johnson, C. Harth, and J. Chen. 1999. Ecosystem control over temperature and energy flux in northern peatlands. Ecological Applications 9: 1345-1358.

Hale, C. M., J. Pastor, and K. Rusterholz. 1999. Comparison of structural and compositional characteristics in old-growth versus mature hardwood forests of Minnesota, U.S.A. Canadian Journal of Forest Research 29: 1479-1489.

Pastor, J., K. Standke, K. Farnsworth, R. Moen, and Y. Cohen. 1999. Further development of the Spalinger-Hobbs mechanistic foraging model for free-ranging moose. Canadian Journal of Zoology 77: 1505-1512.

Terwilliger, J. and J. Pastor. 1999. Small mammals, ectomycorrhizae, and conifer succession in beaver meadows. Oikos 85: 83-94.

Hershey, A. E., G. Gettel, M. E. McDonald, M. C. Miller, H. Mooers, W. J. O'Brien, J. Pastor, C. Richards, S. K. Hamilton, and J. Schuldt. 1999. A geomorphic-trophic model for landscape control of Arctic food webs. BioScience 49: 887-897.

Brown, T.N., J. Pastor, C.A. Johnston, and H.D. Mooers. 2000. A finite difference type algorithm with pro rata resource allocation. Ecological Modelling 126: 1-8.

Cohen, Y., J. Pastor, and T. Vincent. 2000. Nutrient cycling in evolutionary stable ecosystems. Evolutionary Ecology Research 6: 719-743.

Weltzin, J.F., J. Pastor, C. Harth, S.D. Bridgham, K. Updegraff, and C.T. Chapin. 2000. Response of bog and fen plant communities to warming and water-table manipulations. Ecology 81: 3464-3478.

Hershey, A. E., G. Gettel, M. E. McDonald, M. C. Miller, H. Mooers, W. J. O'Brien, J. Pastor, C. Richards, and J. Schuldt. 2000. The geomorphic-trophic hypothesis for arctic lake food webs. Verh. Int. Verein. Limnol. 27: 3269-3274.

Bridgham, S.D., K. Updegraff, and J. Pastor. 2001. A comparison of nutrient availability indices along an ombrotrophic-minerotrophic gradient in Minnesota wetlands. Soil Science Society of America Journal 65: 259-269.

Updegraff, K., S.D. Bridgham, J. Pastor, P. Weishampel, and C. Harth. 2001. Response of CO_2 and CH_4 emissions from peatlands to warming and water-table manipulations in peatland mesocosms. Ecological Applications 11: 311-326.

Weltzin, J.F., C. Harth, S.D. Bridgham, J. Pastor, and M. Vonderharr. 2001. Production and microtopography of bog bryophytes: response to warming and water-table manipulations. Oecologia 128: 557-565.

Pastor, J., B. Peckham, S.D. Bridgham, J.F. Weltzin, and J. Chen. 2002. Plant community composition, nutrient cycling, and alternative stable equilibria in peatlands. American Naturalist 160: 553-568.

Weltzin, J.F., S.D. Bridgham, J. Pastor, J. Chen, C. Harth. 2003. Potential effects of warming and drying on peatland plant community composition. Global Change Biology 9: 141-151.

Pastor, J., J. Solin, S.D. Bridgham, K. Updegraff, C. Harth, P. Weishampel, and B. Dewey. 2003. Global warming and DOC export from boreal peatlands. Oikos 100: 380-386.

Pastor, J. and K. Danell. 2003. Moose-vegetation-soil interactions: a dynamic system. Alces 39:177-192.

Chapin, C.T., S.D. Bridgham, J. Pastor, and K. Updegraff. 2003. Nitrogen, phosphorus, and carbon mineralization in response to nutrient and lime additions in peatlands. Soil Science 168: 409-420.

Noormets, A., J. Chen, S. D. Bridgham, J.F. Weltzin, J. Pastor, B. Dewey, and J. LeMoine. 2004. The effects of infrared loading and water table on soil energy fluxes in northern peatlands. Ecosystems 7: 573-582.

Keller, J.K., J.R. White, S.D. Bridgham, and J. Pastor. 2004. Climate change effects on carbon and nitrogen mineralization in peatlands through changes in soil quality. Global Change Biology 10: 1053-1064.

Chapin, C.T., S.D. Bridgham, and J. Pastor. 2004. pH and nutrient effects on above-ground net primary production in a Minnesota USA bog and fen. Wetlands 24: 186-201.

Persson, I-L., J. Pastor., K. Danell, and R. Bergström. 2005. Impact of moose population density and forest productivity on the production and composition of litter in boreal forests. Oikos 108: 297-306.

Weltzin, J. F., J. K. Keller, S. D. Bridgham, J. Pastor, P. B. Allen, and J. Chen. 2005. Litter controls plant community composition in a northern fen. Oikos 110: 537-546.

Hale, C.M., L.E. Frelich, P.B. Reich, and J. Pastor. 2005. Effects of European earthworm invasion on soil characteristics in northern hardwood forests of Minnesota, U.S.A. Ecosystems 8: 911-927.

Pastor, J., A, Sharp, and P. Wolter. 2005. An application of Markov models to the dynamics of Minnesota's forests. Canadian Journal of Forest Research 35: 3011-3019.

Pastor, J. and R. D. Walker. 2006. Delays in nutrient cycling and plant population oscillations. Oikos 112: 698-705.

Walker, R. D., J. Pastor, and B. Dewey. 2006. Effects of wild rice (*Zizania palustris* L.) straw on biomass and seed production in northern Minnesota. Canadian Journal of Botany 84: 1019-1024.

Knowles, R. D., J. Pastor, and D. D. Biesboer. 2006. Increased soil nitrogen associated with the dinitrogen fixing, terricolous lichens of the genus *Peltigera* in northern Minnesota. Oikos 114: 37-48.

Graves, W., B. Peckham, and J. Pastor. 2006. A bifurcation analysis of a simple differential equations model for mutualism. Bulletin of Mathematical Ecology 68: 1851-1872.

Hale, C. M., L. E. Frelich, P. B. Reich, and J. Pastor. 2008. Exotic earthworm effects on hardwood forest floor, nutrient availability and native plants: a mesocosm study. Oecologia 155: 509-518.

Suominen, O., I.-L. Persson, K. Danell, R. Bergström, and J. Pastor. 2008. Impact of moose densities on abundance and richness of different trophic levels along a productivity gradient. Ecography 31: 636-645.

De Jager, N. and J. Pastor. 2008. Effects of moose *Alces alces* population density and site productivity on the canopy geometry of birch *Betula pubescens* and *B. pendula* and Scots pine *Pinus sylvestris*. Wildlife Biology 14: 251-262.

Chen, J., S. Bridgham, J. Keller, J. Pastor, A. Noormets, and J. Weltzin. 2008. Temperature responses to infra-red loading and water table manipulations in peatland mesocosms. Journal of Integrative Plant Biology 50: 1484-1496.

Bridgham, S. D., J. Pastor, B. Dewey, J. F. Weltzin, and K. Updegraff. 2008. Rapid carbon response of peatlands to climate change. Ecology 89: 3041-3048.

De Jager, N., J. Pastor, and A. Hodgson. 2009. Scaling the effects of moose browsing on forage distribution, from the geometry of plant canopies to the landscape. Ecological Monographs 79: 281-297.

De Jager, N. and J. Pastor. 2009. Declines in moose population density at Isle Royale National Park, MI, USA and accompanied changes in landscape patterns. Landscape Ecology 24: 1389-1403.

Persson, I-L., M. B. Nilsson, J. Pastor, T. Eriksson, R. Bergström, and K. Danell. 2009. Depression of belowground respiration rates at simulated high moose population densities in boreal forests. Ecology 90: 2724-2733.

Walker, R. D., J. Pastor, and B. Dewey. 2010. Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.*)* in northern Minnesota. Ecosystems 13: 485-498.

De Jager, N. and J. Pastor. 2010. Effects of simulated moose browsing on the morphology of rowan (*Sorbus aucuparia*). Wildlife Biology 16: 301-307.

Sharp, A. and J. Pastor. 2011. Stable limit cycles and the paradox of enrichment in a model of chronic wasting disease. Ecological Applications 21: 1024-1030.

Pastor, J. 2011. Landscape nutrition: seeing the forest instead of the trees. Journal of Animal Ecology 80: 707-709.

Lin, L., B. Peckham, H. Stech, and J. Pastor. 2012. Enrichment in a stoichiometric model of two producers and one consumer. Journal of Biological Dynamics 6: 97-116.

Hildebrandt, L., J. Pastor, B. Dewey. 2012. Effects of external and internal nutrient supplies on decomposition of wild rice, *Zizania palustris*. Aquatic Botany 97: 35-43.

Sims, L., J. Pastor, T. Lee, and B. Dewey. 2012. Nitrogen, phosphorus, and light effects on growth and allocation of biomass and nutrient in wild rice. Oecologia 170: 65-76.

Stech, H., B. Peckham, and J. Pastor. 2012. Enrichment in a general class of stoichiometric producer-consumer population growth models. Theoretical Population Biology 81: 210-222.

Stech, H., B. Peckham, and J. Pastor. 2012. Quasi-equilibrium reduction in a general class of stoichiometric producer-consumer models. Journal of Biological Dynamics 6: 992-1018.

Sims, L., J. Pastor, T. Lee, and B. Dewey. 2012. Nitrogen, phosphorus and light effects on reproduction and fitness of wild rice. Botany 90: 876–883.

Stech, H., B. Peckham, and J. Pastor. 2012. Enrichment effects in a simple stoichiometric producer-consumer model. Communications in Applied Analysis 16: 687-702.

Pastor, J. and N. De Jager. 2013. Simulated responses of moose populations to browsing-induced changes in plant architecture and forage production. Oikos 122: 575-582.

Dahlberg, N. and J. Pastor. Desirable host plant qualities in wild rice (*Zizania palustris*) for infection by the rice worm *Apamea apamiformis* (Lepidoptera: Noctuidae). Ecological Entomology: submitted.

Peer-Reviewed Book Chapters

Pastor, J. 1986. Reciprocally linked carbon-nitrogen cycles in forests: biological feedbacks within geological constraints. Pages 131-140 in *Predicting consequences of intensive forest harvesting on long-term productivity*, G.I. Ågren, editor. Swedish University of Agricultural Sciences Report No. 26: 131-140.

Pastor, J. 1986. Nutrient regimes in northern hardwoods: harvest intensity and nutrient status. Pages 98-108 in *The Northern Hardwood Resource: Management and Potential*, G.D. Mroz and D.D Reed, editors. Michigan Technological University, Houghton, MI.

Emanuel, W.R., J. Pastor, and R.V. O'Neill. 1987. Maintaining the integrity of global cycles: requirements for long-term research. Pages 23-40 in *Preserving Ecological Systems, the Agenda for Long-term Research and Development*, S. Draggen, J.J. Cohrssen, and R.E. Morrison, editors. Praeger, New York.

Pastor, J. 1989. Nutrient cycling in aspen ecosystems. Pages 21-38 in *Aspen Symposium '89*, R.D. Adams, editor. U.S. Forest Service General Technical Report NC-140.

Post, W.M. and J. Pastor. 1990. An individual-based forest ecosystem model for projecting forest response to nutrient cycling and climate changes. Pages 61-74 in *Forest Simulation Systems*, L.C. Wensel and G.S. Biging, editors. University of California, Division of Agriculture and Life Sciences, Bulletin 1927.

Cook, E.R., L.J. Graumlich, P. Martin, J. Pastor, I.C. Prentice, T.R. Swetnam, K. Valentin, M. Verstraete, T. Webb III, J. White, and I. Woodward. 1991. Biosphere-climate interactions during the past 18,000 years: Towards a global model of the terrestrial biosphere. Pages 25-42 in *Global Changes of the Past*, R.S. Bradley, editor. University Corporation for Atmospheric Research, Boulder Colorado.

Pastor, J. and D.J. Mladenoff. 1992. The southern boreal-northern hardwood forest border. Pages 216-240 in *A Systems Analysis of the Global Boreal Forest*, H.H. Shugart, R. Leemans, and G.B. Bonan, editors. Cambridge University Press.

Pastor, J. and C.A. Johnston. 1992. Using simulation models and geographic information systems to integrate ecosystem and landscape ecology. Pages 324-346 in *Watershed Management: Balancing Sustainability with Environmental Change*, R.J. Naiman, editor. Springer-Verlag, New York.

Post, W.M., F. Chavez, P.J. Mulholland, J. Pastor, T.-H. Peng, K. Prentice, and T. Webb III. 1992. Climatic feedbacks in the global carbon cycle. Pages 392-412 in *The Science of Global Change*, D.A. Dunnette and R.J. O'Brien, editors. American Chemical Society, Washington, DC.

Johnston, C.A., J. Pastor, and R.J. Naiman. 1992. Effects of beaver and moose on boreal forest landscapes. Pages 237-254 in *Landscape Ecology and Geographical Information Systems*, S.H. Cousins, R. Haines-Young, and D. Green, editors. Taylor and Francis, London.

Johnston, C. A., J. Pastor, and G. Pinay. 1992. Quantitative methods for studying landscape boundaries. Pages 107-125 in *Landscape Boundaries*, A. Hansen and F. diCastri, editors. Springer-Verlag, New York.

Pastor, J., J. Bonde, C.A. Johnston, and R.J. Naiman. 1993. A Markovian analysis of the spatially dependent dynamics of beaver ponds. Pages 5-28 in *Theoretical approaches for predicting spatial effects in ecological systems*. Lectures on Mathematics in the Life Sciences, Vol. 23, R.H. Gardner, editor. American Mathematical Society.

Mladenoff, D.J. and J. Pastor. 1993. Sustainable forest ecosystems in the northern hardwood and conifer region: Concepts and management. Pages 145-180 in: *Defining Sustainable Forestry*, G.H. Aplet, J.T. Olson, N. Johnson, and V.A. Sample, editors. Island Press and The Wilderness Society, Washington, DC.

Updegraff, K., S.D. Bridgham, J. Pastor, and C.A. Johnston. 1993. A method to determine long-term anaerobic carbon and nutrient mineralization in soils. Pages 209-219 in: *Defining Soil Quality for a Sustainable Environment*, J. Doran, D. Bezdicek, and D. Coleman, editors. Soil Science Society of America Special Publication, Madison, WI.

Pastor, J. and D.J. Mladenoff. 1993. Modelling the effects of timber management on population dynamics, diversity, and ecosystem processes. Pages 16-29 in *Modelling Sustainable Forest Ecosystems*, D.C. Le Master and R.A. Sedjo, editors. American Forests, Washington, DC.

Pastor, J. 1995. Diversity of biomass and nitrogen distribution among species in arctic and alpine tundra. Pages 255-270 in: *Arctic and Alpine Biodiversity: Patterns, Causes, and Ecosystem Consequences*, F.S. Chapin, III and C. Körner, editors. Springer-Verlag, Heidelberg.

Pastor, J., D. Mladenoff, Y. Haila, J. Bryant, and S. Payette. 1996. Biodiversity and ecosystem processes in boreal regions. Pages 33-70 in: *Functional Roles of Biodiversity: A Global Perspective*, H.A. Mooney, J.H. Cushman, E. Medina, O.E. Sala, and E-D. Schulze, editors. Wiley Press, New York.

Pastor, J. and Y. Cohen. 1997. Nitrogen cycling and the control of chaos in a boreal forest model. Pages 304-319 in: *Control and Chaos*, K. Judd, A. Mees, K. Teo, and T. Vincent, editors. Mathematical Modelling Series, Birkhäuser, Boston.

Pastor, J. 2003. The Mass Balances of Nutrients in Ecosystem Theory and Experiments: Implications for Coexistence of Species. Pages 272-295 in *Models in Ecosystem Science*, C. D. Canham, J. J. Cole, and W. K. Lauenroth, editors. Princeton University Press.

Pastor, J. 2005. Thoughts on the generation of spatial heterogeneity in ecosystems and landscapes and its importance. Pages 49-66 in: *Ecosystem Function in Heterogeneous Landscapes*, G.M. Lovett, C.G. Jones, M.G. Turner, and K.C. Weathers, editors. Springer-Verlag, NY.

Pastor, J., Y. Cohen, and N.T. Hobbs. 2006. The role of large herbivores in ecosystem nutrient cycles. Chapter 10 in: *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*, K. Danell, R. Bergström, P. Duncan, and J. Pastor, editors. Cambridge University Press.

Pastor, J., K. Danell, R. Bergström, and P. Duncan. 2006. Themes and Future Directions in Herbivore-Ecosystem Interactions and Conservation. Chapter 15 in: *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*, K. Danell, R. Bergström, P. Duncan, and J. Pastor, editors. Cambridge University Press.

Map

Wolter, P.T., B.R. Sturtevant, B.R. Miranda, S.M. Lietz, P.A. Townsend, and J. Pastor. 2012. Forest Land Cover Change (1975-2000) in the Greater Border Lakes Region. Research Map NRS-3. U.S. Forest Service, Newtown Square, Pennsylvania.

Commentary and Invited Essays

Pastor, J. 1993. Northward march of spruce. Nature (News and Views) 361: 208-209.

Pastor, J. 1995. Ecosystem management, ecological risk, and public policy. BioScience 45: 286-288.

Pastor, J. 1996. Unsolved problems of boreal regions. Climatic Change 33: 343-350.

Pastor, J. and R. Moen. 2004. The ecology of ice-age extinctions. Nature (News and Views) 431: 639-640.

Pastor, J. 2008. The ethical basis of the null hypothesis. Nature (Correspondence) 453: 1177.

De Jager, N. and J. Pastor. 2012. On architecture and moose populations. Oikos Blog, October 25, 2012. http://oikosjournal.wordpress.com/2012/10/25/on-architecture-and-moose-populations/

Book Reviews

Pastor, J. 1988. Soil-plant relationships: A Gordian knot remains tied. Ecology 69: 874.

Pastor, J. 1992. Dynamics of nutrient cycling and food webs. Trends in Ecology and Evolution 7: 247-248.

Pastor, J. 1994. Vegetation dynamics and climate change. Ecology 75: 2145-2146.

Pastor, J. 1996. The poetry of ecoregions. The Prairie Naturalist 28(4): 1-2.

Pastor, J. 1998. Theoretical ecosystem ecology. EcoScience 5: 283-284.

Pastor, J. 2007. Images of a complex world: the art and poetry of chaos. The Mathematical Intelligencer 29(4): 87-89.

Pastor, J. 2008. Evolutionary dynamics. The Mathematical Intelligencer 30(3): 64-66.

Pastor, J. 2012. The Mathematics of Life. The Mathematical Intelligencer 34(1): 69-71.

Other Non-refereed Scientific Publications

Pastor, J., and W.M. Post. 1985. Development of a linked forest productivity-soil process model. ORNL Technical Manual 9519.

Pastor, J. and M. Huston. 1986. Predicting ecosystem properties from physical data: a case study of nested soil moisture-climatic gradients along the Appalachian chain. Pages 82-95 in M.I. Dyer and D.A. Crossley, editors. *MAB Workshop on coupling of ecological studies on three U.S. Forest Service Research Sites with remote sensing studies*, Athens Georgia.

Pastor, J. 1987. The Lake States forests of the future. Pages 89-93 in Proceedings, Society of American Foresters Annual Meeting, Minneapolis, MN.

Keenan, R.J., J.P. Kimmins, and J. Pastor. 1995. Modeling carbon and nitrogen dynamics in western red cedar and western hemlock forests. In: Proceedings, North American Forest Soils Conference 3, Gainesville, Fl.

Scientific Drawings

Black spruce cone and twig. Nature 361: 208 (1993).

Osprey at nest. Raptor Research Foundation 1995 Annual Meeting Logo.

Brachycentrus americanus. North American Benthological Society 1999 Annual Meeting Logo.

White pine cone and twig. Third North American Forest Ecology Conference (2001) Meeting Logo.

Blanding's turtle. Society of Conservation Biology 2003 Annual Meeting Logo.

Popular articles

Loggers, caterpillars, and aspens. Minnesota Forests, Fall 1988.

Minnesota forests on a Chinese Mt. St. Helens. Minnesota Forests, Winter 1989.

Aspen, the valuable weed tree. Minnesota Forests, Spring 1989.

How long should a leaf live? Minnesota Forests, Summer 1989.

The moose and the forest. Minnesota Forests, Fall 1989.

What the greenhouse effect means for northern Minnesota. Wolf Ridge Almanac, Spring 1990.

Diversity of biodiversity. BWCA Wilderness News, Winter 1992.

White pine, Douglas-fir, and old growth management. BWCA Wilderness News, Summer 1992.

Warblers, spruce budworm, and Acts of Congress. BWCA Wilderness News, Fall 1993.

The ecology of the Kabetogama Peninsula. Voyageurs Region National Park Association Newsletter, Winter 1994.

New England violets, the evolution of species, and National Parks. Voyageurs Region National Park Association Newsletter, Winter 1996.

How should a clever moose eat? Voyageurs Region National Park Association Newsletter, Spring 1996.

How a beaver pond works. Voyageurs Region National Park Association Newsletter, Fall 1996.

Cosmic reflections on a PreCambrian rock. Voyageurs Region National Park Association Newsletter, Winter 1996.

Skunk cabbage, blowflies, and the smells of Spring. Voyageurs Region National Park Association Newsletter, Spring 1997.

A fire at Little Trout Lake. Voyageurs Region National Park Association Newsletter, Summer 1997.

The spectacular Spring warbler migration. Voyageurs Region National Park Association Newsletter, Winter 1997.

Ancient plants of the North Woods. Voyageurs Region National Park Association Newsletter, Spring 1998.

Linnaeus's flower. Voyageurs Region National Park Association Newsletter, Summer 1998.

Ice. Voyageurs Region National Park Association Newsletter, Winter 1998.

The bedrock of a continent. Voyageurs Region National Park Association Newsletter, Spring 1999.

The habitats of birds. Voyageurs Region National Park Association Newsletter, Summer 1999.

Seas of muck. Voyageurs Region National Park Association Newsletter, Fall 2000.

Everyone's favorite berry. Voyageurs Region National Park Association Newsletter, Spring 2001.

The ups and downs of wild rice. Voyageurs Region National Park Association Newsletter, Winter 2002.

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Attachment B

(16 pages)

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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.

Key words: hydroponics; life cycles; sulfate; sulfide; toxicity; wetlands; wild rice; Zizania palustris.

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

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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. 2012*a*) 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. 2012*b*).

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. 2012*a*, *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

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

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

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

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

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

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

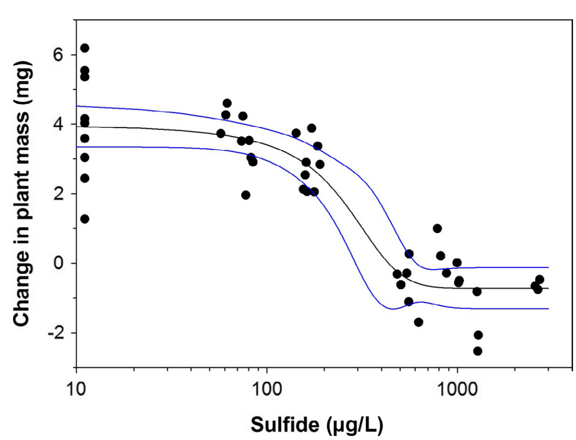


FIG. 1. Growth of wild rice seedlings declines with increasing sulfide concentrations in hydroponic solutions. Individual data points are from three separate experimental runs (see *Methods* and *Results* sections). Fitted sigmoidal response curve (Eq. 1) is shown in black, 95% confidence intervals in 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.)

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.

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.

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

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

	Measured growing season mean sulfate concentrations (mg/L)					
Target sulfate concentration	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.

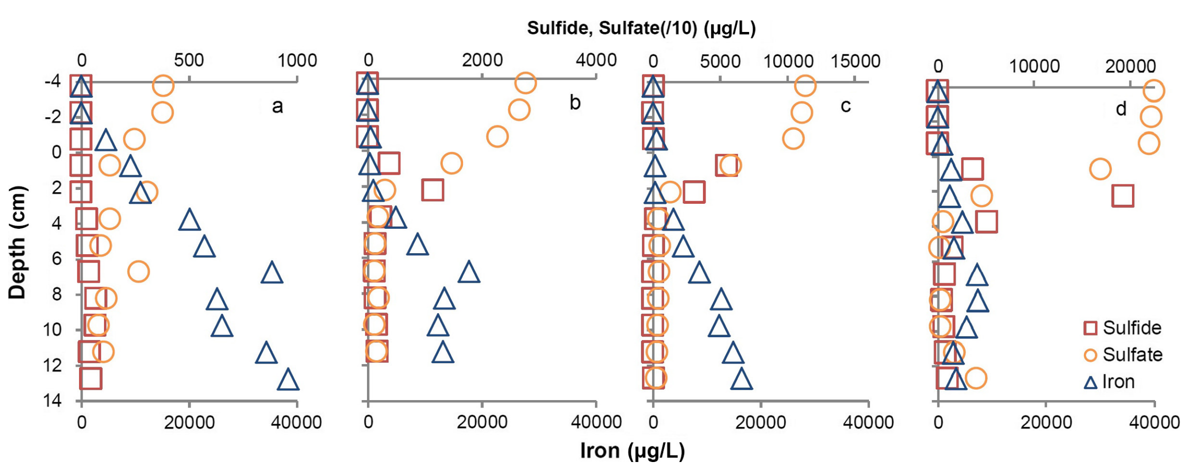


Fig. 2. Vertical profiles of sulfate, sulfide, and iron in mesocosms with different measured sulfate concentrations in the overlying water measured during August 2013. Average annual overlying water sulfate concentrations were (a) 7.05 mg/L, (b) 37.2 mg/L, (c) 127 mg/L, and (d) 268 mg/L. Note different scales for sulfate and sulfide in panels b, c, and d. (Color figure can be viewed at wileyonlinelibrary.com.)

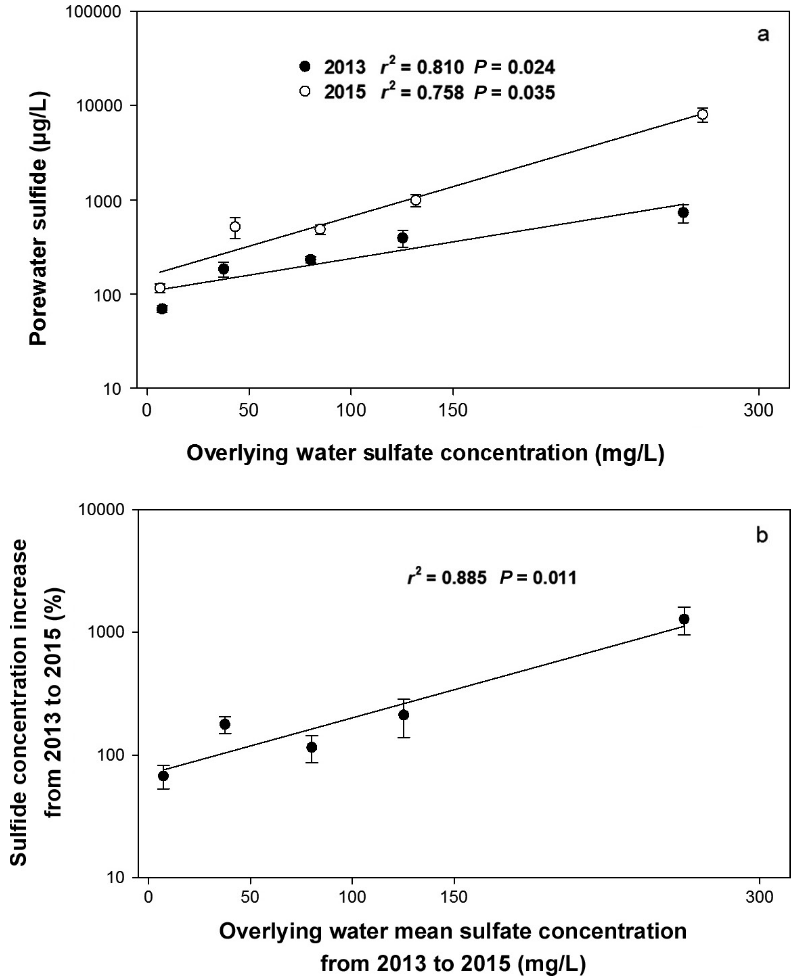


FIG. 3. (a) Porewater sulfide concentrations are strongly correlated with measured concentrations of sulfate in overlying water in the mesocosms and (b) the sulfide concentrations increased from 2013 to 2015 in proportion to sulfate concentrations. Symbols are means and standard errors.

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.

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.

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 $P = 0.598$).

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).

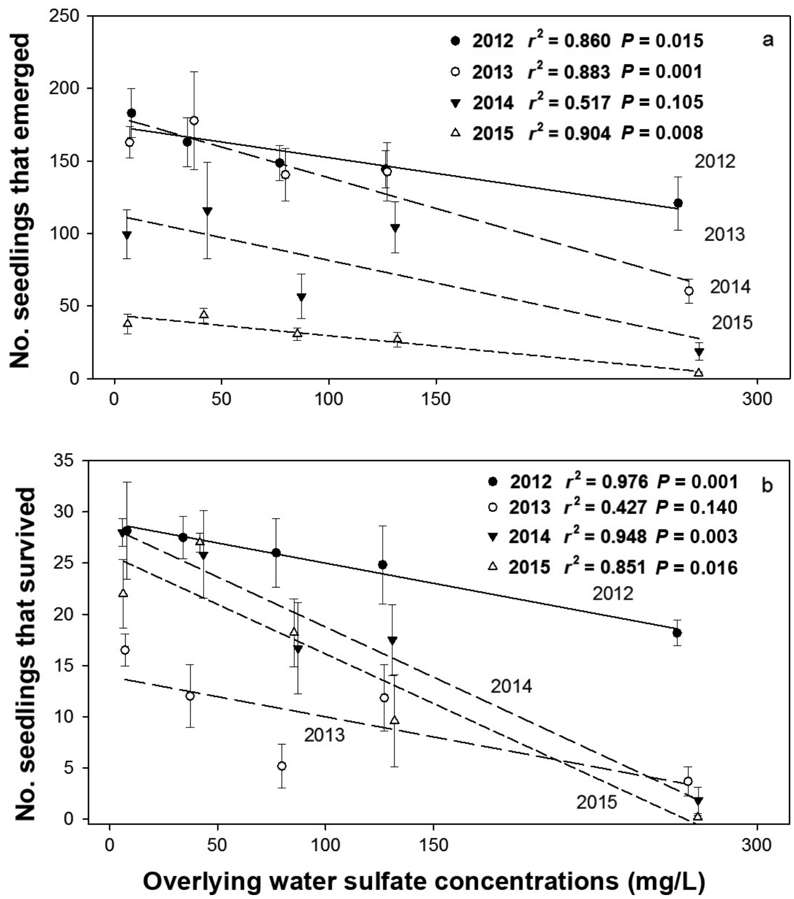


FIG. 4. Emergence (a) and survival (b) of seedlings in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

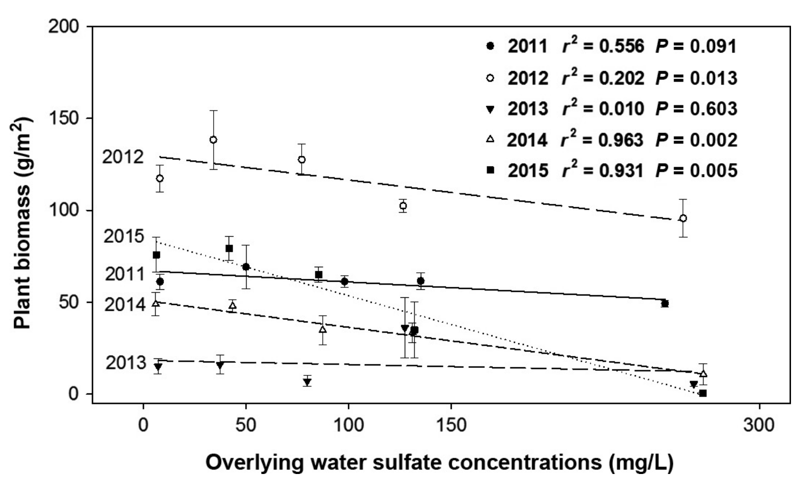


FIG. 5. Vegetative biomass in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

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

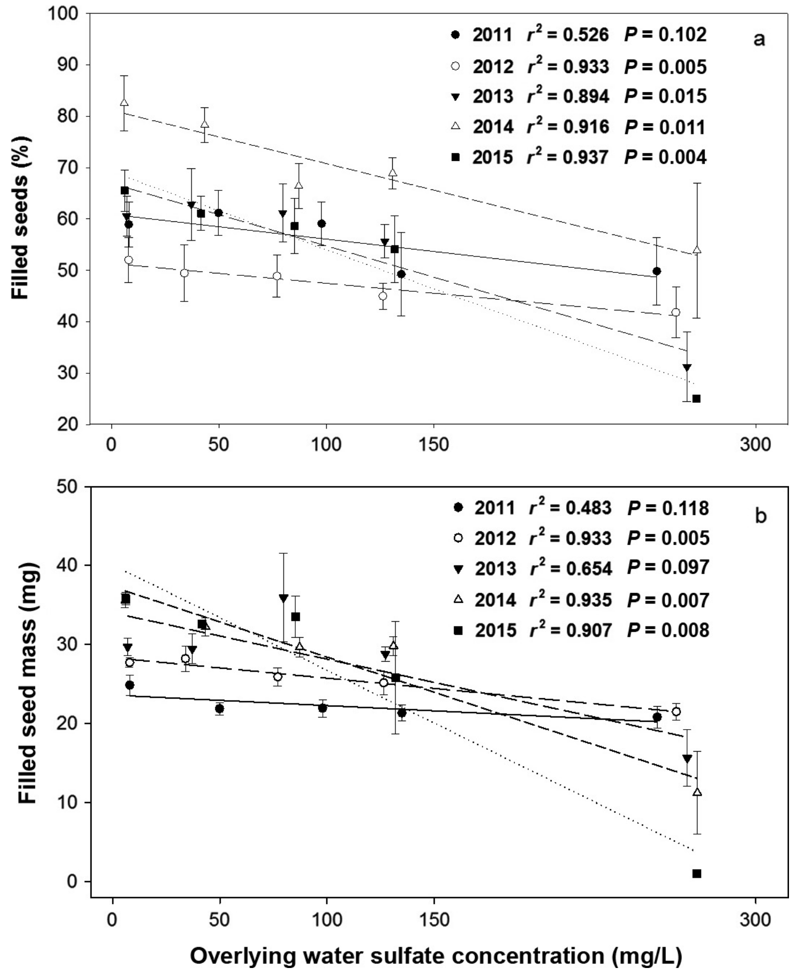


FIG. 6. (a) The proportion of seeds that were filled and (b) the mean seed mass in mesocosms both declined with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

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.

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.

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

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

	Effects of increased sulfate and/or sulfide	
Wild rice life cycle stage	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

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.

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, 1974*a, 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. 2012*a*). 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.

Acknowledgments

This research was supported by the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa with reservation funds and water quality funds provided by the Environmental Protection Agency; by Minnesota's Clean Water Fund; and by Minnesota SeaGrant. The Grand Portage Reservation provided seeds and the Fond du Lac Reservation provided sediment from a wild rice lake for the mesocosm experiment, and Nancy Schuldt and Margaret Watkins, Water Quality Specialists from these reservations, provided advice on the design of this experiment. Russ Erickson at EPA's Mid-Continent Ecology Division in Duluth, Minnesota provided advice on statistical analysis of the hydroponic data. A Review Panel convened by the Minnesota Pollution Control Agency also made excellent suggestions for data analysis and interpretation. We thank all these people for their assistance. We thank Leon Lamers and an anonymous reviewer for many helpful comments on an earlier version of the manuscript.

Literature Cited

Aiken, S. G. 1986. The distinct morphology and germination of the grains of two species of wild rice (*Zizania*, Poaceae). Canadian Field Naturalist 100:237–240.

Allen, H. E., G. Fu, W. Boothman, D. M. DiToro, and J. D. Mahony. 1991. Draft analytical method for determination of acid volatile sulfide in sediment. U.S. Environmental Protection Agency, Washington, D.C., USA.

Armstrong, J., and W. Armstrong. 2005. Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. Annals of Botany 86:625–638.

Balogh, S. J., D. R. Engstrom, J. E. Almendinger, C. McDermott, J. Hu, Y. H. Nollet, M. L. Meyer, and D. K. Johnson. 2009. A sediment record of trace metal loadings in the Upper Mississippi River. Journal of Paleolimnology 41: 623–639.

Campiranon, S., and W. L. Koukkari. 1977. Germination of wild rice, *Zizania aquatica*, seeds and the activity of alcohol dehydrogenase in young seedlings. Physiologia Plantarum 41:293–297.

Caraco, N. F., J. J. Cole, and G. E. Likens. 1989. Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature 341:316–318.

Colmer, T. D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell and Environment 26:17–36.

Cottingham, K. L., J. T. Lennon, and B. L. Brown. 2005. Knowing when to draw the line: designing more informative ecological experiments. Frontiers in Ecology and the Environment 3:145–152.

Day, W. R., and P. F. Lee. 1990. Ecological relationships of wild rice, *Zizania aquatica.* 9. Production in organic-flocculent sediments. Canadian Journal of Botany 68:1542–1548.

DeLaune, R. D., C. J. Smith, and W. H. Patrick Jr. 1983. Relationship of marsh elevation, redox potential, and sulfide to *Spartina alternifolia* productivity. Soil Science Society of America Journal 47:930–935.

Eaton, A. D., L. S. Clesceri, E. W. Rice, A. E. Greenberg, and M. A. H. Franson. 2005. Standard methods for the examination of water and wastewater. Centennial Edition. Twenty-first edition. American Public Health Association, Washington, D.C., USA.

Eldridge, P. M., and J. W. Morse. 2000. A diagenetic model for sediment-seagrass interactions. Marine Chemistry 70:89–103.

Ferren, W. R., and R. E. Good. 1977. Habitat, morphology, and phenology of southern wild rice (*Zizania aquatica* L.) from the Wading River in New Jersey. Bulletin of the Torrey Botanical Club 104:392–396.

Fort, D. J., M. B. Mathis, R. Walker, L. K. Tuominen, M. Hansel, S. Hall, R. Richards, S. R. Grattan, and K. Anderson. 2014. Toxicity of sulfate and chloride to early life stages of wild rice (*Zizania palustris*). Environmental Toxicology and Chemistry 33:2802–2809.

Gao, S., K. K. Tanji, and S. C. Scardaci. 2003. Incorporating straw may induce sulfide toxicity in paddy rice. California Agriculture 57:55–59.

Garrels, R., and C. L. Christ. 1965. Solutions, minerals, and equilibria. Harper and Row, New York, New York, USA.

Geurts, J. J. M., J. M. Sarneel, B. J. C. Willers, J. G. M. Roelofs, J. T. A. Verhoeven, and L. P. M. Lamers. 2009. Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: a mesocosm experiment. Environmental Pollution 157:2072–2081.

Giblin, A. E., G. E. Likens, D. White, and R. W. Howarth. 1990. Sulfur storage and alkalinity generation in New England lake sediments. Limnology and Oceanography 35:852–869.

Grava, J., and K. A. Raisanen. 1978. Growth and nutrient accumulation and distribution in wild rice. Agronomy Journal 70:1077–1081.

Heijs, S. K., H. M. Jonkers, H. van Gemerden, B. E. M. Schaub, and L. J. Stal. 1999. The buffering capacity towards free sulphide in sediments of a coastal lagoon (Bassin d'Arcachon, France)—the relative importance of chemical and biological processes. Estuarine, Coastal and Shelf Science 49:21–35.

Hildebrandt, L., J. Pastor, and B. Dewey. 2012. Effects of external and internal nutrient supplies on decomposition of wild rice, *Zizania palustris*. Aquatic Botany 97:35–43.

Jacq, V. A., K. Prade, and J. C. G. Ottow. 1991. Iron sulphide accumulations in the rhizosphere of wetland rice (*Oryza sativa* L.) as the result of microbial activities. Pages 453–468 *in* J. Berthelin, editor. Diversity of environmental biogeochemistry. Elsevier, Amsterdam, The Netherlands.

Jorgenson, K. D., P. F. Lee, and N. Kanavillil. 2012. Ecological relationships of wild rice *Zizania* spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*). Botany-Botanique 91:189–201.

Joshi, M. M., I. K. A. Ibrahim, and J. P. Hollis. 1975. Hydrogen sulfide: effects on the physiology of rice plants and relation to straighthead disease. Phytopathology 65:1165–1170.

Keenan, T. J., and P. F. Lee. 1988. Ecological relationships of wild rice, Zizania aquatica. 7. Sediment nutrient depletion following introduction of wild rice to a shallow boreal lake. Canadian Journal of Botany 66:236–241.

Koch, M. S., and I. A. Mendelssohn. 1989. Sulphide as a soil phytotoxin: differential responses in two marsh species. Journal of Ecology 77:565–578.

Koch, M. S., I. A. Mendelssohn, and K. L. McKee. 1990. Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnology and Oceanography 35:399–408.

Kordan, H. A. 1972. Rice seedlings germinated in water with normal and impeded environmental gas exchange. Journal of Applied Ecology 9:527–533.

Kordan, H. A. 1974*a*. The rice shoot in relation to oxygen supply and root growth in seedlings germinating under water. New Phytologist 73:695–697.

Kordan, H. A. 1974*b*. Patterns of shoot and root growth in rice seedlings germinating under water. Journal of Applied Ecology 11:685–690.

Koretsky, C. M., M. Haveman, L. Beuving, A. Cuellar, T. Shattuck, and M. Wagner. 2007. Spatial variation of redox and trace metal geochemistry in a minerotrophic fen. Biogeochemistry 86:33–62.

Krabbenhoft, D. P., C. C. Gilmour, J. M. Benoit, C. L. Babiarz, A. W. Andren, and J. P. Hurley. 1998. Methyl mercury dynamics in littoral sediments of a temperate seepage lake. Canadian Journal of Fisheries and Aquatic Sciences 55:835–844.

Lamers, L. P. M., S.-J. Fala, E. M. Samborska, I. A. R. van Dulken, G. van Hengstum, and J. G. M. Roelofs. 2002. Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. Limnology and Oceanography 47:585–593.

Lamers, L. P. M., L. L. Govers, I. C. J. M. Janssen, J. J. M. Geurts, M. E. W. Van der Welle, M. M. Van Katwijk, T. Van der Heide, J. G. M. Roelofs, and A. J. P. Smolders. 2013. Sulfide as a soil phytotoxin. Frontiers in Plant Science 4:1–14.

Lee, P. F. 2002. Ecological relationships of wild rice, *Zizania* spp. 10. Effects of sediment and among-population variations on plant density in *Zizania palustris*. Canadian Journal of Botany 80:1283–1294.

Li, S., I. A. Mendelssohn, H. Chen, and W. H. Orem. 2009. Does sulphate enrichment promote the expansion of *Typha* *domingensis* (cattail) in the Florida Everglades? Freshwater Biology 54:1909–1923.

Malvick, D. K., and J. A. Percich. 1993. Hydroponic culture of wild rice (*Zizania palustris* L.) and its application to studies of silicon nutrition and fungal brown spot disease. Canadian Journal of Plant Science 73:969–975.

Martin, N. M., and B. R. Maricle. 2015. Species-specific enzymatic tolerance of sulfide toxicity in plant roots. Plant Physiology and Biochemistry 88:36–41.

Meeker, J. M. 1996. Wild-rice and sedimentation processes in a Lake Superior coastal wetland. Wetlands 16:219–231.

Mendelsson, I. A., and K. L. McKee. 1988. Spartina alterniflora dieback in Louisiana: time course investigation of soil waterlogging effects. Journal of Ecology 76:509–521.

Minnesota Department of Natural Resources. 2008. Bulletin 25 – An Inventory of Minnesota Lakes. http://www.dnr.state.mn.us/waters/watermgmt_section/pwi/bulletin25.html

Moyle, J. B. 1944. Wild rice in Minnesota. Journal of Wildlife Management 8:177–184.

Moyle, J. B. 1945. Some chemical factors influencing the distribution of aquatic plants in Minnesota. American Midland Naturalist 34:402–420.

MPCA. 2016. National Lakes Assessment 2012. Water chemistry, lake morphometry, and watershed characteristics of Minnesota's 2012 NLA lakes. Minnesota Pollution Control Agency. https://www.pca.state.mn.us/sites/default/files/wq-nlap1-14.pdf

Nichols, S. A., and B. Shaw. 2002. The influence of groundwater flow on the distribution and abundance of aquatic plants in some Wisconsin Lakes. Journal of Freshwater Ecology 17:283–295.

Oelke, E. A., J. Grava, D. Noetzel, D. Barron, J. Percich, C. Schertz, J. Strait, and R. Stucker. 1982. Wild rice production in Minnesota. University of Minnesota Extension Bulletin 464:39.

Pastor, J., and R. D. Walker. 2006. Delays in nutrient cycling and plant population oscillations. Oikos 112:698–705.

Pezeshki, S. R. 2001. Wetland plant responses to soil flooding. Environmental and Experimental Botany 46:299–312.

Schlesinger, W. H. 1991. Biogeochemistry: an analysis of global change. Academic Press, New York, New York, USA.

Sims, L., J. Pastor, T. Lee, and B. Dewey. 2012*a*. Nitrogen, phosphorus, and light effects on growth and allocation of biomass and nutrient in wild rice. Oecologia 170:65–76.

Sims, L., J. Pastor, T. Lee, and B. Dewey. 2012*b*. Nitrogen, phosphorus and light effects on reproduction and fitness of wild rice. Botany-Botanique 90:876–883.

Stover, E. L. 1928. The roots of wild rice. *Zizania aquatica* L. Ohio Journal of Science 28:43–49.

Sun, L., C. Zheng, J. Yang, C. Peng, C. Xu, Y. Wang, J. Feng, and J. Shi. 2015. Impact of sulfur (S) fertilization in paddy soils on copper (Cu) accumulation in rice (*Oryza sativa* L.) plants under flooding conditions. Biology and Fertility of Soils. http://dx.doi.org/10.1007/s00374-015-1050-z

U.S. EPA. 2004. Method 9060A. Total organic carbon. U.S. Environmental Protection Agency, Washington, DC.

Vennum, T. 1998. Wild rice and the Ojibway people. Minnesota Historical Society Press, St. Paul, Minnesota, USA.

Vogt, D. J. 2010. Wild rice monitoring and abundance in the 1854 ceded territory (1998–2010). 1854 Treaty Authority, Duluth, Minnesota, USA.

Walker, R. D., J. Pastor, and B. Dewey. 2010. Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.) in northern Minnesota. Ecosystems 13:485–498.

Wang, Y., and P. Van Cappellen. 1996. A multicomponent reactive transport model of early diagenesis: application to redox cycling in coastal marine sediments. Geochimica et Cosmochimica Acta 60:2993–3014.

Yang, C., X. Zhang, J. Li, M. Bao, D. Ni, and J. L. Seago Jr. 2014. Anatomy and histochemistry of roots and shoots in wild rice (*Zizania latifolia* Griseb.). Journal of Botany. http://dx.doi.org/10.1155/2014/181727

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1452/full

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment C

(25 pages)


Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*)
John Pastor
Dept. of Biology
University of Minnesota Duluth

Does Iron Control Sulfide Toxicity to Wild Rice?

- **Long term Mesocosm Experiment**

- **Bucket Experiment**

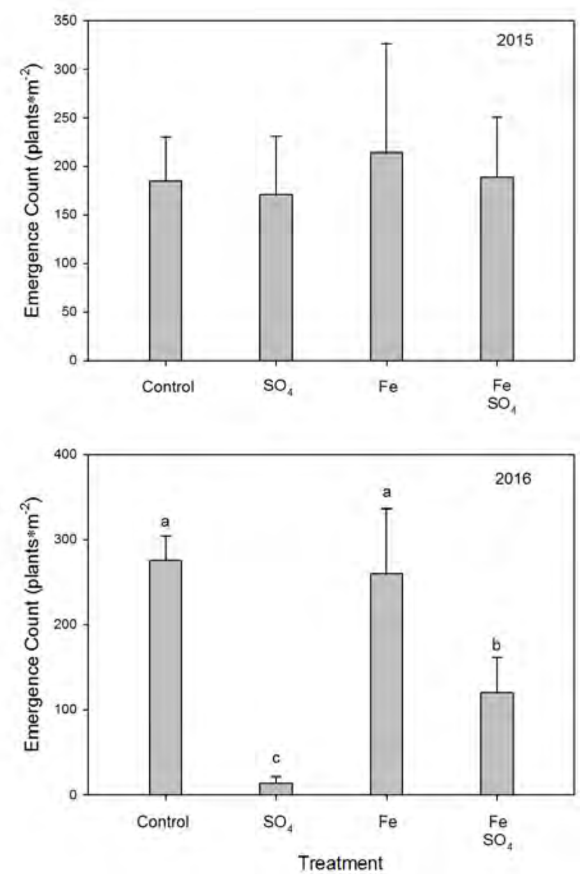
Mesocosm Experimental Design:

- 40 stock tanks
- Sulfate – control (c. 7 mg/L) & 300 mg/L
  added as Na_2SO_4 to water column
- Fe – control & tripled extractable Fe in sediment (220 g/ m^2 added as $FeCl_2$ in four aliquots into sediment in July and August 2014)
- Litter – present or removed (no significant effect)
- Thinned to 30 plants per tank
- Sediment from Rice Portage Lake

- 6 plants marked and harvested for seeds, plant growth, and allocation to roots and shoots
- Rest of tank harvested and weighed but returned to tank (or not if no litter)
- 2014 & 2015

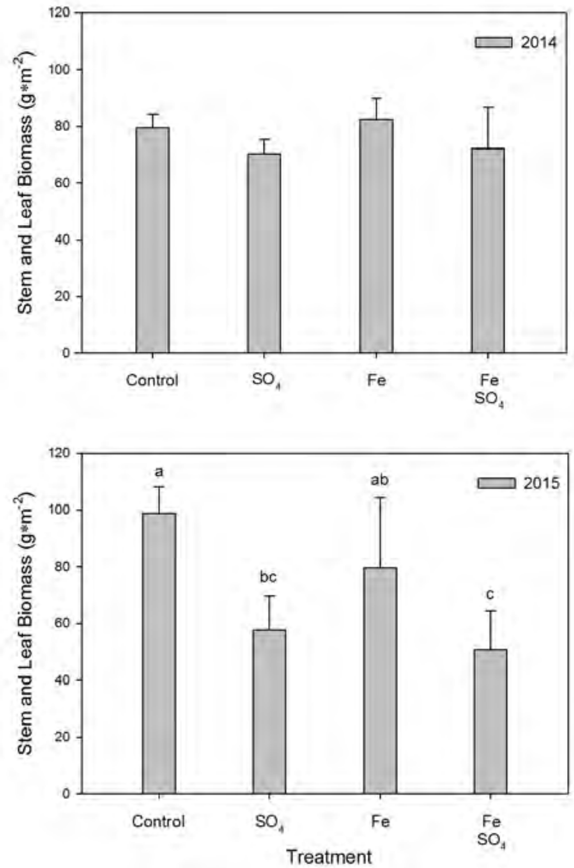
Seedling emergence depressed in the presence of sulfate by 2015

Fe partly compensated for the effect of sulfate/sulfide



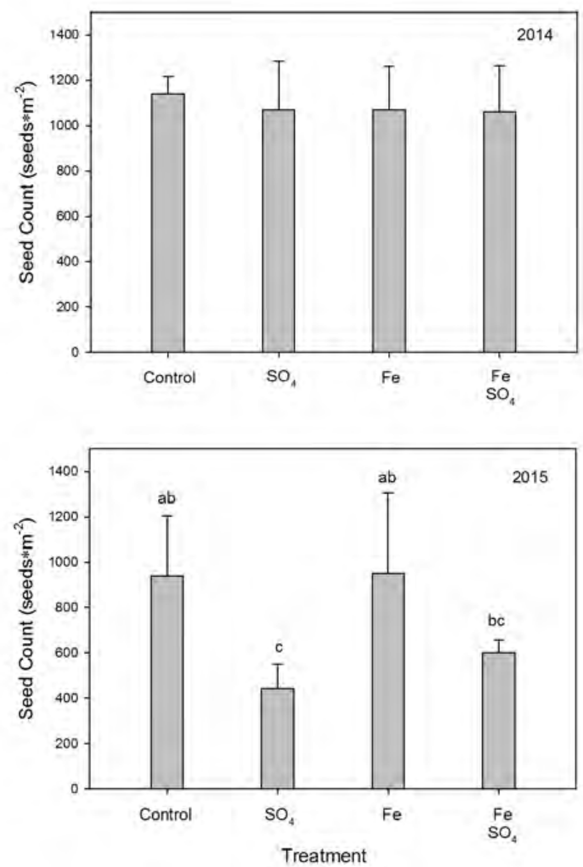
Vegetative growth depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



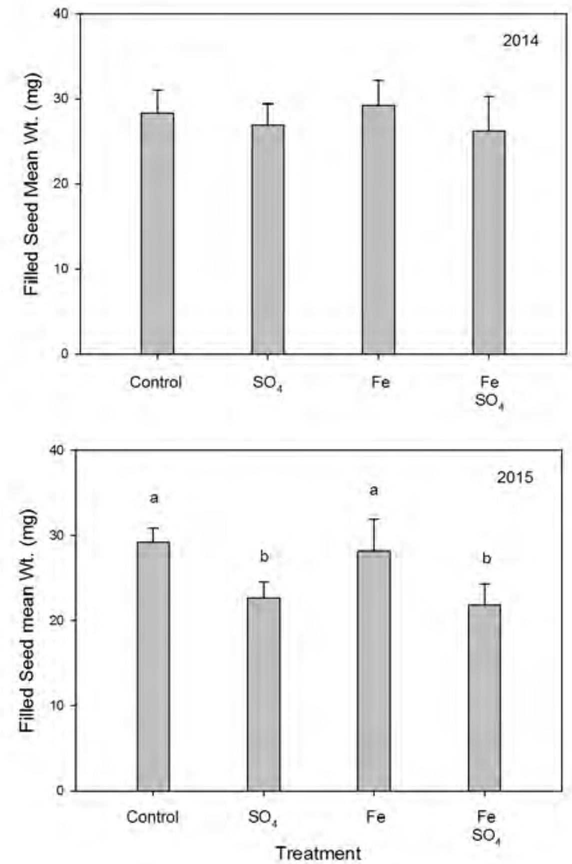
Seed count depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



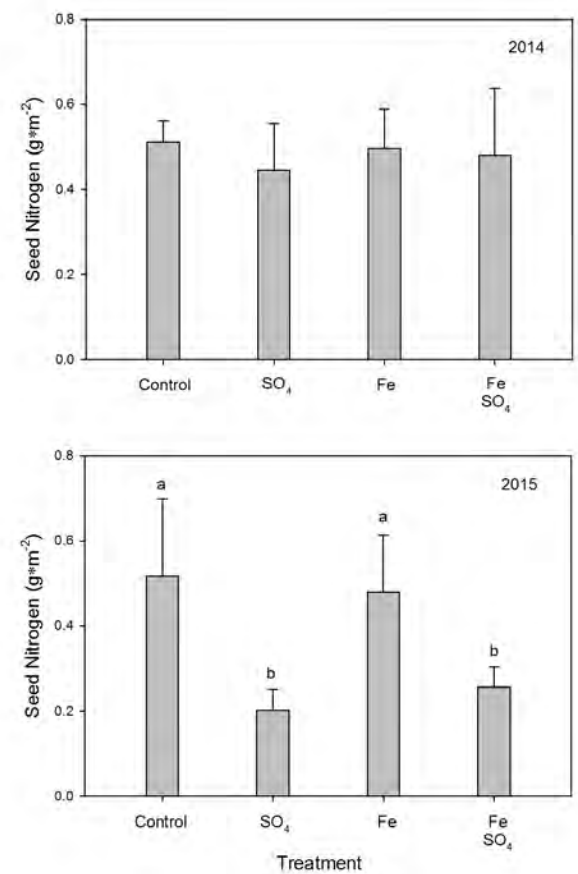
Seed weight depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



Seed nitrogen depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



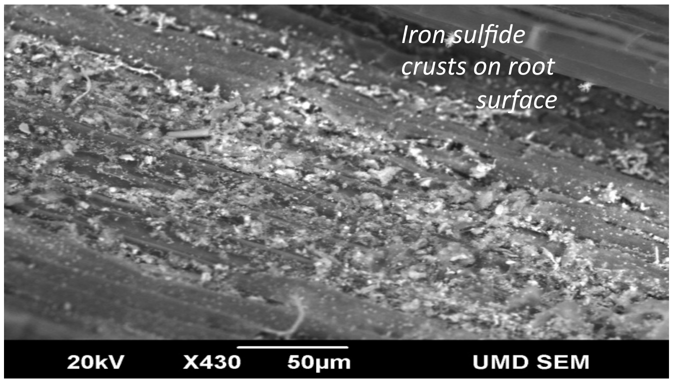
Preliminary Conclusions – Mesocosm Experiment

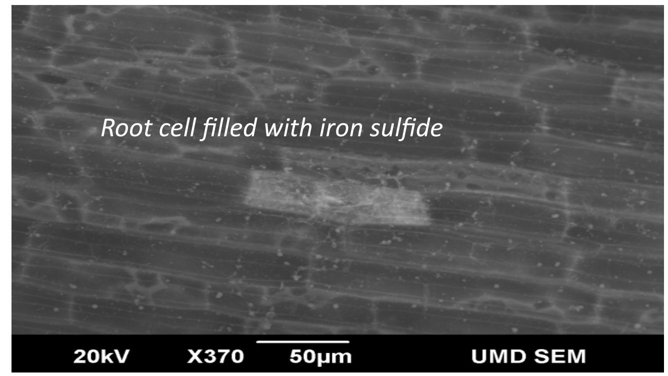
- Fe additions partly compensated for toxic effect of sulfide on seedling emergence, possibly by precipitating FeS

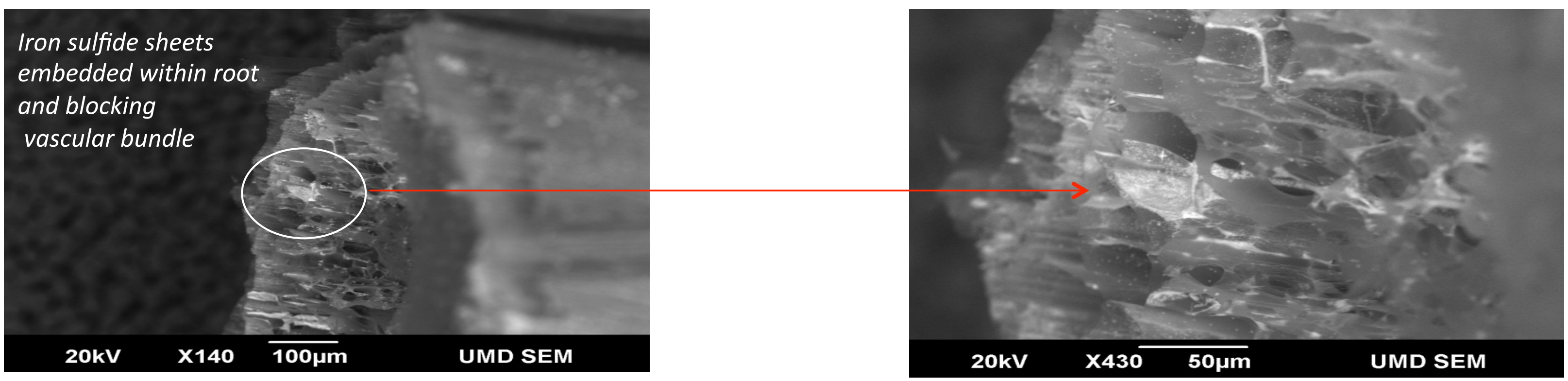
- Fe additions did not compensate for depression of vegetative growth or seed production and nitrogen content

Iron plaques

SEM Scans of Iron Sulfide Precipitates on Roots







Scans courtesy of Dr. Bryan Bandli, UMD

What geochemical conditions are associated with iron sulfide plaque formation?

How do iron sulfide plaques change seasonally?

Do iron sulfide plaques inhibit nitrogen uptake?

Bucket Experimental Design:

- 40 buckets: 300 mg/L SO_4
- 40 buckets: control
- 1 wild rice plant per bucket
- Sediment from Rice Portage Lake

- 8 plants harvested per sample date
  - every 2 weeks during flowering
  - weekly during seed production
- Pore water sampled one day prior to harvest
- Sediment sampled start and end of growing season

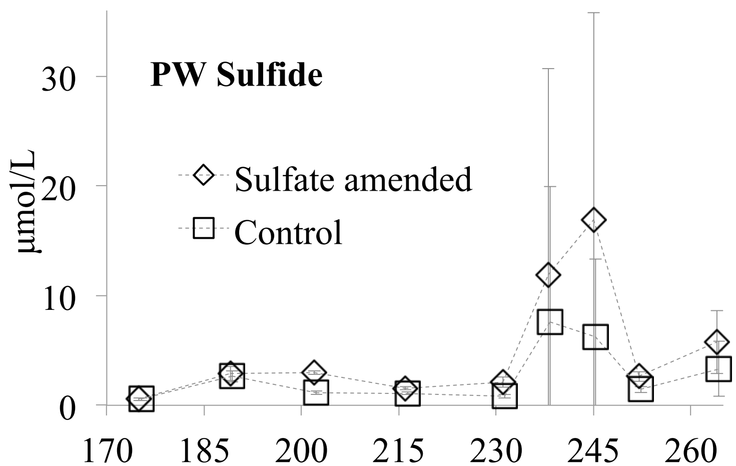
Methods: Pore water collection & analysis

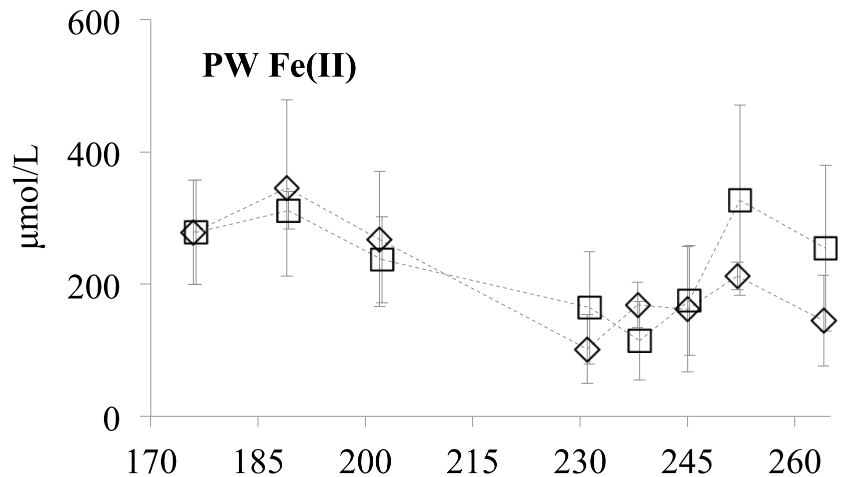
- Sampling procedure: rhizons attached to preloaded, vacuumed bottles

Analyte	Analysis
Sulfide	spectrophotometry (methylene blue)
Sulfate	ion chromatrography
Fe2+	spectrophotometry (phenanthroline)
pH	electrode

Methods: Root AVS & Fe

- Root collection
  - Placed in jar underwater in degassed DI water
- AVS quantification
  - Extracted for 4 hours with 1M HCl
  - Quantified with a sulfide ion-selective electrode
- Fe quantification
  - Aliquot of acid analyzed on AA
  - Ferrous iron quantified on spec

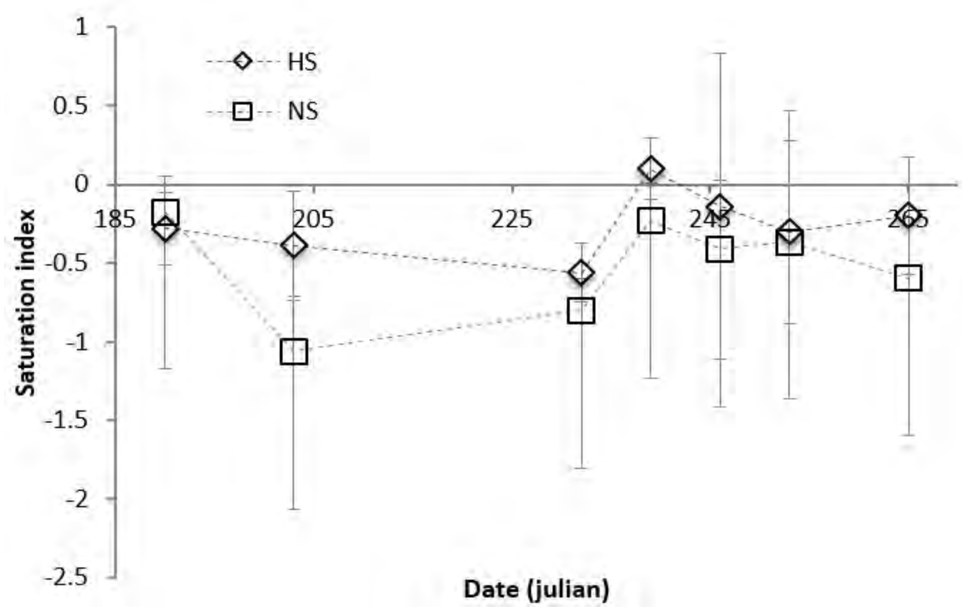

PW Sulfide
µmol/L
Sulfate amended
Control
0
10
20
30
170
185
200
215
230
245
260

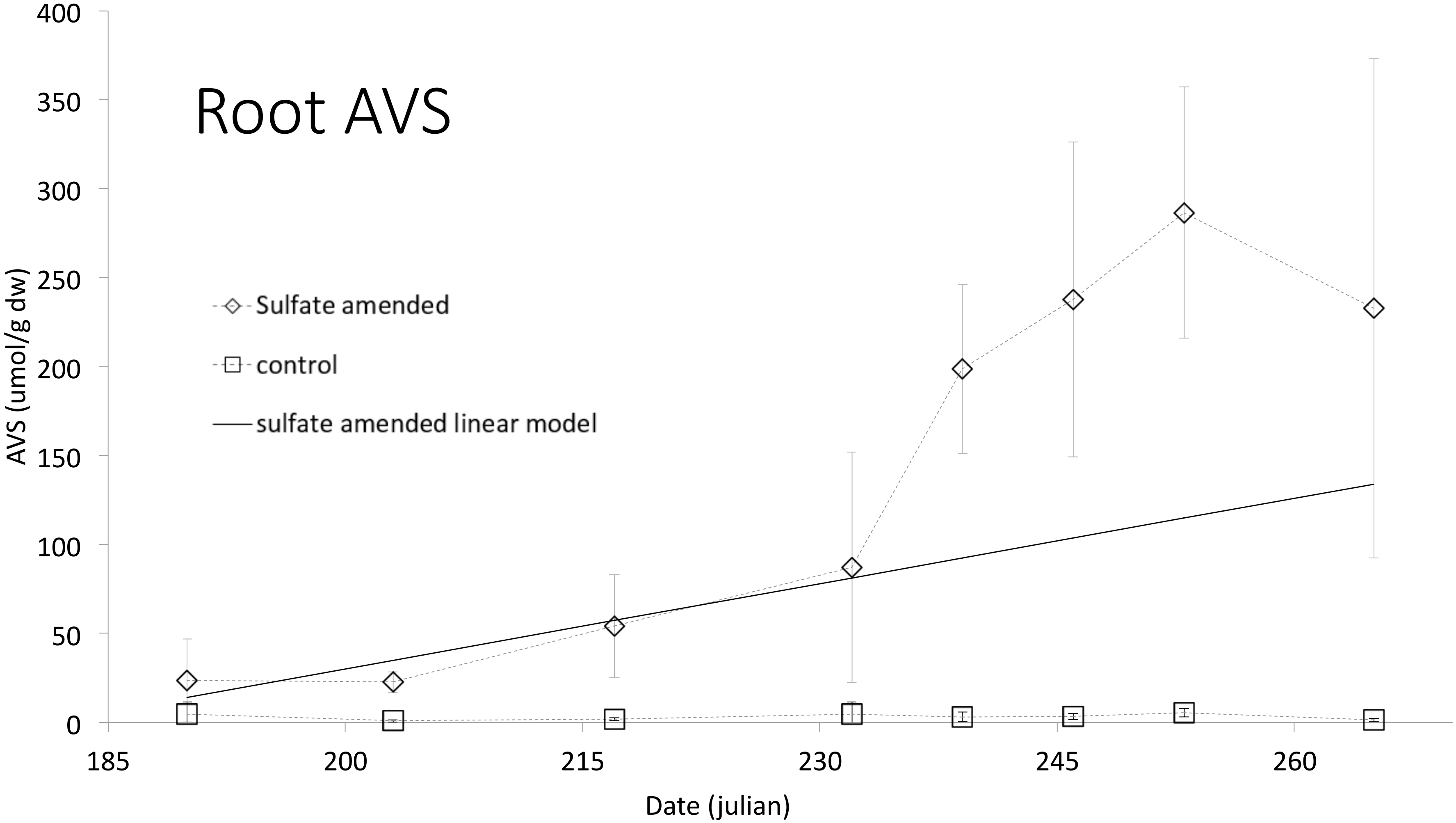

PW Fe(II)
µmol/L
0
200
400
600
170
185
200
215
230
245
260

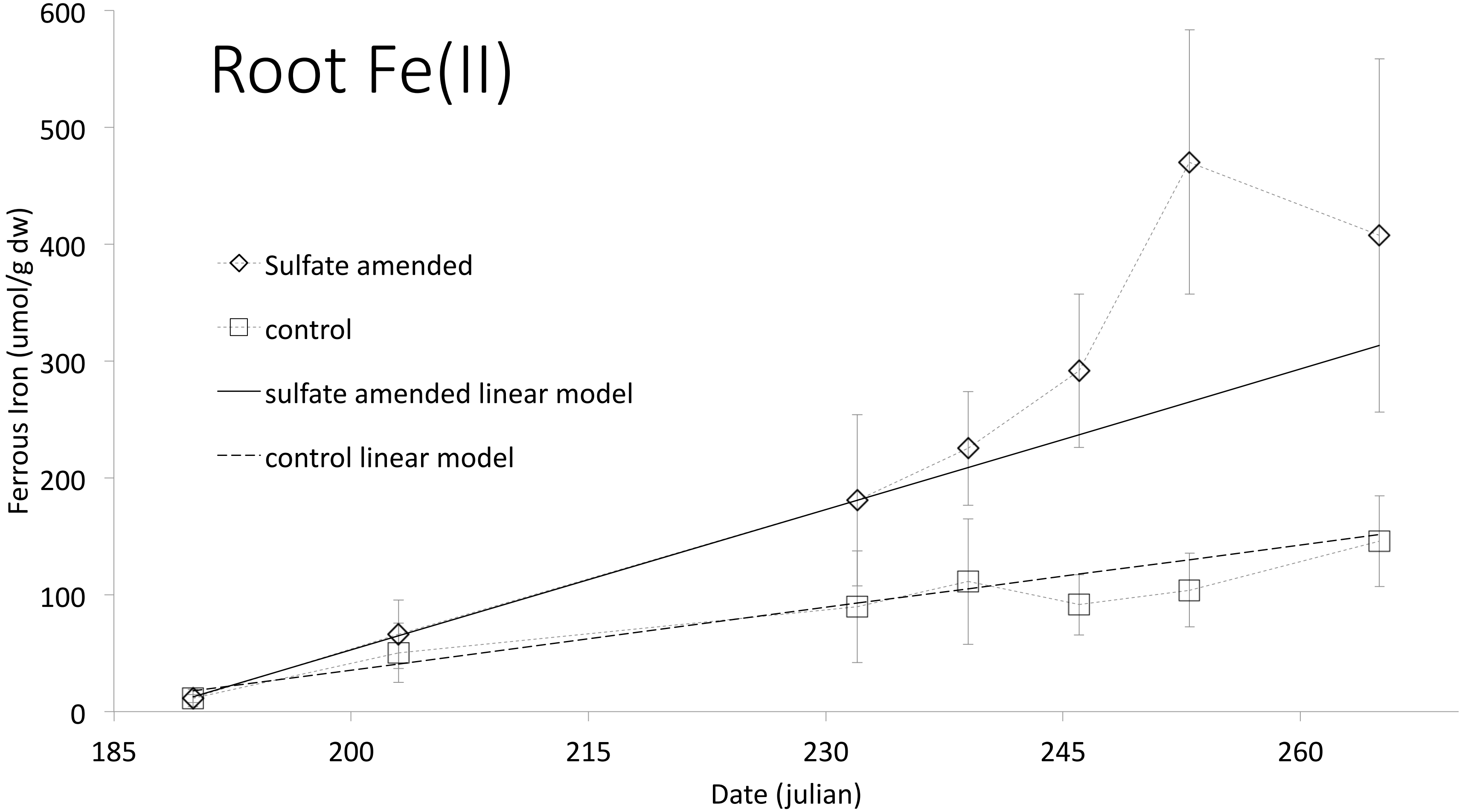
Saturation Index in Bulk Sediment

Pore water 2 cm from roots is *undersaturated* with respect to FeS

$SI = \log[IAP]/K_{sp}$, where $IAP = [Fe^{2+}][HS^{-}]/[H^{+}]$ and $K_{sp} = 10^{-2.95}$

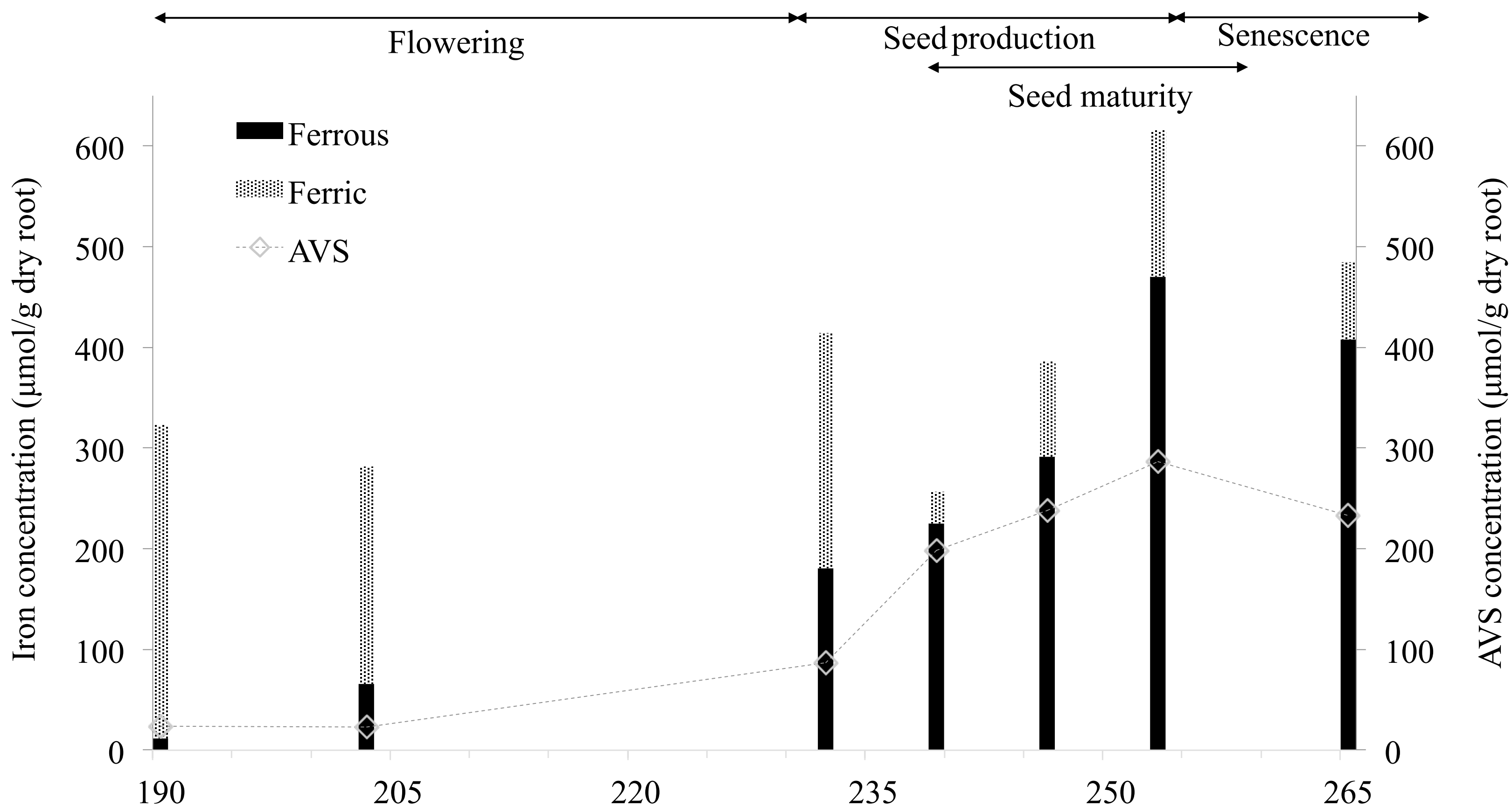


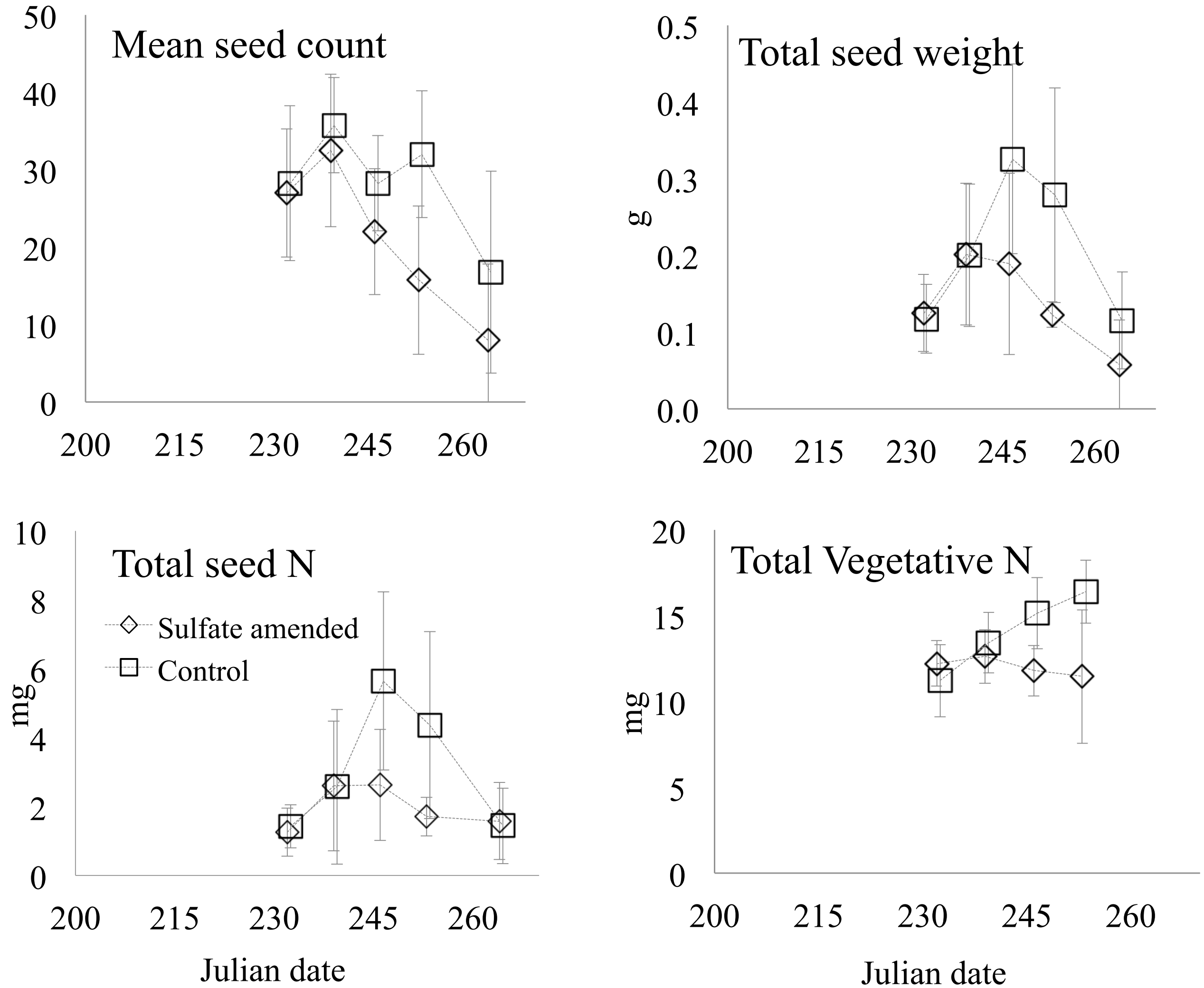

Root AVS
Sulfate amended
control
sulfate amended linear model
AVS (umol/g dw)
400
350
300
250
200
150
100
50
0
185
200
215
230
245
260
Date (julian)



Root Fe(II)
Ferrous Iron (umol/g dw)
Date (julian)
Sulfate amended
control
sulfate amended linear model
control linear model
0
100
200
300
400
500
600
185
200
215
230
245
260

Root iron speciation on amended roots




Mean seed count
50
40
30
20
10
0
200 215 230 245 260
Total seed weight
g
0.5
0.4
0.3
0.2
0.1
0.0
200 215 230 245 260
Total seed N
Sulfate amended
Control
mg
10
8
6
4
2
0
200 215 230 245 260
Julian date
Total Vegetative N
mg
20
15
10
5
0
200 215 230 245 260
Julian date

Repeated measures ANOVA (F values)	Sulfate	d.f.	Time	Sulfate x Time	d.f.
Pore water geochemistry					
Iron	5.16	1, 5	5.51***	1.14	6, 35
pH	3.25	1, 6	12.5***	1.45	6, 36
Saturation index	2.68	1, 4	2.19*	0.50	6, 34
Sulfide	239***	1, 3	8.17***	1.09	5, 27
Root geochemistry					
AVS (during flowering)	66.1***	1, 5	1.10	0.40	3, 17
AVS (during seed production)	148***	1, 6	5.46**	1.76	4, 24
Weak acid extractable iron	0.53	1, 6	2.65**	2.42**	7, 42
Ferrous Iron	127***	1, 6	57.2***	3.34**	6, 36
% Ferrous Iron	235***	1, 6	41.5***	4.91***	6, 36
Biological variables (during seed maturity)					
Plant weight	5.00*	1, 6	0.40	0.31	3, 18
Seed N (total mass)	5.84*	1, 6	1.10	1.22	2, 12
Seed weight	4.88*	1, 6	0.59	0.94	2, 12
Seed count	5.00*	1, 6	1.89	0.70	2, 12
Vegetative N (plant+seed mass)	5.43*	1, 6	0.32	1.71	2, 12

Significance levels

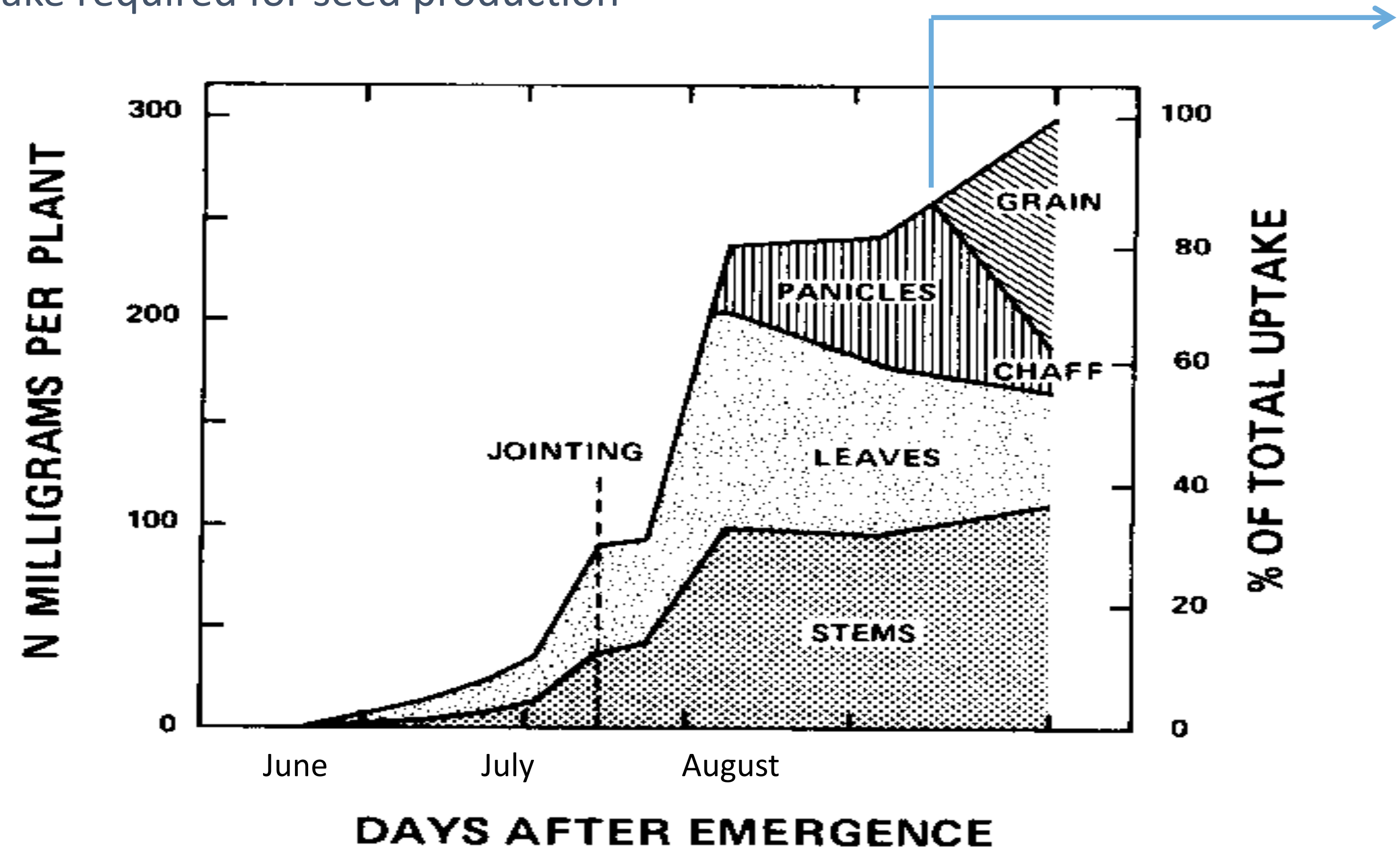
* 0.05 < p <0.10

** 0.001 <p<0.05

*** p <0.001

FeS_x on roots late in season impedes nitrogen uptake required for seed production

Period of FeS Precipitation On Roots



Grava and Raisanen 1978

Preliminary Conclusions – Bucket Experiment

- Iron oxides act as oxidized buffer during early-mid season
- Iron oxide buffer is overwhelmed by sulfide around the start of seed production
- Seed stage may be disproportionately harmed by sulfide because it coincides with iron sulfide precipitation on roots


Acknowledgements
NAH-GAH-CHI-WA-NONG
FOND DU LAC
RESERVATION
Minnesota Pollution Control Agency
Sea Grant
Minnesota

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment D

(4 pages)

[redacted]

MINNESOTA SEA GRANT COLLEGE PROGRAM RESEARCH ANNUAL REPORT

PI NAME: John Pastor

PROJECT NUMBER: R/CE-04-14
Chart String: 1000 10340 20857 00041968

PROJECT END DATE: June 30, 2016

REPORT DATE: May 5, 2016

PROJECT TITLE: The Biogeochemical Habitat of Wild Rice

PROGRESS TOWARD OBJECTIVES: (summarize your progress over the last 12 months)

With Sea Grant funding, we continued one long-term experiment and initiated two others. The long-term experiment consisted of adding sulfate to tanks containing wild rice grown in wild rice sediment to achieve surface water concentrations of ambient (7), 50, 100, 150, and 300 ppm SO4. After five years (two under SeaGrant funding, the wild rice populations in the 300 ppm tanks have gone extinct and the populations in the 150 ppm tanks are nearing extinction (Pastor et al. submitted). Extinction was caused by toxic levels of sulfide (from sulfate reduction) to seedlings and from reduced seed production. Proportional decreases in population productivity have happened in the other amended tanks.

During the course of these experiments, wild rice roots in tanks with more than 50 mg/L sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle (Fig. 1). Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides.

To sort out these two potential effects of FeS precipitation in roots and on sediments, we initiated two additional experiments. One is a large scale tank experiment in which additions of sulfate to 300 ppm, a tripling of sediment iron, and removal of litter (to reduced labile carbon for microbes) were applied in a crossed factorial design. After two years, sulfate amendments had the greatest effect, reducing production as in the first experiment regardless of iron amendment and litter removal. Iron amendment had no statistically significant effect, but plants grown under both sulfate and iron amendments had the lowest vegetative and seed production of all. Litter removal had no effect. While we cannot yet conclude from this experiment that iron has a strong depressive effect on wild rice growth via FeS plaques on roots, we can conclude that iron has no beneficial effect by reducing the toxicity of sulfide.

We also initiated a third experiments aimed at quantifying the development of these FeS root plaques. In this experiment, wild rice was grown individually in buckets with and without sulfate amendments (to 300 ppm). We sampled plants every two weeks to determine the phenology of the development of FeS plaques on the roots. We made two surprising observations. First, accumulation of FeS plaques on roots of plants grown under high sulfate concentrations increased very rapidly and suddenly in midsummer even while porewater sulfide in the bulk sediment remained unchanged. And second, by the end of the growing season, FeS concentrations were two orders of magnitude higher on black root surfaces than in the surrounding sediment; after a single annual growing season, the black roots contained approximately 5% (by mass) of the total amount of sulfur in the experimental sediments. FeS in the bulk sediment also increased during the growing season but much more slowly and without an obvious breakpoint in accumulation rate. These observations suggest an overwhelmingly dominant, plant-induced change towards conditions more conducive to FeS precipitation in the immediate vicinity of the roots that begins in the middle of the growing season and controls the rates and location of sulfur transformations.

Plants with the black FeS plaques on their roots produced fewer and less viable seeds, perhaps because the plaques potentially impair the uptake of phosphorus and nitrogen (Pastor et al. submitted). The rapid accumulation of FeS plaques occurs at the time that wild rice plants are beginning to flower and take up additional nutrients for the ripening seeds. This suggests that even if the precipitation of FeS in the bulk sediment reduces aqueous sulfide, precipitation on the root surfaces somehow impedes seed formation, perhaps by blocking nutrient uptake.

Last summer, we also added ^{15}N periodically throughout the growing season to plants amended with 300 mg/L sulfate and plants without sulfate addition. These experiments are providing a more detailed look at the plant-side nutrient fluxes in the context of the changing rates of sulfur accumulation on root surfaces. Preliminary results suggest that nitrogen uptake by wild rice may be inhibited by plaque formations, especially during the period of seed filling and ripening. If nitrogen uptake is inhibited by FeS plaques, then this may explain why wild rice plants with FeS plaques on roots had smaller seeds and a greater proportion of the seeds were not filled (Pastor et al. submitted).

DIFFICULTIES ENCOUNTERED AND ACTIONS TAKEN TO OVERCOME THEM:

Before we began the ^{15}N experiment last year, we had to spend the previous summer in pilot trials determining how much ^{15}N to add to create a measureable signal in the plants while overcoming the strength of the microbial sink in the sediment. This took up one entire summer The following summer was spent determining the approximate joint phenology of FeS plaque formation and ^{15}N uptake. Now that we know the proper amount of ^{15}N to add and the approximate joint phenology of its uptake in relation to FeS plaque formation, we have devised a sampling schedule wherein we will sample at high frequencies during the time of FeS plaque formation to determine how it coincides with nitrogen uptake. This will allow us to determine whether FeS plaques form at a constant increment controlled entirely by inorganic geochemistry of the sediments, or whether FeS plaques grow exponentially as they progressively cut off radial oxygen losses from the roots. We are, under separate documentation, requesting a no-cost extension of unspent graduate student funds to support Ms. Sophie LaFond-Hudson to continue these experiments which will be part of her Ph.D. thesis in Water Resources Sciences at the University of Minnesota.

RESULTS TO DATE: (please provide a brief summary of your results)

See above. Paper submitted acknowledging SeaGrant support:

Pastor, J., B. Dewey, N. W. Johnson, E.B. Swain, P. Monson, E.B. Peters, and A. Myrbo. Effects of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris*) in hydroponic and mesocosm experiments. Ecological Applications: submitted.

ASSESS PROGRESS RELATIVE TO ORIGINAL SCHEDULE AND FINAL DEADLINE:

We have accomplished all of our original goals involving the tank experiments. The ^{15}N experiments were begun in response to a recommendation of the proposal review panel that we include some isotopic amendments to determine the effect of sulfate amendments on nutrient cycling. However, in order to do that with any precision, we needed to spend two years in pilot experiments to determine the amount of ^{15}N to add and its phenology relative to the growth of FeS plaques at high sulfate concentrations. With one more year's fieldwork we will be able to accomplish this objective.

OUTREACH OR PRODUCTS: Please list any products (Web or print), presentations, articles, media interviews, teacher training, K-12 education, etc. that you or your student(s) have from this research thus far. Is there anything our Communications or Extension staff can do to help you connect your research with stakeholders?

PERFORMANCE MEASURES: We are required to provide performance measures to National Sea Grant each year. You may not have anything at all in some of these categories, and that is expected. All we need at this point is your best guess and an explanation of how you arrived at your answer.

Measure 1: Economic and societal benefits derived from the discovery and application of new sustainable coastal, ocean, and Great Lakes products from the sea.

We are reporting these results to the Minnesota Pollution Control Agency and to the various tribal units of Lake Superior Chippewa who are in discussion about setting sulfate standards for waters entering wild rice beds. Many of these waters also enter Lake Superior and the estuaries of some major rivers such as the St. Louis and Fish Rivers once supported extensive wild rice beds which the states of Minnesota and Wisconsin are trying to restore. These results will help inform these restoration efforts by helping the state agencies determine how many and which acres could be restored to wild rice populations.

Measure 2: Cumulative number of coastal, marine, and Great Lakes issue-based forecast capabilities developed and used for management. *(typically interpreted to include most computer models)*

Not applicable

Measure 3: Percentage/number of tools, technologies, and information services that are used by managers (NOAA and/or its partners and customers) to improve ecosystem-based management.

See answer to Measure 1.

Measure 4: Acres of ecosystems protected or restored as a result of Sea Grant's involvement.

Not directly applicable, but see answer to Measure 1.

Measure 5: Number of environmentally-responsible fisheries and/or aquaculture production or harvesting techniques implemented.

Not applicable.

Measure 6: Number of communities who adopt/implement sustainable, economic and environmental development practices and policies, or hazard resiliency practices.

See answer to Measure 1.

Measure 7: Number of environmental curricula adopted by formal and informal educators.

John Pastor uses these results in his class in Integrated Biological Systems and Nathan Johnson uses these results in his class in Environmental Modelling. In addition, classes from Fond du Lac Community College routinely tour these experiments as part of their curriculum in wild rice management.

OTHER METRICS OF INTEREST TO NOAA: Please answer any that apply to your project (none may, and that is fine).

1. Did or will your project help develop or update sustainable development ordinances, policies, or plans? If so, in what community?

   See answer to Measure 1 above. The communities are the States of Minnesota and Wisconsin and the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa.

2. Did your project help a community implement a sustainable development plan? If so, what community?

   Potentially it will help the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa.

3. Did your project help develop or update a port or waterfront redevelopment ordinance, policy, or plan? If so, what port or community?

Not applicable

4. Did you help a port or waterfront implement a redevelopment plan? If so, what port or community?

Not applicable

5. Did your project help develop or update polluted runoff management ordinances, policies, or plans? If so, for what community?

Potentially the results of this research will help inform the State of Minnesota as it reviews its sulfate criteria for wild rice beds, especially in regard to runoff from iron and copper-nickel mines in northern Minnesota.

6. Did your project help implement a polluted runoff management ordinance, policy, or plan? If so, for what community?

Not applicable (yet).

PLANS FOR THE NEXT 6 MONTHS:

Continue to monitor the changes in wild rice populations in the tank experiments and initiate another ^{15}N addition experiment to distinguish between different models of FeS plaque formation and their effect on nitrogen uptake.

NAMES OF STUDENTS BEING SUPPORTED BY THIS GRANT AND THEIR LEVEL (e.g, grad (MS, PhD), undergrad, etc). For grad students, please indicate whether their thesis research is related to this project.

Ms. Sophie LaFond-Hudson, completed MS - WRS research on this project and is initiating Ph.D. –WRS research on it as well. Advisors: Profs. Nathan Johnson and John Pastor

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November 2017

Attachment E

(3 pages)

June 28, 2017

Progress Report on Experiments on Effects of Sulfate and Sulfide on Wild Rice

John Pastor, Dept. of Biology, University of Minnesota Duluth

This memo is a brief report on our ongoing experiments on the effects of sulfate and sulfide on wild rice, funded by EPA through the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa Water Quality Programs, the State of Minnesota, and Minnesota Sea Grant.

Our hypothesis is that sulfate amendments are detrimental to wild rice populations when it is reduced to the more toxic sulfide. We have initiated several long-term experiments to test this hypothesis and elucidate the underlying mechanisms. The longest experiment consisted of adding sulfate to 100 gallon stock tanks containing wild rice grown in wild rice sediment to achieve surface water concentrations of ambient (7), 50, 100, 150, and 300 mg/l SO_4. Sulfide concentrations in sediments increased in proportion to sulfate concentrations (Pastor et al. 2017). After five years (2011-2015), the wild rice populations in the 300 mg/l tanks have gone extinct and the populations in the 150 mg/l tanks are nearing extinction (Pastor et al. 2016; Fig. 1). Extinction was caused by toxic levels of sulfide (from sulfate reduction) to seedlings (Fig. 1) and from reduced seed production (Fig. 2). Proportional decreases in population productivity have happened in the other amended tanks. Raw data from this experiment has been archived at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1452/full

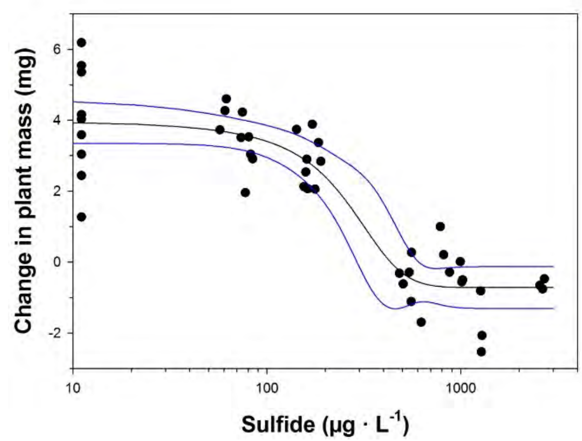

Figure 1. Reduction in seedling growth with increased sulfide concentrations in a hydroponucs experiment (Pastor et al. 2017).

During the course of these experiments, wild rice roots in tanks with more than 50 mg/l sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle. Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides. Precipitation of iron sulfide on roots may inhibit nutrient uptake, thus leading to reduced seed production. On the other hand, precipitation of iron sulfide in sediments could neutralize the toxicity of sulfide to seedlings.

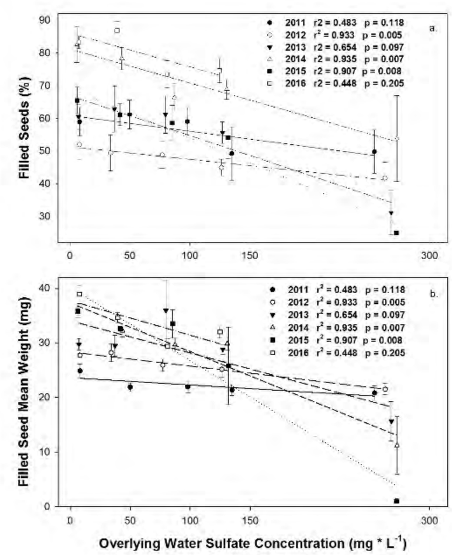

Figure 2. Reduction in seed production with increased sulfate concentrations in stock tank experiments (2011-2015 data from Pastor et al. 2017, with 2016 data added).

To sort out these two potential effects of FeS precipitation in roots and on sediments, we initiated two additional experiments. One is a long-term tank experiment in which additions of sulfate to 300 mg/l, a tripling of sediment iron in the first growing season, and removal of litter (to reduced labile carbon for microbes) were applied in a crossed factorial design. This experiment began at the beginning of the 2015 growing season. During the first three years of this experiment, sulfate amendments had the greatest effect, reducing seedling survival, plant growth, and seed production regardless of iron amendment and litter removal. Litter removal had no effect on seedlings, vegetative growth, or seed production. In the first two growing seasons, adding iron without sulfate had no effect on seedling survival, plant growth, or seed production. Iron amendments in the presence of sulfate increased seedling survival compared with seedlings grown under sulfate amendments alone, but seedling survival in the iron + sulfate tanks was still less than in control tanks. We believe the partially ameliorative effects of iron on seedling survival was due to precipitation of iron sulfide in the sediment, thus partly neutralizing sulfide toxicity to seedlings. However, by the spring of year 3 (2017), the amendment of iron no longer appears to have any effect on seedling survival, possibly because all the iron we added has been titrated out of the tanks by precipitation with sulfide either in the sediment or on the plant roots.

We also initiated a third experiment aimed at quantifying the development of FeS root plaques (Fig. 3). In this experiment, wild rice was grown individually in buckets with and without sulfate amendments (to 300 mg/l). We sampled plants every two weeks to determine the phenology of the development of FeS plaques on the roots. We made two surprising observations. First, accumulation of FeS plaques on roots of plants grown under high sulfate concentrations increased very rapidly and suddenly in midsummer at the time that wild rice plants are beginning to flower and take up additional nutrients for the ripening seeds (Fig. 4). And second, by the end of the growing season, FeS concentrations were two orders of magnitude higher on black root surfaces than in the surrounding sediment; after a single annual growing season, the black roots contained approximately 5% (by mass) of the total amount of sulfur in the experimental sediments. FeS in the bulk sediment also increased during the growing season but much more slowly and without an obvious breakpoint in accumulation rate. These observations suggest an overwhelmingly dominant, plant-induced change towards conditions more conducive to FeS

Figure 3. Orange iron (hydr(oxide) stains on healthy wild rice roots in low sulfate environments (left) and black iron sulfide plaques on roots in high sulfate environments (right).

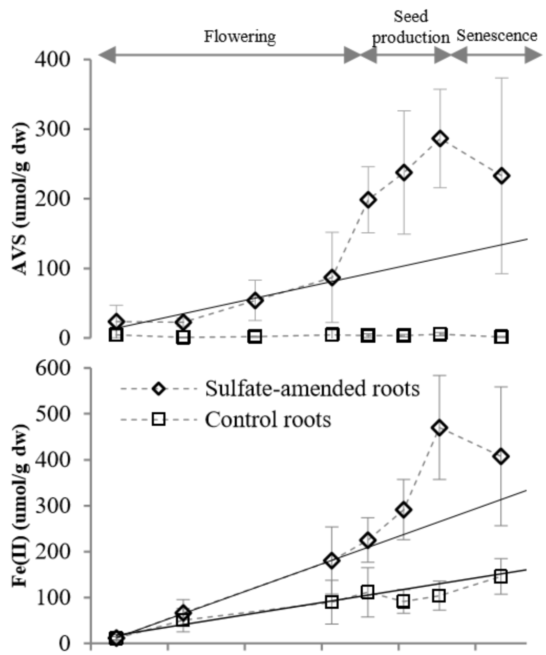

Figure 4. Time course of (top) sulfide and (middle) ferrous iron accumulation on plant roots in sulfate amended and control conditions (LaFond-Hudson et al. submitted).

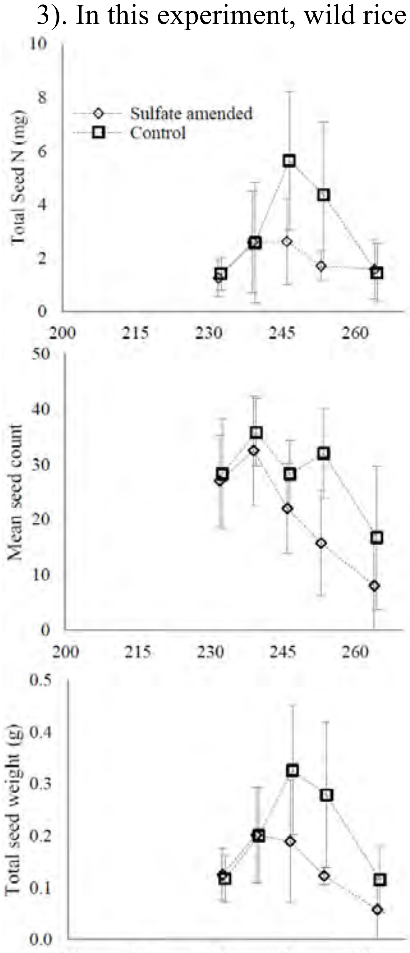

Figure 5. Seed nitrogen, seed count, and seed weight are higher in control plants with orange roots compared with plants with black roots grown under 300 mg/L sulfate (Lafond-Hudson et al. submitted).

precipitation in the immediate vicinity of the roots that begins in the middle of the growing season and controls the rates and location of sulfur transformations.

Plants with the black FeS plaques on their roots produced fewer and smaller seeds containing less nitrogen (Fig. 5), perhaps because the plaques potentially impair the uptake of nitrogen. This suggests that even if the precipitation of FeS in the bulk sediment reduces aqueous sulfide and partly ameliorates sulfide toxicity to seedlings, precipitation on the root surfaces somehow impedes seed formation, perhaps by blocking nutrient uptake.

In summary, our long-term experiments on the biogeochemistry of sulfate in wild rice habitat demonstrates that sulfate is not toxic in and of itself to wild rice, but when reduced to sulfide is directly toxic to seedlings. Iron additions may partly ameliorate sulfide toxicity to seedlings in spring, but precipitation of iron sulfide plaques on roots during the flowering and seed production period of wild rice's life cycle appears to block uptake of nitrogen, leading to fewer and smaller seeds with reduced nitrogen content. The net effect of sulfate additions to wild rice populations is to drive the populations to extinction within 4 or 5 years at high concentrations of sulfate (300 mg/l) and to greatly reduce population viability at lower concentrations.

PUBLICATIONS TO DATE:

LaFond-Hudson, S., N. Johnson, J. Pastor, and B. Dewey. Submitted. Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*). Nature Geosciences.

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 wild rice (*Zizania palustris*) in hydroponic and mesocosm experiments. Ecological Applications 27: 321-336.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment F

(39 pages)

Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*)

A thesis
SUBMITTED TO FACULTY OF THE
UNIVERSITY OF MINNESOTA
BY

Sophia LaFond-Hudson

IN PARTIAL FULFILLMENT OF THE REQUIERMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

Nathan Johnson, John Pastor

May 2016

Acknowledgements

I would like to acknowledge several people who contributed substantially to this project. The members of my committee, Dr. Nathan Johnson, Dr. John Pastor and Dr. Elizabeth Austin-Minor provided intellectual guidance during the experimental setup, data analysis, and writing process. Brad Dewey played an important role in this project by sampling the biological data, assisting with harvesting and cleaning roots, and answering question after question about the experimental setup and methods. Dan Fraser was likewise very helpful in answering any questions about equipment. I am very grateful for the help I received from Marissa Samuelson, who assisted considerably with geochemical sampling and cleaning roots. Finally, I would like to again thank my advisors, Dr. Johnson and Dr. Pastor, for being generous with their excellent advice and constant encouragement.

Abstract

Iron (hydr)oxides typically form on roots of many wetland plants, including wild rice (*Zizania palustris*), an annual macrophyte with significant cultural, economic, and ecological value. Iron (hydr)oxides are thought to protect macrophytes from toxic reduced species, such as sulfide, by providing an oxidized barrier around the roots. However, wild rice grown under high sulfate loading develops a black iron sulfide precipitate on the root surface, and produces fewer and lighter seeds, leading to a decreased population in the long term. In order to investigate the role of iron sulfide root precipitates in impaired seed production, wild rice plants grown in buckets were exposed to sulfate loading of 300 mg/L, and harvested biweekly for extraction of root acid volatile sulfide (AVS) and weak acid extractable iron and analysis of plant and seed N. In sulfate-amended plants, AVS on roots accumulated over the course of the growing season, and accumulated rapidly just prior to seed production. Simultaneously, iron speciation of the root precipitate shifted from Fe(III) to Fe(II), consistent with a transition from iron (hydr)oxide to iron sulfide. A mechanism is herein proposed by which sulfide-induced suberization of roots decreases radial oxygen loss that keeps the rhizosphere oxidized, leading to reduction of iron (hydr)oxides and subsequent iron sulfide accumulation. Plants amended with sulfate produced fewer, lighter seeds with less nitrogen. We suggest that sulfide inhibits N uptake, and seeds are disproportionately harmed because rapid AVS accumulation occurs during the reproductive life stage.

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Introduction

Iron (hydr)oxide plaques have been observed on the roots of wild rice (*Zizania palustris*), a culturally significant macrophyte that forms large monotypic stands in the lakes and rivers of Minnesota, Wisconsin, northern Michigan, and Ontario (Lee and McNaughton 2004, Jorgenson et al. 2013). Iron (hydr)oxide plaques commonly form on the roots of wetland plants growing in anoxic, reduced sediments as a result of a redox gradients found in the rooting zone (Mendelssohn and Postek 1982, Jacq et al. 1991, Snowden and Wheeler 1995, Christensen and Sand-Jensen 1998). Redox gradients in the rhizosphere are caused by radial oxygen loss, a process in which wetland plants release oxygen into the rhizosphere through their roots via arenchyma tissue (Armstrong and Armstrong 2005, Schmidt et al. 2011). When Fe(II) is transported from anoxic sediment into the oxygenated rhizosphere, it is oxidized to Fe(III), which combines with oxygen from the roots to form insoluble iron oxides or hydroxides. Iron plaque formation can occur abiotically, but it is also associated with iron-oxidizing bacteria in many cases (St. Cyr 1993, Neubauer et al. 2007). Iron plaques have been proposed as a mechanism to protect plants from reduced toxic substances such as hydrogen sulfide, because they form an oxidized barrier around the roots (Koch and Mendelssohn 1989, Mendelssohn et al. 1995). However, during previous sulfur addition experiments, black iron sulfide root coatings, characteristic of iron sulfide minerals, have been observed on wild rice roots (Pastor et al., in review). Black root coatings have also been observed in white rice grown in surface water with high sulfate concentrations (Jacq et al. 1991, Gao et al. 2003, Sun et al. 2015).

The iron and sulfur chemistry of aquatic plant rooting zones involves a set of interrelated biogeochemical processes. Sulfate and iron (III) oxides are both redox active species that play a role in degradation of organic matter in aquatic sediments. During aerobic respiration, electrons are transferred from organic compounds to oxygen, but in anaerobic respiration alternative electron acceptors are used, including nitrate, ferric iron, sulfate, and carbon dioxide. Organisms use the more thermodynamically favorable electron acceptors first; nitrate is used before ferric iron, and carbon dioxide is used only when more favorable electron acceptors have been consumed. This thermodynamic ordering manifests itself as stratified microbial communities with distance away from an oxic-anoxic boundary (Boudreau 1996, Van Cappellen and Wang 1996). Anaerobic respiration produces reactive reduced species as byproducts, including ammonia, ferrous iron, sulfide, and methane. Iron-reducing and sulfate-reducing bacteria facilitate production of ferrous iron and sulfide respectively, after which ferrous iron and sulfide can combine to produce iron monosulfide (FeS) or pyrite (FeS_2). Alternatively, ferrous iron and sulfide can undergo oxidization back to ferric iron and sulfate abiotically via bioturbation or water level fluctuations (Thamdrup et al. 1994, Eimers et al. 2003) or biotically via iron or sulfide oxidizing bacteria (lithoautotrophy). Despite the predictability of the sequence of electron acceptors used in anaerobic respiration, coincident iron reduction and sulfate reduction in close proximity has been documented, during which the subsequently produced sulfide reacts abiotically with nearby iron (hydr)oxides to produce reduced iron and elemental sulfur (Hansel et al. 2014, Kwon et al. 2013).

Macrophytes can accelerate iron and sulfur cycling by enhancing redox gradients when radial oxygen loss creates an oxic layer around the root surface. Oxidation of Fe(II) to Fe(III) oxides immobilizes iron on or very near the root surface. Conversely, oxidation of sediment FeS by radial oxygen loss mobilizes previously bound sulfur as soluble sulfate (Choi et al. 2006). Cycling is dynamic near the rhizosphere because oxidation potential (Eh) changes abruptly over just a few millimeters. Just outside the oxic layer, the sediment can be strongly reducing. Heterotrophic iron and sulfate reduction can be stimulated by root exudates released by the plant (Kimura et al., 1981), and, in the case of an annual plant like wild rice, senesced plant material at the end of the growing season each year (Jacq et al. 1991). Several studies have compared sediment with and without vegetation and found higher sulfide or FeS concentrations in sites with plants (Holmer & Nielsen, 1997, Jacq et al. 1991, Lee & Dunton 2000). The increase in reduced species is attributed to larger pools of organic matter to drive reduction.

In Minnesota, surface water sulfate concentrations are regulated in wild rice waters because high surface water sulfate concentrations are associated with decreased wild rice abundance (Moyle, 1945, MPCA Analysis of the Wild Rice Sulfate Standard Study, 2014). It has recently been shown that sulfide, the reduced form of sulfate, is toxic to wild rice seedlings (Pastor et al., in review). In other wetland plants, sulfide is thought to interrupt metabolism by inhibiting metallo-enzymes in the electron transport chain during respiration (Allam and Hollis 1972, Koch and Mendelssohn 1989, Koch et al. 1990, Lamers et al. 2013; Armstrong and Armstrong 2005, Martin and Maricle 2015). Inhibition of ATP production deprives a plant of energy required for nutrient uptake. Sulfide has been shown to reduce nutrient uptake in white rice (*Oryza sativa*), a plant physiologically similar to wild rice (Joshi et al. 1975), so it is plausible that sulfide may also inhibit nutrient uptake in wild rice.

Pastor et al. (in review) found that exposure to sulfide decreased mean seed weight and the proportion of filled seeds more significantly than by having immediate toxic effects on plant growth and physiology. Wild rice takes up nitrogen, its limiting nutrient, in three main bursts: 30% is taken up during early season vegetative growth, 50% is taken up during early flowering, and 20% is taken up during late flowering and seed production (Grava and Raisanen, 1978). The effects of sulfide exposure on wild rice are consistent with nitrogen limitation during seed production, but it is not well understood why the seed production life stage is disproportionately harmed by sulfide. Is iron sulfide plaque accumulation a geochemical mechanism that controls the impact of sulfide on nitrogen uptake?

The objective of this study is to understand how iron and sulfur cycle near root surfaces and how this cycling affects nitrogen uptake by wild rice during its life stages, especially seed production. We investigate the drivers of iron sulfide plaque formation and seek to answer if plant and seed nitrogen uptake are adversely affected by iron sulfide accumulation on root surfaces.

Methods

Experimental Design

Sediment was collected from Rice Portage Lake (MN Lake ID 09003700, 46.703810, -92.682921) on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota in late May, 2015 and placed in a 400L Rubbermaid stock tank where it was homogenized by shovel. Initial carbon in the sediment was 14.8 ± 1.70% and initial nitrogen was 1.12 ± 0.13 %. Eighty 4 L plastic pails were then filled with 3 L of the sediment. Each 4 L pail was placed inside of a 20 L bucket which was filled with 12 L of water to provide a 12-15 cm water column. The overlying water of 40 randomly chosen buckets was then amended with an aliquot of stock solution (5.15g of Na_2SO_4 dissolved in 200ml of deionized water) to result in 300 mg/L (3.125 mM) sodium sulfate. The amendment concentration was chosen as such because when used in previous mesocosm experiments, wild rice populations went extinct within five years (Pastor et al. in review), but it is only slightly higher than the EPA drinking water secondary standard (250mg/L) and is a concentration found in some Minnesota lakes (MPCA Analysis of the Wild Rice Sulfate Standard Study, 2014). The overlying water was sampled twice throughout the trial and adjusted to 300mg/L SO_4 with appropriate amounts of Na_2SO_4 stock solution. The other 40 buckets did not receive any sulfate and on 6/23/15 (day 174, Julian date) had an average surface water sulfate concentration of 14.44 ± 1.01 mg/L, consistent with the local groundwater sulfate concentration. In each bucket, two seeds which were harvested in 2014 from Swamp Lake on the Grand Portage Reservation (MN Lake ID 16000900, 47.951856, -89.856844) were planted on 5/15/15 (Julian day 135). Once shoots reached a height of approximately 20 cm during the aerial stage, plants were thinned to one plant per bucket.

Sampling of pore water, roots, and stems began midsummer (63 days after planting/germination), at the start of flowering and the second burst of nitrogen uptake (Grava and Raisanen, 1978), and continued until plants had thoroughly senesced, for a total of eight sample dates, not including initial sediment and pore water sampling. Sampling occurred every two weeks for the first four sample dates, (flowering, days 189-232) and weekly for the last four sample dates (seed production, days 238-265), for a total of eight sample dates. One week prior to each sampling date, 40 ml of enriched ^{15}N

solution were injected into the sediment of four randomly selected sulfate-amended buckets and four control buckets. For the first two sample dates, the labeling solution was prepared by adding 0.88 mg of 10% ^{15}N-NH_4Cl to 500 ml DI water. For all other sample dates, 2.2mg of 10% ^{15}N-NH_4Cl were added to 500 ml of DI water to account for an increase in plant biomass later in the growing season. The solution was injected into the sediment of the 4L pail in four locations uniformly spaced around the center of the pail, approximately 2 cm from the outer edge and 2 cm from the bottom. Immediately before injection, the overlying water was removed from the outer pail, leaving 2-5 cm above the sediment in the internal pail, to keep the ^{15}N-NH_4Cl contained in the sediment for uptake by the wild rice roots. On each sample date, one week after injection of ^{15}N, the four sulfate-amended and four control buckets were sampled for pore water sulfide, pore water sulfate, pore water iron, and pH. After pore water sampling, the wild rice plant was destructively harvested for analysis of vegetative ^{15}N, vegetative total N, and root AVS and weak acid extractable iron. The bulk sediment was sampled for solid phase S and Fe analysis at the beginning and at the end of the growing season.

Pore water sampling and analysis

Prior to extracting pore water samples, pH was measured *in-situ* with a ThermoScientific Orion pH electrode at a depth of 5 cm below the sediment surface and 2 cm from the stem of the wild rice plant. Pore water was sampled using 5-cm length, 2-mm diameter tension lysimeter filters (Rhizons, Seeberg-Elverfeldt et al., 2005) attached with a hypodermic needle to an evacuated, oxygen-free serum bottle sealed with a 20 mm thick butyl-rubber stopper (Bellco Glass, Inc). The entire filter end of the Rhizon was inserted vertically into the sediment just below the surface. The goal was to draw water from approximately the upper 5 cm of sediment without drawing surface water. The filter was placed with minimal jostling to avoid creating a cavity around the filter that would allow surface water to enter the sediment and contaminate the pore water. The Rhizon was placed approximately 2 cm away from the stem of the wild rice plant and on the opposite side from where pH was measured.

Pore water sulfide samples were drawn into 50-mL serum bottles preloaded with 0.2% 1 M ZnAc and 0.2% 6 M NaOH to preserve sulfide. Sulfide bottles were left to fill overnight, then stored at 4C in the sealed serum bottles used for sample collection for approximately 30 days before sulfide was quantified. Samples for pore water sulfate analysis were withdrawn from sulfide sampling bottles and filtered through a Dionex 1cc metal cartridge and a 0.45 μm polyethersulfone filter approximately three months after they were collected. Pore water iron was collected in 8-mL serum bottles preloaded with 40% deionized water, 40% phenanthroline, 20% acetate buffer, and 1% concentrated hydrochloric acid. Iron bottles were filled until the solution turned light red, approximately ten minutes. If the solution turned red before 8 mL were collected, samples were diluted with deionized water to bring the total solution to 8 mL. Iron samples were quantified within two hours of sampling. Iron and sulfide were quantified colorimetrically using the phenanthroline and methylene blue methods, respectively, on a HACH DR5000 UV-Vis spectrophotometer (Eaton et al., 2005). Sulfate was quantified using a Dionex ICS-1100 Integrated IC system (AS-DV Autosampler) (Eaton et al., 2005).

Solid phase sampling and analysis

Samples for the bulk sediment initial conditions were obtained after homogenization of the sediment prior to placement in the buckets (day 152). Five replicate samples were placed in jars and analyzed for AVS and simultaneously extracted iron. At the end of the season, mini-cores of intact sediment were retrieved immediately before wild rice plants were sampled.

On each sample date throughout the summer, wild rice roots were collected for AVS and weak acid extractable iron. Each plant was removed from the sediment and immediately rinsed in buckets of deoxygenated water continuously bubbled with nitrogen. While submerged in deoxygenated water, the stem was cut just above the root ball so that the shoots and seeds could be saved for ^{15}N analysis. Roots were then placed in jars full of deoxygenated water, which were immediately placed in a plastic bag flushed with nitrogen and transported to an oxygen-free glove box. In the glove box, the roots were cleaned of extra organic matter prior to removing a 1-2 g section of wet root mass for AVS and iron analysis. From both sediment and roots, AVS was extracted using 7.5 ml 1 N HCl for 4 hours using a modified diffusion method (Brouwer and Murphy 1994). During a room temperature acid incubation with gentle mixing, sulfide was trapped in an inner vial containing Sulfide Antioxidant Buffer (SAOB) and subsequently quantified using a ThermoScientific sulfide ion-selective electrode with a detection limit ranging from 0.01-40 mmol/L. Ferrous iron was quantified colorimetrically using the phenanthroline method on a HACH DR5000 UV-Vis spectrophotometer (Eaton et al., 2005), and weak acid extractable iron was quantified using a Varian fast sequential flame atomic absorption spectrometer with an acetylene torch.

A subset of roots was tested for chromium(II)-reducible sulfur (CRS) to determine whether AVS was extracting all total reduced inorganic sulfur on the roots. A diffusion-based CRS method was used, which can fully extract amorphous iron sulfide and pyrite and can partially extract elemental sulfur (Burton et al. 2008). Chromic acid for CRS analysis was prepared according to Burton et al. (2008). Inside an oxygen-free glove box, a section of root from a plant previously analyzed for AVS was placed in the analysis bottle. An inner vial containing SAOB was also placed inside the bottle prior to sealing. Bottles were taken out of the glove box and injected with chromic acid. CRS was extracted for 48 hours and quantified using a ThermoScientific sulfide ion-selective electrode.

Isotope sampling and analysis

For analysis of ^{15}N uptake, the plants were sub-sampled by cutting at the stem to root transition. If seeds were present, they were removed prior to sampling the plant and saved for separate analysis. The plants and seeds were rinsed with deionized water and dried in paper bags for seven days at 65C. The dried plants were weighed, placed in polycarbonate vials with stainless steel balls, and shaken in a SPEX 800M mixer mill until the samples were in a powdered form. Seeds were counted, weighed, and powdered using the same method. The samples were transferred to glass vials and dried again overnight at 65C with caps loosely covering the vials. Samples were quantified for total N and $\delta^{15}N$ on a Finnigan Delta Plus XP isotope ratio monitoring mass spectrometer.

Data analysis

Geochemical parameters and measured attributes of plants were analyzed using repeated measures analysis of variance to determine differences between sulfate amendments and controls. A paired *t* test was used to determine differences between AVS and CRS concentrations on roots. A two-factor ANOVA was used to compare preplanting and post-senescence sediment concentrations of iron and AVS between treatments. Analyses were performed using the statistical software SAS. Logarithmic transformations were used when data was non-normal. A reciprocal transformation was used for dry weight of plants, as a logarithmic transformation was not effective. Data for root AVS were split into pre-seed production and post-seed production because the full-season data was not able to be transformed.

The saturation index was calculated to determine if the pore water was saturated enough to precipitate iron sulfide (equation 1). A positive saturation index value indicates precipitation, and a negative value indicates dissolution. The K_{sp} value used was $10^{-2.95}$ (Stumm and Morgan, 1995).

$SI = log\frac{[IAP]}{K_{sp}}$ where $IAP = \frac{[Fe^{2+}][HS^{-}]}{[H^{+}]}$ Equation 1

Changes in the accumulation rates of root AVS and ferrous iron were tested by fitting linear regressions to the concentrations of root AVS and Fe^{2+} prior to seed production (days 189-231). The model was extrapolated to late season sample dates (days 232-264) to test if accumulation rates changed between flowering and seed production.

A mixing model was used to determine the proportion of seed nitrogen originating from the pore water and the proportion translocated from the stems (equations 2 and 3). The $\delta^{15}N$ of the seeds was measured, and the $\delta^{15}N$ of the pore water and the stems were approximated. In equation 2, δ_{sample} is the isotopic signature of nitrogen in the seed, $\delta_{source1}$ is the isotopic signature of the pore water ammonium, f_1 is the proportion of nitrogen coming from the pore water, $\delta_{source2}$ is the isotopic signature of nitrogen in the plant stem, and f_2 is the proportion of the nitrogen sourced from the plant stem. Seed nitrogen can be sourced only from the pore water or the stems, so the proportions from both components must sum to one (equation 3).

$\partial_{sample} = \partial_{source1} \times f_1 + \partial_{source2} \times f_2$ Equation 2

$f_1 + f_2 = 1$ Equation 3

Results

Pore water

Although sulfate was 40x higher in the overlying water of sulfate-amended plants, pore water sulfide concentrations were only approximately twice as high in the in the rooting zone of sulfate-amended plants compared to the control over the entire growing season. Sulfide concentration and variability increased in the pore water of both amended and control rooting zones one week after the first seeds were produced (day 238, Julian date) and returned to initial concentrations two weeks later (day 245, Fig. 1a). Pore water sulfide data did not fit any parametric model, so a repeated measures ANOVA was not performed.

Pore water iron concentrations were not correlated with sulfate amendment (Table 1). Pore water iron decreased until shortly after seed production began (day 238) in both amendments. The minimum iron concentration occurred at the same time that a peak in pore water sulfide developed (Fig 1b). Shortly before senescence (days 252 and 264), the iron concentrations returned to values similar to concentrations during the first month of data collection.

The pore water pH and saturation index were not correlated with sulfate amendment (Table 1). The pH of the pore water peaked at the start of seed production (days 231-238, Fig.1c). This peak occurred approximately one week before the iron minimum and the sulfide maximum. The saturation index peaked one week after the first seeds were produced, when pH and sulfide were elevated and iron was low (day 238, Appendix Table 1). The average saturation index was above zero only in the sulfate-amended buckets on day 238. The saturation index gradually declined for the rest of the growing season.

Sulfate concentrations ranged from 10-30 times higher in the pore water of plants amended with sulfate (Table 1). Sulfate increased in the amended pore water until seed production began, when it declined precipitously from 2300 μmol/L to 770 μmol/L over 15 days (Fig 1d). In the pore water of control plants, sulfate concentrations followed a similar trend, but at lower concentrations. Control sulfate peaked at 230 μmol/L before decreasing to 34 μmol/L. Sulfate declined just prior to an increase in pore water sulfide.

Table 1. Results of repeated measures ANOVA testing effect of sulfate, time and interaction of sulfate and time on geochemical and biological variables. Tests for pore water and root parameters include data from the entire growing season, whereas tests for biological parameters only include data from mature seed production. *F* values and degrees of freedom (*d.f.*) are given. Tests for time and sulfate x time have the same number of degrees of freedom. Significance levels are shown using asterisks (***indicates $p < 0.001$, **indicates $0.001 < p < 0.05$, *indicates $0.05 < p < 0.10$).

Repeated measures ANOVA (F values)	Sulfate	d.f.	Time	Sulfate x Time	d.f.
Pore water geochemistry					
Iron	5.16	1, 5	5.51***	1.14	6, 35
pH	3.25	1, 6	12.5***	1.45	6, 36
Saturation index	2.68	1, 4	2.19*	0.50	6, 34
Sulfate	239***	1, 3	8.17***	1.09	5, 27
Root geochemistry					
AVS (during flowering)	66.1***	1, 5	1.10	0.40	3, 17
AVS (during seed production)	148***	1, 6	5.46**	1.76	4, 24
Weak acid extractable iron	0.53	1, 6	2.65	2.42**	7, 42
Ferrous Iron	127***	1, 6	57.2***	3.34**	6, 36
% Ferrous Iron	235***	1, 6	41.5***	4.91***	6, 36
Biological variables (during seed maturity)					
Plant N (total mass)	1.53	1, 6	0.35	0.25	2, 12
Plant weight	5.00*	1, 6	0.40	0.31	3, 18
Seed N (total mass)	5.84*	1, 6	1.10	1.22	2, 12
Seed weight	4.88*	1, 6	0.59	0.94	2, 12
Seed count	5.00*	1, 6	1.89	0.70	2, 12
Seed $\delta 15N$	1.47	1, 6	2.45	0.05	2, 12
Seed N%	1.70	1, 6	3.04*	0.40	2, 12
Vegetative N (plant+seed mass)	5.43*	1, 6	0.32	1.71	2, 12

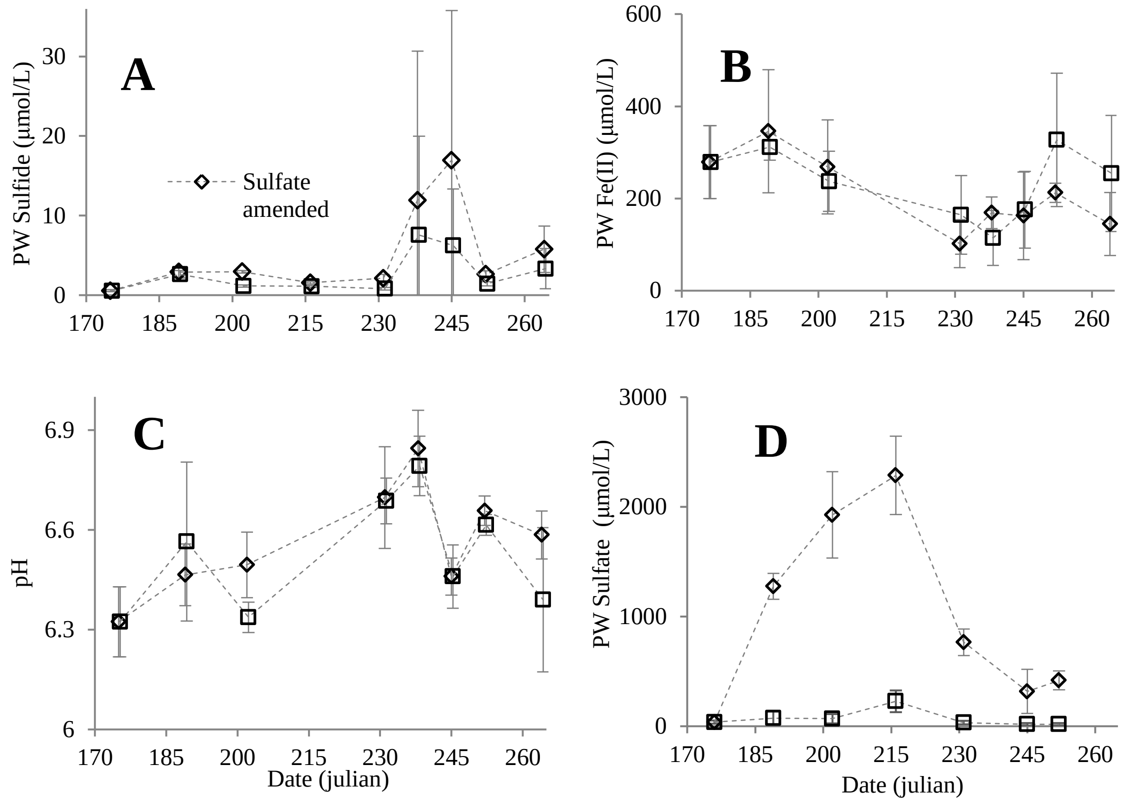


Figure 1. Pore water (PW) data measured in buckets during summer of 2015. Diamonds indicate data from buckets amended with 300 mg/L sulfate. Squares represent data from control buckets. Time is shown in Julian days. Error bars indicate one standard deviation. Control data points are slightly offset to show overlap in error bars.

Roots

Wild rice plants grown in sediment with high overlying water sulfate concentrations developed a black coating on their root surfaces (Appendix Fig. 1). A SEM scan of the roots showed that the root precipitate contained iron and sulfur in approximately a 1:1 ratio (Dan Jones, unpublished data). The oxic/anoxic interface was often recorded on the root; the black coating started on the stem just above the root ball and extended downwards along the entire length of the roots. Adventitious roots that grew at the surface of the sediment remained white, the natural color of wild rice root tissue. Control plants, grown in sediment with low overlying water sulfate, formed very little black color on their roots, instead appearing amber, a color characteristic of iron (hydr)oxides.

Roots grown under elevated sulfate (hereafter “amended roots”) accumulated AVS concentrations up to two orders of magnitude higher than the control roots by late summer. Amended root AVS peaked at 298 ± 74 umol/g dw immediately prior to senescence (Fig 2a). Concentrations of AVS on roots grown under control surface water sulfate (hereafter “control roots”) did not consistently increase, and averaged of 3.2±1.7 umol/g dw. For amended roots, the rate of accumulation of root AVS appeared relatively constant (linear) until the first day seeds were produced (day 232), when the rate of AVS accumulation appeared to increase abruptly. During seed production, AVS concentrations were greater than that predicted by a linear model (constant accumulation rate), suggesting that the net rate of AVS accumulation on amended roots increased rapidly when seed production began. Points after the first day of seed production (day 231) fell outside of a 95% CI of a linear regression on the points during flowering (days 190-231, Appendix Fig. 2). Concentrations of CRS on both amended and control roots did not differ from AVS concentrations on the same roots, indicating that crystalline forms of FeS did not make up a significant proportion of reduced sulfur (paired *t* test, p=0.27, t=0.63, n=20).

Ferrous iron accumulation paralleled AVS accumulation on amended roots (Fig 2b). Root ferrous iron concentrations were elevated and accumulated faster on the amended roots compared to the control (Table 1). Ferrous iron on control roots and amended roots increased linearly, but ferrous iron on amended roots increased at a higher rate until the first seeds were produced (day 232). During seed production, ferrous iron concentrations on amended roots were greater than those predicted by a linear model, while Fe(II) accumulation on control roots appeared to slow.

Weak acid extractable iron (sum of Fe(II) + Fe(III) concentrations on roots, hereafter "total extractable iron") was variable, but did not differ significantly between treatments (Table 1). The average total extractable iron remained relatively constant in both treatments during flowering; however, during the first week of seed production (days 232 and 239) the total extractable iron dropped by about 150-250 umol/g on both the amended and control roots, and then gradually increased over the following three weeks (Fig. 3). Total extractable iron changed seasonally from mostly Fe(III) to mostly Fe(II) on sulfate-amended roots, especially during the first week of seed production (days 232 and 239). This abrupt shift in iron speciation occurred the same week that total extractable iron decreased and at about the same time as the increase in AVS accumulation rate (Fig. 3). Immediately prior to seed production, total extractable iron on the amended roots was 46 ± 11% Fe(II), and after one week of seed production, the composition of iron was 87 ± 10% Fe (II). During this same week, control root Fe(II) increased from 20 ± 11% to 48 ± 16%.

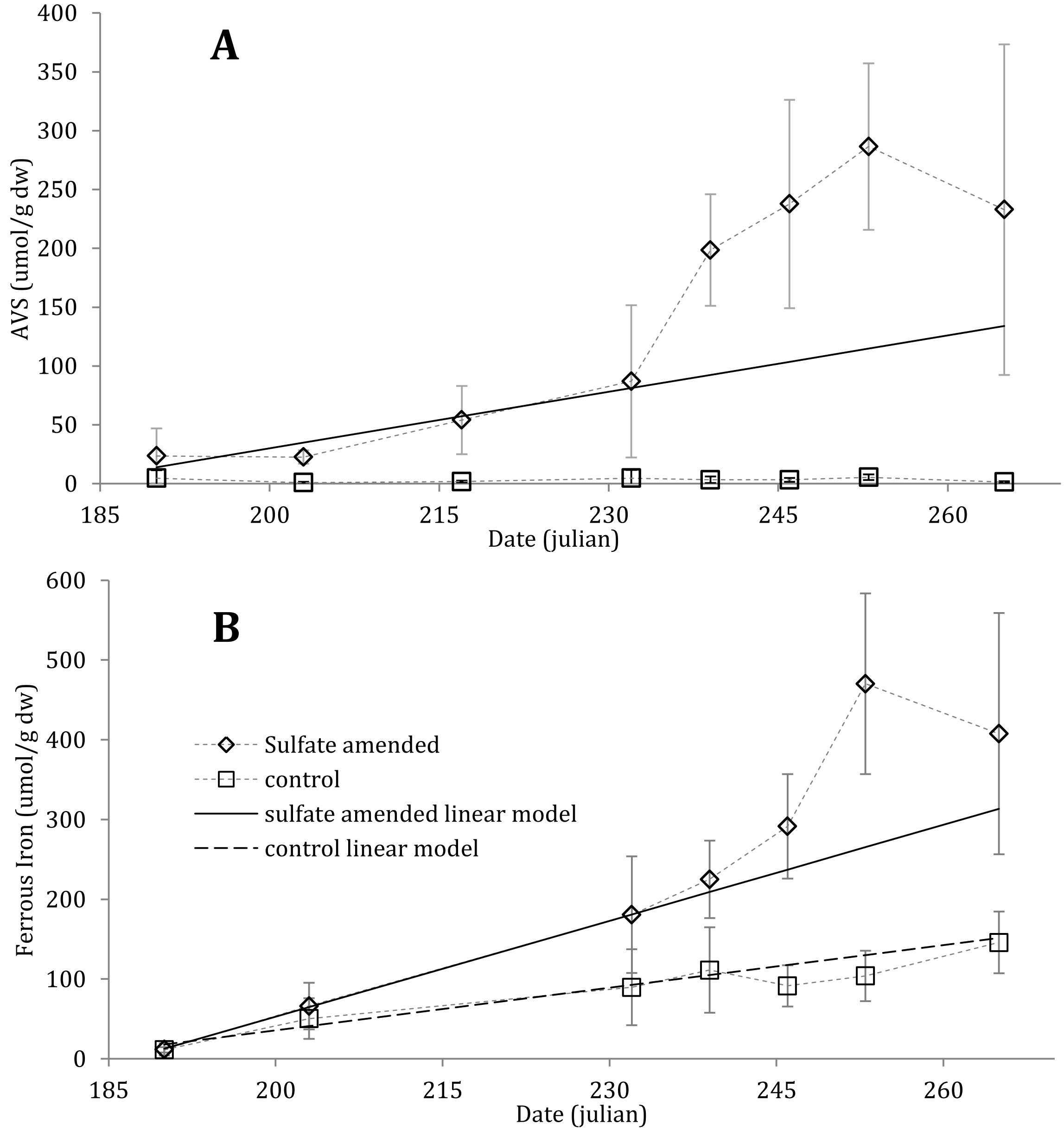


Figure 2. Solid phase acid volatile sulfide (A) and ferrous iron (B) concentrations on roots. Diamonds represent the average concentration on roots of four sulfate-amended plants, and squares represent the average of four control plants. The dashed line shows a linear model fit to the data from day 190 to day 232. Time is expressed in Julian dates. Error bars show one standard deviation.

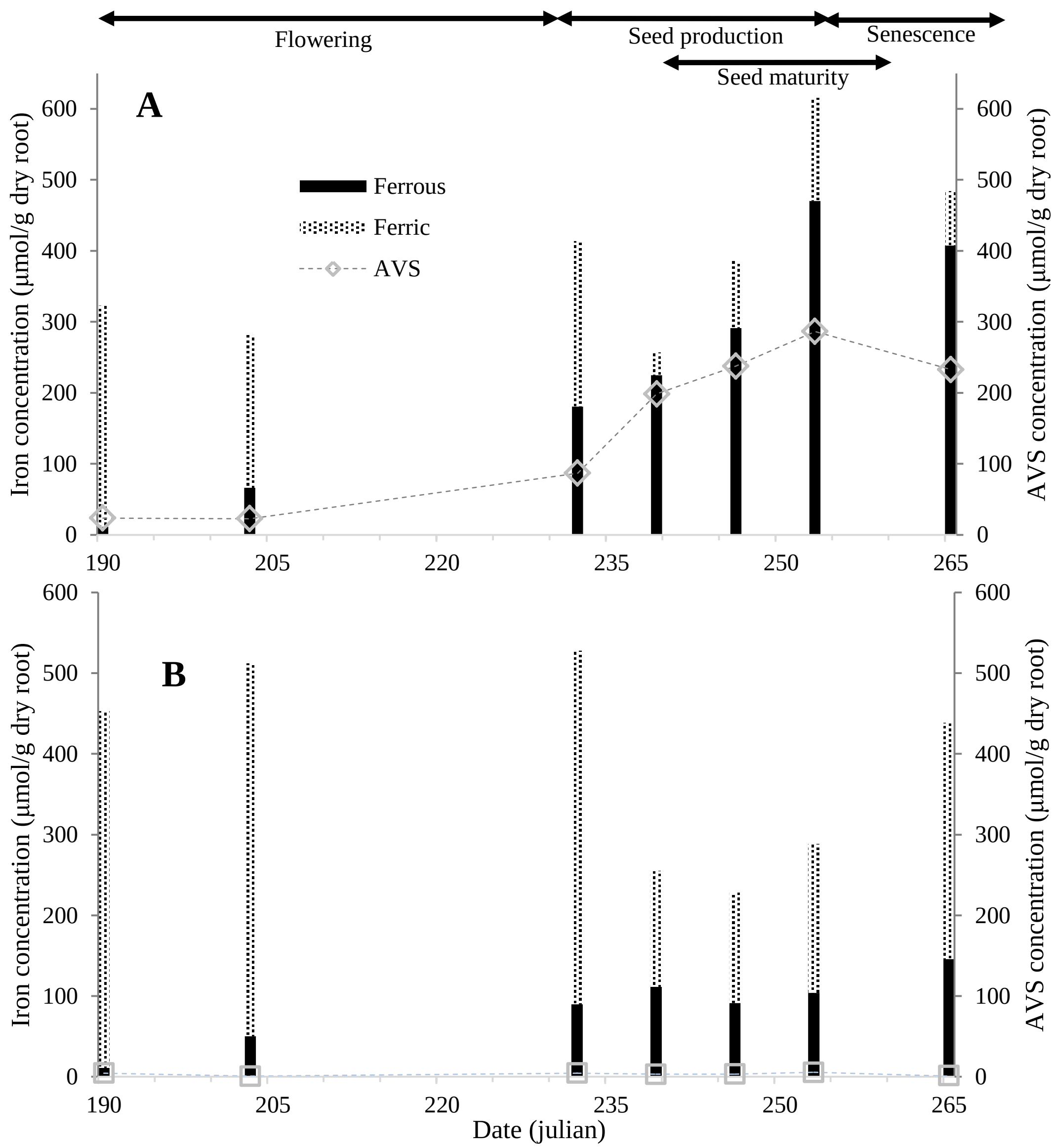


Figure 3. Seasonal iron speciation with root AVS overlain in sulfate-amended bucket. The dotted pattern indicates ferric iron and the solid black represents ferrous iron. A). Sulfate-amended bucket iron. Grey diamonds show root AVS concentrations in sulfate-amended buckets. B). Control bucket iron. Grey squares show root AVS concentrations in control buckets. Error bars are omitted for clarity.

Sediment

Sediment AVS was significantly different between treatments, but total extractable iron was not. In both the sulfate-amended and control sediment, AVS increased during the growing season, but more AVS accumulated in the amended sediment (2-factor ANOVA, time x treatment interaction, f=5.08, df=1,18, p=0.037). Amended sediment AVS increased from 0.39 umol/g in early summer to 4.7 μmol/g at the end of the growing season, whereas the control sediment only increased from 0.39 μmol/g to 0.88 umol/g. There was no difference in total extractable iron between the amended and control sediment at the beginning or end of the growing season (2-factor ANOVA, f=0.65, df=1,18, p=0.429).

Biological effects

Plant sampling began at the start of the flowering stage (days 190-230). The first seeds were collected on 8/20/15 (day 232), but were unripe and not yet filled. In this paper, seed production is referred to as days 230 to day 264, but mature seeds were not produced until one week after the start of seed production (day 239). On the last sample date (day 265) seeds were collected, but were unfilled. Stems and leaves were no longer green, indicating that the plants had senesced. Of the four replicates in the sulfate amendment on this date, two plants did not produce seeds. Thus, “mature seed production” refers to dates 239-253.

Total seed nitrogen, total seed weight, and seed count were all lower in sulfate-amended plants during mature seed production, a time that coincided with elevated FeS on roots (days 239-253, Table 1, Fig 4). Sulfate addition was not correlated with seed $\delta^{15}N$ or seed N %. During mature seed production and senescence, the dry weight of the sulfate-amended plants was lower than that of control plants. Total vegetative (plant + seeds) N was unaffected by sulfate until the last two sample dates prior to senescence, when it was lower in sulfate-amended plants (Fig 4d, two-sample t test, p=0.031, p=0.047, n=8 for both dates).

A mixing model was used to determine the fraction of total seed nitrogen coming from the pore water and the fraction translocated from the stem (Appendix Fig. 3). In the days following a spike of enriched nitrogen to sediment pore water, there were two possible sources of nitrogen in the seeds; wild rice can translocate nitrogen from its stem or take nitrogen up from the pore water. The plant $\delta^{15}N$ was estimated to be 4.5‰ from the average of 12 unlabeled plants harvested on the first two sample dates. The pore water $\delta^{15}N$ was approximated to be 180‰ and calculated from the percent by mass of $^{15}NH_4$ added ($\delta^{15}N$ =26,200‰) and the percent by mass of ammonia already present in the pore water ($\delta^{15}N$ assumed to be 0‰). The two-component mixing model showed no difference in fraction of nitrogen uptake from pore water between the amended and control plants (repeated measures ANOVA, $p=0.83$, $f=0.05$, $df=1,6$). In both control and amended plants, the fraction of total seed nitrogen originating from the pore water increased two weeks into seed production (day 246) from 27 ± 18 % to 51 ± 19%, but returned to 29 ± 19 % a week later (day 253). The elevated proportion coming from the pore water coincides with the day seeds contained the most nitrogen (Fig 4c). On this day, total seed nitrogen was significantly lower in the sulfate amended plants than in the control plants (two-sample t test, $p=0.047$, $n=8$). Plant N (excluding seeds), however, was not different between amended and control plants on this day (two-sample t test, $p=0.41$, $n=8$).

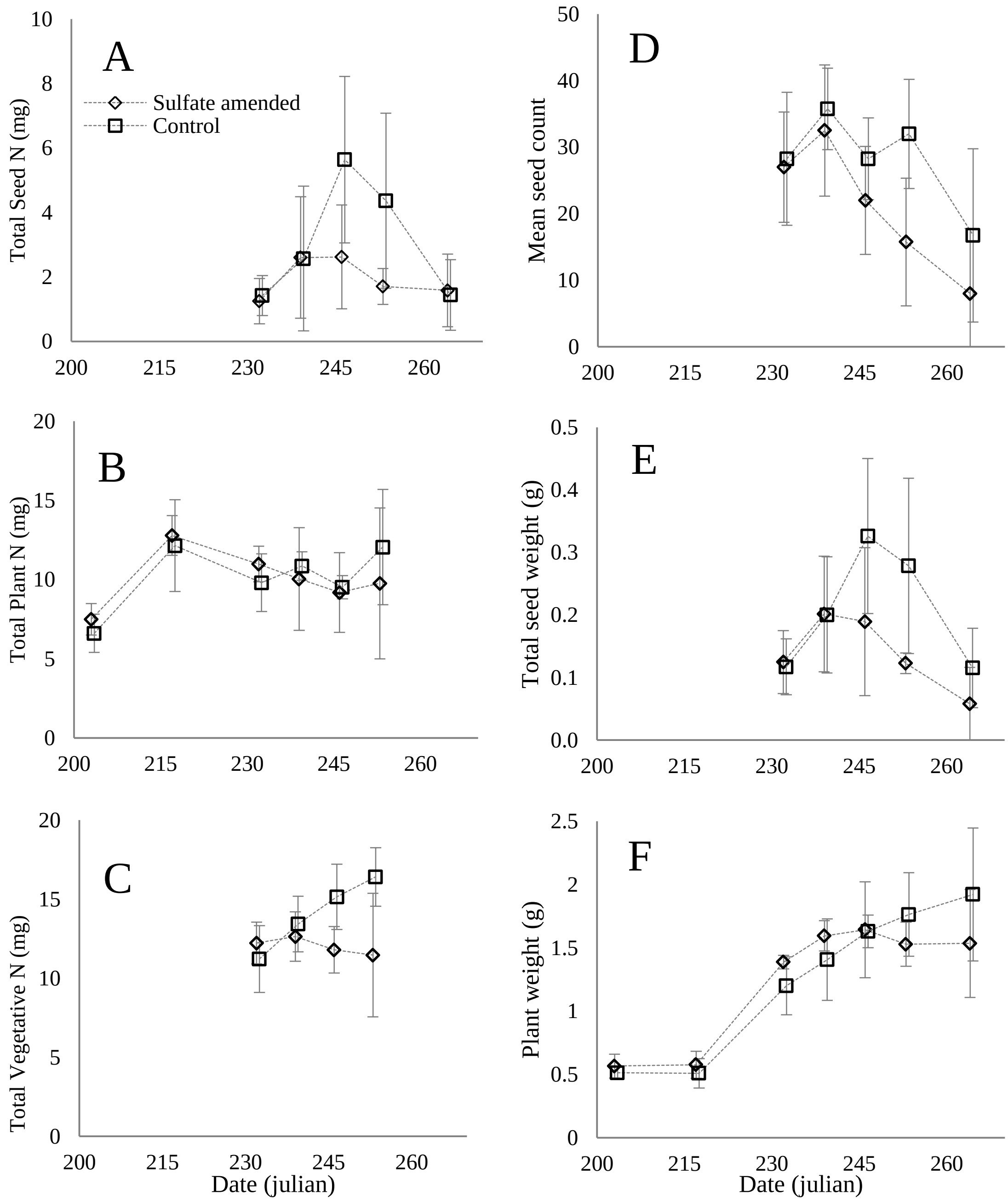


Figure 4. Biological endpoints. Diamonds represent plants grown in surface water with 300 mg/L sulfate added while squares show data from control plants. Each data point represents four replicates. Error bars represent one standard deviation. A) Weekly total mass of nitrogen in seeds of sulfate amended and control plants. B) Total mass of nitrogen in the plant (stems+leaves), excluding seeds, over the course of the growing season. C) Weekly total vegetative nitrogen in amended and control plants. Total vegetative nitrogen was calculated by summing nitrogen from seeds, stems, and leaves. D) Weekly seed count in amended plants and control plants. E) Weekly total seed mass in amended plants and control plants. F) Dry mass of plants over the course of the growing season.

Discussion

Our observations suggest a tight coupling of iron and sulfur cycling in the rooting zone of wild rice. Iron (hydr)oxides form on wild rice roots early in the growing season, but roots that are exposed to high sulfate loading (300 mg/L) develop iron sulfides later in the growing season. An inflection point in iron sulfide accumulation occurs at the start of seed production, shortly after rapid depletion of sulfate in the pore water, and defines an increase in the net rate of FeS accumulation. The rapid increase in net FeS accumulation suggests a change in a process that controls the way iron and sulfur cycle in the rhizosphere, and the timing suggests that this process may be tied to and have important implications for rice physiology. Previous research has suggested that an accumulation of FeS occurs after plant senescence (Jacq 1991), but our observations clearly show accumulation of FeS during the reproductive life stage of wild rice.

The change in FeS accumulation rate is consistent with an inhibition of radial oxygen loss. Sulfate accumulation in the pore water during the flowering stage suggests that the rhizosphere is relatively oxidized. The initially linear FeS accumulation rate on plant roots suggests constant rates of sulfide production and sulfide oxidation, with a higher rate of sulfide production than oxidization (net accumulation). However, sulfide exposure in white rice leads to the formation of suberin in the cell walls of roots which is hypothesized to create a barrier that limits diffusion of toxic solutes into the plant (Armstrong and Armstrong, 2005). The barrier not only excludes toxic solutes like sulfide, but also traps oxygen inside the roots, suppressing radial oxygen loss (Krishnamurthy et al. 2009, Soukup et al. 2006). A relatively rapid transition to anoxia of the rhizosphere appears to have occurred at the onset of seed production, possibly as a result of suberin-induced suppression of radial oxygen loss. Under the anoxic conditions, the net accumulation of reduced species likely increased because fewer reduced species cycled back to their oxidized form.

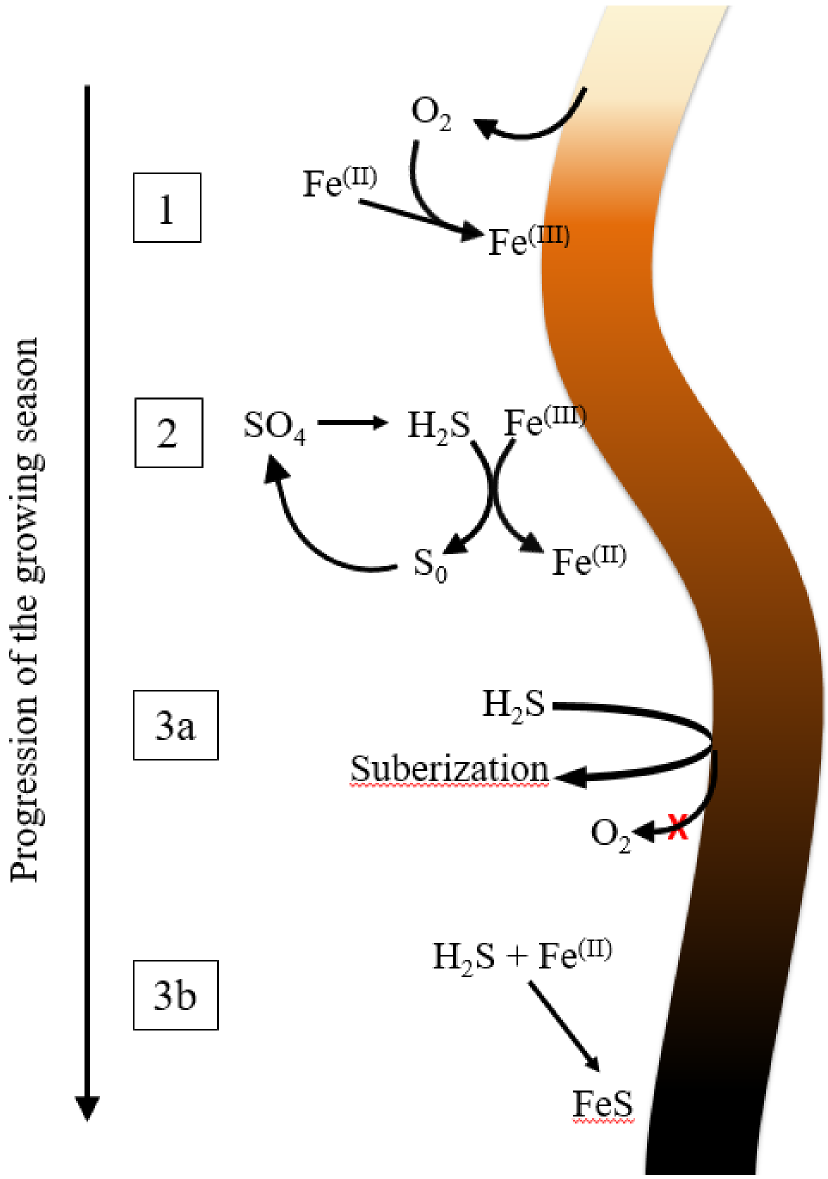


Figure 5. Proposed mechanism of iron sulfide formation on wild rice roots. Roots are protected by iron (hydr)oxides [1], but reduced by sulfide [2]. Exposure of roots to sulfide induces suberization of root cells, which leads to decreased radial oxygen loss [3a]. Rhizosphere anoxia allows iron sulfides to precipitate [3b].

A hypothesized pathway for how the rice roots might transition from iron (hydr)oxide plaques to iron sulfide plaques over the growing season is outlined in Figure 5. Initially, radial oxygen loss creates oxic conditions in the rooting zone, causing ferrous iron within the rhizosphere to precipitate as iron (hydr)oxides and accumulate on root surfaces (Fig. 5, [1] label). At this initial stage, the root is protected from reduced species by both radial oxygen loss and iron (hydr)oxide plaques, an electron accepting sink. Before sulfide can penetrate to the root, the iron (hydr)oxide plaques, effectively acting as an electron accepting buffer, must be reduced (Fig. 5, [2] label). As sulfide erodes the accumulated ferric iron barrier (Hansel et al. 2014, Kwon et al. 2013), sulfide can then reach the root surface and cause suberization (Fig. 5, [3a] label). Once radial oxygen loss is suppressed by suberin formation, the electron accepting buffer capacity of iron (hydr)oxides can no longer be replenished. The remaining quantity of iron (hydr)oxides can be more rapidly reduced due to a net change in the flow of electrons to the rooting zone. Upon depletion of iron (hydr)oxides, sulfide accumulates rapidly, since neither iron (hydr)oxides or a supply of radial oxygen loss are available to oxidize sulfide (Fig 5, [3b] label). As sulfide penetrates closer to the root surface, it precipitates with available iron, and the redox potential of the rhizosphere shifts to more reducing conditions.

The rapid accumulation of sulfur on roots in amended plants seems inconsistent with the relatively small difference in sulfur and iron concentrations in pore water. The saturation index (SI), which is calculated from pore water concentrations two centimeters from the stem, indicates that the pore water is undersaturated with respect to iron sulfide. The thermodynamic understanding of mineral precipitation and dissolution is that minerals precipitate when pore water is saturated and dissolve when pore waters are undersaturated (Stumm & Morgan, 1995). The rapid accumulation of iron sulfide on roots in the setting of undersaturated pore water suggests that the transition of iron (hydr)oxide to iron sulfide on the roots occurs very close to the surface of the root, and thus depends on near-root-surface processes more than on pore water concentrations. Sulfide on root surfaces must be supplied externally, either from reduction of surface water sulfate, or from mobilization of AVS on sediment, but ferrous iron in the FeS plaques could be sourced from the reduction of iron (hydr)oxides already accumulated on the root surface earlier in the season. Indeed, a decrease in solid-phase iron on the roots, a shift in iron speciation, and an accumulation of pore water iron all occur simultaneously, which is consistent with loss of soluble ferrous iron off of the root surface during the redox transition. Thus, if the ferrous iron in FeS plaques is sourced from the iron (hydr)oxides on the root, saturation index calculations based on pore water iron concentrations may not be relevant to understanding FeS formation on roots. Additionally, the decline of pore water sulfate followed by rapid accumulation of AVS on the root surfaces suggests that a large amount of sulfur passes through the pore water pool very quickly. Iron sulfide formation is strongly favorable thermodynamically and kinetically rapid (Rickard, 1995). Using pore water sulfide concentrations to calculate the saturation index may underestimate the amount of sulfur available to precipitate on root surfaces, as pore water sulfide may act as a transient phase between pore water sulfate and root AVS. The transience of sulfide in pore waters near rice rhizospheres was noted by Hara (2013) who observed black iron sulfide zones around white rice seeds grown in sulfate-amended sediment, but was unable to quantify any sulfide, despite measuring redox potentials low enough to support sulfide production.

In this experiment, iron sulfide plaques occurred concomitantly with lower seed nitrogen and fewer seeds. Less nitrogen was present in the total seed mass of the amended plants, and fewer seeds were produced. This is likely a strategy for optimizing reproduction; amended plants produce fewer filled seeds but each filled seed is fully viable (Pastor et al., in reveiw). The two-component isotope mixing model suggests that the amended plants were not able to compensate for inhibition of nitrogen uptake by translocating a greater percentage of seed nitrogen from the stem and leaves. Between the sulfate and control, no difference was observed in the fraction of N uptake from the pore water. The decreased total seed N in sulfate amended plants appears to be an equally proportioned result of decreased uptake from pore water and decreased translocation from the plant.

Biological variables were only affected during seed production. During the biomass growth life stages, little difference in total plant weight and total plant N was observed. Biomass may not have been impacted because sulfide can produce a fertilization effect by sequestering iron bound with phosphate, releasing free phosphate

(Geurts et al. 2009, Caraco et al. 1989, Smolders et al. 2003, Lamers et al. 2002). However, nitrogen, rather than phosphorus, is the limiting nutrient for wild rice (Sims et al. 2012), so the fertilization effect is likely minimal in wild rice. In the long term, Pastor et al. (in review) showed that sulfide takes several years to affect a population of wild rice, because although sulfide showed no effect on germination and very little effect on biomass of wild rice, sulfide greatly decreased the number of juvenile seedlings that survive and the number of filled seeds produced by the plant. The results from our study suggest that during seed production, the buffering capacity of iron (hydr)oxides has been overwhelmed by sulfide and no longer protects the plant from sulfide. Similarly, juvenile seedlings may be vulnerable to sulfide because they have not yet grown out of the water column and are thus unable to transport oxygen from the atmosphere to their roots. The life stages of wild rice affected by sulfide are consistent with times during which an oxic barrier around the roots is absent.

Accumulation of FeS on roots may have implications for wetland cycling of iron and sulfide. After senescence, roots coated with FeS decay and become incorporated into the bulk sediment. Jacq et al. (1991) found significant accumulation of FeS on white rice roots after senescence, likely because the dead root material stimulated continued iron and sulfate reduction. Additionally, Jacq et al. (1991) found that sediment in a planted rice paddy contained higher FeS concentrations than an unplanted rice paddy. Because wild rice is an annual plant, the amount of root FeS that accumulates over a growing season is added to the sediment each year. Choi et al. (2006) likewise found that in a riparian wetland containing *Phragmites australis* and *Zizania latifolia*, AVS concentrations were higher in the top 6 cm of non-vegetated sediment, but vegetated sediment had higher concentrations of AVS 6-14 cm below the sediment-water interface. If AVS on roots is supplied mainly from reduction of surface water sulfate, burial of FeS coated roots may be supplying sulfide to the sediment faster than pore water precipitation of iron sulfide in the bulk sediment. If root AVS is supplied largely by mobilization of sediment AVS, which Choi et al. suggests can be caused by radial oxygen loss, then sediment AVS concentration may be an important parameter in determining iron sulfide accumulation and concomitant inhibition of nitrogen uptake in wild rice. Knowledge of the main sources of sulfur for root AVS will be crucial in managing wild rice in sulfur-impacted systems.

Conclusion & Directions for Future Work

The timing of our observations of rhizosphere AVS accumulation in conjunction with decreased total seed N in sulfate-amended plants suggests that nitrogen uptake by wild rice is affected only after significant sulfide accumulation on root surfaces. In this experiment, elevated sulfide on plant roots coincides with the plant's reproductive stage. We propose that root surface iron (hydr)oxides delay sulfide from entering the plant, effectively acting as a buffer against early and mid-season sulfide exposure. When the oxic barrier on the root surface is overwhelmed, iron sulfide accumulates rapidly, as shown by the doubling of AVS and the shift in iron speciation from about 50% Fe(II) to 90% Fe(II) within just one week. In this experiment, the oxic barrier was overwhelmed just prior to seed production; concurrently, reduced seed count, total seed weight, and total seed nitrogen were observed.

Many questions remain about the cause of the redox shift in the rhizosphere. We propose a mechanism in which sulfide-induced suberization of roots facilitates reduction of the oxic barrier, but a seasonal change in wild rice physiology could also facilitate a rapid transition to anoxia. Control roots, like sulfate-amended roots, lost about half of their total extractable iron at the start of seed production, and accumulated some ferrous iron even in the absence of significant S accumulation. Is there a seasonal shift in redox potential in wild rice rhizospheres, regardless of the presence of sulfur? Seasonal measurements of redox potential and magnitude of radial oxygen loss may provide insight into the comparative influence of plant processes and sulfur loading on shifting redox conditions in the rhizosphere. Is the bacterial community affected more by rhizosphere geochemistry or by life stages of the plant? Seasonal microbial community analysis could also elucidate the relative causes of the rhizosphere anoxia, as a significant seasonal shift in the microbial community of control plants would indicate plant controlled redox conditions. If the redox conditions of the rhizosphere are controlled by iron and sulfur geochemistry as proposed, would a lower initial concentration of iron on roots result in erosion of the iron (hydr)oxide barrier and subsequent inhibition of nitrogen uptake earlier in the growing season? If so, would plant biomass and nitrogen also be decreased? A similar study to this one could be done in which total iron concentrations of the sediment were varied to produce different initial concentrations of iron (hydr)oxides on roots.

Finally, from a management perspective, it would be useful to understand the sources of sulfur on root surfaces and the sediment parameters that control those sources. Is the sulfide on the roots sourced primarily from surface water sulfate or from mobilization of sediment AVS? Could a lake that has previously received high sulfur loads but currently has low surface water sulfate contain wild rice with significant iron sulfide plaques? This question has implications for restoration of wild rice in sulfur-impacted lakes.

References

Allam, A.I., and Hollis, J.P. 1972, "Sulfide inhibition of oxidases in rice roots", *Phytopathology,* vol. 62, pp. 634-639.

Armstrong, J., AfreenZobayed, F. & Armstrong, W. 1996, "Phragmites die-back: Sulphide- and acetic acid-induced bud and root death, lignifications, and blockages within aeration and vascular systems", *New Phytologist,* vol. 134, no. 4, pp. 601-614.

Armstrong, J. & Armstrong, W. 2005, "Rice: Sulfide-induced barriers to root radial oxygen loss, Fe2+ and water uptake, and lateral root emergence", *Annals of Botany,* vol. 96, no. 4, pp. 625-638.

Beck, B.F. & Johnson, N.W. 2014, "Geochemical factors influencing the production and transport of methylmercury in St. Louis River Estuary sediment", *Applied Geochemistry,* vol. 51, pp. 44-54.

Brouwer, H. & Murphy, T. 1994, "Diffusion Method for the Determination of Acid-Volatile Sulfides (Avs) in Sediment", *Environmental Toxicology and Chemistry,* vol. 13, no. 8, pp. 1273-1275.

Boudreau, B. P. *Diagenetic Models and their Implementation:Modeling Transport and Reactions in Aquatic Sediments*; Springer: New York, 1996.

Burton, E.D., Sullivan, L.A., Bush, R.T., Johnston, S.G. & Keene, A.F. 2008, "A simple and inexpensive chromium-reducible sulfur method for acid-sulfate soils", *Applied Geochemistry,* vol. 23, no. 9, pp. 2759-2766.

Caracao, N.F., Cole, J.J., and Likens, G.E. 1989. "Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems", *Letters to Nature*, vol 341, pp. 316-318.

Choi, J., Park, S. & Jaffe, P. 2006, "The effect of emergent macrophytes on the dynamics of sulfur species and trace metals in wetland sediments", *Environmental Pollution,* vol. 140, no. 2, pp. 286-293.

Christensen, K. & Sand-Jensen, K. 1998, "Precipitated iron and manganese plaques restrict root uptake of phosphorus in *Lobelia dortmanna*", *Canadian Journal of Botany-Revue Canadienne De Botanique,* vol. 76, no. 12, pp. 2158-2163.

Eaton, A.D., L.S. Clesceri, E.W. Rice, A.E. Greenberg, eds. 2005, Standard Methods for the Examination of Water and Wastewater, 21st ed. American Public Health Association.

Eimers, M. C., Dillon, P.J., Schiff, S.L., Jeffries, D.S. 2003. “The effects of drying and re-wetting and increased temperature on sulphate release from upland and wetland material”, *Soil Biology and Biochemistry,* vol. 35, no. 12, pp. 1663–1673

Gao, S., Tanji K.K., and Scardaci, S.C. 2003, “Incorporating straw may induce sulfide toxicity in paddy rice”, *California Agriculture* vol. 57, pp. 55-59

Geurts, J.J.M., Sarneel, J.M., Willers, B.J.C., Roelofs, J.G.M., Verhoeven, J.T.A. & Lamers, L.P.M. 2009, "Interacting effects of sulphate pollution, sulphide toxicity and eutrophication on vegetation development in fens: A mesocosm experiment", *Environmental Pollution,* vol. 157, no. 7, pp. 2072-2081.

Grava, J. and Raisanen, K.A. 1978, “Growth and nutrient accumulation and distribution in wild rice”, *Agronomy Journal* vol 70, pp. 1077-1081.

Hansel, C.M., Lentini, C.J., Tang, Y., Johnston, D.T., Wankel, S.D. & Jardine, P.M. 2015, "Dominance of sulfur-fueled iron oxide reduction in low-sulfate freshwater sediments", *Isme Journal,* vol. 9, no. 11, pp. 2400-2412.

Hara, Y. 2013, “Suppressive effect of sulfate on establishment of rice seedlings in submerged soil may be due to sulfide generation around the seeds”, *Plant Production Science*, vol 16, no. 1, pp. 50-60.

Holmer, M and S. Nielsen. 1997, “Sediment sulfur dynamics related to biomass-density patterns in *Zostera marina* (eelgrass) beds”, *Marine Ecology Progress Series* vol. 146, pp.163-171.

Jacq, V.A., Prade, K., and Ottow, J.C.G. 1991, “Iron sulphide accumulation in the rhizosphere of wetland rice (Oryza sativa L.) as the result of microbial activities”, in *Diversity of Environmental Biogeochemistry* (ed. J. Berthelin) pp. 453-468.

Jorgenson, K.D., Lee, P.F. & Kanavillil, N. 2013, "Ecological relationships of wild rice, Zizania spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*)", *Botany,* vol. 91, no. 3, pp. 189-201.

Joshi, M.M., Ibrahim, I.K.A., and Hollis, J.P. 1975, “Hydrogen sulfide: effects on the physiology of rice plants and relation to straighthead disease”, *Phytopathology*, vol 65, pp. 1165-1170.

Koch, M.S., and Mendelssohn, I.A. 1989, “Sulphide as a soil phototoxin: differential responses in two marsh species”, *British Ecological Society*, vol 77, no. 2, pp.565-578.

Koch, M.S., Mendelssohn, I.A., and McKee, K.L. 1990 “Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes”, *Limnology and Oceanography*, vol. 35, no. 2, pp. 399-408.

Krishnamurthy, P., Ranathunge, K., Franke, R., Prakash, H.S., Schreiber, L. & Mathew, M.K. 2009, "The role of root apoplastic transport barriers in salt tolerance of rice (*Oryza sativa L.*)", *Planta,* vol. 230, no. 1, pp. 119-134.

Kwon, M.J., Boyanov, M.I., Antonopoulos, D.A., Brulc, J.M., Johnston, E.R., Skinner, K.A., Kemner, K.M. & O'Loughlin, E.J. 2014, "Effects of dissimilatory sulfate reduction on Fe-III (hydr)oxide reduction and microbial community development", *Geochimica et Cosmochimica Acta,* vol. 129, pp. 177-190.

Lamers, L.P.M., Govers, L.L., Janssen, I.C.J.M., Geurts, J.J.M., Van der Welle, M.E.W., Van Katwijk, M.M., Van der Heide, T., Roelofs, J.G.M. & Smolders, A.J.P. 2013, "Sulfide as a soil phytotoxin-a review", *Frontiers in Plant Science,* vol. 4, pp. UNSP 268.

Lamers, L., Falla, S., Samborska, E., van Dulken, L., van Hengstum, G. & Roelofs, J. 2002, "Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands", *Limnology and Oceanography,* vol. 47, no. 2, pp. 585-593.

Lee, P. & McNaughton, K. 2004, "Macrophyte induced microchemical changes in the water column of a northern Boreal Lake", *Hydrobiologia,* vol. 522, no. 1-3, pp. 207-220.

Lee, K., and Dunton, K.H. 2000, “Diurnal changes in pore water sulfide concentrations in the seagrass *Thalassia testudinum* beds: the effects of seagrasses on sulfide dynamics”, *Journal of Experimental Marine Biology and Ecology*, vol 255, pp. 201-214.

Martin, N.M. & Maricle, B.R. 2015, "Species-specific enzymatic tolerance of sulfide toxicity in plant roots", *Plant Physiology and Biochemistry,* vol. 88, pp. 36-41.

Mendelssohn, I., Kleiss, B. & Wakeley, J. 1995, "Factors Controlling the Formation of Oxidized Root Channels - a Review", *Wetlands,* vol. 15, no. 1, pp. 37-46.

Mendelssohn, I.A., and Postek, M.T. 1982, “Elemental analysis of deposits on the roots of *Spartina alterniflora Loisel*.”, *American Journal of Botany* vol 69, no. 6, pp. 904-912.

Minnesota Pollution Control Agency. 2014. Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review.

Moyle, J. 1944. Wild rice in Minnesota. *Journal of Wildlife Management* 8:177-184.

Neubauer, S.C., Toledo-Duran, G.E., Emerson, D. & Megonigal, J.P. 2007, "Returning to their roots: Iron-oxidizing bacteria enhance short-term plaque formation in the wetland-plant rhizosphere", *Geomicrobiology Journal,* vol. 24, no. 1, pp. 65-73.

Rickard, D. 1995, “Kinetics of FeS Precipitation, Part 1. Competing Reaction Mechanisms”, Geochimica et Cosmochimica Acta vol. 59, no. 21, pp. 4367-4379.

Ruiz-Halpern, S., Macko, S.A. & Fourqurean, J.W. 2008, "The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment", *Biogeochemistry,* vol. 87, no. 2, pp. 113-126.

Schmidt, H., Eickhorst, T. & Tippkoetter, R. 2011, "Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis", *Plant and Soil,* vol. 341, no. 1-2, pp. 221-232.

Seeberg-Elverfeldt, J., Koelling, M., Schluter, M. & Feseker, T. 2005, "Rhizon in situ sampler (RISS) for pore water sampling from aquatic sediments", *Abstracts of Papers of the American Chemical Society,* vol. 230, pp. U1763-U1764.

Smolders, A., Lamers, L., den Hartog, C. & Roelofs, J. 2003, "Mechanisms involved in the decline of *Stratiotes aloides L*. in The Netherlands: sulphate as a key variable", *Hydrobiologia,* vol. 506, no. 1-3, pp. 603-610.

Smolders, A. & Roelofs, J. 1996, "The roles of internal iron hydroxide precipitation, sulphide toxicity and oxidizing ability in the survival of *Stratiotes aloides* roots at different iron concentrations in sediment pore water", *New Phytologist,* vol. 133, no. 2, pp. 253-260.

Snowden, R. & Wheeler, B. 1995, "Chemical changes in selected wetland plant species with increasing Fe supply, with specific reference to root precipitates and Fe tolerance", *New Phytologist,* vol. 131, no. 4, pp. 503-520.

Soukup, A., Armstrong, W., Schreiber, L., Franke, R. & Votrubova, O. 2007, "Apoplastic barriers to radial oxygen loss and solute penetration: a chemical and functional comparison of the exodermis of two wetland species, *Phragmites australis* and *Glyceria maxima*", *New Phytologist,* vol. 173, no. 2, pp. 264-278.

St Cyr, L., Fortin, D. & Campbell, P. 1993, "Microscopic Observations of the Iron Plaque of a Submerged Aquatic Plant (*Vallisneria-Americana michx*)", *Aquatic Botany,* vol. 46, no. 2, pp. 155-167.

Stumm, W., and Morgan, J.J. 1995, Aquatic Chemistry, 3rd edition.

Sun, M., Xiao, T., Ning, Z., Xiao, E. & Sun, W. 2015, "Microbial community analysis in rice paddy soils irrigated by acid mine drainage contaminated water", *Applied Microbiology and Biotechnology,* vol. 99, no. 6, pp. 2911-2922.

Thamdrup, B., Fossing, H., & Jorgenson, B.B. 1994, "Manganese, iron, and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark", *Geochimica et Cosmochimica Acta*, vol. 58, no. 23, pp. 5115-5129.

VanCappellen, P. V., Wang, Y. 1996. "Cycling of iron and manganese in surficial sediments", *Am. J. Sci.* vol. *296*, pp. 197–243.

Wang, M., Glass, A., Shaff, J. & Kochian, L. 1994, "Ammonium Uptake by Rice Roots .3. Electrophysiology", *Plant Physiology*, vol. 104, no. 3, pp. 899-906.

Appendix

Table 1. Average and standard deviation of the saturation index in sulfate amended and control pore waters. The K_{sp} value used was $10^{-2.95}$.

Date (julian)	Sulfate-amended	Control
177	-1.436 ± 0.228	-1.436 ± 0.228
190	-0.282 ± 0.346	-0.175 ± 0.354
203	-0.390 ± 0.189	-1.061 ± 0.204
232	-0.560 ± 0.195	-0.802 ± 0.242
239	0.099 ± 0.969	-0.232 ± 0.435
245	-0.140 ± 0.580	-0.410 ± 0.837
256	-0.302 ± 0.376	-0.365 ± 0.333
263	-0.199 ± 0.198	-0.597 ±0.581

Figure 1. Sulfate-amended root (left) and control root (right). Sulfate-amended root has black color extending from about 0.5 cm above the root ball down to the tips of the roots (not shown). Control root has amber color characteristic of iron (hydr)oxides, especially 2-3 cm below root ball.

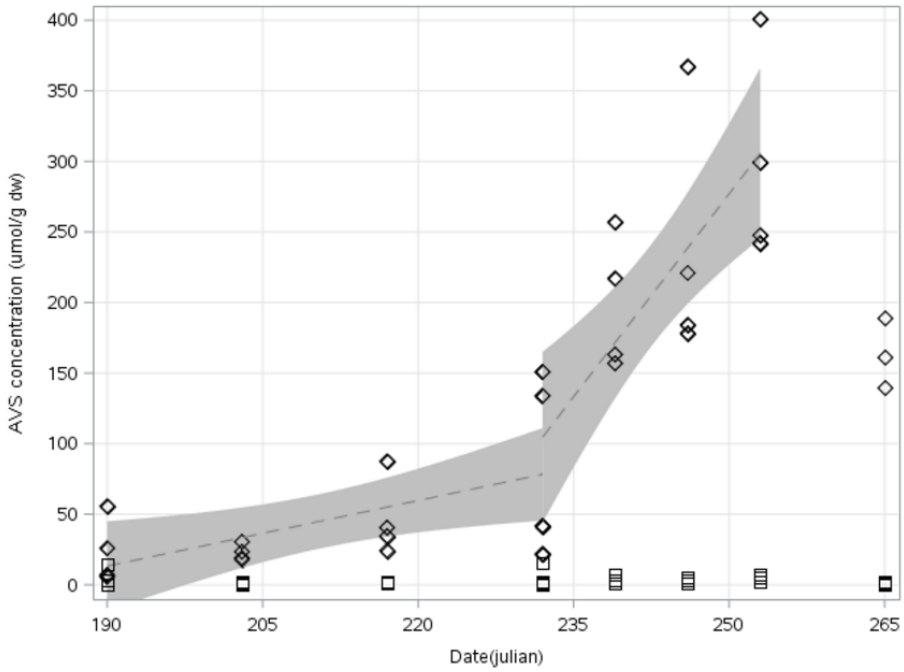


Figure 2. A 95% confidence interval around a regression of time and AVS on sulfate amended roots depicting the change in rate of sulfide accumulation. Diamonds represent sulfate amended plants, and squares represent control plants. The plant is in the flowering stage until day 232, when it starts producing seeds. The last sample date was during senescence, and is therefore not included in the 95% confidence interval.

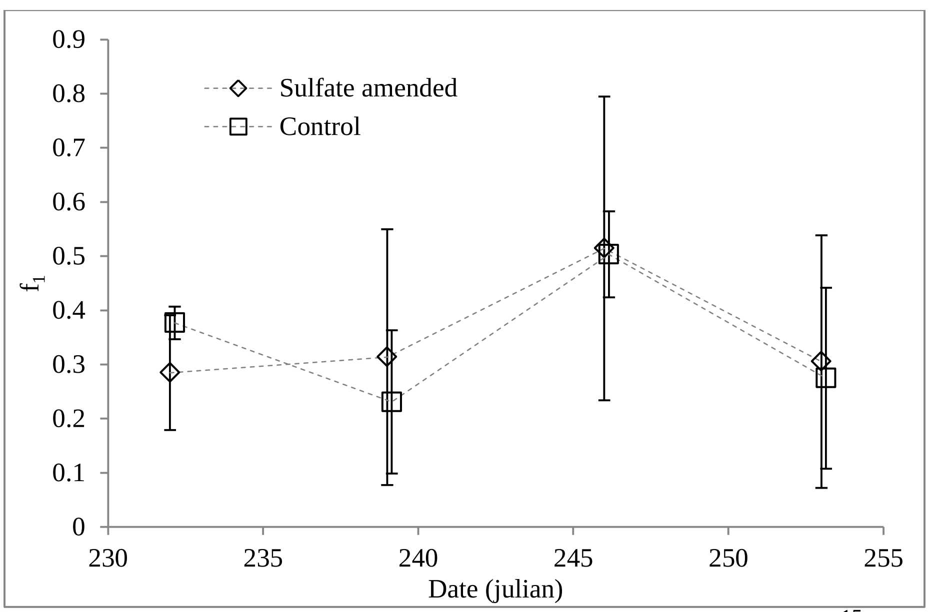


Figure 3. Isotopic mixing model showing the proportion (f_1) of $\delta^{15}N$ in seeds that originated from ammonium in the pore water during seed production. Diamonds represent sulfate amended plants, and squares represent control plants. Each data point is the average of four replicates. Error bars are one standard deviation.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment G
(13 pages)

LacCore_field_ID	Site_name	Unique site ID	DNR/State ID	Date	Lat	Long	Calculated Wild rice ave stems/m2	surface water SO4 (mg SO4/L)	pore water Total Sulfide (TS, mg S/L)	Sediment Fe (µg/g)	Sediment TOC (%)	potential SO4 standard CPSC120
P-35	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7377	3.0	2.23	0.493	2170	14.84	1.2
FS-192	Anka	26	21-0353-00-202	8/29/12	46.07689	-95.7292	2.3	8.44	0.53	1498	22.85	0.4
P-34	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7292	25.9	2.23	0.671	1485	23.57	0.3
FS-134	Bass	43	31-0576-00-207	9/18/12	47.2844	-93.6276	64.0	1.01	0.0664	3740	26.12	1.8
FS-85	Bean	8	03-0411-00-201	8/21/12	46.9337	-95.8706	0.0	85	16	1967	11.85	1.4
FS-87	Bee	60	60-0192-00-202	8/23/12	47.6527	-96.0504	39.8	11	0.67	3054	13.62	2.7
FS-193	Big Mud	79	71-0085-00-201	8/30/12	45.4529	-93.7418	14.3	< 0.5	0.0308	12943	18.63	29.5
FS-216	Big Sucker	39	31-0124-00-203	9/12/12	47.3919	-93.2658	3.8	7.78	0.145	3559	21.45	2.1
FS-205	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.7418	56.3	5.47	0.0527	1719	4.81	3.1
FS-204	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.742	133.7	5.49	0.0914	1731	5.94	2.4
FS-89	Birch	67	69-0003-00-205	9/10/12	47.7358	-91.943	33.1	8.61	0.1	16938	31.2	26.7
P-12	Birch	67	69-0003-00-205	8/30/11	47.7357	-91.9428	68.6	3.58	0.104	12431	26.8	17.7
FS-52	Blaamyhre	48	34-0345-00-203	8/1/12	45.364	-95.186	102.2	0.62	0.078	3517	9.33	5.5
FS-214	Bowstring	116	S007-219	9/11/12	47.7024	-94.0608	69.7	1.34	0.256	1974	24.34	0.6
FS-126	Bray	58	56-0472-00-202	8/20/12	46.4518	-95.8783	7.6	1.65	0.072	3937	21.95	2.5
FS-63	Caribou	72	69-0489-00-206	9/3/12	46.8913	-92.3135	0.0	1.21	0.0938	13791	29.44	19.3
P-53	Carlos Avery Pool 9	4	02-0504-00-201	8/19/11	45.3179	-93.0587	43.0	0.35	0.029	37965	16.51	270.0
FS-109	Carlos Avery Pool 9	4	02-0504-00-202	7/3/12	45.3192	-93.0611	52.8	< 0.5	< 0.011	14736	12.51	61.0
FS-339	Christina	28	21-0375-00-315	7/31/13	46.0734	-95.7567	0.6	14.6	1.93	1741	8.96	1.5
FS-373	Clearwater	96	S002-121	9/9/13	47.9372	-95.6909	3.2	34.4	0.0354	5315	3.33	41.8
FS-189	Clearwater	96	S002-121	8/28/12	47.9372	-95.6906	4.5	23.8	0.117	2856	1.27	40.2

FS-327	Clearwater	96	S002-121	7/17/13	47.9371	-95.6906	0.3	23.7	0.117	3521	1.82	39.1
FS-314	Clearwater	96	S002-121	6/24/13	47.9372	-95.6907	0.6	28	0.0664	3946	2.68	30.6
FS-337	Clearwater	98	S004-204	7/29/13	47.5175	-95.3906	69.1	0.95	0.0608	14564	24.58	26.6
FS-88	Clearwater	98	S004-204	8/24/12	47.5174	-95.3904	148.3	2.04	0.0488	9874	22.17	14.2
P-31	Cloquet	52	38-0539-00-201	9/14/11	47.4313	-91.4844	74.4	0.81	0.024	4252	6.58	12.1
FS-128	Cromwell	14	14-0103-00-201	8/22/12	46.9651	-96.3171	0.0	41.2	1.22	2948	2.85	16.2
FS-369	Dark	77	69-0790-00-202	9/5/13	47.6389	-92.7781	11.8	176	0.052	2037	0.82	35.4
FS-352	Dark	77	69-0790-00-202	8/15/13	47.6388	-92.7782	2.9	173	0.136	5120	3.61	35.3
FS-368	Dark	77	69-0790-00-202	9/5/13	47.6387	-92.7782	11.1	175	0.305	3354	1.94	33.0
FS-322	Dark	77	69-0790-00-202	7/10/13	47.6389	-92.7781	3.2	175	0.131	2480	1.48	25.5
FS-64	Dead Fish	12	09-0051-00-202	9/4/12	46.7454	-92.6865	0.0	0.71	0.0608	14387	22.4	29.0
P-44	Dead Fish	12	09-0051-00-202	9/20/11	46.7451	-92.6863	48.7	0.3	0.056	9685	16.6	19.4
FS-378	Duck Lake WMA	22	18-0178-00-202	9/12/13	46.7521	-93.8851	113.0	< 0.5	0.0251	12151	26.57	17.1
FS-86	Eighteen	61	60-0199-00-202	8/22/12	47.6397	-96.0607	40.1	4.29	0.164	1860	3.1	6.1
FS-309	Eighteen	62	60-0199-00-203	6/13/13	47.6369	-96.0599	0.0	4.36	0.127	4478	16.52	4.4
FS-328	Eighteen	62	60-0199-00-203	7/18/13	47.6369	-96.0599	44.2	3.34	0.25	5106	24.65	3.5
FS-359	Eighteen	62	60-0199-00-203	8/20/13	47.6367	-96.06	21.0	2.83	0.118	5500	30.88	3.1
P-6	Elk	15	15-0010-00-203	8/25/11	47.1946	-95.2254	25.9	0.28	0.04	8480	10.24	26.8
FS-137	Elk	15	15-0010-00-204	9/19/12	47.1952	-95.2249	42.7	< 0.5	0.0936	6334	10.07	15.6
FS-333	Embarrass	73	69-0496-00-203	7/26/13	47.5333	-92.2976	0.0	18.2	0.0866	11179	0.47	1821.2
FS-95	Embarrass	73	69-0496-00-203	9/14/12	47.5334	-92.2979	0.0	18.8	0.0298	21847	1.89	1248.9
FS-76	Field	45	34-0151-00-201	7/25/12	45.2964	-94.9058	0.0	< 0.5	0.0687	7586	8.68	26.3
FS-195	Fisher	78	70-0087-00-201	8/31/12	44.7942	-93.4061	20.7	6.85	0.136	11140	5.76	90.1
FS-81	Flowage	1	01-0061-00-204	8/7/12	46.688	-93.337	0.0	0.78	0.134	12470	32.34	14.2
P-51	Flowage	1	01-0061-00-205	9/22/11	46.6896	-93.338	160.2	0.56	0.014	5627	20.1	5.4
P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	0.56	0.018	4641	18.1	4.2
P-52	Flowage	1	01-0061-00-205	9/22/11	46.6895	-93.338	123.1	0.56	0.018	3706	16.52	3.1

P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	0.56	0.018	4302	21.79	2.9
FS-194	Gilchrist	91	86-0064-00-201	8/31/12	45.2309	-93.824	0.0	6.98	0.355	3117	20.81	1.7
FS-51	Glesne Slough	49	34-0353-00-201	7/31/12	45.3514	-95.1887	99.6	< 0.5	0.061	7983	3.01	103.2
P-23	Gourd	10	04-0253-00-201	9/7/11	47.812	-94.9654	38.4	0.69	0.038	2675	27.4	0.9
FS-104	Gourd	10	04-0253-00-201	6/27/12	47.8121	-94.965	0.0	0.27		1776	36.87	0.3
FS-213	Gull	9	04-0120-00-204	9/10/12	47.6558	-94.6945	9.5	1.14	0.0778	3527	16.01	2.9
P-20	Gull	9	04-0120-00-203	9/6/11	47.6559	-94.6944	15.6	0.78	0.103	1608	5.08	2.5
FS-367	Hay	33	31-0037-00-202	9/4/13	47.287	-93.1009	141.0	22.1	0.0447	15436	3.44	312.7
P-45	Hay	33	31-0037-00-201	9/21/11	47.2874	-93.1017	0.0	10.24	0.087	12403	4.36	154.6
P-46	Hay	33	31-0037-00-201	9/21/11	47.2869	-93.1018	0.0	10.24	0.026	16139	7.69	130.0
FS-130	Hay	33	31-0037-00-202	9/6/12	47.2874	-93.102	141.0	31.7	0.0738	13154	5.79	123.3
FS-221	Hay Creek Flowage	59	58-0005-00-202	9/17/12	46.0894	-92.4104	97.7	1.95	0.119	9456	22.05	13.2
FS-375	Height of Land	5	03-0195-00-210	9/10/13	46.913	-95.6111	117.5	< 0.5	< 0.011	1795	0.86	26.2
FS-127	Height of Land	5	03-0195-00-210	8/21/12	46.9133	-95.6095	111.1	< 0.5	< 0.011	2112	1.32	21.5
FS-318	Height of Land	5	03-0195-00-210	6/26/13	46.9135	-95.6124	43.0	1.21	0.0658	1349	1.13	10.9
FS-338	Height of Land	5	03-0195-00-210	7/30/13	46.913	-95.6116	94.2	< 0.5	0.0554	2641	4.58	7.4
P-1	Height of Land	5	03-0195-00-209	8/22/11	46.9129	-95.6095	62.9	0.24	0.053	1298	1.76	6.0
FS-131	Hinken	113	S007-207	9/5/12	47.7271	-93.9923	46.8	< 0.5	0.0876	2960	4.53	9.4
FS-185	Hoffs Slough	85	76-0103-00-201	8/1/12	45.3255	-95.7059	0.0	273	0.0343	3512	0.75	112.3
FS-353	Holman	42	31-0227-00-202	8/12/13	47.3009	-93.3444	0.0	68	0.583	5094	30.6	2.7
FS-218	Holman	42	31-0227-00-202	9/13/12	47.3005	-93.3445	0.0	24.2	1.01	3035	29.74	1.0
FS-182	Hunt	65	66-0047-00-208	7/27/12	44.3275	-93.4443	0.0	17.1	0.0729	2412	1.21	30.8
FS-191	Ina	27	21-0355-00-202	8/29/12	46.0715	-95.7281	30.2	7.08	0.274	2216	9.09	2.3
FS-136	Itasca	16	15-0016-00-208	9/19/12	47.2343	-95.2049	23.6	< 0.5	0.0636	1496	2.23	5.9
P-7	Itasca	16	15-0016-00-207	8/25/11	47.2332	-95.1985	20.1	0.26	0.064	1650	6.01	2.2
P-5	Itasca	16	15-0016-00-208	8/25/11	47.2381	-95.2065	45.8	0.26	0.056	1355	7.4	1.2
FS-207	Kelly Lake	64	66-0015-00-204	8/13/12	44.3542	-93.3743	0.0	1.92	0.0927	4387	27.33	2.3

FS-79	Lady Slipper	53	42-0020-00-203	7/27/12	44.5723	-95.6216	0.0	330	1.63	3314	1.85	34.1
FS-78	Lady Slipper	53	42-0020-00-202	7/27/12	44.5699	-95.6275	0.0	335	1.68	2719	1.66	26.5
P-55	Lady Slipper	53	42-0020-00-204	9/22/11	44.5702	-95.6274	0.0	107.71	14.84	2814	2.09	21.5
P-61	Lily	90	81-0067-00-202	9/28/11	44.194	-93.6469	51.5	0.66	0.041	6180	14.06	10.0
P-62	Lily	90	81-0067-00-202	9/28/11	44.194	-93.6469	0.0	0.64		5069	13.39	7.2
FS-180	Lily	90	81-0067-00-202	7/26/12	44.1947	-93.647	38.2	< 0.5	0.0295	5095	28.07	3.0
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.05	4503	4.46	21.4
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	2236	1.75	17.1
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	3544	5.11	11.5
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	2253	8.37	2.7
FS-54	Little Birch	87	77-0089-00-207	8/3/12	45.7779	-94.7978	70.0	7.4	0.0353	1794	6.02	2.6
P-4	Little Flat	6	03-0217-00-201	8/24/11	46.9981	-95.6641	83.1	0.22	0.011	7479	33.13	5.2
FS-250	Little Rice	75	69-0612-00-201	9/20/12	47.7086	-92.4389	29.3	1.03	0.0293	9488	26.45	10.7
FS-342	Little Round	7	03-0302-00-203	8/5/13	46.9721	-95.7358	58.3	< 0.5	0.0676	4447	25.16	2.6
FS-138	Little Round	7	03-0302-00-203	9/20/12	46.9726	-95.735	78.0	< 0.5	0.128	3069	27.48	1.2
FS-374	Little Round	7	03-0302-00-202	9/10/13	46.9745	-95.738	37.6	0.12	0.0391	2018	14.8	1.1
FS-319	Little Round	7	03-0302-00-203	6/27/13	46.9724	-95.735	17.5	< 0.5	0.117	3579	39.84	1.0
P-3	Little Round	7	03-0302-00-202	8/24/11	46.9759	-95.7404	57.2	0.46	0.032	1689	20.91	0.5
FS-223	Little Sucker	40	31-0126-00-202	9/14/12	47.3765	-93.246	0.0	13.7	0.534	6297	16.56	8.5
FS-203	Long Prairie	110	S007-203	8/9/12	45.9729	-95.1603	58.3	6.66	0.0391	5074	4.35	27.8
FS-202	Long Prairie	110	S007-204	8/9/12	46.0072	-95.2634	13.4	7.71	0.0793	2897	2.85	15.7
FS-200	Louisa	94	86-0282-00-205	8/8/12	45.2998	-94.258	0.0	7.04	0.192	7824	8.76	27.6
FS-226	Louise	25	21-0094-00-202	8/14/12	45.9331	-95.4148	46.5	4.09	0.0746	1833	0.83	28.5
FS-60	Lower Panasa	38	31-0112-00-205	8/29/12	47.3018	-93.2521	0.0	33.6	0.243	8048	14.12	16.5
FS-357	Lower Panasa	38	31-0112-00-204	8/15/13	47.3026	-93.2561	0.0	28.5	1.26	2347	2.42	12.7
P-25	Lower Rice	107	S006-985	9/8/11	47.3793	-95.4834	114.4	1.02	0.097	2337	17.76	1.2
P-26	Lower Rice	109	S007-164	9/8/11	47.3817	-95.4926	120.1	0.55	0.07	2364	6.76	3.8

FS-133	Mahnomen	21	18-0126-02-201	9/17/12	46.4985	-93.9958	0.0	16.9	0.308	18746	7.7	173.2
FS-377	Mahnomen	21	18-0126-02-201	9/11/13	46.4986	-93.9956	0.0	21.1	0.0283	16540	7.47	141.1
FS-175	Maloney	88	79-0001-00-201	7/23/12	44.2251	-91.9321	0.0	3.15	0.0608	15126	4.57	214.0
P-64	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	0.0	1.83		10382	4.05	119.9
P-63	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	148.7	1.83	0.01	10269	4.24	111.2
FS-187	McCormic	81	73-0273-00-203	8/2/12	45.722	-94.9121	8.9	1.54	0.144	1512	1.1	14.0
FS-230	Mill Pond	23	21-0034-00-202	8/16/12	46.0715	-95.2218	80.9	7.36	0.192	3969	3.14	25.6
FS-229	Mill Pond	23	21-0034-00-202	8/16/12	46.0716	-95.2218	102.2	7.16	0.109	5143	7.86	14.0
FS-225	Miltona	24	21-0083-00-205	8/13/12	46.0496	-95.4217	0.0	4.11	0.0694	2624	1.77	22.9
FS-201	Mink	92	86-0229-00-206	8/8/12	45.274	-94.0269	0.0	1.31	0.0373	1740	1.53	12.4
FS-129	Mink	92	86-0229-00-207	8/23/12	45.2767	-94.0299	0.0	1.22	0.182	4247	13.63	5.0
FS-80	Mission	95	S001-646	8/6/12	45.8623	-93.0011	87.5	0.62	0.0485	9231	4.83	77.5
FS-83	Mississippi Crow Win	111	S007-205	8/8/12	46.4386	-94.1251	0.0	3.13	0.127	13451	3.88	207.8
FS-211	Mississippi Pool 4/Ro	89	79-0005-02-201	8/16/12	44.3611	-91.9897	57.6	17.7	0.0714	9265	1.55	304.2
FS-336	Mississippi Pool 4/Ro	89	79-0005-02-201	7/30/13	44.3613	-91.9901	46.5	55.3	0.0602	8193	1.41	269.0
FS-210	Mississippi Pool 4/Ro	89	79-0005-02-202	8/16/12	44.3593	-91.9881	35.3	15.7	0.07	6450	1.16	214.5
FS-371	Mississippi Pool 5 / S	123	S007-660	9/10/13	44.2016	-91.8443	39.8	34.4	0.069	3582	0.11	1161.0
FS-335	Mississippi Pool 5 / S	123	S007-660	7/30/13	44.1953	-91.841	63.0	47.7	0.0342	4362	0.25	634.7
FS-212	Mississippi Pool 5 / S	123	S007-660	8/17/12	44.1993	-91.8461	29.6	17.2	0.0224	3674	0.22	531.7
FS-372	Mississippi Pool 5 / S	123	S007-660	9/10/13	44.2016	-91.8443	26.7	34.8	0.0536	3330	0.33	270.9
FS-312	Mississippi Pool 5 / S	123	S007-660	6/21/13	44.2018	-91.8444	35.7	28.3	0.0844	3563	0.67	132.2
FS-370	Mississippi Pool 8 at	118	S007-222	9/9/13	43.5765	-91.2337	17.8	33.3	0.062	6558	1.43	172.4
FS-208	Mississippi Pool 8 at	118	S007-222	8/14/12	43.5758	-91.2334	41.4	18	0.176	2178	0.41	92.3
FS-334	Mississippi Pool 8 at	118	S007-222	7/29/13	43.5758	-91.2344	52.8	44.2	0.102	1969	0.4	78.3
FS-311	Mississippi Pool 8 at	118	S007-222	6/20/13	43.5766	-91.2341	12.7	29.3	0.107	1544	0.62	29.0
FS-209	Mississippi Pool 8 at	122	S007-556	8/15/12	43.6025	-91.2686	72.3	18.1	0.0711	9187	2.29	187.6
P-14	Mississippi River abo	108	S007-163	9/1/11	47.2379	-93.7196	163.2	1.09	0.053	7964	6.43	41.4

FS-354	Mississippi River abov	108	S007-163	8/13/13	47.2376	-93.7187	132.7	1.18	0.0532	7052	5.76	37.4
FS-58	Mississippi River abov	108	S007-163	8/28/12	47.2386	-93.7197	0.0	1.19	0.0806	8636	9.08	32.0
FS-57	Mississippi River belo	103	S006-923	8/28/12	47.2551	-93.6342	0.0	10.3	0.134	4225	1.2	91.3
P-15	Mississippi River belo	103	S006-923	9/1/11	47.2547	-93.6344	100.2	3.65	0.035	8667	6.07	52.2
FS-355	Mississippi River belo	103	S006-923	8/13/13	47.2553	-93.634	78.3	10.2	0.0819	10479	8.98	47.1
FS-313	Monongalia	46	34-0158-01-203	6/23/13	45.3334	-94.9293	50.0	34.7	0.0941	6028	19.44	6.4
FS-340	Monongalia	46	34-0158-02-203	7/31/13	45.3331	-94.9292	87.9	33.6	0.122	5530	22.1	4.7
FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.3332	-94.9292	154.4	34.6	0.242	5436	26.42	3.7
P-42	Monongalia (Middle F	45.5	34-0158-01-201	9/20/11	45.3481	-94.9509	5.7	16.51	0.042	46471	14.76	455.4
FS-77	Monongalia (near hw	46	34-0158-02-204	7/26/12	45.3331	-94.9268	121.3	21.7	1.37	4953	18.66	4.6
FS-75	Mortenson	44	34-0150-02-201	7/24/12	45.3	-94.9062	0.0	< 0.5	0.103	9071	12.09	25.0
FS-176	North Geneva	29	24-0015-00-209	7/24/12	43.7876	-93.271	0.0	15.6	1.54	2212	13.45	1.5
FS-132	Ox Hide	35	31-0106-00-203	9/7/12	47.335	-93.2134	10.5	26.4	0.042	14936	14.43	52.7
FS-198	Ox Hide	35	31-0106-00-203	9/7/12	47.335	-93.2134	0.6	26.4	0.0751	8743	24.51	10.0
FS-350	Ox Hide	35	31-0106-00-203	8/14/13	47.3351	-93.2132	0.0	25.9	0.119	3889	12.12	4.9
FS-344	Padua	82	73-0277-00-202	8/6/13	45.6231	-95.0187	9.5	< 0.5	0.0806	4520	12.61	6.2
P-29	Padua	82	73-0277-00-203	9/13/11	45.6202	-95.0192	3.4	0.76	0.13	4927	20.15	4.2
FS-220	Padua	82	73-0277-00-202	8/7/12	45.623	-95.0186	0.0	0.86	0.23	2291	9.77	2.3
FS-92	Partridge	119	S007-443	9/12/12	47.5207	-92.1909	4.1	36.3	0.0741	29463	5.87	571.7
P-13	Partridge	119	S007-443	8/31/11	47.5212	-92.1899	65.9	10.39	0.075	11026	1.44	464.3
FS-331	Partridge	119	S007-443	7/24/13	47.5212	-92.1904	60.5	14.6	0.112	10082	1.68	325.0
FS-366	Partridge	119	S007-443	9/3/13	47.5213	-92.19	47.7	34.2	0.057	7671	1.79	178.1
FS-365	Partridge	119	S007-443	9/3/13	47.5212	-92.1901	76.7	34.1	0.0393	9179	2.5	168.6
FS-301	Partridge	119	S007-443	5/28/13	47.5213	-92.1903	0.0	14.8	0.125	9491	3.94	104.3
FS-302	Partridge	121	S007-513	5/30/13	47.5153	-92.1894	0.0	43.1	0.0624	24784	6.27	378.8
FS-364	Partridge	121	S007-513	8/30/13	47.5138	-92.1894	105.7			28890	8.19	369.5
FS-332	Partridge	121	S007-513	7/24/13	47.5137	-92.1894	79.6	54.4	0.102	20512	8.34	187.1

FS-316	Partridge	121	S007-513	6/28/13	47.5137	-92.1899	0.0	24.9	0.098	6291	2.6	77.8
FS-55	Pelkey	55	49-0030-00-202	8/26/12	45.9962	-94.2273	0.0	3.42	0.0522	30642	17.32	168.8
P-10	Pike	104	S006-927	8/30/11	47.7325	-92.3468	43.0	8.31	0.063	15572	10.9	80.0
FS-91	Pike	104	S006-927	9/11/12	47.7327	-92.3473	3.5	14.2	0.0656	6565	4.72	41.4
FS-190	Pine	18	15-0149-00-205	8/28/12	47.6841	-95.5414	114.9	14.7	0.368	4477	7.08	12.2
FS-84	Pleasant	13	11-0383-00-207	8/10/12	46.9228	-94.4874	0.0	< 0.5	0.0218	7065	23.99	6.8
P-27	Pleasant	13	11-0383-00-206	9/9/11	46.928	-94.4757	28.6	0.49		5331	30.37	3.0
FS-215	Popple	101	S006-188	9/11/12	47.7254	-94.0817	36.3	< 0.5	0.0269	2971	14.42	2.4
FS-196	Prairie	115	S007-209	9/3/12	47.2519	-93.4884	44.6	9.63	0.0709	15071	10.51	78.4
FS-82	Rabbit	20	18-0093-02-204	8/8/12	46.5313	-93.9285	0.0	15.3	0.22	10903	11.79	36.7
P-28	Raymond	83	73-0285-00-203	9/12/11	45.629	-95.0234	68.6	0.82	0.094	3922	10.06	6.2
FS-343	Raymond	83	73-0285-00-203	8/6/13	45.629	-95.0233	61.4	1.92	0.0903	3270	7.59	6.1
FS-53	Raymond	83	73-0285-00-203	8/2/12	45.6286	-95.0225	61.1	< 0.5	0.0787	1905	4.79	3.8
FS-56	Rice	19	18-0053-00-203	8/27/12	46.3389	-93.8915	19.4	< 0.5	0.0259	83421	31.88	558.1
FS-376	Rice	19	18-0053-00-203	9/11/13	46.3394	-93.8918	46.5	< 0.5	0.0451	65261	33.36	329.7
P-69	Rice	19	18-0053-00-203	9/27/11	46.3394	-93.8913	43.0	0.23	0.021	50389	35.55	185.8
FS-304	Rice	19	18-0053-00-203	6/10/13	46.3387	-93.8906	5.7	< 0.5	0.0236	48287	33.61	183.1
FS-324	Rice	19	18-0053-00-203	7/15/13	46.3392	-93.8918	56.7	< 0.5	0.11	44704	33.18	160.3
FS-181	Rice	66	66-0048-00-203	7/27/12	44.3332	-93.4734	0.0	5.22	0.777	3829	21.67	2.4
FS-345	Rice	80	73-0196-00-216	8/7/13	45.3865	-94.6313	0.0	6.85	2.08	2012	14.83	1.1
FS-184	Rice	80	73-0196-00-216	7/30/12	45.3864	-94.6309	0.0	2.58	2.97	1523	15.03	0.6
FS-179	Rice	84	74-0001-00-201	7/25/12	44.0842	-93.0737	0.0	3.84	0.217	4152	19.07	3.2
FS-199	Rice	102	S006-208	9/5/12	47.6742	-93.6547	75.4	1.57	0.0552	3273	10.88	4.0
FS-231	Rice	2	02-0008-00-206	8/17/12	45.1604	-93.121	0.0	3.6	0.145	2159	7.98	2.6
P-11	Sand	97	S003-249	8/30/11	47.6348	-92.4235	14.4	7.69	0.046	22677	17.49	93.5
FS-90	Sand	97	S003-249	9/11/12	47.6351	-92.4234	2.9	15.9	0.152	7287	9.68	21.4
FS-321	Sandy-1	76	69-0730-00-203	7/9/13	47.6255	-92.5885	0.0	122	0.189	36502	29.51	124.9

FS-306	Sandy-1	76	69-0730-00-203	6/11/13	47.6255	-92.5884	0.0	11	0.0918	35357	28.53	122.3
FS-251	Sandy-1	76	69-0730-00-203	9/21/12	47.6254	-92.5886	3.8	3.05	0.123	35905	33.08	105.5
FS-382	Sandy-1	76	69-0730-00-203	9/17/13	47.6255	-92.5885	0.0	67.9	0.135	26645	32.28	61.2
FS-320	Sandy-2	76	69-0730-00-204	7/9/13	47.6188	-92.5936	0.0	118	3.08	19749	15.43	83.3
FS-348	Sandy-2	76	69-0730-00-204	8/13/13	47.6186	-92.5934	0.0	123	0.305	13216	8.23	81.6
FS-381	Sandy-2	76	69-0730-00-204	9/17/13	47.6187	-92.5931	0.0	126	0.0342	16172	11.67	79.2
FS-305	Sandy-2	76	69-0730-00-204	6/11/13	47.6187	-92.5937	0.0	135	1.08	19094	22.23	50.4
FS-380	Sandy-2	76	69-0730-00-204	9/17/13	47.6187	-92.5939	0.6	126	0.0342	17868	22.7	43.3
FS-349	Sandy-3	76	69-0730-00-205	8/13/13	47.6191	-92.5898	0.0	122	0.0697	14897	20.46	34.6
P-24	Second	17	15-0091-00-201	9/7/11	47.8255	-95.3635	37.3	0.87	0.139	3813	25.67	1.9
FS-105	Second	17	15-0091-00-202	6/27/12	47.8258	-95.3637	48.4	0.74	0.119	2527	33.3	0.6
FS-310	Second	117	S007-220	6/14/13	47.5205	-92.1925	57.6	316	0.0927	31190	4.22	946.8
FS-384	Second	117	S007-220	9/19/13	47.5204	-92.1925	27.7		0.104	22634	3.42	657.3
FS-303	Second	117	S007-220	5/30/13	47.5204	-92.1925	0.0	303	0.0991	13086	2.2	388.6
FS-323	Second	117	S007-220	7/11/13	47.5204	-92.1925	76.4	405	0.067	10036	2.91	166.9
FS-351	Second	117	S007-220	8/15/13	47.5205	-92.1925	66.8	838	0.0447	7088	1.84	148.0
FS-197	Snowball	36	31-0108-00-202	9/4/12	47.3355	-93.244	0.0	8.4	0.0936	4213	6	13.2
FS-347	Snowball	36	31-0108-00-202	8/12/13	47.3356	-93.2439	0.0	8.2	0.097	1136	1.19	7.4
FS-177	South Geneva	30	24-0015-02-208	7/24/12	43.7709	-93.2851	0.0	14.1	3.19	1618	16.71	0.6
P-16	St. Louis	106	S006-929	9/1/11	47.4015	-92.3773	0.0	24.5	0.025	1488	0.1	240.3
FS-69	St. Louis	114	S007-208	9/7/12	47.4671	-91.9279	0.0	1.33	0.181	11429	27.16	14.8
P-17	St. Louis	114	S007-208	9/1/11	47.4668	-91.9355	68.6	1.23	0.04	9654	30.4	9.3
FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.6545	-92.2739	0.0	16	0.0445	6169	1.73	122.0
FS-330	St. Louis Estuary	120	S007-444	7/22/13	46.6518	-92.2372	11.8	6.71	0.0901	5817	1.55	124.3
FS-315	St. Louis Estuary	120	S007-444	6/24/13	46.6516	-92.2373	0.0	8.1	0.147	6056	1.68	122.0
FS-300	St. Louis Estuary	120	S007-444	5/27/13	46.6515	-92.2376	0.0	9.4	0.0713	4499	1.26	97.2
FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.6518	-92.2372	31.2			4761	1.4	95.5

FS-67	St. Louis Estuary Poke	105	S006-928	9/5/12	46.6859	-92.1606	0.0	9.97	0.112	14015	3.66	241.1
FS-341	Stella	54	47-0068-00-205	8/1/13	45.066	-94.4339	57.6	24.7	0.0884	1786	1.35	15.1
P-30	Stella	54	47-0068-00-203	9/14/11	45.0659	-94.4339	31.6	7.59	0.08	2159	2.88	8.8
FS-188	Stella	54	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	18.1	1.79	1257	2.34	4.0
FS-224	Stone Lake	68	69-0046-00-201	9/19/12	47.5039	-91.8857	21.0	3.26	0.0533	5225	18.87	5.1
FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	1.62	0.0659	2505	0.65	69.6
FS-61	Swan	34	31-0067-02-206	8/30/12	47.2888	-93.2127	12.4	12.5	0.332	5827	22.71	5.0
FS-62	Swan	34	31-0067-02-206	8/30/12	47.289	-93.2124	3.8	14	0.221	4821	22.53	3.5
FS-125	Tamarac	56	56-0192-00-203	8/19/12	46.3637	-95.5714	0.0	2.33	0.0768	21908	18.41	82.3
FS-356	Trout	41	31-0216-00-212	8/14/13	47.2591	-93.3942	0.0	39.1	0.103	11992	12.59	40.7
FS-219	Trout	41	31-0216-00-212	9/13/12	47.2592	-93.3942	0.0	38.6	0.117	12535	15	35.9
FS-93	Turpela	71	69-0427-00-201	9/12/12	47.4613	-92.2371	1.0	3.3	0.115	6979	31.08	4.9
FS-183	Unnamed	50	34-0611-00-201	7/30/12	45.2675	-94.865	64.9	16.8	0.15	2157	5.61	4.0
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.286	2311	6.48	3.8
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	2193	8.1	2.6
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	1946	13.8	1.1
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	1689	12.6	0.9
FS-383	Upper Panasa	37	31-0111-00-204	9/18/13	47.3059	-93.2676	0.0	33.6	0.0399	19148	2.86	590.3
FS-59	Upper Panasa	37	31-0111-00-202	8/29/12	47.306	-93.2652	0.0	29.6	0.126	895	0.43	15.8
FS-139	Welby family farm	93	86-0231-00-202	9/21/12	45.3592	-94.0782	17.2	< 0.5	0.118	7267	30.76	5.3
FS-228	West battle	57	56-0239-00-204	8/15/12	46.2906	-95.6049	144.8	4.03	0.189	3108	17.37	2.1
FS-186	Westport	63	61-0029-00-204	8/1/12	45.6897	-95.217	0.0	7.11	1.79	4917	20.15	4.2
FS-346	Westport	63	61-0029-00-205	8/8/13	45.7042	-95.203	6.7	6.3	0.205	3262	19.66	2.0
FS-65	Wild Rice	11	09-0023-00-202	9/4/12	46.6712	-92.6055	0.0	< 0.5	0.083	13650	28.82	19.4
P-36	Wild Rice Reservoir	70	69-0371-00-204	9/16/11	46.9098	-92.1636	17.2	1.13	0.023	5555	3.75	39.5
FS-68	Wolf	69	69-0143-00-101	9/6/12	47.2564	-91.963	8.9	2.01	0.119	9526	17.19	18.0
P-19	Wolf	69	69-0143-00-202	9/2/11	47.2586	-91.9618	128.8	1.54	0.139	8240	25.1	8.7

LacCore_field_ID	Site_name	UniqID	DNRStateID	Date	Lat	Long	WRavestemM2	WRpresent	SO4mg_L	TSmgL	SedFeµgg	SedTOCpct	CPSC120
P-34	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7292	25.9	YES	2.23	0.671	1485	23.57	0.3
FS-134	Bass	43	31-0576-00-207	9/18/12	47.2844	-93.6276	64.0	YES	1.01	0.0664	3740	26.12	1.8
FS-85	Bean	8	03-0411-00-201	8/21/12	46.9337	-95.8706	0.0	NO	85	16	1967	11.85	1.4
FS-87	Bee	60	60-0192-00-202	8/23/12	47.6527	-96.0504	39.8	YES	11	0.67	3054	13.62	2.7
FS-193	Big Mud	79	71-0085-00-201	8/30/12	45.4529	-93.7418	14.3	YES	< 0.5	0.0308	12943	18.63	29.5
FS-216	Big Sucker	39	31-0124-00-203	9/12/12	47.3919	-93.2658	3.8	YES	7.78	0.145	3559	21.45	2.1
FS-204	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.742	133.7	YES	5.49	0.0914	1731	5.94	2.4
P-12	Birch	67	69-0003-00-205	8/30/11	47.7357	-91.9428	68.6	YES	3.58	0.104	12431	26.8	17.7
FS-52	Blaamyhre	48	34-0345-00-203	8/1/12	45.364	-95.186	102.2	YES	0.62	0.078	3517	9.33	5.5
FS-214	Bowstring	116	S007-219	9/11/12	47.7024	-94.0608	69.7	YES	1.34	0.256	1974	24.34	0.6
FS-126	Bray	58	56-0472-00-202	8/20/12	46.4518	-95.8783	7.6	YES	1.65	0.072	3937	21.95	2.5
FS-63	Caribou	72	69-0489-00-206	9/3/12	46.8913	-92.3135	0.0	NO	1.21	0.0938	13791	29.44	19.3
FS-109	Carlos Avery Pool 9	4	02-0504-00-202	7/3/12	45.3192	-93.0611	52.8	YES	< 0.5	< 0.011	14736	12.51	61.0
FS-339	Christina	28	21-0375-00-315	7/31/13	46.0734	-95.7567	0.6	YES	14.6	1.93	1741	8.96	1.5
FS-314	Clearwater	96	S002-121	6/24/13	47.9372	-95.6907	0.6	YES	28	0.0664	3946	2.68	30.6
FS-88	Clearwater	98	S004-204	8/24/12	47.5174	-95.3904	148.3	YES	2.04	0.0488	9874	22.17	14.2
P-31	Cloquet	52	38-0539-00-201	9/14/11	47.4313	-91.4844	74.4	YES	0.81	0.024	4252	6.58	12.1
FS-128	Cromwell	14	14-0103-00-201	8/22/12	46.9651	-96.3171	0.0	NO	41.2	1.22	2948	2.85	16.2
FS-322	Dark	77	69-0790-00-202	7/10/13	47.6389	-92.7781	3.2	YES	175	0.131	2480	1.48	25.5
P-44	Dead Fish	12	09-0051-00-202	9/20/11	46.7451	-92.6863	48.7	YES	0.3	0.056	9685	16.6	19.4
FS-378	Duck Lake WMA	22	18-0178-00-202	9/12/13	46.7521	-93.8851	113.0	YES	< 0.5	0.0251	12151	26.57	17.1
FS-86	Eighteen	61	60-0199-00-202	8/22/12	47.6397	-96.0607	40.1	YES	4.29	0.164	1860	3.1	6.1
FS-137	Elk	15	15-0010-00-204	9/19/12	47.1952	-95.2249	42.7	YES	< 0.5	0.0936	6334	10.07	15.6
FS-95	Embarrass	73	69-0496-00-203	9/14/12	47.5334	-92.2979	0.0	NO	18.8	0.0298	21847	1.89	1248.9
FS-76	Field	45	34-0151-00-201	7/25/12	45.2964	-94.9058	0.0	NO	< 0.5	0.0687	7586	8.68	26.3

FS-195	Fisher	78	70-0087-00-201	8/31/12	44.7942	-93.4061	20.7	YES	6.85	0.136	11140	5.76	90.1
P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	YES	0.56	0.018	4302	21.79	2.9
FS-194	Gilchrist	91	86-0064-00-201	8/31/12	45.2309	-93.824	0.0	NO	6.98	0.355	3117	20.81	1.7
FS-51	Glesne Slough	49	34-0353-00-201	7/31/12	45.3514	-95.1887	99.6	YES	< 0.5	0.061	7983	3.01	103.2
FS-104	Gourd	10	04-0253-00-201	6/27/12	47.8121	-94.965	0.0	NO	0.27		1776	36.87	0.3
P-20	Gull	9	04-0120-00-203	9/6/11	47.6559	-94.6944	15.6	YES	0.78	0.103	1608	5.08	2.5
FS-130	Hay	33	31-0037-00-202	9/6/12	47.2874	-93.102	141.0	YES	31.7	0.0738	13154	5.79	123.3
FS-221	Hay Creek Flowage	59	58-0005-00-202	9/17/12	46.0894	-92.4104	97.7	YES	1.95	0.119	9456	22.05	13.2
P-1	Height of Land	5	03-0195-00-209	8/22/11	46.9129	-95.6095	62.9	YES	0.24	0.053	1298	1.76	6.0
FS-131	Hinken	113	S007-207	9/5/12	47.7271	-93.9923	46.8	YES	< 0.5	0.0876	2960	4.53	9.4
FS-185	Hoffs Slough	85	76-0103-00-201	8/1/12	45.3255	-95.7059	0.0	NO	273	0.0343	3512	0.75	112.3
FS-218	Holman	42	31-0227-00-202	9/13/12	47.3005	-93.3445	0.0	NO	24.2	1.01	3035	29.74	1.0
FS-182	Hunt	65	66-0047-00-208	7/27/12	44.3275	-93.4443	0.0	NO	17.1	0.0729	2412	1.21	30.8
FS-191	Ina	27	21-0355-00-202	8/29/12	46.0715	-95.7281	30.2	YES	7.08	0.274	2216	9.09	2.3
P-5	Itasca	16	15-0016-00-208	8/25/11	47.2381	-95.2065	45.8	YES	0.26	0.056	1355	7.4	1.2
FS-207	Kelly Lake	64	66-0015-00-204	8/13/12	44.3542	-93.3743	0.0	NO	1.92	0.0927	4387	27.33	2.3
P-55	Lady Slipper	53	42-0020-00-204	9/22/11	44.5702	-95.6274	0.0	NO	107.71	14.84	2814	2.09	21.5
FS-180	Lily	90	81-0067-00-202	7/26/12	44.1947	-93.647	38.2	YES	< 0.5	0.0295	5095	28.07	3.0
FS-54	Little Birch	87	77-0089-00-207	8/3/12	45.7779	-94.7978	70.0	YES	7.4	0.0353	1794	6.02	2.6
P-4	Little Flat	6	03-0217-00-201	8/24/11	46.9981	-95.6641	83.1	YES	0.22	0.011	7479	33.13	5.2
FS-250	Little Rice	75	69-0612-00-201	9/20/12	47.7086	-92.4389	29.3	YES	1.03	0.0293	9488	26.45	10.7
P-3	Little Round	7	03-0302-00-202	8/24/11	46.9759	-95.7404	57.2	YES	0.46	0.032	1689	20.91	0.5
FS-223	Little Sucker	40	31-0126-00-202	9/14/12	47.3765	-93.246	0.0	NO	13.7	0.534	6297	16.56	8.5
FS-202	Long Prairie	110	S007-204	8/9/12	46.0072	-95.2634	13.4	YES	7.71	0.0793	2897	2.85	15.7
FS-200	Louisa	94	86-0282-00-205	8/8/12	45.2998	-94.258	0.0	NO	7.04	0.192	7824	8.76	27.6
FS-226	Louise	25	21-0094-00-202	8/14/12	45.9331	-95.4148	46.5	YES	4.09	0.0746	1833	0.83	28.5
FS-357	Lower Panasa	38	31-0112-00-204	8/15/13	47.3026	-93.2561	0.0	NO	28.5	1.26	2347	2.42	12.7
P-26	Lower Rice	109	S007-164	9/8/11	47.3817	-95.4926	120.1	YES	0.55	0.07	2364	6.76	3.8
P-25	Lower Rice	107	S006-985	9/8/11	47.3793	-95.4834	114.4	YES	1.02	0.097	2337	17.76	1.2
FS-377	Mahnomen	21	18-0126-02-201	9/11/13	46.4986	-93.9956	0.0	NO	21.1	0.0283	16540	7.47	141.1
P-63	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	148.7	YES	1.83	0.01	10269	4.24	111.2

FS-187	McCormic	81	73-0273-00-203	8/2/12	45.722	-94.9121	8.9	YES	1.54	0.144	1512	1.1	14.0
FS-229	Mill Pond	23	21-0034-00-202	8/16/12	46.0716	-95.2218	102.2	YES	7.16	0.109	5143	7.86	14.0
FS-225	Miltona	24	21-0083-00-205	8/13/12	46.0496	-95.4217	0.0	NO	4.11	0.0694	2624	1.77	22.9
FS-129	Mink	92	86-0229-00-207	8/23/12	45.2767	-94.0299	0.0	NO	1.22	0.182	4247	13.63	5.0
FS-80	Mission	95	S001-646	8/6/12	45.8623	-93.0011	87.5	YES	0.62	0.0485	9231	4.83	77.5
FS-83	Mississippi Crow Wing	111	S007-205	8/8/12	46.4386	-94.1251	0.0	NO	3.13	0.127	13451	3.88	207.8
FS-210	Mississippi Pool 4/Robinson Lake	89	79-0005-02-202	8/16/12	44.3593	-91.9881	35.3	YES	15.7	0.07	6450	1.16	214.5
FS-312	Mississippi Pool 5 / Spring	123	S007-660	6/21/13	44.2018	-91.8444	35.7	YES	28.3	0.0844	3563	0.67	132.2
FS-311	Mississippi Pool 8 at Genoa	118	S007-222	6/20/13	43.5766	-91.2341	12.7	YES	29.3	0.107	1544	0.62	29.0
FS-209	Mississippi Pool 8 at Reno Bottoms	122	S007-556	8/15/12	43.6025	-91.2686	72.3	YES	18.1	0.0711	9187	2.29	187.6
FS-58	Mississippi River above Clay Boswe	108	S007-163	8/28/12	47.2386	-93.7197	0.0	NO	1.19	0.0806	8636	9.08	32.0
FS-355	Mississippi River below Clay Boswe	103	S006-923	8/13/13	47.2553	-93.634	78.3	YES	10.2	0.0819	10479	8.98	47.1
FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.3332	-94.9292	154.4	YES	34.6	0.242	5436	26.42	3.7
P-42	Monongalia (Middle Fork Crow R)	45.5	34-0158-01-201	9/20/11	45.3481	-94.9509	5.7	YES	16.51	0.042	46471	14.76	455.4
FS-75	Mortenson	44	34-0150-02-201	7/24/12	45.3	-94.9062	0.0	NO	< 0.5	0.103	9071	12.09	25.0
FS-176	North Geneva	29	24-0015-00-209	7/24/12	43.7876	-93.271	0.0	NO	15.6	1.54	2212	13.45	1.5
FS-350	Ox Hide	35	31-0106-00-203	8/14/13	47.3351	-93.2132	0.0	NO	25.9	0.119	3889	12.12	4.9
FS-220	Padua	82	73-0277-00-202	8/7/12	45.623	-95.0186	0.0	NO	0.86	0.23	2291	9.77	2.3
FS-301	Partridge	119	S007-443	5/28/13	47.5213	-92.1903	0.0	NO	14.8	0.125	9491	3.94	104.3
FS-316	Partridge	121	S007-513	6/28/13	47.5137	-92.1899	0.0	NO	24.9	0.098	6291	2.6	77.8
FS-55	Pelkey	55	49-0030-00-202	8/26/12	45.9962	-94.2273	0.0	NO	3.42	0.0522	30642	17.32	168.8
FS-91	Pike	104	S006-927	9/11/12	47.7327	-92.3473	3.5	YES	14.2	0.0656	6565	4.72	41.4
FS-190	Pine	18	15-0149-00-205	8/28/12	47.6841	-95.5414	114.9	YES	14.7	0.368	4477	7.08	12.2
P-27	Pleasant	13	11-0383-00-206	9/9/11	46.928	-94.4757	28.6	YES	0.49		5331	30.37	3.0
FS-215	Popple	101	S006-188	9/11/12	47.7254	-94.0817	36.3	YES	< 0.5	0.0269	2971	14.42	2.4
FS-196	Prairie	115	S007-209	9/3/12	47.2519	-93.4884	44.6	YES	9.63	0.0709	15071	10.51	78.4
FS-82	Rabbit	20	18-0093-02-204	8/8/12	46.5313	-93.9285	0.0	NO	15.3	0.22	10903	11.79	36.7
FS-53	Raymond	83	73-0285-00-203	8/2/12	45.6286	-95.0225	61.1	YES	< 0.5	0.0787	1905	4.79	3.8
FS-324	Rice	19	18-0053-00-203	7/15/13	46.3392	-93.8918	56.7	YES	< 0.5	0.11	44704	33.18	160.3
FS-199	Rice	102	S006-208	9/5/12	47.6742	-93.6547	75.4	YES	1.57	0.0552	3273	10.88	4.0
FS-179	Rice	84	74-0001-00-201	7/25/12	44.0842	-93.0737	0.0	NO	3.84	0.217	4152	19.07	3.2

FS-181	Rice	66	66-0048-00-203	7/27/12	44.3332	-93.4734	0.0	NO	5.22	0.777	3829	21.67	2.4
FS-184	Rice	80	73-0196-00-216	7/30/12	45.3864	-94.6309	0.0	NO	2.58	2.97	1523	15.03	0.6
FS-231	Rice	2	02-0008-00-206	8/17/12	45.1604	-93.121	0.0	NO	3.6	0.145	2159	7.98	2.6
FS-349	Sandy-3	76	69-0730-00-205	8/13/13	47.6191	-92.5898	0.0	NO	122	0.0697	14897	20.46	34.6
FS-351	Second	117	S007-220	8/15/13	47.5205	-92.1925	66.8	YES	838	0.0447	7088	1.84	148.0
FS-105	Second	17	15-0091-00-202	6/27/12	47.8258	-95.3637	48.4	YES	0.74	0.119	2527	33.3	0.6
FS-347	Snowball	36	31-0108-00-202	8/12/13	47.3356	-93.2439	0.0	NO	8.2	0.097	1136	1.19	7.4
FS-177	South Geneva	30	24-0015-02-208	7/24/12	43.7709	-93.2851	0.0	NO	14.1	3.19	1618	16.71	0.6
P-16	St. Louis	106	S006-929	9/1/11	47.4015	-92.3773	0.0	NO	24.5	0.025	1488	0.1	240.3
P-17	St. Louis	114	S007-208	9/1/11	47.4668	-91.9355	68.6	YES	1.23	0.04	9654	30.4	9.3
FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.6545	-92.2739	0.0	NO	16	0.0445	6169	1.73	122.0
FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.6518	-92.2372	31.2	YES			4761	1.4	95.5
FS-67	St. Louis Estuary Pokegama Bay	105	S006-928	9/5/12	46.6859	-92.1606	0.0	NO	9.97	0.112	14015	3.66	241.1
FS-188	Stella	54	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	YES	18.1	1.79	1257	2.34	4.0
FS-224	Stone Lake	68	69-0046-00-201	9/19/12	47.5039	-91.8857	21.0	YES	3.26	0.0533	5225	18.87	5.1
FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	YES	1.62	0.0659	2505	0.65	69.6
FS-62	Swan	34	31-0067-02-206	8/30/12	47.289	-93.2124	3.8	YES	14	0.221	4821	22.53	3.5
FS-125	Tamarac	56	56-0192-00-203	8/19/12	46.3637	-95.5714	0.0	NO	2.33	0.0768	21908	18.41	82.3
FS-219	Trout	41	31-0216-00-212	9/13/12	47.2592	-93.3942	0.0	NO	38.6	0.117	12535	15	35.9
FS-93	Turpela	71	69-0427-00-201	9/12/12	47.4613	-92.2371	1.0	YES	3.3	0.115	6979	31.08	4.9
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	YES	6.42	0.065	1689	12.6	0.9
FS-59	Upper Panasa	37	31-0111-00-202	8/29/12	47.306	-93.2652	0.0	NO	29.6	0.126	895	0.43	15.8
FS-139	Welby family farm	93	86-0231-00-202	9/21/12	45.3592	-94.0782	17.2	YES	< 0.5	0.118	7267	30.76	5.3
FS-228	West battle	57	56-0239-00-204	8/15/12	46.2906	-95.6049	144.8	YES	4.03	0.189	3108	17.37	2.1
FS-346	Westport	63	61-0029-00-205	8/8/13	45.7042	-95.203	6.7	YES	6.3	0.205	3262	19.66	2.0
FS-65	Wild Rice	11	09-0023-00-202	9/4/12	46.6712	-92.6055	0.0	NO	< 0.5	0.083	13650	28.82	19.4
P-36	Wild Rice Reservoir	70	69-0371-00-204	9/16/11	46.9098	-92.1636	17.2	YES	1.13	0.023	5555	3.75	39.5
P-19	Wolf	69	69-0143-00-202	9/2/11	47.2586	-91.9618	128.8	YES	1.54	0.139	8240	25.1	8.7

Memorandum Regarding Proposed Wild Rice Rule Change

Joel Roberts, Ph.D.
Professor Emeritus of Mathematics
University of Minnesota
November 22, 2017

1. My past involvement with the Wild Rice Rule process.

I am a Professor Emeritus of Mathematics, having retired from the University of Minnesota faculty. My most relevant field of expertise is Applications of Mathematics. My *curriculum vitae* is summarized in Section 7 of this report.

In December 2015, I submitted a memorandum about the statistical calculations in MPCA's March 2015 Proposed Approach. A copy of that memorandum is attached (See Attachment 1), because I will refer to some parts of it. I did this submission in cooperation with WaterLegacy and at the urging of Len Anderson, who was a member of the Wild Rice Advisory Committee. On September 30, 2016 I attended a meeting of that Committee at the MPCA office in St. Paul. At the meeting I learned that MPCA staff had devoted significant effort to dealing with some observations made in that memorandum and to overcoming some criticisms.

Some sections of the December 2015 memorandum remain relevant to my analysis of the current Technical Support Document (TSD) and the proposed change in the Wild Rice Rule, particularly:

- §2 Ratios and log-log scale
- §3 Identification of the Main Outlying Cluster

  This is a set of sites where the formula under-predicts the porewater sulfide level. Almost all of these sites exhibit extremely high levels of porewater sulfide. A significant number also have high levels of sediment iron, but the interaction between these two variables is quite complex.

Some progress has been made toward achieving a better fit of the formula to a large subset of the data; however problems presented by the Main Outlying Cluster have not been resolved.

2. New and Old elements in the 2017 TSD.

The March 2015 Proposed Approach was based on an equation, derived from Structural Equation Modeling (SEM), that directly relates (surface water) sulfate and (porewater) sulfide levels, along with sediment iron and sediment carbon. The approach used in the 2017 TSD is based on a formula that calculates the *probability* of the sulfide level being above an assigned protective level, taken as 120 μg/L (micrograms per liter) for purposes of the proposed rule change. This formula is obtained by Multiple Binary Linear Regression (MBLR).

Strict reliance on this current model would make it difficult to do quantitative assessments of goodness of fit, comparable to what I did in my December 2015 memorandum. It is, of course, possible to compare the actual surface water sulfate level with the calculated protective sulfate level (CPSC) for a given site.

Some comparisons also are done in MPCA memos referenced here. The calculation involved in these comparisons uses the assigned protective porewater sulfide level as an input, rather than the actual porewater sulfide level at the site under consideration.

An inverse version of the 2015 SEM equation yields a predicted value of the porewater sulfide level, based on the sulfate, sediment iron, and sediment carbon levels. In my December 2015 memorandum, this predicted value was compared with the measured value from the Wild Rice Field Study. On the other hand, the 2017 MBLR approach does not provide a means for doing a direct comparison of a predicted sulfide value with the measured sulfide value.

Some limited comparisons can be made using the tools provided in the 2017 TSD. I verified the CPSC calculated by the MPCA for each site and sampling event in the field survey and made a comparison of the CPSC with the actual surface water sulfate level at each site. (See Attachment 2.)

- Among these 237 sites (all Minnesota non-paddy data), more than 140 (or about 58%) have CPSC values that exceed the current 10 mg/L Wild Rice Standard for sulfate, some of them by very substantial amounts. The proposed new standard would therefore weaken protection of wild rice, compared to the present standard.

- Reviewing data for all field survey sampling events other than those paddy rice sites, for 170 of the 238 (71 %) sampling events, the CPSC calculated was higher than the existing sulfate level. For 156 of the sites, the CPSC exceeds the actual sulfate level of the site by 20% or more. For about 60 of the sites, the CPSC is less than 80% of the sulfate level of the site. Three sites have a missing measurement, and for the remaining 18 sites, the CPSC is within 20% of the actual sulfate level.

Reviewing data for all field survey sampling events other than those paddy rice sites, for 170 of the 238 (71 %) sampling events, the CPSC calculated was higher than the existing sulfate level.

According to the 2017 TSD, an elevated level of porewater sulfide is the environmental variable which is most directly toxic to wild rice plants. The fact that the output of the MBLR equation cannot be accurately compared to the measured level of porewater sulfate therefore is a serious obstacle to an impartial verification of the MBLR equation.

A more thorough reading of the TSD shows, however, that equations from the SEM approach were extensively used to guide development of the MBLR-based equation. (This is mentioned in the TSD, and in a more detailed way in the MPCA Data Analysis Unit Memos listed in the references.) This means that comments about the previous approach still are highly relevant. Among other things, the Main Outlying Cluster identified in my earlier memorandum still is present in the data, even though it has been de-emphasized by restriction to the Class B data set rather than using all of the data.

3. Advantages and Disadvantages of equation-based approaches to the Wild Rice Standard.

Some of the points mentioned below are briefly discussed in the TSD, although firm conclusions are not always drawn there.

(a) The following statement is found on page 49 of the TSD:

> 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.

This is indeed a significant question, but no answer is given. In addition to the added cost, it should be pointed out that implementation of the equation-based standard also involves the possibility of sampling error. Here is a diagram from the March 2015 Proposed Approach that indicates that the sampling error problem likely would be widespread:

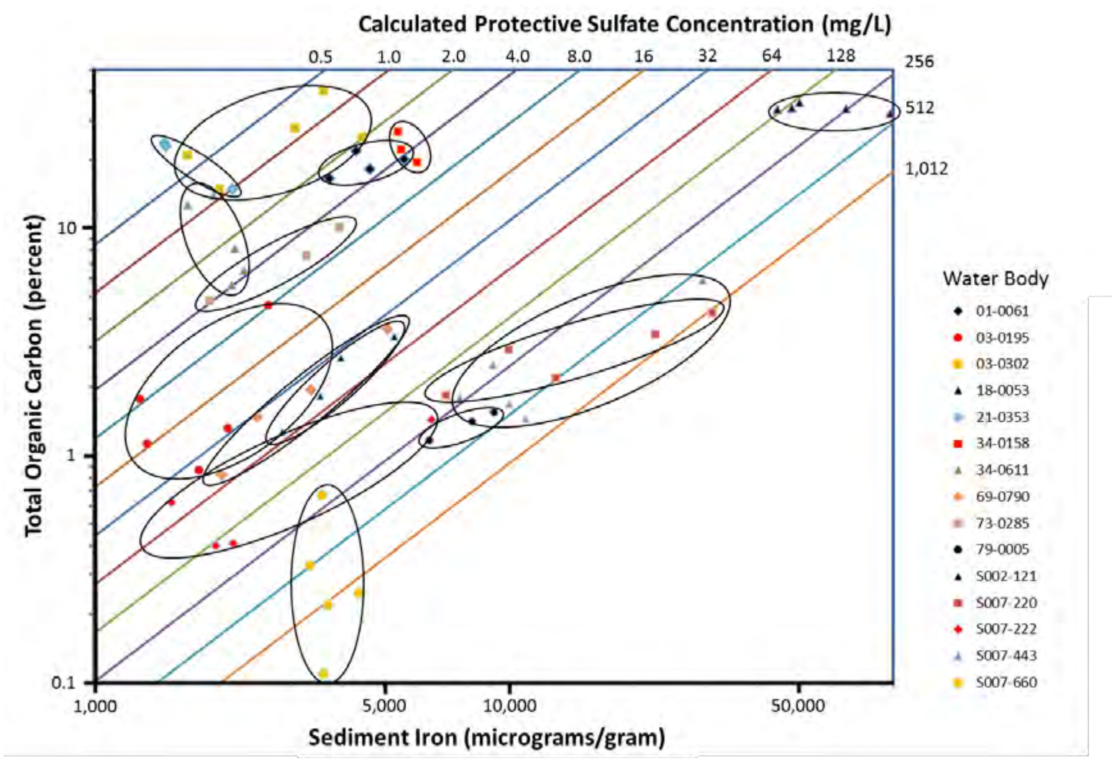


Figure 14. Data from water bodies with wild rice where three or more sediment samples were taken within 1,000 feet of each other. Ellipses encompass the range in calculated protective sulfate concentrations, which are based on sediment iron and total organic carbon data as modeled with Equation 1. The log-log display allows both a greater range of sediment concentrations and better separation of the sites than in Figure 10. Additional water body information is given in Table 6.

Each oval encloses all of the data points for a given water body with wild rice. The sloped lines represent values of the Calculated Protective Sulfate Concentration (CPSC). The number of sloped lines crossed by each oval indicates the size of the uncertainty in the CPSC. For instance, in the water body that corresponds to the small oval at the upper right, the CPSC values range from 128 mg/L to 512 mg/L. Thus, the largest value in this case is 4 times as large as the smallest one, which is a large degree of uncertainty.

(b) For data sets where the formula fits reasonably well, the CPSC levels do not diverge widely from the current 10 mg/L standard. (This statement does not appear explicitly in the TSD, but it is evident in many of the comparisons that are made.)

(c) Although there is no similar illustration in the 2017 TSD of the divergence in CPSC calculated on the basis of sampling the same site at various times, this information can be reviewed using the CPSC for the water bodies identified with the numbers at the right side of the above chart.

Reviewing the 2017 calculated CPSC for sites where multiple samples were taken (Attachment 3), a wide divergence is found at sites where the calculated CPSC is higher than the current 10 mg/L standard. At Second Creek (S007-220), based on sampling dates within the same year, the CPSC ranged from 166.92 mg/L to a CPSC of 657.30, nearly four times higher. At Mississippi Pool 5 (S007-660), again within the same sampling year, the CPSC ranged from a low of 132.16 to a high of 1160.97, a level 8.78 times higher. For Lake Monongalia (34-0158), where various locations within the water body were sampled, calculated CPSC ranged from 3.66 mg/L to 455.39, more than two orders of magnitude of variation.

(d) It is doubtful that the added expense and effort of implementing an equation-based standard is justified. The potential for divergent calculations of CPSC levels calls into question the reliability of the methodology and could create many issues regarding the time and location of sampling. In addition, because an equation-based standard is more complicated, it becomes excessively difficult for many stakeholders and for the general public to have any kind of concrete understanding of what the standard involves.

Despite the fact that the merits of an equation-based standard are debatable, we still should look at the equation that is proposed. We have observed that the SEM equation (whose inputs are sulfate, iron, and organic carbon) provides serious under-prediction of the sulfide level at sites where the measured sulfide level is high. Although not all of these sites have high level of sediment iron, I still believe that the iron exponent provides a significant part of the explanation. Here are the two formulas.

- From the 2015 SEM approach:

  $$\text{Calculated Sulfate Standard} = 0.0000136\left(\text{Sediment Iron}^{1.956} / \text{Organic Carbon}^{1.410}\right)$$

- (Sulfate and sulfide are expressed in mg/L; organic carbon is percent total organic carbon in the sediment; iron is micrograms extractable iron per gram sediment).

- And from the 2017 MBLR approach:

  $$\text{Calculated Sulfate Standard} = 0.0000121\left(\text{Sediment Iron}^{1.923} / \text{Organic Carbon}^{1.197}\right)$$

  (The units are the same as in the previous equation)

In the first case the iron exponent is 1.956, and in the second case the iron exponent is 1.923. Since the exponents nearly are equal to 2, we are close to a situation where the equation (with carbon value held constant) is a quadratic function of the iron value. (This would correspond to the graph being a parabola rather than a straight line.) This means, for instance, that if the iron value is increased by a factor of 10, then the CPSC is increased by a factor of 100. Such an increase in iron values actually is not especially large in the Wild Rice Field Study on which this study is based. Indeed, the values in the data range from 895 µg/g (micrograms of iron per gram of sediment) to 83,421 µg/g. Thus, the ratio of highest to lowest is about 93. The 10^{th} percentile is around 1,800 µg/g while the 90^{th} percentile is around 19,000 µg/g. The ratio between these two values is about 10.6.

If an exponent close to 2 is needed to make the model fit the data in the middle of the range, it would be highly likely to lead to inflated estimates of the CPSC at the upper end of the range. Indeed, this is the natural consequence of moving to the right along a parabola.

In addition, recent research by Prof. John Pastor at the University of Minnesota at Duluth has shown that iron sulfide plaques can form on wild rice roots in sediments where iron and sulfides are present. (Several of his papers are referenced in the TSD, and some other aspects of his research are discussed there. This aspect of his research is not discussed in the TSD, however.) It is relevant to mention this finding here, because it indicates the possibility of a significant interaction between two of the independent variables, specifically iron and carbon. The type of model used in the TSD, however, doesn't make sufficient allowance for complex interactions between the different inputs.

4. Comparisons of the 2015 Formula and the 2017 Formula.

(a) In the 2015 Proposed Approach, protective sulfate levels were calculated by means of the deterministic SEM equation. The adjective "deterministic" is being used to emphasize the fact that the SEM equation relates an actual value of the porewater sulfide level to the values of the other variables. The protective sulfate concentration was based on an EC20 sulfide level, which was estimated to be 165 µg/L. (The designation EC20 refers to the Effect Concentration of sulfide at which there is a 20% negative effect on the growth of wild rice – see page 31 of the TSD). The probabilistic MBLR formula of the 2017 TSD and the proposed rule change is based on an EC10 sulfide level, which is estimated to be 120 µg/L. The EC10 value, which would correspond to a 10% negative effect on the growth of wild rice, was adopted at the recommendation of a peer review panel as stated in the TSD. The EC10 value seemingly should be more protective of wild rice. Thus, we might have expected that the CPSC values obtained from the new approach to be somewhat lower than the corresponding values obtained from the old approach. What happened, however, was that the values from the 2017 formula are only very slightly changed from the values obtained from the 2015 formula. Indeed, a spreadsheet calculation showed a seemingly random pattern of mostly small changes.

A more robust comparison emerges if we use the deterministic 2015 SEM equations to calculate EC10 values of the protective sulfate concentration (CPSC). Indeed, the March 2015 Proposed Approach provides a straightforward means to modify the CPSC equation: one changes the relevant parameter value from 165 to 120, and then proceeds in a completely similar way to what is done in the March 2015 Proposed Approach. A spreadsheet calculation is included (See Attachment 4). All of the CPSC values from this calculation are between 26% and 94% of the corresponding values obtained from the probabilistic 2017 MBLR equation. Nearly four fifths of the values from the deterministic calculation are below 50% of the corresponding values from the probabilistic calculation. Using the same proposed EC10 threshold of 120 µg/L, the 2015 SEM equation would have resulted in lower sulfate standards in every case, and in sulfate standards less than half those currently proposed by MPCA in almost 80% of the cases.

It is reasonable to ask why the 2017 MBLR equation did not lead to more protective calculated sulfate levels even as the sulfide threshold was changed. One plausible explanation is related to the fact that there is a probabilistic aspect to this equation. Clearly, the 2017 MBLR equation does not immediately appear to be probabilistic. It is, however, derived from an equation which calculates the probability of the porewater sulfide level being greater than 120 µg/L. At a certain stage, this probability is assigned a specific value. The issues associated with this assignment are somewhat technical (see subsection (c) below), but I believe that the choice is rather arbitrary.

(b) The reasons for changing from a deterministic equation to a probabilistic one are not fully explained in the TSD. The main reason given in the TSD is that it is supposed to avoid a phenomenon called *re-transformation bias*, sometimes also called back-transformation bias. This phenomenon occurs when a linear equation is fitted to logarithmically transformed data.

- In the linear formula that is fitted to the log-transformed data, the random errors average to zero. Thus, $\log(Y) = A + B\log(X) + E$ in the transformed data, where E is the error term. After the re-transformation, the error term E is transformed to an error ratio 10^E. Even though the error terms average to 0, the error ratios probably will average to some value larger than 1. Thus, re-transformation bias seems unavoidable.

- The TSD provides no explanation of **how** the MBLR approach overcomes this bias. In fact, the claim that the MBLR approach overcomes the re-transformation bias actually is **subject to serious doubt**, because the derivation of the MBLR equation starts from a regression formula applied to log-transformed data. (That regression formula is presented in subsection (c) below.)

- Methods are available for estimating magnitude of the re-transformation bias. I located one of these in a paper from the journal *Environmental Toxicology and Chemistry* that is referenced in one of the TSD references. For a normally distributed variable, the estimate is based on the root-mean square (RMS) error in the logarithmically transformed data. The RMS error undoubtedly could have been extracted from the calculations that were previously done with the SEM model. Despite the real possibility of implementing such estimates, however, there is no mention in the TSD of any effort to implement any estimate of this type.

Since the use of the MBLR approach makes the whole project much more difficult to understand, and since it is not clear that it actually overcomes the re-transformation bias, more effort should have been devoted to seeing whether it could be avoided by using either a deterministic formula or a fixed standard.

(c) The following equation is presented in the TSD, and it is the starting point for deriving the equation that appears in the proposed rule change:

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

$$\text{logit(sulfide>120 µg/L)} = 9.3176 + 1.8962*\log_{10}\text{sulfate} - 3.6443*\log_{10}\text{iron} + 2.2698*\log_{10}\text{TOC}$$
(equation 1)

If p is the probability that sulfide exceeds 120 µg/L, then the expression logit(sulfide>120 µg/L) refers to the quantity $\log_{10}(p/(1-p))$. If you know the value of the logit term, then you can find the probability and vice-versa.

A few steps later in the derivation, it is decided to set $p = 0.5$. Values of p range from 0 to 1, so this decision is equivalent to saying that there is a 50% chance of sulfide exceeding 120 µg/L. Mathematically, this is convenient because setting $p = 0.5$ simplifies the equation. Indeed, the quotient $p/(1-p)$ is then equal to 1. And therefore the logarithm of the quotient is equal to 0, eliminating the expression on the left side of the equation.

Whether or not the decision to set $p = 0.5$ is protective of wild rice is much more debatable, however. Accepting it would mean that we were settling for a 50% chance of wild rice being protected at the EC10 level that was recommended by the peer review panel. This seems inadequate for protecting wild rice. Therefore a lower probability would be needed to be protective of wild rice. The TSD provides no discussion or citation to support the assumption that a 50% chance of protecting wild rice would be sufficiently protective. Absent a compelling rationale to the contrary, simple logic suggests that a lower probability would be needed to be protective of wild rice.

A lower probability alone would not address the concerns raised above regarding sampling error and divergence of CPSC results for the same site, the potential for inflated estimates of CPSC at the higher end of the range, the transformation bias and the potential that the iron exponent misrepresents the role of iron in ecosystems. But, as an illustration, I calculated the outcome applying $p = 0.25$, which would correspond under Equation 1 of the TSD to a 75% probability of wild rice being protected at the EC10 level. With $p = 0.25$, the calculated protected sulfate value (CPSC) for any site would be equal to about 0.56 times the value calculated for that site by the formula in the proposed rule change. This would represent a 44% decrease in the CPSC.

Although a 44% decrease in the CPSC value might initially seem substantial, sites at the higher end of the range, where the equation is most likely to result in prediction errors, would still be substantially above the current standard of 10 mg/L. This modification would lead to more than half of the sites in the all Minnesota non-paddy data set having CPSC values at or below the current standard of 10 mg/L.

5. Conclusions and Recommendations.

The MPCA's proposed one-size-fits-all version of an equation-based standard is inadequate. It is inadequate for explaining the data from the Wild Rice Field Study. It does not resolve all of the concerns raised by analysis of the 2015 SEM equation. And it is inadequate for protecting Minnesota's Wild Rice.

Recommendations:

- If an equation-based approach is specifically sought, more research would need to be done regarding the iron levels in various water bodies and zones of the state, with separate equations adapted to the various zones. The constant iron exponent could also be replaced by a more appropriate mathematical expression. The probability that porewater sulfide exceeds 120 µg/L could be set lower than $p = 0.5$ to make the standard more likely to be protective of wild rice.

- The State of Minnesota could continue to use the existing 10 mg/L sulfate standard, which has been found to be protective of wild rice. This has the additional advantage of being more straightforward to understand and to administer.

6. References

Final Technical Support Document: Refinements to Minnesota's Sulfate Water Quality Standard to Protect Wild Rice, Minnesota Pollution Control Agency, 2017. https://www.pca.state.mn.us/sites/default/files/wq-rule4-15n.pdf

Proposed revision of Minnesota Rule 7050.0224, *in* Proposed Permanent Rules Relating to Wild Rice Sulfate Standard and Wild Rice Waters, MPCA and Revisor of Statutes, July 24, 2017. https://www.pca.state.mn.us/sites/default/files/wq-rule4-15h.pdf

March 2015 proposed approach for Minnesota's sulfate standard to protect wild rice, Minnesota Pollution Control Agency, 2015. https://www.pca.state.mn.us/sites/default/files/wq-s6-43l.pdf

MPCA Data Analysis Unit memos dated 1/22/2016, 1/26/2016, 2/9/2016, 2/16/2016, and 3/10/2016.

Michael C. Newman, Regression Analysis of Log-Transformed Data: Statistical Bias and its Correction, *Environmental Toxicology and Chemistry*, Vol 12 (1993), pp. 1129-1133, Pergamon Press.

7. Curriculum Vitae

Joel Roberts was born in Denver, Colorado, and grew up in the Denver area. He majored in mathematics at M.I.T. and received his Ph.D. in mathematics from Harvard University. After teaching at Purdue University for four years, he joined the University of Minnesota mathematics faculty in 1972. He was a full professor starting in 1980. He became professor emeritus of mathematics in 2009.

MathSciNet lists 25 research papers by Joel Roberts. He has had five Ph.D. students and has worked with numerous other graduate students doing thesis research in mathematics, computer science, physical sciences, and engineering. Prof. Roberts has given three different month-long lecture series at the National University of Mexico. He has visited the University of Bergen, Norway, on several occasions for research collaborations, and has been a Visiting Scholar at the University of California, Berkeley.

In recent years he has become interested in the use of computers for calculation with polynomials and also for visualization of algebraic curves and surfaces. This work included participation in the 2005-2006 Special Year on Applications of Algebraic Geometry, held at the Institute for Mathematics and Its Applications.

Mathematical publications are listed in Section 8 (pages 8-9) of my December 16, 2015 Memorandum Regarding Wild Rice Sulfate Standard Calculations provided in Attachment 1 of this Memorandum.

Roberts Memorandum - Wild Rice Rule
November 2017

Attachment 1

(20 pages)

Memorandum Regarding Wild Rice Sulfate Standard Calculations Comparing Expected and Observed Sulfide Levels in Field Study Data and Interpreting Statistical Analysis

Joel Roberts, Ph.D., Professor Emeritus of Mathematics
University of Minnesota
December 16, 2015

1. INTRODUCTION

I am a Professor Emeritus of Mathematics, having retired from the University of Minnesota faculty. My most relevant field of expertise is Applications of Mathematics. My *curriculum vitae* is summarized in Section 8 of this report. On review of the Minnesota Pollution Control Agency's (MPCA) proposal,[1] I was struck by the degree of scatter reflected in the Figure 9 comparison between the modeled levels of porewater sulfide and the levels of sulfide that were actually observed in the field study. I have reproduced the illustrations of this scatter pattern below and provide some discussion of the significance of the fit of the data shown in the *MPCA Proposal.*

In order to further test the predictive power of the proposed MPCA formula to derive sulfide concentrations, I obtained from the MPCA the wild rice, sulfate and sulfide data on which the *MPCA Proposal* is based and replicated the MPCA's calculations of predicted sulfide to compare them with observed sulfide. The spreadsheet containing this analysis is provided in Attachment A and Attachment B, which illustrate different ways of sorting this data. Comparing observed sulfide concentrations in the MPCA field data with predicted sulfide concentrations obtained by applying the MPCA's equation demonstrated to me the poor predictive power of the proposed equation. The lack of consistency in the ratios of predicted and observed sulfide provides no confidence that the MPCA's Proposal will provide a reliable prediction of sulfide levels. Thus, even setting aside questions about the ecology that these predictions represent (a set of issues that are outside my expertise) the MPCA Proposal seems like an unreliable method to protect wild rice from excess sulfide.

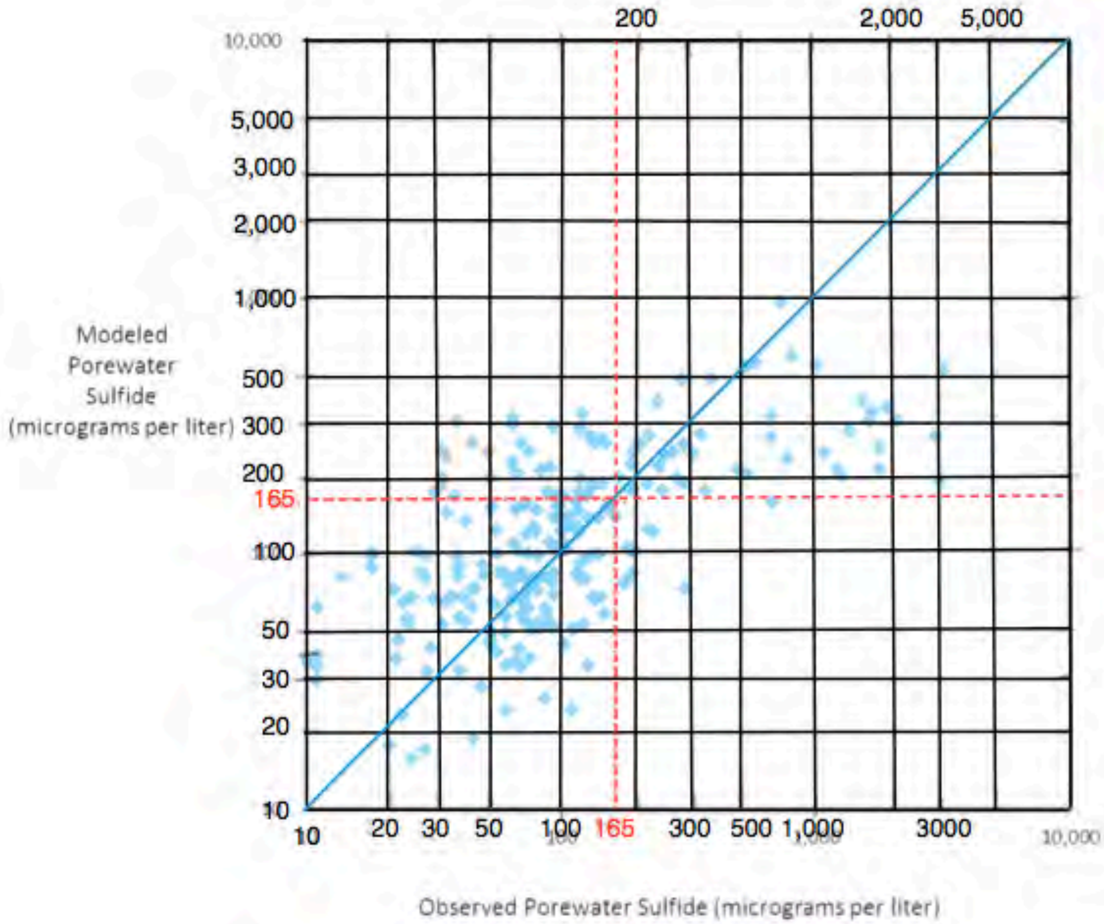
2. GRAPHIC REPRESENTATION AND CHI-SQUARE ANALYSIS OF MPCA DATA

Section 2 of the *MPCA Proposal* is entitled *The relationship between sulfide and sulfate*. The relationship is shown in Figure 9. That figure is reproduced below, with gridlines added to show how the points correspond to actual values. The positions of the gridlines were carefully measured to take account of the logarithmic scale.

The data points tend to cluster around the main diagonal (shown in blue), indicating some degree of relationship. Since this is a log-log plot (logarithmic scale in both variables), however, the relationship is made to appear much closer than what would be seen if the chart had been based on the more commonly used linear scale. Indeed, the measure of closeness actually is the *ratio*

[1] Minnesota Pollution Control Agency, *Proposed Approach for Minnesota's Sulfate Standard to Protect Wild Rice*, March 24, 2015. (hereinafter *MPCA Proposal*)

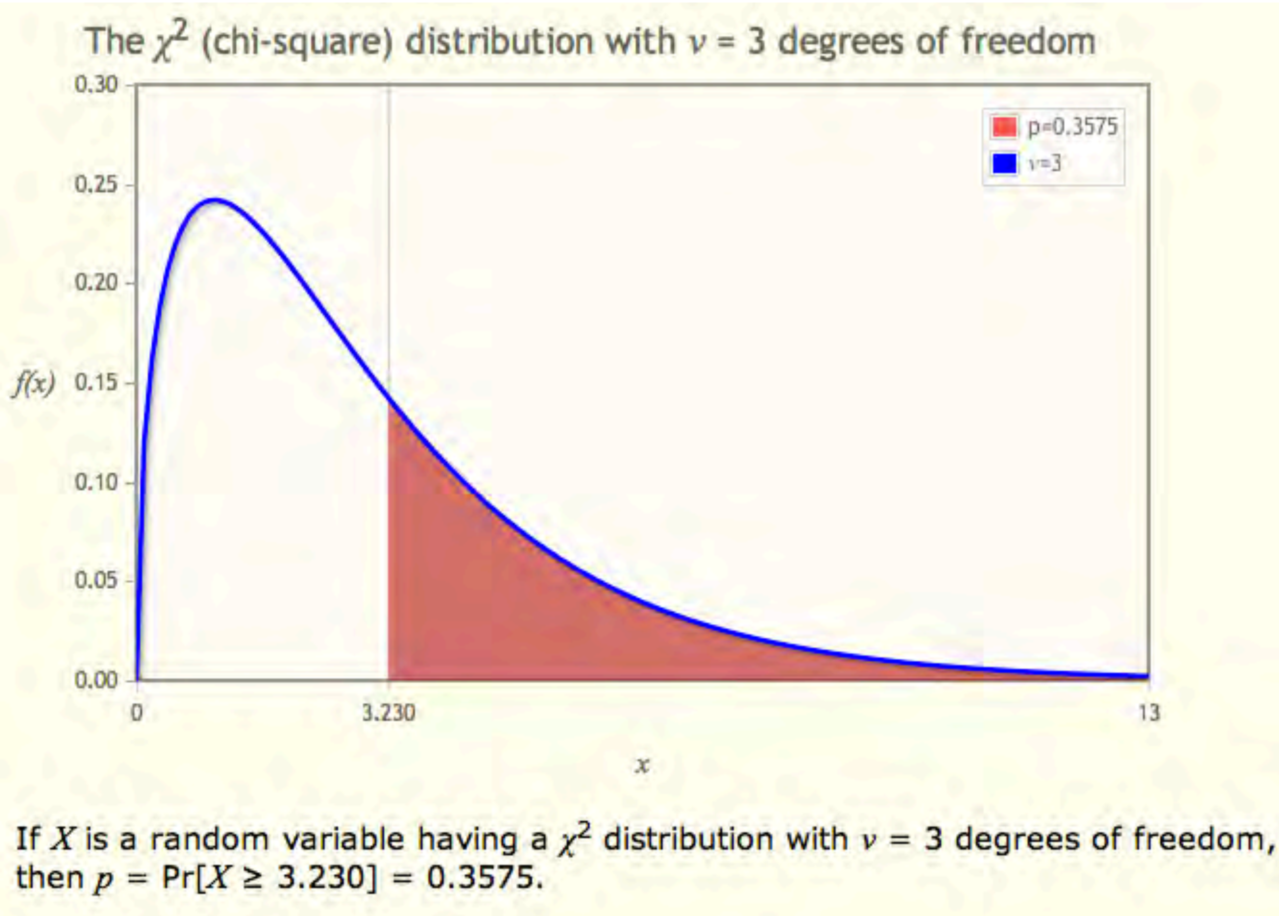
between the expected value and the observed value. This is the ratio that I actually calculated in the spreadsheet reproduced in Attachments A and B.



Some tests of significance are presented in Appendix 1 of the Main Document. The chi-squared statistic is the most basic, since RMSEA (root mean square error of approximation) can be calculated from it. The stated chi squared value is 3.23, with 3 degrees of freedom (essentially the number of independent variables), and N =184. (The number of sites studied in the field study was around 184.) The probability of a chi-squared value with 3 degrees of freedom being greater than 3.23 is given as 0.3572. This means that the value of such a variable being **less** than 3.23 is 0.6428. Now, a better fit to the data corresponds to a **smaller** chi-squared value.

The stated chi-squared value would indicate that the probability of a better fit is 0.6428. In my opinion, the chi-squared calculation presents an inconclusive result. It does not make a compelling case for goodness of fit of the model. In simple terms, using the chi-squared test of the fit of the data, the proposed equation predicts less than half of the variability of the data. Even though it is possible to draw a line through the data points that indicates a potential relationship between the data points, as the MPCA has done, this single line does not provide a powerful predictor of results for specific water bodies/data points.

The figure shown below illustrates the chi-square calculation.



3. Outlying Data and Under-prediction of Porewater Sulfide

I performed some additional analysis to review outlying data points and the potential for under-prediction of porewater sulfide.

In the *MPCA Proposal*, the following formula was presented for calculating pore water sulfide concentration:

. $Sulfide = 7.873\ Sulfate^{0.345}\ Organic\ Carbon^{0.486}\ Sediment\ Iron^{-0.675}$ (Equation 2)
(Sulfate and sulfide are expressed in mg/L; organic carbon is percent total organic carbon in the sediment; iron is micrograms extractable iron per gram sediment).

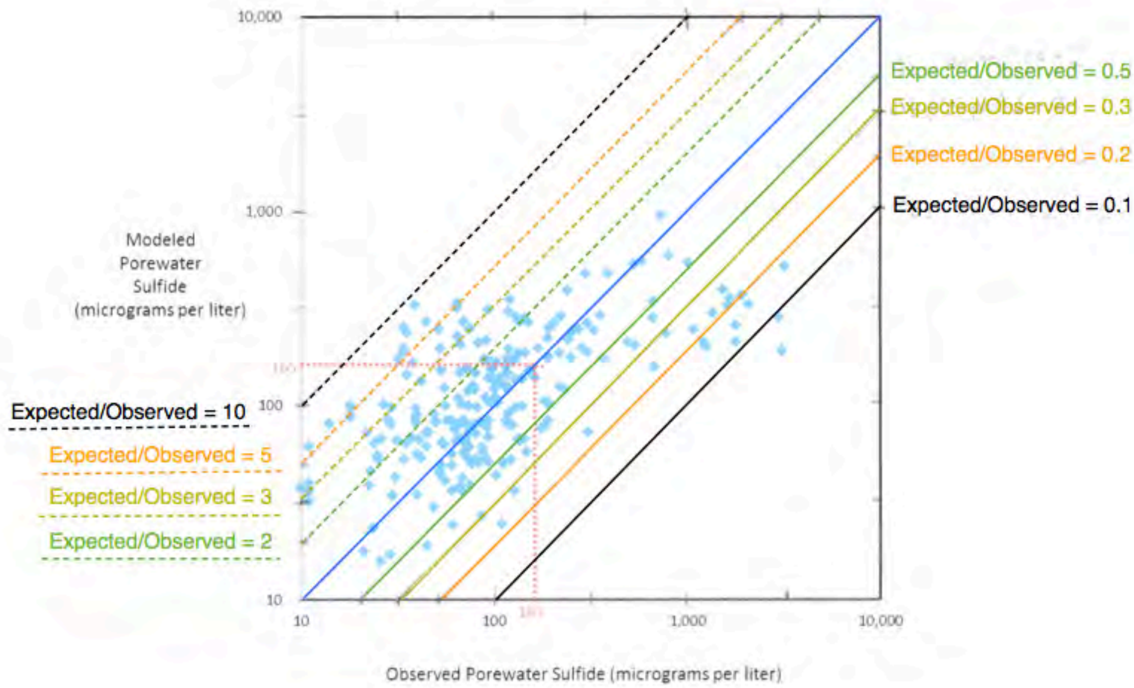
This corresponds to the following logarithmic version:

$$\log(Sulfide) = \log(7.873) + 0.345 \log(Sulfate) + 0.486 \log(Organic\ Carbon) - 0.675 \log(Sediment\ Iron)$$

Thus, the logarithmic version is linear, and the exponents in the original equation are transformed into coefficients in the logarithmic equation. In the linear regression method of fitting an equation to the data, one finds the coefficient values that give the best fit of the equation to the data. In the

MPCA Proposal structural equation modeling was used to derive Equation 2 used to predict expected porewater sulfide, but an equation obtained from linear regression was presented for purposes of comparison.

The following graphic superimposes on Figure 9 of the *MPCA Proposal* diagonal lines corresponding to the ratio of Expected Porewater Sulfide to Observed Porewater Sulfide.

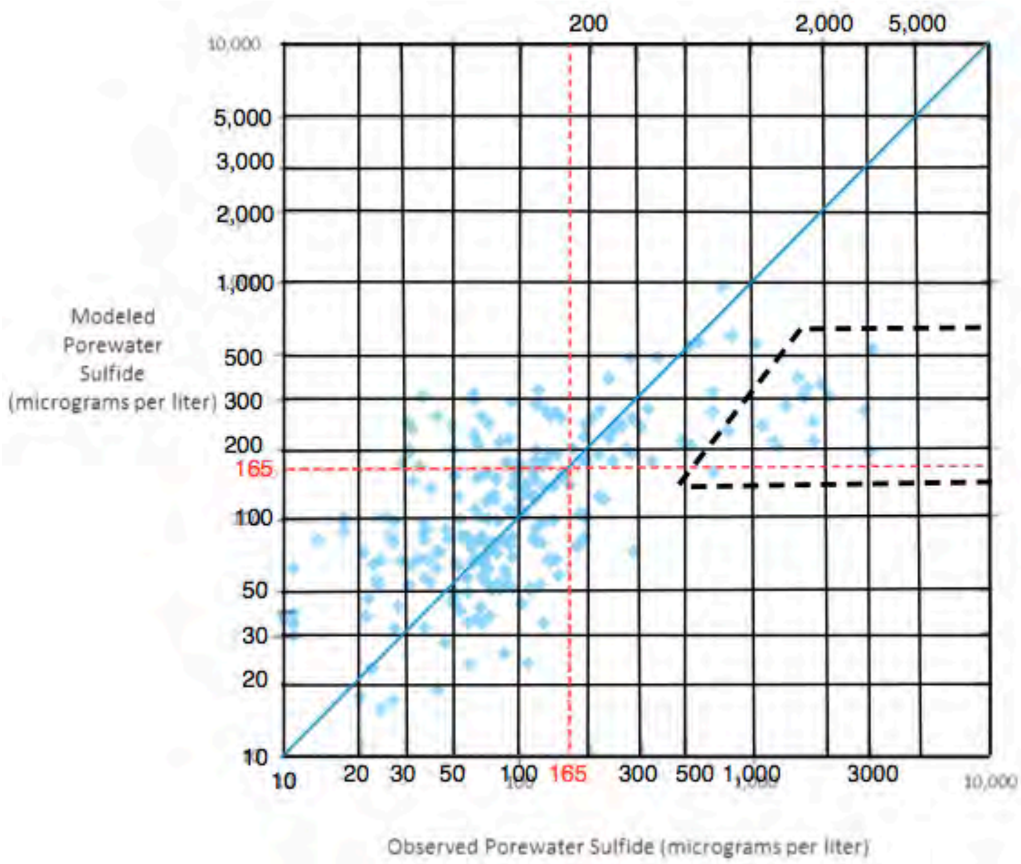


Cases where the Expected/Observed ratio is less than 1 correspond to under-prediction by the model, and cases where the Expected/Observed value is greater than 1 correspond to over-prediction by the model. A solid line and the broken line of the same color, for instance 0.2 and 5, correspond to reciprocal values. The ratio values 3 and 0.3 actually were measured to 3.16 and 0.316 respectively (the square root of 10 and its reciprocal).

A point on the main diagonal (shown in blue) would correspond to a data point where the Observed and Expected (or calculated) values are equal. Points between the solid green line and the dotted green line can be considered to lie in the central region of the diagram. These points correspond to ratio values that are between 0.5 and 2. These values of the ratio correspond to percentage error between 0 and 100% in the case of over-prediction, or between 0 and 50% percent error in the case of under-prediction. These diagonal lines are illustrative and do not consider the range of variation from the ratio of 1 that would be considered acceptable levels of precision in prediction. In addition, it should be noted that for diagonal lines that are farther from the center of the diagram, the logarithmic scale minimizes the distance from the main predictive diagonal (ratio of 1).

Despite this distortion from the use of the logarithmic scale, this graphic representation is useful to identify a cluster where the MPCA equation significantly under-predicts sulfide in waters with high observed sulfide concentrations.

The following diagram graphically shows an outlying cluster where high concentrations of observed porewater sulfide were poorly predicted by the MPCA's equation.



Most of the points with Expected/Observed ratios less than 0.3 have high values of observed porewater sulfide. While it is conceivable that such clustering could happen for purely random reasons, an effort to obtain conclusive results should include investigation of whether or not a peculiarity like this indicates the presence of effects that the model does not account for.

4. Spreadsheet Data Analysis – Methodology and Results

In order to more precisely address the questions raised in Figure 9 of the *MPCA Proposal*, I worked with MPCA staff to obtain the MPCA field study data collected by University of Minnesota researcher Amy Myrbo that provided the basis for Figure 9 and the MPCA Proposal. The specific references used are identified in Section 7 of this report.

I have entered the MPCA field data into my own spreadsheet, and I then programmed Formula 2 of the main document into my spreadsheet, along with a calculation of the ratio of the Expected (or Calculated) Sulfide value to the Observed Sulfide value. My complete spreadsheet is reproduced in Attachment A to my report, which sorts the data according to the Observed Porewater Sulfide level (Column J). A second spreadsheet sorting the data by the Sulfide Ratio Expected to Observed (Column P) for every water body where this calculation could be made is provided in Attachment

B. My methodology in preparing this spreadsheet is described below, then some results of reviewing the spreadsheet data are summarized.

A. METHODS

My complete spreadsheet in Attachment A is explained below.

- Data was provided for every water body on which the MPCA had field data. All quantifiable data was represented.
- The sulfide values (as in the MPCA spreadsheet) are given in milligrams per liter (mg/L) rather than micrograms per liter (μg/L). Hence the EC10 (protective) level of 165 μg/L would appear as 0.165 mg/L.
- Columns A through L give identifying information and observed (measured) values from the Wild Rice Field Survey. Column J, which gives the observed porewater sulfide value is highlighted.
- Column M is the MPCA calculation of the Calculated Protective Sulfate Concentration (CPSC) from its equation. It is the sulfate level that corresponds to the MPCA's proposed EC10 sulfide level of 165 micrograms per liter (ug/L).
- Column N reflects calculation of the CPSC, using the MPCA Equation 1 on page 14 of the MPCA March 2015 proposal (Attachment 2 to this report). This calculation was done to verify accuracy in application of the MPCA formula. As shown by comparing Columns M and N, my results agree closely with those of the MPCA.
- Column O reflects the calculation, using Equation 2 on page 9 of the MPCA March 2015 proposal (Attachment 2) to calculate the porewater sulfide levels that would be predicted from the measured values of sulfate, iron, and total organic carbon. This is the expected sulfide level.
- Column P contains ratios obtained by dividing the calculated porewater sulfide value by the observed value. This is the Expected/Observed sulfide ratio.

In the spreadsheet provided in Attachment B, sorting was done to focus on data points which:

- Sufficient data was given so that the ratio actually could be calculated.
- The value of the Expected/Observed ratio in Column P is 1 or smaller. This allows review of the points where use of the MPCA's equation results in under-prediction of sulfide levels. Column P is also highlighted.
- The data in this spreadsheet is sorted according to the Expected/Observed ratio: wild rice beds with the lowest Expected/Observed ratio value are at the top. Thus, the sites with the highest degree of under-prediction are listed first.

B. RESULTS

Column P ratios of Expected/Observed porewater sulfide levels reflect poor correlation between calculated and observed sulfide levels. Few of the Expected/Observed ratios cluster around the central value of 1, which would be the indicator of a perfect positive correlation. The degree of correlation that would be necessary for this particular application (15% variability, 20% variability or some other percentage variation from perfect correlation) to be deemed protective of wild rice would be a determination that biologists or ecologists would need to make.

However, the spreadsheet results demonstrate a number of situations where the MPCA's Calculated Protective Sulfate Concentration (CPSC) equation would underpredict observed sulfide. In those situations, it is likely that reliance on the formula would insufficiently protect wild rice from elevated sulfide. In Column P, ratios less than 1 correspond to situations where the MPCA formula has under-predicted porewater sulfide.

Nearly every site with Expected/Observed ratios below 0.4 has either no wild rice or very sparse wild rice.[2] For example, applying the MPCA's CPSC equation to Mahnomen Lake (FS-133, line 33 of Attachment A) yields a CPSC of 174.4 mg/L, which suggests that a sulfate limit of 174.4 mg/L of sulfate would be sufficient to protect wild rice in Mahnomen Lake from excess sulfide (levels exceeding 165 ug/L). However, with observed sulfate levels of 16.9 mg/L, porewater sulfide was observed at 308 ug/L. The lake's name suggests this water body once grew wild rice, but MPCA field study data showed no wild rice present.

Sandy Lake in St. Louis County (FS-320, FS-305, FS-348 on Attachment B) was historically a major and abundant ricing site for the Bois Forte Band. Although Sandy Lake has high sediment iron levels, around the 90th percentile among sites that were sampled, Sandy Lake sulfide was significantly underpredicted by the CPSC equation and exceeded the MPCA's proposed protective level of 165 ug/L by more than an order of magnitude: sulfide levels were observed at 3,080 ug/L (FS-320) and at 1,080 ug/L (FS-305). No wild rice was observed at either location.

These two examples of underprediction of sulfide using the MPCA equation do not seem to be anomalies. If one uses a threshold of variability of 20%, for example, the MPCA field data contains at least 77 of the 242 sites for which data was available where the MPCA's CPSC underpredicted sulfide levels or 32% of the sampled sites. Although not specifically analyzed in this report, the MPCA's overprediction of sulfide levels at other sampling sites would also call into questions the use of the proposed CPSC equation.

6. Conclusion

Neither MPCA's graphic representation of field study data in Figure 9 of the *MPCA Proposal*, the chi-square analysis of predictive power nor the analysis of underlying field study data in individual water bodies comparing calculated/expected levels of sulfide with observed levels provides any basis for confidence in the use of MPCA's proposed CPSC equation to predict sulfide levels and protect wild rice from excessive levels of sulfide.

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[2] Monongalia Lake in Kandiyohi County (FS-379, FS-340, in rows 42 and 82, in Attachment A) has divergent ratios, predictions and sulfide observations in sampling, making it difficult to draw conclusions regarding this lake. For Rice Lake (FS-324), despite under-prediction of sulfide, given sulfate levels of 0.5 mg/L and observed sulfide of 0.045, the presence of wild rice is not at all surprising.

7. References

My calculations are based on MPCA data, obtained from the following pages:
http://www.pca.state.mn.us/index.php/water/water-permits-and-rules/water-rulemaking/sulfate-standard-and-wild-rice/wild-rice-study-and-process-of-revising-standard.html

http://www.pca.state.mn.us/index.php/water/water-permits-and-rules/water-rulemaking/sulfate-standard-and-wild-rice/draft-proposal-for-protecting-wild-rice-from-excess-sulfate.html
On this page, follow the link Detailed MPCA proposal for protecting wild rice from excess sulfate (wq-s6-43l), which leads to the .pdf version of the main Wild Rice Study document that is discussed above. The scatter chart in question is figure 9 in that document.

In order to find more detailed MPCA field survey data, I also followed a link labeled ftp://files.pca.state.mn.us/pub/wild_rice/ and connected as a guest with a server called files.pca.state.mn.us. One folder on that server is called Wild Rice Field Survey, which contains spreadsheets used in my analysis, notably
MPCA_Field_Survey_Data_with_calculated_protective_sulfate_concentration.xlsx and
Wild_field_survey_updated_Feb_6_2015.xlsx.

8. Curriculum Vitae

Joel Roberts was born in Denver, Colorado, and grew up in the Denver area. He majored in mathematics at M.I.T. and received his Ph.D. in mathematics from Harvard University. After teaching at Purdue University for four years, he joined the University of Minnesota mathematics faculty in 1972. He has been a full professor since 1980.

Joel Roberts has had five Ph.D. students and has worked with numerous other graduate students doing thesis research in mathematics, computer science, physical sciences, and engineering. Prof. Roberts has given three different month-long lecture series at the National University of Mexico. He has visited the University of Bergen, Norway, on several occasions for research collaborations, and has been a Visiting Scholar at the University of California, Berkeley.

In recent years he has become interested in the use of computers for calculation with polynomials and also for visualization of algebraic curves and surfaces. This work included participation in the 2005-2006 Special Year on Applications of Algebraic Geometry, held at the Institute for Mathematics and Its Applications.

Mathematic publications are listed below:

1. Generic projections of algebraic varieties, Amer. J. Math. 93 (1971), 191-214.
2. The variation of singular cycles in an algebraic family of morphisms, Trans. Amer. Math. Soc. 168 (1972), 153-164.
3. Chow's moving lemma: an appendix to lectures of S. Kleiman, *Algebraic Geometry, Oslo 1970* (F.Oort, ed.), Groningen, Wolters-Noordhoff, 1972, p. 89-96.
4. Singularity subschemes and generic projections [research announcement], Bull. Amer. Math. Soc. 78 (1972), 706-708.
5. (with M. Hochster) Actions of reductive groups on regular rings and Cohen-Macaulay rings[research announcement], Bull. Amer. Math. Soc. 80 (1974), 281-284.
6. (with M. Hochster) Rings of invariants of reductive groups acting on regular rings are Cohen-Macaulay, Advances in Math. 13 (1974), 115-175.

7. Singularity subschemes and generic projections, Trans. Amer. Math. Soc. 212 (1975), 229-268.
8. (with M. Hochster) The purity of the Frobenius and local cohomology, Advances in Math. 21(1976), 117-172.
9. A stratification of the dual variety, preprint, July 1976.
10. Hypersurfaces with nonsingular normalization and their double loci, J. of Algebra 53 (1978), 253-267.
11. (with A. Holme) Pinch points and multiple locus of generic projections of singular varieties,Advances in Math. 33 (1979), 212-256.
12. Some properties of double point schemes, Compositio Math. 41 (1980), 61-94.
13. (with T. Fujita) Varieties with small secant varieties: the extremal case, Amer. J. Math. 103 (1981),953-976.
14. (with R. Speiser) Schubert's enumerative geometry of triangles from a modern viewpoint, *Algebraic Geometry: Proceedings, University of Illinois at Chicago Circle, 1980* , Springer LectureNotes in Mathematics 862 (1981), 272-281.
15. (with R. Zaare-Nahandi) Transversality of generic projections and seminormality of the image hypersurfaces, Compositio Math 52 (1984), 211-220.
16. (with R. Speiser) Enumerative geometry of triangles, I , Comm. in Algebra 12 (1984), 1213-1255.
17. (with R. Speiser) Enumerative geometry of triangles, II , Comm. in Algebra 14 (1986), 155-191.
18. (with R. Speiser) Enumerative geometry of triangles, III , Comm. in Algebra 15 (1987), 1929-1966.
19. Old and new results about the triangle varieties, *Algebraic Geometry Sundance 1986* , SpringerLecture Notes 1311 (1988), 197-219.
20. (with A. Holme) On the embeddings of projective varieties, *Algebraic Geometry Sundance 1986* ,Springer Lecture Notes 1311 (1988), 118-146.
21. Projective embeddings of algebraic varieties (lecture notes), *Monografías del Instituto deMatemáticas*, no. 19 (1988) Universidad Nacional Autónoma de México.
22. (with J. Weyman) A short proof of a theorem of M. Hashimoto, J. Algebra 134 (1990), 144 - 156.
23. Embeddings of algebraic surfaces in P^4, *Seminari di Geometria 1991-93*, Università di Bologna (1994), pp. 169 - 178.
24. (with A. Holme) Zak's theorem on superadditivity, Arkiv för Matematik 32 (1994), 99 - 120.
25. (with J. Gil de Lamadrid) The Jordan canonical form of a matrix related to a second order systemof ordinary differential equations, preprint, January 1997.
26. (with V. Reiner) Resolutions and the homology of matching and chessboard complexes, J.Algebraic Combinatorics 11 (2000), 135-154.
27. (with H Haghighi and R. Zaare-Nahandi) Some properties of finite morphisms on double points,Compositio Math. 121 (2000), 35 - 53.
28. (with J. Eagon) Minimal resolutions derived from bicomplexes and other Wall complexes, work inprogress.
29. (with A. Holme) The enumerative theory of *k*-secant (*k*-1)-spaces, work in progress

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
2	FS-85	Bean	03-0411-00-201	8/21/12	46.9337	-95.8706	0	0.0	85	16.000	1,967	11.85	1.2	1.15	0.725	0.04531
3	P-55	Lady Slipper	42-0020-00-204	9/22/11	44.5702	-95.6274	0		107.71	14.840	2,814	2.09	26.9	26.85	0.266	0.01791
4	FS-177	South Geneva	24-0015-02-208	7/24/12	43.7709	-93.2851	0	0.0	14.1	3.190	1,618	16.71	0.5	0.49	0.526	0.16487
5	FS-320	Sandy	69-0730-00-204	7/9/13	47.6188	-92.5936	0	0.0	118	3.080	19,749	15.43	72.5	72.45	0.195	0.06316
6	FS-184	Rice	73-0196-00-216	7/30/12	45.3864	-94.6309	0	0.0	2.58	2.970	1,523	15.03	0.5	0.50	0.290	0.09752
7	FS-345	Rice	73-0196-00-216	8/7/13	45.3865	-94.6313	0	0.0	6.85	2.080	2,012	14.83	0.9	0.88	0.334	0.16055
8	FS-339	Christina	21-0375-00-315	7/31/13	46.0734	-95.7567	0.3	0.6	14.6	1.930	1,741	8.96	1.3	1.35	0.374	0.1939
9	FS-188	Stella	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	0.3	18.1	1.790	1,257	2.34	4.7	4.73	0.261	0.14607
10	FS-186	Westport	61-0029-00-204	8/1/12	45.6897	-95.217	0	0.0	7.11	1.790	4,917	20.15	3.3	3.28	0.215	0.11999
11	FS-78	Lady Slipper	42-0020-00-202	7/27/12	44.5699	-95.6275	0	0.0	335	1.680	2,719	1.66	34.8	34.74	0.360	0.21415
12	FS-79	Lady Slipper	42-0020-00-203	7/27/12	44.5723	-95.6216	0	0.0	330	1.630	3,314	1.85	43.9	43.92	0.330	0.20251
13	FS-176	North Geneva	24-0015-00-209	7/24/12	43.7876	-93.271	0	0.0	15.6	1.540	2,212	13.45	1.2	1.21	0.397	0.25768
14	FS-77	Monongalia	34-0158-02-204	7/26/12	45.3331	-94.927	38.8	121.3	21.7	1.370	4,953	18.66	3.7	3.70	0.303	0.22086
15	FS-357	Lower Panasa	31-0112-00-204	8/15/13	47.3026	-93.2561	0	0.0	28.5	1.260	2,347	2.42	15.3	15.31	0.204	0.16186
16	FS-128	Cromwell	14-0103-00-201	8/22/12	46.9651	-96.3171	0	0.0	41.2	1.220	2,948	2.85	19.0	18.99	0.215	0.17622
17	FS-305	Sandy	69-0730-00-204	6/11/13	47.6187	-92.5937	0	0.0	135	1.080	19,094	22.23	40.6	40.53	0.249	0.23051
18	FS-218	Holman	31-0227-00-202	9/13/12	47.3005	-93.3445	0	0.0	24.2	1.010	3,035	29.74	0.7	0.74	0.548	0.54303
19	FS-308	Rice paddy	WT00028	6/12/13	47.8056	-95.674	36.3	85.9	57.1	0.802	2,779	17.1	1.4	1.35	0.598	0.74579
20	FS-181	Rice	66-0048-00-203	7/27/12	44.3332	-93.4734	0	0.0	5.22	0.777	3,829	21.67	1.8	1.81	0.237	0.30476
21	FS-103	Rice paddy	WT00028	6/26/12	47.8053	-95.6732	23.8	58.9	279	0.732	3,367	19.01	1.7	1.70	0.956	1.30635
22	FS-102	Rice paddy	WT00027	6/26/12	47.9265	-95.6313	39.3	93.6	1.61	0.677	4,932	31.82	1.7	1.73	0.160	0.23683
23	P-34	Anka	21-0353-00-201	9/16/11	46.0769	-95.7292	11.3		2.23	0.671	1,485	23.57	0.3	0.25	0.349	0.51956
24	FS-87	Bee	60-0192-00-202	8/23/12	47.6527	-96.0504	18.8	39.8	11	0.670	3,054	13.62	2.2	2.24	0.285	0.42488
25	FS-353	Holman	31-0227-00-202	8/12/13	47.3009	-93.3444	0	0.0	68	0.583	5,094	30.6	1.9	1.95	0.560	0.96047
26	FS-223	Little Sucker	31-0126-00-202	9/14/12	47.3765	-93.246	0	0.0	13.7	0.534	6,297	16.56	7.0	7.01	0.207	0.38799
27	FS-192	Anka	21-0353-00-202	8/29/12	46.07689	-95.7292	1		8.44	0.530	1,498	22.85	0.3	0.27	0.540	1.01953
28	P-35	Anka	21-0353-00-201	9/16/11	46.0769	-95.7377	1.3		2.23	0.493	2,170	14.84	1.0	1.02	0.216	0.43718
29	FS-326	Rice paddy	WT00028	7/17/13	47.8055	-95.6732	100	251.8	28.8	0.390	2,842	18.37	1.3	1.28	0.482	1.23517
30	FS-190	Pine	15-0149-00-205	8/28/12	47.6841	-95.5414	47.5	114.9	14.7	0.368	4,477	7.08	11.9	11.92	0.177	0.48053
31	FS-194	Gilchrist	86-0064-00-201	8/31/12	45.2309	-93.824	0	0.0	6.98	0.355	3,117	20.81	1.3	1.28	0.295	0.83071
32	FS-61	Swan	31-0067-02-206	8/30/12	47.2888	-93.2127	3	12.4	12.5	0.332	5,827	22.71	3.9	3.86	0.247	0.74282
33	FS-133	Mahnomen	18-0126-02-201	9/17/12	46.4985	-93.9958	0	0.0	16.9	0.308	18,746	7.7	174.4	174.34	0.074	0.23869
34	FS-348	Sandy	69-0730-00-204	8/13/13	47.6186	-92.5934	0	0.0	123	0.305	13,216	8.23	80.2	80.11	0.191	0.62517
35	FS-368	Dark	69-0790-00-202	9/5/13	47.6387	-92.7782	6.3	11.1	175	0.305	3,354	1.94	42.1	42.05	0.269	0.88268
36	FS-101	Rice paddy	WT00026	6/25/12	48.2161	-94.6188	4.3	8.3	11.3	0.298	3,284	44.21	0.5	0.49	0.485	1.627
37	P-57	Unnamed	34-0611-00-201	9/23/11	45.2675	-94.865	32.5		6.42	0.286	2,311	6.48	3.7	3.71	0.199	0.6954
38	FS-191	Ina	21-0355-00-202	8/29/12	46.0715	-95.7281	8.5	30.2	7.08	0.274	2,216	9.09	2.1	2.12	0.249	0.91044
39	FS-214	Bowstring	S007-219	9/11/12	47.7024	-94.0608	27.5	69.7	1.34	0.256	1,974	24.34	0.4	0.42	0.245	0.95751

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
40	FS-328	Eighteen	60-0199-00-203	7/18/13	47.6369	-96.0599	27.5	44.2	3.34	0.250	5,106	24.65	2.7	2.66	0.178	0.71181
41	FS-60	Lower Panasa	31-0112-00-205	8/29/12	47.3018	-93.2521	0	0.0	33.6	0.243	8,048	14.12	14.2	14.18	0.221	0.91117
42	FS-379	Monongalia	34-0158-02-203	9/13/13	45.3332	-94.929	62.5	154.4	34.6	0.242	5,436	26.42	2.7	2.72	0.395	1.63325
43	FS-220	Padua	73-0277-00-202	8/7/12	45.623	-95.0186	0	0.0	0.86	0.230	2,291	9.77	2.0	2.04	0.122	0.53075
44	FS-62	Swan	31-0067-02-206	8/30/12	47.289	-93.2124	0.8	3.8	14	0.221	4,821	22.53	2.7	2.69	0.290	1.31367
45	FS-82	Rabbit	18-0093-02-204	8/8/12	46.5313	-93.9285	0	0.0	15.3	0.220	10,903	11.79	33.1	33.12	0.126	0.5726
46	FS-179	Rice	74-0001-00-201	7/25/12	44.0842	-93.0737	0	0.0	3.84	0.217	4,152	19.07	2.5	2.54	0.190	0.87338
47	FS-346	Westport	61-0029-00-205	8/8/13	45.7042	-95.203	4.5	6.7	6.3	0.205	3,262	19.66	1.5	1.52	0.269	1.3099
48	FS-107	Rice paddy	WT00030	6/28/12	47.8521	-95.4953	80	134.3	9.46	0.194	5,647	28.09	2.7	2.69	0.254	1.3078
49	FS-230	Mill Pond	21-0034-00-202	8/16/12	46.0715	-95.2218	21.5	80.9	7.36	0.192	3,969	3.14	29.7	29.64	0.102	0.53004
50	FS-200	Louisa	86-0282-00-205	8/8/12	45.2998	-94.258	0	0.0	7.04	0.192	7,824	8.76	26.3	26.31	0.104	0.54356
51	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	2,253	8.37	2.5	2.46	0.180	0.94344
52	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	3,544	5.11	12.0	11.95	0.104	0.54672
53	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	2,236	1.75	22.0	22.00	0.085	0.44321
54	FS-228	West battle	56-0239-00-204	8/15/12	46.2906	-95.6049	35	144.8	4.03	0.189	3,108	17.37	1.6	1.65	0.224	1.18475
55	FS-321	Sandy	69-0730-00-203	7/9/13	47.6255	-92.5885	0	0.0	122	0.189	36,502	29.51	96.6	96.55	0.178	0.94257
56	FS-129	Mink	86-0229-00-207	8/23/12	45.2767	-94.0299	0	0.0	1.22	0.182	4,247	13.63	4.3	4.27	0.107	0.58651
57	FS-69	St. Louis	S007-208	9/7/12	47.4671	-91.9279	0	0.0	1.33	0.181	11,429	27.16	11.2	11.20	0.079	0.43544
58	FS-208	Miss.R. Pool 8/Genoa	S007-222	8/14/12	43.5758	-91.2334	43.8	41.4	18	0.176	2,178	0.41	161.8	161.72	0.077	0.43885
59	FS-106	Rice paddy	WT00029	6/28/12	47.8523	-95.4732	25	50.6	7.14	0.169	3,242	9.75	4.0	4.04	0.200	1.18478
60	FS-86	Eighteen	60-0199-00-202	8/22/12	47.6397	-96.0607	23.8	40.1	4.29	0.164	1,860	3.1	6.9	6.85	0.140	0.85384
61	FS-90	Sand	S003-249	9/11/12	47.6351	-92.4234	0.8	2.9	15.9	0.152	7,287	9.68	19.9	19.89	0.152	1.0016
62	FS-183	Unnamed	34-0611-00-201	7/30/12	45.2675	-94.865	16.3	64.9	16.8	0.150	2,157	5.61	4.0	3.97	0.271	1.80479
63	FS-315	St. Louis Estuary	S007-444	6/24/13	46.6516	-92.2373	0	0.0	8.1	0.147	6,056	1.68	163.7	163.61	0.058	0.39699
64	FS-231	Rice	02-0008-00-206	8/17/12	45.1604	-93.121	0	0.0	3.6	0.145	2,159	7.98	2.4	2.42	0.189	1.30152
65	FS-216	Big Sucker	31-0124-00-203	9/12/12	47.3919	-93.2658	1.3	3.8	7.78	0.145	3,559	21.45	1.6	1.59	0.284	1.95924
66	FS-187	McCormic	73-0273-00-203	8/2/12	45.722	-94.9121	1.3	8.9	1.54	0.144	1,512	1.1	19.7	19.70	0.068	0.47467
67	P-24	Second	15-0091-00-201	9/7/11	47.8255	-95.3635	16.3		0.87	0.139	3,813	25.67	1.4	1.42	0.139	0.99974
68	P-19	Wolf	69-0143-00-202	9/2/11	47.2586	-91.9618	56.3		1.54	0.139	8,240	25.1	6.6	6.60	0.100	0.71583
69	FS-352	Dark	69-0790-00-202	8/15/13	47.6388	-92.7782	1.3	2.9	173	0.136	5,120	3.61	40.1	40.07	0.273	2.0041
70	FS-195	Fisher	70-0087-00-201	8/31/12	44.7942	-93.4061	25	20.7	6.85	0.136	11,140	5.76	94.9	94.85	0.066	0.48846
71	FS-382	Sandy	69-0730-00-203	9/17/13	47.6255	-92.5885	0	0.0	67.9	0.135	26,645	32.28	46.0	45.97	0.188	1.39268
72	FS-81	Flowage	01-0061-00-204	8/7/12	46.688	-93.337	0	0.0	0.78	0.134	12,470	32.34	10.4	10.38	0.067	0.50215
73	FS-57	Miss. R./ bel. Clay Boswell	S006-923	8/28/12	47.2551	-93.6342	0	0.0	10.3	0.134	4,225	1.2	130.1	130.02	0.069	0.51229
74	FS-322	Dark	69-0790-00-202	7/10/13	47.6389	-92.7781	1.3	3.2	175	0.131	2,480	1.48	34.1	34.12	0.289	2.20907
75	P-29	Padua	73-0277-00-203	9/13/11	45.6202	-95.0192	1.5		0.76	0.130	4,927	20.15	3.3	3.29	0.099	0.76289

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76	FS-138	Little Round	03-0302-00-203	9/20/12	46.9726	-95.735	46.3	78.0	0.5	0.128	3,069	27.48	0.8	0.84	0.137	1.07328
77	FS-309	Eighteen	60-0199-00-203	6/13/13	47.6369	-96.0599	0	0.0	4.36	0.127	4,478	16.52	3.6	3.61	0.175	1.38175
78	FS-83	Miss.R Crow Wing	S007-205	8/8/12	46.4386	-94.1251	0	0.0	3.13	0.127	13,451	3.88	239.5	239.40	0.037	0.29011
79	FS-59	Upper Panasa	31-0111-00-202	8/29/12	47.306	-93.2652	0	0.0	29.6	0.126	895	0.43	26.6	26.55	0.171	1.35753
80	FS-301	Partridge	S007-443	5/28/13	47.5213	-92.1903	0	0.0	14.8	0.125	9,491	3.94	118.5	118.44	0.080	0.64224
81	FS-251	Sandy	69-0730-00-203	9/21/12	47.6254	-92.5886	1.3	3.8	3.05	0.123	35,905	33.08	79.6	79.59	0.053	0.43361
82	FS-340	Monongalia	34-0158-02-203	7/31/13	45.3331	-94.929	60	87.9	33.6	0.122	5,530	22.1	3.6	3.62	0.355	2.90672
83	FS-105	Second	15-0091-00-202	6/27/12	47.8258	-95.3637	13	48.4	0.74	0.119	2,527	33.3	0.439	0.44	0.197	1.65435
84	FS-350	Ox Hide	31-0106-00-203	8/14/13	47.3351	-93.2132	0	0.0	25.9	0.119	3,889	12.12	4.2	4.24	0.307	2.58002
85	FS-221	Hay Creek Flowage	58-0005-00-202	9/17/12	46.0894	-92.4104	58.8	97.7	1.95	0.119	9,456	22.05	10.4	10.37	0.092	0.77616
86	FS-68	Wolf	69-0143-00-101	9/6/12	47.2564	-91.963	2.3	8.9	2.01	0.119	9,526	17.19	15.0	14.95	0.082	0.69147
87	FS-359	Eighteen	60-0199-00-203	8/20/13	47.6367	-96.06	5.5	21.0	2.83	0.118	5,500	30.88	2.2	2.23	0.178	1.51134
88	FS-139	Welby family farm	86-0231-00-202	9/21/12	45.3592	-94.0782	2	17.2	0.5	0.118	7,267	30.76	3.9	3.88	0.081	0.68731
89	FS-319	Little Round	03-0302-00-203	6/27/13	46.9724	-95.735	5	17.5	0.5	0.117	3,579	39.84	0.7	0.67	0.148	1.26784
90	FS-219	Trout	31-0216-00-212	9/13/12	47.2592	-93.3942	0	0.0	38.6	0.117	12,535	15	31.0	30.99	0.177	1.51591
91	FS-189	Clearwater	S002-121	8/28/12	47.9372	-95.6906	1.8	4.5	23.8	0.117	2,856	1.27	55.8	55.80	0.123	1.04878
92	FS-327	Clearwater	S002-121	7/17/13	47.9371	-95.6906	0.3	0.3	23.7	0.117	3,521	1.82	50.6	50.60	0.127	1.08302
93	FS-93	Turpela	69-0427-00-201	9/12/12	47.4613	-92.2371	0.8	1.0	3.3	0.115	6,979	31.08	3.5	3.53	0.161	1.39674
94	FS-325	Rice paddy	WT00046	7/16/13	47.8481	-95.4865	51.3	79.6	0.46	0.115	4,673	19.28	3.2	3.16	0.085	0.73566
95	FS-67	St. Louis Est. Pok. Bay	S006-928	9/5/12	46.6859	-92.1606	0	0.0	9.97	0.112	14,015	3.66	281.8	281.69	0.052	0.46385
96	FS-331	Partridge	S007-443	7/24/13	47.5212	-92.1904	30	60.5	14.6	0.112	10,082	1.68	443.6	443.39	0.051	0.45262
97	FS-324	Rice	18-0053-00-203	7/15/13	46.3392	-93.8918	27.5	56.7	0.5	0.110	44,704	33.18	121.7	121.67	0.025	0.22442
98	FS-229	Mill Pond	21-0034-00-202	8/16/12	46.0716	-95.2218	30	102.2	7.16	0.109	5,143	7.86	13.5	13.49	0.132	1.21273
99	FS-311	Miss. R Pool 8/Genoa	S007-222	6/20/13	43.5766	-91.2341	10	12.7	29.3	0.107	1,544	0.62	46.1	46.05	0.141	1.31702
100	P-12	Birch	69-0003-00-205	8/30/11	47.7357	-91.9428	30		3.58	0.104	12,431	26.8	13.5	13.45	0.104	1.0011
101	FS-384	Second	S007-220	9/19/13	47.5204	-92.1925	15	27.7		0.104	22,634	3.42		791.54	0.000	0
102	P-20	Gull	04-0120-00-203	9/6/11	47.6559	-94.6944	6.8		0.78	0.103	1,608	5.08	2.6	2.57	0.109	1.05897
103	FS-356	Trout	31-0216-00-212	8/14/13	47.2591	-93.3942	0	0.0	39.1	0.103	11,992	12.59	36.4	36.38	0.169	1.63668
104	FS-75	Mortenson	34-0150-02-201	7/24/12	45.3	-94.9062	0	0.0	0.5	0.103	9,071	12.09	22.3	22.31	0.044	0.43062
105	FS-334	Miss. R Pool 8/Genoa	S007-222	7/29/13	43.5758	-91.2344	28.8	52.8	44.2	0.102	1,969	0.4	137.5	137.46	0.111	1.09193
106	FS-332	Partridge	S007-513	7/24/13	47.5137	-92.1894	53.8	79.6	54.4	0.102	20,512	8.34	185.9	185.77	0.108	1.05537
107	FS-89	Birch	69-0003-00-205	9/10/12	47.7358	-91.943	26.3	33.1	8.61	0.100	16,938	31.2	19.9	19.88	0.123	1.23138
108	FS-303	Second	S007-220	5/30/13	47.5204	-92.1925	0	0.0	303	0.099	13,086	2.2	505.1	504.89	0.138	1.39231
109	FS-316	Partridge	S007-513	6/28/13	47.5137	-92.1899	0	0.0	24.9	0.098	6,291	2.6	95.3	95.22	0.104	1.05717
110	FS-347	Snowball	31-0108-00-202	8/12/13	47.3356	-93.2439	0	0.0	8.2	0.097	1,136	1.19	10.1	10.08	0.153	1.58114
111	P-25	Lower Rice	S006-985	9/8/11	47.3793	-95.4834	50		1.02	0.097	2,337	17.76	0.9	0.91	0.171	1.76084
112	FS-360	Rice paddy	WT00046	8/21/13	47.8479	-95.4866	33.8	66.5		0.094	4,221	14.94		3.71	0.000	0

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113	FS-313	Monongalia	34-0158-01-203	6/23/13	45.3334	-94.929	32.5	50.0	34.7	0.094	6,028	19.44	5.1	5.13	0.318	3.37795
114	P-28	Raymond	73-0285-00-203	9/12/11	45.629	-95.0234	30		0.82	0.094	3,922	10.06	5.6	5.61	0.085	0.90141
115	FS-63	Caribou	69-0489-00-206	9/3/12	46.8913	-92.3135	0	0.0	1.21	0.094	13,791	29.44	14.4	14.43	0.070	0.74503
116	FS-137	Elk	15-0010-00-204	9/19/12	47.1952	-95.2249	7.3	42.7	0.5	0.094	6,334	10.07	14.3	14.30	0.052	0.55252
117	FS-197	Snowball	31-0108-00-202	9/4/12	47.3355	-93.244	0	0.0	8.4	0.094	4,213	6	13.4	13.37	0.140	1.49735
118	FS-207	Kelly Lake	66-0015-00-204	8/13/12	44.3542	-93.3743	0	0.0	1.92	0.093	4,387	27.33	1.7	1.71	0.171	1.84732
119	FS-310	Second	S007-220	6/14/13	47.5205	-92.1925	25	57.6	316	0.093	31,190	4.22	1102.4	1,101.9	0.107	1.15317
120	FS-306	Sandy	69-0730-00-203	6/11/13	47.6255	-92.5884	0	0.0	11	0.092	35,357	28.53	95.2	95.14	0.078	0.85042
121	FS-204	Big Swan	77-0023-00-207	8/10/12	45.8795	-94.742	55	133.7	5.49	0.091	1,731	5.94	2.4	2.38	0.220	2.40191
122	FS-343	Raymond	73-0285-00-203	8/6/13	45.629	-95.0233	25	61.4	1.92	0.090	3,270	7.59	5.8	5.85	0.112	1.24071
123	FS-330	St. Louis Estuary	S007-444	7/22/13	46.6518	-92.2372	8.8	11.8	6.71	0.090	5,817	1.55	169.5	169.40	0.054	0.59974
124	FS-341	Stella	47-0068-00-205	8/1/13	45.066	-94.4339	28.8	57.6	24.7	0.088	1,786	1.35	20.4	20.44	0.176	1.98834
125	FS-131	Hinken	S007-207	9/5/12	47.7271	-93.9923	18.8	46.8	0.5	0.088	2,960	4.53	10.0	9.96	0.059	0.66915
126	P-45	Hay	31-0037-00-201	9/21/11	47.2874	-93.1017	0		10.24	0.087	12,403	4.36	173.4	173.30	0.062	0.71258
127	FS-333	Embarrass	69-0496-00-203	7/26/13	47.5333	-92.2976	0	0.0	18.2	0.087	11,179	0.47	3271.6	3,270.1	0.027	0.31719
128	FS-312	Miss. R Pool 5/Spring	S007-660	6/21/13	44.2018	-91.8444	23.8	35.7	28.3	0.084	3,563	0.67	212.0	211.90	0.082	0.97423
129	FS-65	Wild Rice	09-0023-00-202	9/4/12	46.6712	-92.6055	0	0.0	0.5	0.083	13,650	28.82	14.6	14.58	0.051	0.61859
130	FS-358	Turtle River, North Branch	S007-662	8/19/13	47.9952	-97.6276	22.5	121.0	198	0.083	4,262	1.52	94.8	94.77	0.212	2.5574
131	FS-355	Miss. R./bel.Clay Boswell	S006-923	8/13/13	47.2553	-93.634	33.8	78.3	10.2	0.082	10,479	8.98	45.0	45.00	0.099	1.20337
132	FS-344	Padua	73-0277-00-202	8/6/13	45.6231	-95.0187	2.5	9.5	0.5	0.081	4,520	12.61	5.4	5.38	0.072	0.89883
133	FS-58	Miss. R/ ab. Clay Boswell	S007-163	8/28/12	47.2386	-93.7197	0	0.0	1.19	0.081	8,636	9.08	30.4	30.34	0.054	0.66756
134	P-30	Stella	47-0068-00-203	9/14/11	45.0659	-94.4339	13.8		7.59	0.080	2,159	2.88	10.2	10.18	0.149	1.85942
135	FS-202	Long Prairie	S007-204	8/9/12	46.0072	-95.2634	8.8	13.4	7.71	0.079	2,897	2.85	18.4	18.36	0.122	1.53865
136	FS-53	Raymond	73-0285-00-203	8/2/12	45.6286	-95.0225	19	61.1	0.5	0.079	1,905	4.79	3.9	3.89	0.081	1.03044
137	FS-52	Blaamyhre	34-0345-00-203	8/1/12	45.364	-95.186	15	102.2	0.62	0.078	3,517	9.33	5.0	5.04	0.080	1.02357
138	FS-213	Gull	04-0120-00-204	9/10/12	47.6558	-94.6945	4.5	9.5	1.14	0.078	3,527	16.01	2.4	2.37	0.128	1.64297
139	FS-125	Tamarac	56-0192-00-203	8/19/12	46.3637	-95.5714	0	0.0	2.33	0.077	21,908	18.41	69.2	69.19	0.051	0.66439
140	FS-198	Ox Hide	31-0106-00-203	9/7/12	47.335	-93.2134	0.3	0.6	26.4	0.075	8,743	24.51	7.7	7.66	0.252	3.35393
141	P-13	Partridge	S007-443	8/31/11	47.5212	-92.1899	28.8		10.39	0.075	11,026	1.44	656.8	656.47	0.039	0.52498
142	FS-226	Louise	21-0094-00-202	8/14/12	45.9331	-95.4148	17	46.5	4.09	0.075	1,833	0.83	42.7	42.70	0.073	0.98284
143	FS-92	Partridge	S007-443	9/12/12	47.5207	-92.1909	1.5	4.1	36.3	0.074	29,463	5.87	619.3	618.96	0.062	0.83423
144	FS-130	Hay	31-0037-00-202	9/6/12	47.2874	-93.102	53.8	141.0	31.7	0.074	13,154	5.79	130.4	130.33	0.101	1.36851
145	FS-182	Hunt	66-0047-00-208	7/27/12	44.3275	-93.4443	0	0.0	17.1	0.073	2,412	1.21	42.9	42.93	0.120	1.64409
146	FS-126	Bray	56-0472-00-202	8/20/12	46.4518	-95.8783	1.8	7.6	1.65	0.072	3,937	21.95	1.9	1.88	0.157	2.18293
147	FS-211	Miss. R Pool 4/Rob'n Lake	79-0005-02-201	8/16/12	44.3611	-91.9897	51.3	57.6	17.7	0.071	9,265	1.55	421.2	421.04	0.055	0.77246
148	FS-300	St. Louis Estuary	S007-444	5/27/13	46.6515	-92.2376	0	0.0	9.4	0.071	4,499	1.26	137.3	137.25	0.065	0.9156
149	FS-209	Miss.R Pool 8/Reno Bot.	S007-556	8/15/12	43.6025	-91.2686	46.3	72.3	18.1	0.071	9,187	2.29	239.0	238.86	0.068	0.9504

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
150	FS-196	Prairie	S007-209	9/3/12	47.2519	-93.4884	16.3	44.6	9.63	0.071	15,071	10.51	73.4	73.37	0.082	1.15106
151	FS-210	Miss. R Pool 4/Rob'n Lake	79-0005-02-202	8/16/12	44.3593	-91.9881	21.3	35.3	15.7	0.070	6,450	1.16	312.1	312.00	0.059	0.8385
152	P-26	Lower Rice	S007-164	9/8/11	47.3817	-95.4926	52.5		0.55	0.070	2,364	6.76	3.7	3.65	0.086	1.22352
153	FS-349	Sandy	69-0730-00-205	8/13/13	47.6191	-92.5898	0	0.0	122	0.070	14,897	20.46	28.1	28.04	0.273	3.91706
154	FS-225	Miltona	21-0083-00-205	8/13/12	46.0496	-95.4217	0	0.0	4.11	0.069	2,624	1.77	29.6	29.61	0.083	1.20025
155	FS-371	Miss. R Pool 5/Spring	S007-660	9/10/13	44.2016	-91.8443	26.3	39.8	34.4	0.069	3,582	0.11	2736.9	2,735.55	0.036	0.52782
156	FS-76	Field	34-0151-00-201	7/25/12	45.2964	-94.9058	0	0.0	0.5	0.069	7,586	8.68	25.1	25.09	0.043	0.62007
157	FS-342	Little Round	03-0302-00-203	8/5/13	46.9721	-95.7358	18.8	58.3	0.5	0.068	4,447	25.16	2.0	1.97	0.102	1.51579
158	FS-323	Second	S007-220	7/11/13	47.5204	-92.1925	45	76.4	405	0.067	10,036	2.91	202.6	202.54	0.209	3.11915
159	FS-134	Bass	31-0576-00-207	9/18/12	47.2844	-93.6276	32.5	64.0	1.01	0.066	3,740	26.12	1.3	1.33	0.149	2.25125
160	FS-314	Clearwater	S002-121	6/24/13	47.9372	-95.6907	0.3	0.6	28	0.066	3,946	2.68	36.7	36.64	0.150	2.25896
161	FS-94	Sturgeon	S004-870	9/13/12	47.656	-92.9315	13.8	37.9	1.62	0.066	2,505	0.65	111.1	111.02	0.038	0.58132
162	FS-318	Height of Land	03-0195-00-210	6/26/13	46.9135	-95.6124	22.5	43.0	1.21	0.066	1,349	1.13	15.2	15.17	0.069	1.04596
163	FS-91	Pike	S006-927	9/11/12	47.7327	-92.3473	23.8	3.5	14.2	0.066	6,565	4.72	44.7	44.65	0.111	1.68919
164	P-57	Unnamed	34-0611-00-201	9/23/11	45.2675	-94.865	32.5		6.42	0.065	1,689	12.6	0.8	0.79	0.340	5.22344
165	P-57	Unnamed	34-0611-00-201	9/23/11	45.2675	-94.865	32.5		6.42	0.065	1,946	13.8	0.9	0.91	0.323	4.96177
166	P-57	Unnamed	34-0611-00-201	9/23/11	45.2675	-94.865	32.5		6.42	0.065	2,193	8.1	2.4	2.44	0.230	3.53304
167	P-7	Itasca	15-0016-00-207	8/25/11	47.2332	-95.1985	8.8		0.26	0.064	1,650	6.01	2.1	2.13	0.080	1.24411
168	FS-136	Itasca	15-0016-00-208	9/19/12	47.2343	-95.2049	7.5	23.6	0.5	0.064	1,496	2.23	7.1	7.12	0.066	1.0352
169	P-10	Pike	S006-927	8/30/11	47.7325	-92.3468	18.8		8.31	0.063	15,572	10.9	74.3	74.30	0.077	1.2258
170	FS-302	Partridge	S007-513	5/30/13	47.5153	-92.1894	0	0.0	43.1	0.062	24,784	6.27	402.3	402.15	0.076	1.21971
171	FS-370	Miss. R Pool 8/Genoa	S007-222	9/9/13	43.5765	-91.2337	11.3	17.8	33.3	0.062	6,558	1.43	240.1	239.95	0.083	1.34327
172	FS-51	Glesne Slough	34-0353-00-201	7/31/12	45.3514	-95.1887	22.5	99.6	0.5	0.061	7,983	3.01	123.5	123.42	0.025	0.40325
173	FS-64	Dead Fish	09-0051-00-202	9/4/12	46.7454	-92.6865	0	0.0	0.71	0.061	14,387	22.4	23.1	23.05	0.049	0.81379
174	FS-175	Maloney	79-0001-00-201	7/23/12	44.2251	-91.9321	0	0.0	3.15	0.061	15,126	4.57	239.2	239.11	0.037	0.60752
175	FS-337	Clearwater	S004-204	7/29/13	47.5175	-95.3906	52.5	69.1	0.95	0.061	14,564	24.58	20.7	20.71	0.057	0.9336
176	FS-336	Miss. R Pool 4/Rob'n Lake	79-0005-02-201	7/30/13	44.3613	-91.9901	30	46.5	55.3	0.060	8,193	1.41	378.5	378.30	0.085	1.40841
177	FS-366	Partridge	S007-443	9/3/13	47.5213	-92.19	17.5	47.7	34.2	0.057	7,671	1.79	237.7	237.57	0.084	1.4795
178	P-44	Dead Fish	09-0051-00-202	9/20/11	46.7451	-92.6863	21.3		0.3	0.056	9,685	16.6	16.2	16.22	0.042	0.74116
179	P-5	Itasca	15-0016-00-208	8/25/11	47.2381	-95.2065	20		0.26	0.056	1,355	7.4	1.1	1.08	0.101	1.79682
180	FS-338	Height of Land	03-0195-00-210	7/30/13	46.913	-95.6116	36.3	94.2	0.5	0.055	2,641	4.58	7.9	7.85	0.064	1.14885
181	FS-199	Rice	S006-208	9/5/12	47.6742	-93.6547	29	75.4	1.57	0.055	3,273	10.88	3.5	3.52	0.124	2.25424
182	FS-372	Mississippi Pool 5 / Spring	S007-660	9/10/13	44.2016	-91.8443	13.8	26.7	34.8	0.054	3,330	0.33	504.1	503.89	0.066	1.22227
183	FS-224	Stone Lake	69-0046-00-201	9/19/12	47.5039	-91.8857	6.3	21.0	3.26	0.053	5,225	18.87	4.1	4.05	0.153	2.86279
184	FS-354	Miss. R/ ab. Clay Boswell	S007-163	8/13/13	47.2376	-93.7187	75	132.7	1.18	0.053	7,052	5.76	38.8	38.78	0.049	0.92679
185	P-1	Height of Land	03-0195-00-209	8/22/11	46.9129	-95.6095	27.5		0.24	0.053	1,298	1.76	7.5	7.53	0.050	0.94601
186	P-14	Miss. R/ ab. Clay Boswell	S007-163	9/1/11	47.2379	-93.7196	71.3		1.09	0.053	7,964	6.43	42.1	42.13	0.047	0.87964
187	FS-205	Big Swan	77-0023-00-207	8/10/12	45.8795	-94.7418	17.5	56.3	5.47	0.053	1,719	4.81	3.2	3.16	0.199	3.77265

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1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
188	FS-55	Pelkey	49-0030-00-202	8/26/12	45.9962	-94.2273	0	0.0	3.42	0.052	30,642	17.32	145.4	145.35	0.045	0.86373
189	FS-369	Dark	69-0790-00-202	9/5/13	47.6389	-92.7781	12.8	11.8	176	0.052	2,037	0.82	53.4	53.39	0.249	4.77951
190	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.050	4,503	4.46	23.1	23.13	0.083	1.66307
191	FS-88	Clearwater	S004-204	8/24/12	47.5174	-95.3904	61.3	148.3	2.04	0.049	9,874	22.17	11.2	11.20	0.091	1.87198
192	FS-80	Mission	S001-646	8/6/12	45.8623	-93.0011	52.3	87.5	0.62	0.049	9,231	4.83	84.2	84.18	0.030	0.6232
193	P-11	Sand	S003-249	8/30/11	47.6348	-92.4235	6.3		7.69	0.046	22,677	17.49	79.6	79.57	0.073	1.59589
194	FS-376	Rice	18-0053-00-203	9/11/13	46.3394	-93.8918	22.5	46.5	0.5	0.045	65,261	33.36	253.2	253.08	0.019	0.42511
195	FS-367	Hay	31-0037-00-202	9/4/13	47.287	-93.1009	83.8	141.0	22.1	0.045	15,436	3.44	371.5	371.33	0.062	1.39045
196	FS-351	Second	S007-220	8/15/13	47.5205	-92.1925	52.5	66.8	838	0.045	7,088	1.84	195.9	195.78	0.272	6.08065
197	FS-66	St. Louis Estuary	S007-206	9/5/12	46.6545	-92.2739	0	0.0	16	0.045	6,169	1.73	162.8	162.76	0.074	1.66149
198	FS-132	Ox Hide	31-0106-00-203	9/7/12	47.335	-93.2134	4	10.5	26.4	0.042	14,936	14.43	46.1	46.11	0.136	3.22953
199	P-42	Monongalia	34-0158-01-201	9/20/11	45.3481	-94.951	2.5		16.51	0.042	46,471	14.76	411.5	411.28	0.054	1.29075
200	P-61	Lily	81-0067-00-202	9/28/11	44.194	-93.6469	22.5		0.66	0.041	6,180	14.06	8.5	8.51	0.068	1.65998
201	P-6	Elk	15-0010-00-203	8/25/11	47.1946	-95.2254	11.3		0.28	0.040	8,480	10.24	24.7	24.71	0.035	0.87637
202	P-17	St. Louis	S007-208	9/1/11	47.4668	-91.9355	30		1.23	0.040	9,654	30.4	6.9	6.87	0.091	2.27037
203	FS-383	Upper Panasa	31-0111-00-204	9/18/13	47.3059	-93.2676	0	0.0	33.6	0.040	19,148	2.86	734.7	734.34	0.057	1.42271
204	FS-365	Partridge	S007-443	9/3/13	47.5212	-92.1901	31.3	76.7	34.1	0.039	9,179	2.5	210.8	210.70	0.088	2.23388
205	FS-374	Little Round	03-0302-00-202	9/10/13	46.9745	-95.738	21.3	37.6	0.12	0.039	2,018	14.8	0.9	0.89	0.082	2.10963
206	FS-203	Long Prairie	S007-203	8/9/12	45.9729	-95.1603	46.3	58.3	6.66	0.039	5,074	4.35	30.3	30.26	0.098	2.49607
207	FS-307	Rice paddy	WT00046	6/12/13	47.8482	-95.4865	4.3	8.3	16.6	0.039	4,292	22.33	2.2	2.17	0.332	8.50213
208	P-23	Gourd	04-0253-00-201	9/7/11	47.812	-94.9654	16.8		0.69	0.038	2,675	27.4	0.6	0.65	0.168	4.42652
209	FS-201	Mink	86-0229-00-206	8/8/12	45.274	-94.0269	0	0.0	1.31	0.037	1,740	1.53	16.3	16.28	0.069	1.85046
210	FS-373	Clearwater	S002-121	9/9/13	47.9372	-95.6909	5	3.2	34.4	0.035	5,315	3.33	48.3	48.30	0.146	4.13469
211	FS-54	Little Birch	77-0089-00-207	8/3/12	45.7779	-94.7978	11.3	70.0	7.4	0.035	1,794	6.02	2.5	2.50	0.239	6.77336
212	P-15	Miss. R./bel.Clay Boswell	S006-923	9/1/11	47.2547	-93.6344	43.8		3.65	0.035	8,667	6.07	53.9	53.92	0.065	1.85623
213	FS-185	Hoffs Slough	76-0103-00-201	8/1/12	45.3255	-95.7059	0	0.0	273	0.034	3,512	0.75	175.8	175.71	0.192	5.58924
214	FS-380	Sandy	69-0730-00-204	9/17/13	47.6187	-92.5939	0.3	0.6	126	0.034	17,868	22.7	34.6	34.56	0.257	7.50964
215	FS-381	Sandy	69-0730-00-204	9/17/13	47.6187	-92.5931	0	0.0	126	0.034	16,172	11.67	72.7	72.66	0.199	5.8133
216	FS-335	Miss. R Pool 5/Spring	S007-660	7/30/13	44.1953	-91.841	42.5	63.0	47.7	0.034	4,362	0.25	1264.4	1,263.8	0.053	1.5553
217	P-3	Little Round	03-0302-00-202	8/24/11	46.9759	-95.7404	25		0.46	0.032	1,689	20.91	0.4	0.38	0.175	5.46618
218	FS-108	Rice paddy	WT00031	6/29/12	46.246	-94.2548	33.8	54.7	0.25	0.031	7,874	37.88	3.4	3.38	0.067	2.13826
219	FS-193	Big Mud	71-0085-00-201	8/30/12	45.4529	-93.7418	4.3	14.3	0.5	0.031	12,943	18.63	24.3	24.30	0.043	1.39776
220	FS-95	Embarrass	69-0496-00-203	9/14/12	47.5334	-92.2979	0	0.0	18.8	0.030	21,847	1.89	1705.2	1,704.4	0.035	1.16622
221	FS-180	Lily	81-0067-00-202	7/26/12	44.1947	-93.647	18.8	38.2	0.5	0.030	5,095	28.07	2.2	2.20	0.099	3.34185
222	FS-250	Little Rice	69-0612-00-201	9/20/12	47.7086	-92.4389	8.8	29.3	1.03	0.029	9,488	26.45	8.1	8.08	0.081	2.75681
223	P-53	Carlos Avery Pool 9	02-0504-00-201	8/19/11	45.3179	-93.0587	18.8		0.35	0.029	37,965	16.51	236.6	236.48	0.017	0.59867
224	FS-377	Mahnomen	18-0126-02-201	9/11/13	46.4986	-93.9956	0	0.0	21.1	0.028	16,540	7.47	142.5	142.43	0.085	3.00714
225	FS-215	Popple	S006-188	9/11/12	47.7254	-94.0817	11.8	36.3	0.5	0.027	2,971	14.42	2.0	1.96	0.103	3.81575

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226	P-46	Hay	31-0037-00-201	9/21/11	47.2869	-93.1018	0		10.24	0.026	16,139	7.69	130.4	130.31	0.068	2.63005
227	FS-56	Rice	18-0053-00-203	8/27/12	46.3389	-93.8915	3.5	19.4	0.5	0.026	83,421	31.88	436.3	436.11	0.016	0.61353
228	FS-378	Duck Lake WMA	18-0178-00-202	9/12/13	46.7521	-93.8851	42.5	113.0	0.5	0.025	12,151	26.57	13.0	13.02	0.053	2.12693
229	P-16	St. Louis	S006-929	9/1/11	47.4015	-92.3773	0		24.5	0.025	1,488	0.1	561.5	561.24	0.056	2.23849
230	P-31	Cloquet	38-0539-00-201	9/14/11	47.4313	-91.4844	32.5		0.81	0.024	4,252	6.58	12.0	11.95	0.065	2.70841
231	FS-304	Rice	18-0053-00-203	6/10/13	46.3387	-93.8906	2.5	5.7	0.5	0.024	48,287	33.61	139.0	138.93	0.024	0.9992
232	P-36	Wild Rice Reservoir	69-0371-00-204	9/16/11	46.9098	-92.1636	7.5		1.13	0.023	5,555	3.75	44.6	44.54	0.046	2.01392
233	FS-212	Miss. R Pool 5/Spring	S007-660	8/17/12	44.1993	-91.8461	17.5	29.6	17.2	0.022	3,674	0.22	1082.3	1,081.8	0.039	1.76237
234	FS-84	Pleasant	11-0383-00-207	8/10/12	46.9228	-94.4874	0	0.0	0.5	0.022	7,065	23.99	5.2	5.21	0.073	3.36026
235	P-69	Rice	18-0053-00-203	9/27/11	46.3394	-93.8913	18.8		0.23	0.021	50,389	35.55	139.6	139.52	0.018	0.85773
236	P-52	Flowage	01-0061-00-205	9/22/11	46.6895	93.338	53.8		0.56	0.018	3,706	16.52	2.5	2.49	0.098	5.45675
237	P-52	Flowage	01-0061-00-206	9/22/11	46.6895	93.338	53.8		0.56	0.018	4,302	21.79	2.3	2.26	0.102	5.64492
238	P-52	Flowage	01-0061-00-206	9/22/11	46.6895	93.338	53.8		0.56	0.018	4,641	18.1	3.4	3.40	0.088	4.90074
239	P-51	Flowage	01-0061-00-205	9/22/11	46.6896	93.338	70		0.56	0.014	5,627	20.1	4.3	4.28	0.082	5.82175
240	FS-109	Carlos Avery Pool 9	02-0504-00-202	7/3/12	45.3192	-93.0611	23.8	52.8	0.5	0.011	14,736	12.51	54.9	54.92	0.033	2.95463
241	FS-127	Height of Land	03-0195-00-210	8/21/12	46.9133	-95.6095	70	111.1	0.5	0.011	2,112	1.32	29.3	29.28	0.040	3.67555
242	FS-375	Height of Land	03-0195-00-210	9/10/13	46.913	-95.6111	63.8	117.5	0.5	0.011	1,795	0.86	39.0	38.98	0.037	3.33093
243	P-4	Little Flat	03-0217-00-201	8/24/11	46.9981	-95.6641	36.3		0.22	0.011	7,479	33.13	3.7	3.69	0.062	5.64764
244	P-63	Maloney	79-0001-00-201	9/29/11	44.2243	-91.9328	65		1.83	0.010	10,269	4.24	124.7	124.60	0.038	3.83534
245	P-22	Ham	02-0053-00-201	9/6/11	45.2572	-93.2264	0		0.95							
246	FS-104	Gourd	04-0253-00-201	6/27/12	47.8121	-94.965	0	0.0	0.27		1,776	36.87	0.2	0.19	0.185	
247	P-43	Wild Rice	09-0023-00-201	9/20/11	46.6735	-92.6023	0		0.37							
248	P-27	Pleasant	11-0383-00-206	9/9/11	46.928	-94.4757	12.5		0.49		5,331	30.37	2.2	2.15	0.099	
249	P-56	Rice	18-0053-00-203	9/23/11	46.3396	-93.8901	0		0.38							
250	P-37	Ina	21-0355-00-201	9/16/11	46.0822	-95.726	0		2.17							
251	FS-178	Bear	24-0028-00-206	7/25/12	43.5465	-93.5028	0	0.0	18.3							
252	P-33	Pelican	26-0002-00-219	9/15/11	46.0616	-95.8296	0		5.79							
253	P-8	Pelican	26-0002-00-219	8/26/11	46.0616	-95.8296	0									
254	FS-50	Swan	34-0223-00-201	7/30/12	45.326	-95.067	0	0.0	11.7							
255	P-18	Lax	38-0406-00-203	9/2/11	47.3508	-91.2921	0		1.43							
256	P-32	Caribou	69-0489-00-205	9/15/11	46.8991	-92.3217	0		0.63							
257	P-9	Embarrass	69-0496-00-202	8/29/11	47.534	-92.3164	0		6.35							
258	P-39	Grand	69-0511-00-203	9/17/11	46.8872	-92.3988	0		0.83							
259	P-64	Maloney	79-0001-00-201	9/29/11	44.2243	-91.9328	0		1.83		10,382	4.05	135.9	135.79	0.037	
260	P-62	Lily	81-0067-00-202	9/28/11	44.194	-93.6469	0		0.64		5,069	13.39	6.2	6.19	0.075	
261	P-2	Mud	S004-735	8/23/11	46.6266	-95.5751	0									
262	P-41	St. Louis Est. Pok. Bay	S006-928	9/19/11	46.6855	-92.1619	0		2.33							
263	FS-70	St. Louis	S006-929	9/7/12	47.4015	-92.3772	0	0.0	73.8							

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
264	FS-317	Partridge	S007-443	6/26/13	47.5215	-92.1903	0	0.0	7.65							
265	FS-363	St. Louis Estuary	S007-444	8/26/13	46.6518	-92.2372	18.8	31.2			4,761	1.4		132.15		
266	P-40	St. Louis Estuary	S007-444	9/19/11	46.6588	-92.2819	0		4.9							
267	FS-364	Partridge	S007-513	8/30/13	47.5138	-92.1894	57.5	105.7			28,890	8.19		372.42		
268	FS-361	Rice paddy	WT00028	8/21/13	47.8054	-95.6744	68.8	78.6			3,089	12.46		2.60		

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
2	P-55	Lady Slipper	42-0020-00-204	9/22/11	44.5702	-95.6274	0		107.71	14.840	2,814	2.09	26.9	26.85	0.266	0.01791
3	FS-85	Bean	03-0411-00-201	8/21/12	46.9337	-95.8706	0	0.0	85	16.000	1,967	11.85	1.2	1.15	0.725	0.04531
4	FS-320	Sandy	69-0730-00-204	7/9/13	47.6188	-92.5936	0	0.0	118	3.080	19,749	15.43	72.5	72.45	0.195	0.06316
5	FS-184	Rice	73-0196-00-216	7/30/12	45.3864	-94.6309	0	0.0	2.58	2.970	1,523	15.03	0.5	0.50	0.290	0.09752
6	FS-186	Westport	61-0029-00-204	8/1/12	45.6897	-95.217	0	0.0	7.11	1.790	4,917	20.15	3.3	3.28	0.215	0.11999
7	FS-188	Stella	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	0.3	18.1	1.790	1,257	2.34	4.7	4.73	0.261	0.14607
8	FS-345	Rice	73-0196-00-216	8/7/13	45.3865	-94.6313	0	0.0	6.85	2.080	2,012	14.83	0.9	0.88	0.334	0.16055
9	FS-357	Lower Panasa	31-0112-00-204	8/15/13	47.3026	-93.2561	0	0.0	28.5	1.260	2,347	2.42	15.3	15.31	0.204	0.16186
10	FS-177	South Geneva	24-0015-02-208	7/24/12	43.7709	-93.2851	0	0.0	14.1	3.190	1,618	16.71	0.5	0.49	0.526	0.16487
11	FS-128	Cromwell	14-0103-00-201	8/22/12	46.9651	-96.3171	0	0.0	41.2	1.220	2,948	2.85	19.0	18.99	0.215	0.17622
12	FS-339	Christina	21-0375-00-315	7/31/13	46.0734	-95.7567	0.3	0.6	14.6	1.930	1,741	8.96	1.3	1.35	0.374	0.1939
13	FS-79	Lady Slipper	42-0020-00-203	7/27/12	44.5723	-95.6216	0	0.0	330	1.630	3,314	1.85	43.9	43.92	0.330	0.20251
14	FS-78	Lady Slipper	42-0020-00-202	7/27/12	44.5699	-95.6275	0	0.0	335	1.680	2,719	1.66	34.8	34.74	0.360	0.21415
15	FS-77	Monongalia	34-0158-02-204	7/26/12	45.3331	-94.927	38.8	121.3	21.7	1.370	4,953	18.66	3.7	3.70	0.303	0.22086
16	FS-324	Rice	18-0053-00-203	7/15/13	46.3392	-93.8918	27.5	56.7	0.5	0.110	44,704	33.18	121.7	121.67	0.025	0.22442
17	FS-305	Sandy	69-0730-00-204	6/11/13	47.6187	-92.5937	0	0.0	135	1.080	19,094	22.23	40.6	40.53	0.249	0.23051
18	FS-102	Rice paddy	WT00027	6/26/12	47.9265	-95.6313	39.3	93.6	1.61	0.677	4,932	31.82	1.7	1.73	0.160	0.23683
19	FS-133	Mahnomen	18-0126-02-201	9/17/12	46.4985	-93.9958	0	0.0	16.9	0.308	18,746	7.7	174.4	174.34	0.074	0.23869
20	FS-176	North Geneva	24-0015-00-209	7/24/12	43.7876	-93.271	0	0.0	15.6	1.540	2,212	13.45	1.2	1.21	0.397	0.25768
21	FS-83	Mississippi Crow Wing	S007-205	8/8/12	46.4386	-94.1251	0	0.0	3.13	0.127	13,451	3.88	239.5	239.40	0.037	0.29011
22	FS-181	Rice	66-0048-00-203	7/27/12	44.3332	-93.4734	0	0.0	5.22	0.777	3,829	21.67	1.8	1.81	0.237	0.30476
23	FS-333	Embarrass	69-0496-00-203	7/26/13	47.5333	-92.2976	0	0.0	18.2	0.087	11,179	0.47	3271.6	3,270.1	0.027	0.31719
24	FS-223	Little Sucker	31-0126-00-202	9/14/12	47.3765	-93.246	0	0.0	13.7	0.534	6,297	16.56	7.0	7.01	0.207	0.38799
25	FS-315	St. Louis Estuary	S007-444	6/24/13	46.6516	-92.2373	0	0.0	8.1	0.147	6,056	1.68	163.7	163.61	0.058	0.39699
26	FS-51	Glesne Slough	34-0353-00-201	7/31/12	45.3514	-95.1887	22.5	99.6	0.5	0.061	7,983	3.01	123.5	123.42	0.025	0.40325
27	FS-87	Bee	60-0192-00-202	8/23/12	47.6527	-96.0504	18.8	39.8	11	0.670	3,054	13.62	2.2	2.24	0.285	0.42488
28	FS-376	Rice	18-0053-00-203	9/11/13	46.3394	-93.8918	22.5	46.5	0.5	0.045	65,261	33.36	253.2	253.08	0.019	0.42511
29	FS-75	Mortenson	34-0150-02-201	7/24/12	45.3	-94.9062	0	0.0	0.5	0.103	9,071	12.09	22.3	22.31	0.044	0.43062
30	FS-251	Sandy	69-0730-00-203	9/21/12	47.6254	-92.5886	1.3	3.8	3.05	0.123	35,905	33.08	79.6	79.59	0.053	0.43361
31	FS-69	St. Louis	S007-208	9/7/12	47.4671	-91.9279	0	0.0	1.33	0.181	11,429	27.16	11.2	11.20	0.079	0.43544
32	P-35	Anka	21-0353-00-201	9/16/11	46.0769	-95.7377	1.3		2.23	0.493	2,170	14.84	1.0	1.02	0.216	0.43718
33	FS-208	Mississippi Pool 8 at Genoa	S007-222	8/14/12	43.5758	-91.2334	43.8	41.4	18	0.176	2,178	0.41	161.8	161.72	0.077	0.43885
34	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	2,236	1.75	22.0	22.00	0.085	0.44321
35	FS-331	Partridge	S007-443	7/24/13	47.5212	-92.1904	30	60.5	14.6	0.112	10,082	1.68	443.6	443.39	0.051	0.45262
36	FS-67	St. Louis Estuary Pokegama Bay	S006-928	9/5/12	46.6859	-92.1606	0	0.0	9.97	0.112	14,015	3.66	281.8	281.69	0.052	0.46385
37	FS-187	McCormic	73-0273-00-203	8/2/12	45.722	-94.9121	1.3	8.9	1.54	0.144	1,512	1.1	19.7	19.70	0.068	0.47467
38	FS-190	Pine	15-0149-00-205	8/28/12	47.6841	-95.5414	47.5	114.9	14.7	0.368	4,477	7.08	11.9	11.92	0.177	0.48053

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1	LacCore field ID	Site name	DNR/State ID	Date	Lat	Long	WR ring % cover	Ave. stems /m2	Obs surf water sulfate (mg SO4/L)	Obs pore water Tot Sulfide (TS, mg S/L)	Observed Sediment Fe (µg/g)	Observed Sediment TOC (%)	CPSC (mg/L)	CPSC check	Direct sulfide calc (expect)	Sulfide ratio expect to obs
39	FS-195	Fisher	70-0087-00-201	8/31/12	44.7942	-93.4061	25	20.7	6.85	0.136	11,140	5.76	94.9	94.85	0.066	0.48846
40	FS-81	Flowage	01-0061-00-204	8/7/12	46.688	-93.337	0	0.0	0.78	0.134	12,470	32.34	10.4	10.38	0.067	0.50215
41	FS-57	Mississippi River below Clay Boswell	S006-923	8/28/12	47.2551	-93.6342	0	0.0	10.3	0.134	4,225	1.2	130.1	130.02	0.069	0.51229
42	P-34	Anka	21-0353-00-201	9/16/11	46.0769	-95.7292	11.3		2.23	0.671	1,485	23.57	0.3	0.25	0.349	0.51956
43	P-13	Partridge	S007-443	8/31/11	47.5212	-92.1899	28.8		10.39	0.075	11,026	1.44	656.8	656.47	0.039	0.52498
44	FS-371	Mississippi Pool 5 / Spring	S007-660	9/10/13	44.2016	-91.8443	26.3	39.8	34.4	0.069	3,582	0.11	2736.9	2,735.55	0.036	0.52782
45	FS-230	Mill Pond	21-0034-00-202	8/16/12	46.0715	-95.2218	21.5	80.9	7.36	0.192	3,969	3.14	29.7	29.64	0.102	0.53004
46	FS-220	Padua	73-0277-00-202	8/7/12	45.623	-95.0186	0	0.0	0.86	0.230	2,291	9.77	2.0	2.04	0.122	0.53075
47	FS-218	Holman	31-0227-00-202	9/13/12	47.3005	-93.3445	0	0.0	24.2	1.010	3,035	29.74	0.7	0.74	0.548	0.54303
48	FS-200	Louisa	86-0282-00-205	8/8/12	45.2998	-94.258	0	0.0	7.04	0.192	7,824	8.76	26.3	26.31	0.104	0.54356
49	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	3,544	5.11	12.0	11.95	0.104	0.54672
50	FS-137	Elk	15-0010-00-204	9/19/12	47.1952	-95.2249	7.3	42.7	0.5	0.094	6,334	10.07	14.3	14.30	0.052	0.55252
51	FS-82	Rabbit	18-0093-02-204	8/8/12	46.5313	-93.9285	0	0.0	15.3	0.220	10,903	11.79	33.1	33.12	0.126	0.5726
52	FS-94	Sturgeon	S004-870	9/13/12	47.656	-92.9315	13.8	37.9	1.62	0.066	2,505	0.65	111.1	111.02	0.038	0.58132
53	FS-129	Mink	86-0229-00-207	8/23/12	45.2767	-94.0299	0	0.0	1.22	0.182	4,247	13.63	4.3	4.27	0.107	0.58651
54	P-53	Carlos Avery Pool 9	02-0504-00-201	8/19/11	45.3179	-93.0587	18.8		0.35	0.029	37,965	16.51	236.6	236.48	0.017	0.59867
55	FS-330	St. Louis Estuary	S007-444	7/22/13	46.6518	-92.2372	8.8	11.8	6.71	0.090	5,817	1.55	169.5	169.40	0.054	0.59974
56	FS-175	Maloney	79-0001-00-201	7/23/12	44.2251	-91.9321	0	0.0	3.15	0.061	15,126	4.57	239.2	239.11	0.037	0.60752
57	FS-56	Rice	18-0053-00-203	8/27/12	46.3389	-93.8915	3.5	19.4	0.5	0.026	83,421	31.88	436.3	436.11	0.016	0.61353
58	FS-65	Wild Rice	09-0023-00-202	9/4/12	46.6712	-92.6055	0	0.0	0.5	0.083	13,650	28.82	14.6	14.58	0.051	0.61859
59	FS-76	Field	34-0151-00-201	7/25/12	45.2964	-94.9058	0	0.0	0.5	0.069	7,586	8.68	25.1	25.09	0.043	0.62007
60	FS-80	Mission	S001-646	8/6/12	45.8623	-93.0011	52.3	87.5	0.62	0.049	9,231	4.83	84.2	84.18	0.030	0.6232
61	FS-348	Sandy	69-0730-00-204	8/13/13	47.6186	-92.5934	0	0.0	123	0.305	13,216	8.23	80.2	80.11	0.191	0.62517
62	FS-301	Partridge	S007-443	5/28/13	47.5213	-92.1903	0	0.0	14.8	0.125	9,491	3.94	118.5	118.44	0.080	0.64224
63	FS-125	Tamarac	56-0192-00-203	8/19/12	46.3637	-95.5714	0	0.0	2.33	0.077	21,908	18.41	69.2	69.19	0.051	0.66439
64	FS-58	Mississippi River above Clay Boswell	S007-163	8/28/12	47.2386	-93.7197	0	0.0	1.19	0.081	8,636	9.08	30.4	30.34	0.054	0.66756
65	FS-131	Hinken	S007-207	9/5/12	47.7271	-93.9923	18.8	46.8	0.5	0.088	2,960	4.53	10.0	9.96	0.059	0.66915
66	FS-139	Welby family farm	86-0231-00-202	9/21/12	45.3592	-94.0782	2	17.2	0.5	0.118	7,267	30.76	3.9	3.88	0.081	0.68731
67	FS-68	Wolf	69-0143-00-101	9/6/12	47.2564	-91.963	2.3	8.9	2.01	0.119	9,526	17.19	15.0	14.95	0.082	0.69147
68	P-57	Unnamed	34-0611-00-201	9/23/11	45.2675	-94.865	32.5		6.42	0.286	2,311	6.48	3.7	3.71	0.199	0.6954
69	FS-328	Eighteen	60-0199-00-203	7/18/13	47.6369	-96.0599	27.5	44.2	3.34	0.250	5,106	24.65	2.7	2.66	0.178	0.71181
70	P-45	Hay	31-0037-00-201	9/21/11	47.2874	-93.1017	0		10.24	0.087	12,403	4.36	173.4	173.30	0.062	0.71258
71	P-19	Wolf	69-0143-00-202	9/2/11	47.2586	-91.9618	56.3		1.54	0.139	8,240	25.1	6.6	6.60	0.100	0.71583
72	FS-325	Rice paddy	WT00046	7/16/13	47.8481	-95.4865	51.3	79.6	0.46	0.115	4,673	19.28	3.2	3.16	0.085	0.73566
73	P-44	Dead Fish	09-0051-00-202	9/20/11	46.7451	-92.6863	21.3		0.3	0.056	9,685	16.6	16.2	16.22	0.042	0.74116
74	FS-61	Swan	31-0067-02-206	8/30/12	47.2888	-93.2127	3	12.4	12.5	0.332	5,827	22.71	3.9	3.86	0.247	0.74282
75	FS-63	Caribou	69-0489-00-206	9/3/12	46.8913	-92.3135	0	0.0	1.21	0.094	13,791	29.44	14.4	14.43	0.070	0.74503

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76	FS-308	Rice paddy	WT00028	6/12/13	47.8056	-95.674	36.3	85.9	57.1	0.802	2,779	17.1	1.4	1.35	0.598	0.74579
77	P-29	Padua	73-0277-00-203	9/13/11	45.6202	-95.0192	1.5		0.76	0.130	4,927	20.15	3.3	3.29	0.099	0.76289
78	FS-211	Mississippi Pool 4/Robinson Lake	79-0005-02-201	8/16/12	44.3611	-91.9897	51.3	57.6	17.7	0.071	9,265	1.55	421.2	421.04	0.055	0.77246
79	FS-221	Hay Creek Flowage	58-0005-00-202	9/17/12	46.0894	-92.4104	58.8	97.7	1.95	0.119	9,456	22.05	10.4	10.37	0.092	0.77616
80	FS-64	Dead Fish	09-0051-00-202	9/4/12	46.7454	-92.6865	0	0.0	0.71	0.061	14,387	22.4	23.1	23.05	0.049	0.81379
81	FS-194	Gilchrist	86-0064-00-201	8/31/12	45.2309	-93.824	0	0.0	6.98	0.355	3,117	20.81	1.3	1.28	0.295	0.83071
82	FS-92	Partridge	S007-443	9/12/12	47.5207	-92.1909	1.5	4.1	36.3	0.074	29,463	5.87	619.3	618.96	0.062	0.83423
83	FS-210	Mississippi Pool 4/Robinson Lake	79-0005-02-202	8/16/12	44.3593	-91.9881	21.3	35.3	15.7	0.070	6,450	1.16	312.1	312.00	0.059	0.8385
84	FS-306	Sandy	69-0730-00-203	6/11/13	47.6255	-92.5884	0	0.0	11	0.092	35,357	28.53	95.2	95.14	0.078	0.85042
85	FS-86	Eighteen	60-0199-00-202	8/22/12	47.6397	-96.0607	23.8	40.1	4.29	0.164	1,860	3.1	6.9	6.85	0.140	0.85384
86	P-69	Rice	18-0053-00-203	9/27/11	46.3394	-93.8913	18.8		0.23	0.021	50,389	35.55	139.6	139.52	0.018	0.85773
87	FS-55	Pelkey	49-0030-00-202	8/26/12	45.9962	-94.2273	0	0.0	3.42	0.052	30,642	17.32	145.4	145.35	0.045	0.86373
88	FS-179	Rice	74-0001-00-201	7/25/12	44.0842	-93.0737	0	0.0	3.84	0.217	4,152	19.07	2.5	2.54	0.190	0.87338
89	P-6	Elk	15-0010-00-203	8/25/11	47.1946	-95.2254	11.3		0.28	0.040	8,480	10.24	24.7	24.71	0.035	0.87637
90	P-14	Mississippi River above Clay Boswell	S007-163	9/1/11	47.2379	-93.7196	71.3		1.09	0.053	7,964	6.43	42.1	42.13	0.047	0.87964
91	FS-368	Dark	69-0790-00-202	9/5/13	47.6387	-92.7782	6.3	11.1	175	0.305	3,354	1.94	42.1	42.05	0.269	0.88268
92	FS-344	Padua	73-0277-00-202	8/6/13	45.6231	-95.0187	2.5	9.5	0.5	0.081	4,520	12.61	5.4	5.38	0.072	0.89883
93	P-28	Raymond	73-0285-00-203	9/12/11	45.629	-95.0234	30		0.82	0.094	3,922	10.06	5.6	5.61	0.085	0.90141
94	FS-191	Ina	21-0355-00-202	8/29/12	46.0715	-95.7281	8.5	30.2	7.08	0.274	2,216	9.09	2.1	2.12	0.249	0.91044
95	FS-60	Lower Panasa	31-0112-00-205	8/29/12	47.3018	-93.2521	0	0.0	33.6	0.243	8,048	14.12	14.2	14.18	0.221	0.91117
96	FS-300	St. Louis Estuary	S007-444	5/27/13	46.6515	-92.2376	0	0.0	9.4	0.071	4,499	1.26	137.3	137.25	0.065	0.9156
97	FS-354	Mississippi River above Clay Boswell	S007-163	8/13/13	47.2376	-93.7187	75	132.7	1.18	0.053	7,052	5.76	38.8	38.78	0.049	0.92679
98	FS-337	Clearwater	S004-204	7/29/13	47.5175	-95.3906	52.5	69.1	0.95	0.061	14,564	24.58	20.7	20.71	0.057	0.9336
99	FS-321	Sandy	69-0730-00-203	7/9/13	47.6255	-92.5885	0	0.0	122	0.189	36,502	29.51	96.6	96.55	0.178	0.94257
100	P-47	Little Birch	77-0089-00-101	9/21/11	45.7747	-94.7996	11.3		3.2	0.191	2,253	8.37	2.5	2.46	0.180	0.94344
101	P-1	Height of Land	03-0195-00-209	8/22/11	46.9129	-95.6095	27.5		0.24	0.053	1,298	1.76	7.5	7.53	0.050	0.94601
102	FS-209	Mississippi Pool 8 at Reno Bottoms	S007-556	8/15/12	43.6025	-91.2686	46.3	72.3	18.1	0.071	9,187	2.29	239.0	238.86	0.068	0.9504
103	FS-214	Bowstring	S007-219	9/11/12	47.7024	-94.0608	27.5	69.7	1.34	0.256	1,974	24.34	0.4	0.42	0.245	0.95751
104	FS-353	Holman	31-0227-00-202	8/12/13	47.3009	-93.3444	0	0.0	68	0.583	5,094	30.6	1.9	1.95	0.560	0.96047
105	FS-312	Mississippi Pool 5 / Spring	S007-660	6/21/13	44.2018	-91.8444	23.8	35.7	28.3	0.084	3,563	0.67	212.0	211.90	0.082	0.97423
106	FS-226	Louise	21-0094-00-202	8/14/12	45.9331	-95.4148	17	46.5	4.09	0.075	1,833	0.83	42.7	42.70	0.073	0.98284
107	FS-304	Rice	18-0053-00-203	6/10/13	46.3387	-93.8906	2.5	5.7	0.5	0.024	48,287	33.61	139.0	138.93	0.024	0.9992
108	P-24	Second	15-0091-00-201	9/7/11	47.8255	-95.3635	16.3		0.87	0.139	3,813	25.67	1.4	1.42	0.139	0.99974

Roberts Memorandum - Wild Rice Rule
November 2017

Attachment 2

(9 pages)

MPCA Field Data - All MN non-paddy data
CPSC and Sulfate Ratio

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	LacCore _field_ID	Site_name	Unique site ID	DNR/State ID	Date	Lat	Long	Calculated Wild rice ave stems/m2	surface water SO4 (mg SO4/L)	pore water Total Sulfide (TS, mg S/L)	Sediment Fe (µg/g)	Sediment TOC (%)	potential SO4 standard CPSC120	CPSC120 calculated	surface water SO4 (mg SO4/L)	SO4 ratio: CPSC to actual
2	FS-384	Second	117	S007-220	9/19/13	47.52	-92.1925	27.7		0.104	22634	3.42	657.3	657.30	missing	#VALUE!
3	FS-364	Partridge	121	S007-513	8/30/13	47.514	-92.1894	105.7			28890	8.19	369.5	369.49	missing	#VALUE!
4	FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.652	-92.2372	31.2			4761	1.4	95.5	95.52	missing	#VALUE!
5	FS-56	Rice	19	18-0053-00	8/27/12	46.339	-93.8915	19.4	< 0.5	0.0259	83421	31.88	558.1	558.07	0.50	1116.13
6	P-69	Rice	19	18-0053-00	9/27/11	46.339	-93.8913	43.0	0.23	0.021	50389	35.55	185.8	185.79	0.23	807.78
7	P-53	Carlos Avery Pool 9	4	02-0504-00	8/19/11	45.318	-93.0587	43.0	0.35	0.029	37965	16.51	270.0	269.96	0.35	771.31
8	FS-376	Rice	19	18-0053-00	9/11/13	46.339	-93.8918	46.5	< 0.5	0.0451	65261	33.36	329.7	329.66	0.50	659.31
9	FS-304	Rice	19	18-0053-00	6/10/13	46.339	-93.8906	5.7	< 0.5	0.0236	48287	33.61	183.1	183.07	0.50	366.13
10	FS-324	Rice	19	18-0053-00	7/15/13	46.339	-93.8918	56.7	< 0.5	0.11	44704	33.18	160.3	160.29	0.50	320.58
11	FS-51	Glesne Slough	49	34-0353-00	7/31/12	45.351	-95.1887	99.6	< 0.5	0.061	7983	3.01	103.2	103.23	0.50	206.46
12	FS-80	Mission	95	S001-646	8/6/12	45.862	-93.0011	87.5	0.62	0.0485	9231	4.83	77.5	77.50	0.62	124.99
13	FS-109	Carlos Avery Pool 9	4	02-0504-00	7/3/12	45.319	-93.0611	52.8	< 0.5	< 0.011	14736	12.51	61.0	60.98	0.50	121.95
14	FS-333	Embarrass	73	69-0496-00	7/26/13	47.533	-92.2976	0.0	18.2	0.0866	11179	0.47	1821.2	1821.22	18.20	100.07
15	P-6	Elk	15	15-0010-00	8/25/11	47.195	-95.2254	25.9	0.28	0.04	8480	10.24	26.8	26.78	0.28	95.63
16	FS-175	Maloney	88	79-0001-00	7/23/12	44.225	-91.9321	0.0	3.15	0.0608	15126	4.57	214.0	214.03	3.15	67.95
17	FS-95	Embarrass	73	69-0496-00	9/14/12	47.533	-92.2979	0.0	18.8	0.0298	21847	1.89	1248.9	1248.85	18.80	66.43
18	FS-83	Mississippi Crow Wing	111	S007-205	8/8/12	46.439	-94.1251	0.0	3.13	0.127	13451	3.88	207.8	207.75	3.13	66.37
19	P-64	Maloney	88	79-0001-00	9/29/11	44.224	-91.9328	0.0	1.83		10382	4.05	119.9	119.94	1.83	65.54
20	P-44	Dead Fish	12	09-0051-00	9/20/11	46.745	-92.6863	48.7	0.3	0.056	9685	16.6	19.4	19.39	0.30	64.63
21	P-63	Maloney	88	79-0001-00	9/29/11	44.224	-91.9328	148.7	1.83	0.01	10269	4.24	111.2	111.17	1.83	60.75
22	FS-193	Big Mud	79	71-0085-00	8/30/12	45.453	-93.7418	14.3	< 0.5	0.0308	12943	18.63	29.5	29.50	0.50	58.99
23	FS-76	Field	45	34-0151-00	7/25/12	45.296	-94.9058	0.0	< 0.5	0.0687	7586	8.68	26.3	26.34	0.50	52.68
24	FS-375	Height of Land	5	03-0195-00	9/10/13	46.913	-95.6111	117.5	< 0.5	< 0.011	1795	0.86	26.2	26.23	0.50	52.45
25	FS-75	Mortenson	44	34-0150-02	7/24/12	45.3	-94.9062	0.0	< 0.5	0.103	9071	12.09	25.0	24.99	0.50	49.97
26	FS-55	Pelkey	55	49-0030-00	8/26/12	45.996	-94.2273	0.0	3.42	0.0522	30642	17.32	168.8	168.82	3.42	49.36
27	P-13	Partridge	119	S007-443	8/31/11	47.521	-92.1899	65.9	10.39	0.075	11026	1.44	464.3	464.30	10.39	44.69

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
28	FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	1.62	0.0659	2505	0.65	69.6	69.60	1.62	42.97
29	FS-127	Height of Land	5	03-0195-00	8/21/12	46.913	-95.6095	111.1	< 0.5	< 0.011	2112	1.32	21.5	21.47	0.50	42.94
30	FS-64	Dead Fish	12	09-0051-00	9/4/12	46.745	-92.6865	0.0	0.71	0.0608	14387	22.4	29.0	28.99	0.71	40.84
31	FS-65	Wild Rice	11	09-0023-00	9/4/12	46.671	-92.6055	0.0	< 0.5	0.083	13650	28.82	19.4	19.38	0.50	38.76
32	P-14	Mississippi River above Clay Boswell	108	S007-163	9/1/11	47.238	-93.7196	163.2	1.09	0.053	7964	6.43	41.4	41.42	1.09	38.00
33	FS-125	Tamarac	56	56-0192-00	8/19/12	46.364	-95.5714	0.0	2.33	0.0768	21908	18.41	82.3	82.32	2.33	35.33
34	P-36	Wild Rice Reservoir	70	69-0371-00	9/16/11	46.91	-92.1636	17.2	1.13	0.023	5555	3.75	39.5	39.51	1.13	34.96
35	FS-251	Sandy-1	76	69-0730-00	9/21/12	47.625	-92.5886	3.8	3.05	0.123	35905	33.08	105.5	105.54	3.05	34.60
36	FS-378	Duck Lake WMA	22	18-0178-00	9/12/13	46.752	-93.8851	113.0	< 0.5	0.0251	12151	26.57	17.1	17.08	0.50	34.16
37	FS-371	Mississippi Pool 5 / Spring	123	S007-660	9/10/13	44.202	-91.8443	39.8	34.4	0.069	3582	0.11	1161.0	1160.97	34.40	33.75
38	FS-354	Mississippi River above Clay Boswell	108	S007-163	8/13/13	47.238	-93.7187	132.7	1.18	0.0532	7052	5.76	37.4	37.40	1.18	31.69
39	FS-137	Elk	15	15-0010-00	9/19/12	47.195	-95.2249	42.7	< 0.5	0.0936	6334	10.07	15.6	15.59	0.50	31.18
40	FS-212	Mississippi Pool 5 / Spring	123	S007-660	8/17/12	44.199	-91.8461	29.6	17.2	0.0224	3674	0.22	531.7	531.70	17.20	30.91
41	FS-337	Clearwater	98	S004-204	7/29/13	47.518	-95.3906	69.1	0.95	0.0608	14564	24.58	26.6	26.56	0.95	27.96
42	P-42	Monongalia (Middle Fork Crow R)	45.5	34-0158-01	9/20/11	45.348	-94.9509	5.7	16.51	0.042	46471	14.76	455.4	455.39	16.51	27.58
43	FS-58	Mississippi River above Clay Boswell	108	S007-163	8/28/12	47.239	-93.7197	0.0	1.19	0.0806	8636	9.08	32.0	32.02	1.19	26.91
44	P-1	Height of Land	5	03-0195-00	8/22/11	46.913	-95.6095	62.9	0.24	0.053	1298	1.76	6.0	5.97	0.24	24.86
45	FS-67	St. Louis Estuary Pokegama Bay	105	S006-928	9/5/12	46.686	-92.1606	0.0	9.97	0.112	14015	3.66	241.1	241.10	9.97	24.18
46	P-4	Little Flat	6	03-0217-00	8/24/11	46.998	-95.6641	83.1	0.22	0.011	7479	33.13	5.2	5.16	0.22	23.45
47	FS-331	Partridge	119	S007-443	7/24/13	47.521	-92.1904	60.5	14.6	0.112	10082	1.68	325.0	325.02	14.60	22.26
48	FS-131	Hinken	113	S007-207	9/5/12	47.727	-93.9923	46.8	< 0.5	0.0876	2960	4.53	9.4	9.39	0.50	18.78
49	FS-330	St. Louis Estuary	120	S007-444	7/22/13	46.652	-92.2372	11.8	6.71	0.0901	5817	1.55	124.3	124.30	6.71	18.52
50	FS-81	Flowage	1	01-0061-00	8/7/12	46.688	-93.337	0.0	0.78	0.134	12470	32.34	14.2	14.19	0.78	18.19
51	FS-383	Upper Panasa	37	31-0111-00	9/18/13	47.306	-93.2676	0.0	33.6	0.0399	19148	2.86	590.3	590.25	33.60	17.57
52	FS-211	Mississippi Pool 4/Robinson Lake	89	79-0005-02	8/16/12	44.361	-91.9897	57.6	17.7	0.0714	9265	1.55	304.2	304.23	17.70	17.19
53	FS-63	Caribou	72	69-0489-00	9/3/12	46.891	-92.3135	0.0	1.21	0.0938	13791	29.44	19.3	19.27	1.21	15.93
54	FS-92	Partridge	119	S007-443	9/12/12	47.521	-92.1909	4.1	36.3	0.0741	29463	5.87	571.7	571.67	36.30	15.75
55	P-61	Lily	90	81-0067-00	9/28/11	44.194	-93.6469	51.5	0.66	0.041	6180	14.06	10.0	9.97	0.66	15.11
56	P-45	Hay	33	31-0037-00	9/21/11	47.287	-93.1017	0.0	10.24	0.087	12403	4.36	154.6	154.59	10.24	15.10

MPCA Field Data - All MN non-paddy data
CPSC and Sulfate Ratio

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
57	FS-315	St. Louis Estuary	120	S007-444	6/24/13	46.652	-92.2373	0.0	8.1	0.147	6056	1.68	122.0	121.96	8.10	15.06
58	FS-338	Height of Land	5	03-0195-00	7/30/13	46.913	-95.6116	94.2	< 0.5	0.0554	2641	4.58	7.4	7.44	0.50	14.89
59	P-31	Cloquet	52	38-0539-00	9/14/11	47.431	-91.4844	74.4	0.81	0.024	4252	6.58	12.1	12.05	0.81	14.88
60	P-15	Mississippi River below Clay Boswell	103	S006-923	9/1/11	47.255	-93.6344	100.2	3.65	0.035	8667	6.07	52.2	52.22	3.65	14.31
61	FS-367	Hay	33	31-0037-00	9/4/13	47.287	-93.1009	141.0	22.1	0.0447	15436	3.44	312.7	312.66	22.10	14.15
62	FS-210	Mississippi Pool 4/Robinson Lake	89	79-0005-02	8/16/12	44.359	-91.9881	35.3	15.7	0.07	6450	1.16	214.5	214.49	15.70	13.66
63	FS-84	Pleasant	13	11-0383-00	8/10/12	46.923	-94.4874	0.0	< 0.5	0.0218	7065	23.99	6.8	6.80	0.50	13.61
64	FS-335	Mississippi Pool 5 / Spring	123	S007-660	7/30/13	44.195	-91.841	63.0	47.7	0.0342	4362	0.25	634.7	634.70	47.70	13.31
65	FS-195	Fisher	78	70-0087-00	8/31/12	44.794	-93.4061	20.7	6.85	0.136	11140	5.76	90.1	90.10	6.85	13.15
66	P-46	Hay	33	31-0037-00	9/21/11	47.287	-93.1018	0.0	10.24	0.026	16139	7.69	130.0	130.04	10.24	12.70
67	FS-344	Padua	82	73-0277-00	8/6/13	45.623	-95.0187	9.5	< 0.5	0.0806	4520	12.61	6.2	6.22	0.50	12.45
68	P-11	Sand	97	S003-249	8/30/11	47.635	-92.4235	14.4	7.69	0.046	22677	17.49	93.5	93.53	7.69	12.16
69	FS-136	Itasca	16	15-0016-00	9/19/12	47.234	-95.2049	23.6	< 0.5	0.0636	1496	2.23	5.9	5.91	0.50	11.81
70	P-62	Lily	90	81-0067-00	9/28/11	44.194	-93.6469	0.0	0.64		5069	13.39	7.2	7.22	0.64	11.28
71	FS-306	Sandy-1	76	69-0730-00	6/11/13	47.626	-92.5884	0.0	11	0.0918	35357	28.53	122.3	122.32	11.00	11.12
72	FS-69	St. Louis	114	S007-208	9/7/12	47.467	-91.9279	0.0	1.33	0.181	11429	27.16	14.8	14.79	1.33	11.12
73	FS-139	Welby family farm	93	86-0231-00	9/21/12	45.359	-94.0782	17.2	< 0.5	0.118	7267	30.76	5.3	5.33	0.50	10.67
74	FS-209	Mississippi Pool 8 at Reno Bottoms	122	S007-556	8/15/12	43.603	-91.2686	72.3	18.1	0.0711	9187	2.29	187.6	187.61	18.10	10.36
75	FS-250	Little Rice	75	69-0612-00	9/20/12	47.709	-92.4389	29.3	1.03	0.0293	9488	26.45	10.7	10.67	1.03	10.36
76	FS-300	St. Louis Estuary	120	S007-444	5/27/13	46.652	-92.2376	0.0	9.4	0.0713	4499	1.26	97.2	97.18	9.40	10.34
77	FS-133	Mahnomen	21	18-0126-02	9/17/12	46.499	-93.9958	0.0	16.9	0.308	18746	7.7	173.2	173.16	16.90	10.25
78	P-16	St. Louis	106	S006-929	9/1/11	47.402	-92.3773	0.0	24.5	0.025	1488	0.1	240.3	240.27	24.50	9.81
79	P-51	Flowage	1	01-0061-00	9/22/11	46.69	-93.338	160.2	0.56	0.014	5627	20.1	5.4	5.43	0.56	9.69
80	P-10	Pike	104	S006-927	8/30/11	47.733	-92.3468	43.0	8.31	0.063	15572	10.9	80.0	79.96	8.31	9.62
81	FS-201	Mink	92	86-0229-00	8/8/12	45.274	-94.0269	0.0	1.31	0.0373	1740	1.53	12.4	12.40	1.31	9.46
82	FS-187	McCormic	81	73-0273-00	8/2/12	45.722	-94.9121	8.9	1.54	0.144	1512	1.1	14.0	14.04	1.54	9.12
83	FS-374	Little Round	7	03-0302-00	9/10/13	46.975	-95.738	37.6	0.12	0.0391	2018	14.8	1.1	1.09	0.12	9.08
84	FS-318	Height of Land	5	03-0195-00	6/26/13	46.914	-95.6124	43.0	1.21	0.0658	1349	1.13	10.9	10.92	1.21	9.03
85	FS-68	Wolf	69	69-0143-00	9/6/12	47.256	-91.963	8.9	2.01	0.119	9526	17.19	18.0	18.01	2.01	8.96

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86	FS-52	Blaamyhre	48	34-0345-00	8/1/12	45.364	-95.186	102.2	0.62	0.078	3517	9.33	5.5	5.51	0.62	8.89
87	FS-57	Mississippi River below Clay Boswell	103	S006-923	8/28/12	47.255	-93.6342	0.0	10.3	0.134	4225	1.2	91.3	91.30	10.30	8.86
88	FS-302	Partridge	121	S007-513	5/30/13	47.515	-92.1894	0.0	43.1	0.0624	24784	6.27	378.8	378.83	43.10	8.79
89	P-7	Itasca	16	15-0016-00	8/25/11	47.233	-95.1985	20.1	0.26	0.064	1650	6.01	2.2	2.18	0.26	8.37
90	FS-196	Prairie	115	S007-209	9/3/12	47.252	-93.4884	44.6	9.63	0.0709	15071	10.51	78.4	78.43	9.63	8.14
91	FS-372	Mississippi Pool 5 / Spring	123	S007-660	9/10/13	44.202	-91.8443	26.7	34.8	0.0536	3330	0.33	270.9	270.88	34.80	7.78
92	FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.655	-92.2739	0.0	16	0.0445	6169	1.73	122.0	122.02	16.00	7.63
93	P-17	St. Louis	114	S007-208	9/1/11	47.467	-91.9355	68.6	1.23	0.04	9654	30.4	9.3	9.34	1.23	7.59
94	P-52	Flowage	1	01-0061-00	9/22/11	46.69	-93.338	123.1	0.56	0.018	4641	18.1	4.2	4.25	0.56	7.59
95	P-28	Raymond	83	73-0285-00	9/12/11	45.629	-95.0234	68.6	0.82	0.094	3922	10.06	6.2	6.21	0.82	7.57
96	FS-53	Raymond	83	73-0285-00	8/2/12	45.629	-95.0225	61.1	< 0.5	0.0787	1905	4.79	3.8	3.76	0.50	7.53
97	FS-301	Partridge	119	S007-443	5/28/13	47.521	-92.1903	0.0	14.8	0.125	9491	3.94	104.3	104.32	14.80	7.05
98	FS-88	Clearwater	98	S004-204	8/24/12	47.517	-95.3904	148.3	2.04	0.0488	9874	22.17	14.2	14.23	2.04	6.98
99	FS-226	Louise	25	21-0094-00	8/14/12	45.933	-95.4148	46.5	4.09	0.0746	1833	0.83	28.5	28.49	4.09	6.97
100	P-26	Lower Rice	109	S007-164	9/8/11	47.382	-95.4926	120.1	0.55	0.07	2364	6.76	3.8	3.77	0.55	6.86
101	FS-221	Hay Creek Flowage	59	58-0005-00	9/17/12	46.089	-92.4104	97.7	1.95	0.119	9456	22.05	13.2	13.18	1.95	6.76
102	P-47	Little Birch	87	77-0089-00	9/21/11	45.775	-94.7996	25.9	3.2	0.05	4503	4.46	21.4	21.44	3.20	6.70
103	FS-377	Mahnomen	21	18-0126-02	9/11/13	46.499	-93.9956	0.0	21.1	0.0283	16540	7.47	141.1	141.14	21.10	6.69
104	P-27	Pleasant	13	11-0383-00	9/9/11	46.928	-94.4757	28.6	0.49		5331	30.37	3.0	2.99	0.49	6.09
105	FS-180	Lily	90	81-0067-00	7/26/12	44.195	-93.647	38.2	< 0.5	0.0295	5095	28.07	3.0	3.01	0.50	6.01
106	P-19	Wolf	69	69-0143-00	9/2/11	47.259	-91.9618	128.8	1.54	0.139	8240	25.1	8.7	8.66	1.54	5.63
107	FS-225	Miltona	24	21-0083-00	8/13/12	46.05	-95.4217	0.0	4.11	0.0694	2624	1.77	22.9	22.94	4.11	5.58
108	P-29	Padua	82	73-0277-00	9/13/11	45.62	-95.0192	3.4	0.76	0.13	4927	20.15	4.2	4.19	0.76	5.52
109	P-52	Flowage	1	01-0061-00	9/22/11	46.69	-93.338	123.1	0.56	0.018	3706	16.52	3.1	3.07	0.56	5.49
110	P-47	Little Birch	87	77-0089-00	9/21/11	45.775	-94.7996	25.9	3.2	0.191	2236	1.75	17.1	17.10	3.20	5.34
111	FS-342	Little Round	7	03-0302-00	8/5/13	46.972	-95.7358	58.3	< 0.5	0.0676	4447	25.16	2.6	2.64	0.50	5.28
112	P-52	Flowage	1	01-0061-00	9/22/11	46.69	-93.338	123.1	0.56	0.018	4302	21.79	2.9	2.94	0.56	5.25
113	FS-366	Partridge	119	S007-443	9/3/13	47.521	-92.19	47.7	34.2	0.057	7671	1.79	178.1	178.11	34.20	5.21
114	FS-370	Mississippi Pool 8 at Genoa	118	S007-222	9/9/13	43.577	-91.2337	17.8	33.3	0.062	6558	1.43	172.4	172.39	33.30	5.18

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115	FS-208	Mississippi Pool 8 at Genoa	118	S007-222	8/14/12	43.576	-91.2334	41.4	18	0.176	2178	0.41	92.3	92.34	18.00	5.13
116	FS-365	Partridge	119	S007-443	9/3/13	47.521	-92.1901	76.7	34.1	0.0393	9179	2.5	168.6	168.62	34.10	4.94
117	P-12	Birch	67	69-0003-00	8/30/11	47.736	-91.9428	68.6	3.58	0.104	12431	26.8	17.7	17.66	3.58	4.93
118	FS-336	Mississippi Pool 4/Robinson Lake	89	79-0005-02	7/30/13	44.361	-91.9901	46.5	55.3	0.0602	8193	1.41	269.0	268.98	55.30	4.86
119	FS-215	Popple	101	S006-188	9/11/12	47.725	-94.0817	36.3	< 0.5	0.0269	2971	14.42	2.4	2.37	0.50	4.73
120	FS-312	Mississippi Pool 5 / Spring	123	S007-660	6/21/13	44.202	-91.8444	35.7	28.3	0.0844	3563	0.67	132.2	132.16	28.30	4.67
121	FS-355	Mississippi River below Clay Boswell	103	S006-923	8/13/13	47.255	-93.634	78.3	10.2	0.0819	10479	8.98	47.1	47.07	10.20	4.62
122	P-5	Itasca	16	15-0016-00	8/25/11	47.238	-95.2065	45.8	0.26	0.056	1355	7.4	1.2	1.16	0.26	4.47
123	FS-203	Long Prairie	110	S007-203	8/9/12	45.973	-95.1603	58.3	6.66	0.0391	5074	4.35	27.8	27.79	6.66	4.17
124	FS-129	Mink	92	86-0229-00	8/23/12	45.277	-94.0299	0.0	1.22	0.182	4247	13.63	5.0	5.03	1.22	4.12
125	FS-200	Louisa	94	86-0282-00	8/8/12	45.3	-94.258	0.0	7.04	0.192	7824	8.76	27.6	27.65	7.04	3.93
126	FS-130	Hay	33	31-0037-00	9/6/12	47.287	-93.102	141.0	31.7	0.0738	13154	5.79	123.3	123.26	31.70	3.89
127	P-47	Little Birch	87	77-0089-00	9/21/11	45.775	-94.7996	25.9	3.2	0.191	3544	5.11	11.5	11.49	3.20	3.59
128	FS-230	Mill Pond	23	21-0034-00	8/16/12	46.072	-95.2218	80.9	7.36	0.192	3969	3.14	25.6	25.60	7.36	3.48
129	FS-332	Partridge	121	S007-513	7/24/13	47.514	-92.1894	79.6	54.4	0.102	20512	8.34	187.1	187.13	54.40	3.44
130	P-20	Gull	9	04-0120-00	9/6/11	47.656	-94.6944	15.6	0.78	0.103	1608	5.08	2.5	2.53	0.78	3.25
131	FS-343	Raymond	83	73-0285-00	8/6/13	45.629	-95.0233	61.4	1.92	0.0903	3270	7.59	6.1	6.13	1.92	3.19
132	FS-316	Partridge	121	S007-513	6/28/13	47.514	-92.1899	0.0	24.9	0.098	6291	2.6	77.8	77.80	24.90	3.12
133	FS-89	Birch	67	69-0003-00	9/10/12	47.736	-91.943	33.1	8.61	0.1	16938	31.2	26.7	26.69	8.61	3.10
134	FS-310	Second	117	S007-220	6/14/13	47.521	-92.1925	57.6	316	0.0927	31190	4.22	946.8	946.84	316.00	3.00
135	FS-91	Pike	104	S006-927	9/11/12	47.733	-92.3473	3.5	14.2	0.0656	6565	4.72	41.4	41.36	14.20	2.91
136	FS-220	Padua	82	73-0277-00	8/7/12	45.623	-95.0186	0.0	0.86	0.23	2291	9.77	2.3	2.29	0.86	2.66
137	FS-213	Gull	9	04-0120-00	9/10/12	47.656	-94.6945	9.5	1.14	0.0778	3527	16.01	2.9	2.90	1.14	2.55
138	FS-199	Rice	102	S006-208	9/5/12	47.674	-93.6547	75.4	1.57	0.0552	3273	10.88	4.0	3.99	1.57	2.54
139	FS-82	Rabbit	20	18-0093-02	8/8/12	46.531	-93.9285	0.0	15.3	0.22	10903	11.79	36.7	36.68	15.30	2.40
140	FS-138	Little Round	7	03-0302-00	9/20/12	46.973	-95.735	78.0	< 0.5	0.128	3069	27.48	1.2	1.16	0.50	2.33
141	P-24	Second	17	15-0091-00	9/7/11	47.826	-95.3635	37.3	0.87	0.139	3813	25.67	1.9	1.92	0.87	2.20
142	FS-202	Long Prairie	110	S007-204	8/9/12	46.007	-95.2634	13.4	7.71	0.0793	2897	2.85	15.7	15.69	7.71	2.04
143	FS-319	Little Round	7	03-0302-00	6/27/13	46.972	-95.735	17.5	<0.5	0.117	3579	39.84	1.0	1.00	0.50	2.01

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144	FS-132	Ox Hide	35	31-0106-00	9/7/12	47.335	-93.2134	10.5	26.4	0.042	14936	14.43	52.7	52.75	26.40	2.00
145	FS-229	Mill Pond	23	21-0034-00	8/16/12	46.072	-95.2218	102.2	7.16	0.109	5143	7.86	14.0	14.05	7.16	1.96
146	FS-182	Hunt	65	66-0047-00	7/27/12	44.328	-93.4443	0.0	17.1	0.0729	2412	1.21	30.8	30.76	17.10	1.80
147	FS-134	Bass	43	31-0576-00	9/18/12	47.284	-93.6276	64.0	1.01	0.0664	3740	26.12	1.8	1.81	1.01	1.79
148	FS-334	Mississippi Pool 8 at Genoa	118	S007-222	7/29/13	43.576	-91.2344	52.8	44.2	0.102	1969	0.4	78.3	78.33	44.20	1.77
149	FS-189	Clearwater	96	S002-121	8/28/12	47.937	-95.6906	4.5	23.8	0.117	2856	1.27	40.2	40.17	23.80	1.69
150	FS-327	Clearwater	96	S002-121	7/17/13	47.937	-95.6906	0.3	23.7	0.117	3521	1.82	39.1	39.06	23.70	1.65
151	FS-197	Snowball	36	31-0108-00	9/4/12	47.336	-93.244	0.0	8.4	0.0936	4213	6	13.2	13.23	8.40	1.57
152	FS-224	Stone Lake	68	69-0046-00	9/19/12	47.504	-91.8857	21.0	3.26	0.0533	5225	18.87	5.1	5.08	3.26	1.56
153	FS-126	Bray	58	56-0472-00	8/20/12	46.452	-95.8783	7.6	1.65	0.072	3937	21.95	2.5	2.46	1.65	1.49
154	FS-93	Turpela	71	69-0427-00	9/12/12	47.461	-92.2371	1.0	3.3	0.115	6979	31.08	4.9	4.87	3.30	1.48
155	FS-86	Eighteen	61	60-0199-00	8/22/12	47.64	-96.0607	40.1	4.29	0.164	1860	3.1	6.1	6.05	4.29	1.41
156	FS-90	Sand	97	S003-249	9/11/12	47.635	-92.4234	2.9	15.9	0.152	7287	9.68	21.4	21.40	15.90	1.35
157	P-23	Gourd	10	04-0253-00	9/7/11	47.812	-94.9654	38.4	0.69	0.038	2675	27.4	0.9	0.90	0.69	1.30
158	FS-303	Second	117	S007-220	5/30/13	47.52	-92.1925	0.0	303	0.0991	13086	2.2	388.6	388.62	303.00	1.28
159	FS-373	Clearwater	96	S002-121	9/9/13	47.937	-95.6909	3.2	34.4	0.0354	5315	3.33	41.8	41.84	34.40	1.22
160	FS-207	Kelly Lake	64	66-0015-00	8/13/12	44.354	-93.3743	0.0	1.92	0.0927	4387	27.33	2.3	2.33	1.92	1.21
161	P-30	Stella	54	47-0068-00	9/14/11	45.066	-94.4339	31.6	7.59	0.08	2159	2.88	8.8	8.80	7.59	1.16
162	P-25	Lower Rice	107	S006-985	9/8/11	47.379	-95.4834	114.4	1.02	0.097	2337	17.76	1.2	1.16	1.02	1.14
163	P-3	Little Round	7	03-0302-00	8/24/11	46.976	-95.7404	57.2	0.46	0.032	1689	20.91	0.5	0.51	0.46	1.11
164	FS-359	Eighteen	62	60-0199-00	8/20/13	47.637	-96.06	21.0	2.83	0.118	5500	30.88	3.1	3.11	2.83	1.10
165	FS-314	Clearwater	96	S002-121	6/24/13	47.937	-95.6907	0.6	28	0.0664	3946	2.68	30.6	30.60	28.00	1.09
166	FS-104	Gourd	10	04-0253-00	6/27/12	47.812	-94.965	0.0	0.27		1776	36.87	0.3	0.29	0.27	1.06
167	FS-328	Eighteen	62	60-0199-00	7/18/13	47.637	-96.0599	44.2	3.34	0.25	5106	24.65	3.5	3.53	3.34	1.06
168	FS-356	Trout	41	31-0216-00	8/14/13	47.259	-93.3942	0.0	39.1	0.103	11992	12.59	40.7	40.72	39.10	1.04
169	FS-321	Sandy-1	76	69-0730-00	7/9/13	47.626	-92.5885	0.0	122	0.189	36502	29.51	124.9	124.90	122.00	1.02
170	FS-309	Eighteen	62	60-0199-00	6/13/13	47.637	-96.0599	0.0	4.36	0.127	4478	16.52	4.4	4.42	4.36	1.01
171	FS-311	Mississippi Pool 8 at Genoa	118	S007-222	6/20/13	43.577	-91.2341	12.7	29.3	0.107	1544	0.62	29.0	29.04	29.30	0.99
172	FS-219	Trout	41	31-0216-00	9/13/12	47.259	-93.3942	0.0	38.6	0.117	12535	15	35.9	35.95	38.60	0.93

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173	FS-382	Sandy-1	76	69-0730-00	9/17/13	47.626	-92.5885	0.0	67.9	0.135	26645	32.28	61.2	61.24	67.90	0.90
174	FS-347	Snowball	36	31-0108-00	8/12/13	47.336	-93.2439	0.0	8.2	0.097	1136	1.19	7.4	7.38	8.20	0.90
175	FS-105	Second	17	15-0091-00	6/27/12	47.826	-95.3637	48.4	0.74	0.119	2527	33.3	0.6	0.64	0.74	0.86
176	FS-179	Rice	84	74-0001-00	7/25/12	44.084	-93.0737	0.0	3.84	0.217	4152	19.07	3.2	3.22	3.84	0.84
177	P-47	Little Birch	87	77-0089-00	9/21/11	45.775	-94.7996	25.9	3.2	0.191	2253	8.37	2.7	2.66	3.20	0.83
178	FS-190	Pine	18	15-0149-00	8/28/12	47.684	-95.5414	114.9	14.7	0.368	4477	7.08	12.2	12.19	14.70	0.83
179	FS-231	Rice	2	02-0008-00	8/17/12	45.16	-93.121	0.0	3.6	0.145	2159	7.98	2.6	2.60	3.60	0.72
180	FS-320	Sandy-2	76	69-0730-00	7/9/13	47.619	-92.5936	0.0	118	3.08	19749	15.43	83.3	83.30	118.00	0.71
181	FS-348	Sandy-2	76	69-0730-00	8/13/13	47.619	-92.5934	0.0	123	0.305	13216	8.23	81.6	81.64	123.00	0.66
182	FS-381	Sandy-2	76	69-0730-00	9/17/13	47.619	-92.5931	0.0	126	0.0342	16172	11.67	79.2	79.24	126.00	0.63
183	FS-223	Little Sucker	40	31-0126-00	9/14/12	47.377	-93.246	0.0	13.7	0.534	6297	16.56	8.5	8.50	13.70	0.62
184	FS-341	Stella	54	47-0068-00	8/1/13	45.066	-94.4339	57.6	24.7	0.0884	1786	1.35	15.1	15.14	24.70	0.61
185	P-57	Unnamed	50	34-0611-00	9/23/11	45.268	-94.865	74.4	6.42	0.286	2311	6.48	3.8	3.80	6.42	0.59
186	FS-186	Westport	63	61-0029-00	8/1/12	45.69	-95.217	0.0	7.11	1.79	4917	20.15	4.2	4.18	7.11	0.59
187	FS-205	Big Swan	86	77-0023-00	8/10/12	45.88	-94.7418	56.3	5.47	0.0527	1719	4.81	3.1	3.07	5.47	0.56
188	P-35	Anka	26	21-0353-00	9/16/11	46.077	-95.7377	3.0	2.23	0.493	2170	14.84	1.2	1.25	2.23	0.56
189	FS-59	Upper Panasa	37	31-0111-00	8/29/12	47.306	-93.2652	0.0	29.6	0.126	895	0.43	15.8	15.77	29.60	0.53
190	FS-228	West battle	57	56-0239-00	8/15/12	46.291	-95.6049	144.8	4.03	0.189	3108	17.37	2.1	2.06	4.03	0.51
191	FS-60	Lower Panasa	38	31-0112-00	8/29/12	47.302	-93.2521	0.0	33.6	0.243	8048	14.12	16.5	16.48	33.60	0.49
192	FS-181	Rice	66	66-0048-00	7/27/12	44.333	-93.4734	0.0	5.22	0.777	3829	21.67	2.4	2.37	5.22	0.45
193	FS-357	Lower Panasa	38	31-0112-00	8/15/13	47.303	-93.2561	0.0	28.5	1.26	2347	2.42	12.7	12.73	28.50	0.45
194	FS-204	Big Swan	86	77-0023-00	8/10/12	45.88	-94.742	133.7	5.49	0.0914	1731	5.94	2.4	2.42	5.49	0.44
195	FS-214	Bowstring	116	S007-219	9/11/12	47.702	-94.0608	69.7	1.34	0.256	1974	24.34	0.6	0.58	1.34	0.43
196	FS-323	Second	117	S007-220	7/11/13	47.52	-92.1925	76.4	405	0.067	10036	2.91	166.9	166.92	405.00	0.41
197	FS-185	Hoffs Slough	85	76-0103-00	8/1/12	45.326	-95.7059	0.0	273	0.0343	3512	0.75	112.3	112.32	273.00	0.41
198	P-57	Unnamed	50	34-0611-00	9/23/11	45.268	-94.865	74.4	6.42	0.065	2193	8.1	2.6	2.63	6.42	0.41
199	FS-61	Swan	34	31-0067-02	8/30/12	47.289	-93.2127	12.4	12.5	0.332	5827	22.71	5.0	5.02	12.50	0.40
200	FS-128	Cromwell	14	14-0103-00	8/22/12	46.965	-96.3171	0.0	41.2	1.22	2948	2.85	16.2	16.23	41.20	0.39
201	FS-198	Ox Hide	35	31-0106-00	9/7/12	47.335	-93.2134	0.6	26.4	0.0751	8743	24.51	10.0	9.99	26.40	0.38

MPCA Field Data - All MN non-paddy data
CPSC and Sulfate Ratio

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
202	FS-305	Sandy-2	76	69-0730-00	6/11/13	47.619	-92.5937	0.0	135	1.08	19094	22.23	50.4	50.43	135.00	0.37
203	FS-54	Little Birch	87	77-0089-00	8/3/12	45.778	-94.7978	70.0	7.4	0.0353	1794	6.02	2.6	2.55	7.40	0.34
204	FS-380	Sandy-2	76	69-0730-00	9/17/13	47.619	-92.5939	0.6	126	0.0342	17868	22.7	43.3	43.29	126.00	0.34
205	FS-191	Ina	27	21-0355-00	8/29/12	46.072	-95.7281	30.2	7.08	0.274	2216	9.09	2.3	2.34	7.08	0.33
206	FS-346	Westport	63	61-0029-00	8/8/13	45.704	-95.203	6.7	6.3	0.205	3262	19.66	2.0	1.95	6.30	0.31
207	FS-349	Sandy-3	76	69-0730-00	8/13/13	47.619	-92.5898	0.0	122	0.0697	14897	20.46	34.6	34.55	122.00	0.28
208	FS-216	Big Sucker	39	31-0124-00	9/12/12	47.392	-93.2658	3.8	7.78	0.145	3559	21.45	2.1	2.08	7.78	0.27
209	FS-62	Swan	34	31-0067-02	8/30/12	47.289	-93.2124	3.8	14	0.221	4821	22.53	3.5	3.52	14.00	0.25
210	FS-87	Bee	60	60-0192-00	8/23/12	47.653	-96.0504	39.8	11	0.67	3054	13.62	2.7	2.67	11.00	0.24
211	FS-184	Rice	80	73-0196-00	7/30/12	45.386	-94.6309	0.0	2.58	2.97	1523	15.03	0.6	0.62	2.58	0.24
212	FS-194	Gilchrist	91	86-0064-00	8/31/12	45.231	-93.824	0.0	6.98	0.355	3117	20.81	1.7	1.67	6.98	0.24
213	FS-183	Unnamed	50	34-0611-00	7/30/12	45.268	-94.865	64.9	16.8	0.15	2157	5.61	4.0	3.96	16.80	0.24
214	FS-188	Stella	54	47-0068-00	8/27/12	45.068	-94.4334	0.3	18.1	1.79	1257	2.34	4.0	3.99	18.10	0.22
215	FS-77	Monongalia (near hwy embankment)	46	34-0158-02	7/26/12	45.333	-94.9268	121.3	21.7	1.37	4953	18.66	4.6	4.64	21.70	0.21
216	FS-352	Dark	77	69-0790-00	8/15/13	47.639	-92.7782	2.9	173	0.136	5120	3.61	35.3	35.35	173.00	0.20
217	FS-369	Dark	77	69-0790-00	9/5/13	47.639	-92.7781	11.8	176	0.052	2037	0.82	35.4	35.41	176.00	0.20
218	P-55	Lady Slipper	53	42-0020-00	9/22/11	44.57	-95.6274	0.0	107.71	14.84	2814	2.09	21.5	21.51	107.71	0.20
219	FS-350	Ox Hide	35	31-0106-00	8/14/13	47.335	-93.2132	0.0	25.9	0.119	3889	12.12	4.9	4.89	25.90	0.19
220	FS-368	Dark	77	69-0790-00	9/5/13	47.639	-92.7782	11.1	175	0.305	3354	1.94	33.0	32.96	175.00	0.19
221	FS-313	Monongalia	46	34-0158-01	6/23/13	45.333	-94.9293	50.0	34.7	0.0941	6028	19.44	6.4	6.45	34.70	0.19
222	FS-351	Second	117	S007-220	8/15/13	47.521	-92.1925	66.8	838	0.0447	7088	1.84	148.0	148.03	838.00	0.18
223	P-57	Unnamed	50	34-0611-00	9/23/11	45.268	-94.865	74.4	6.42	0.065	1946	13.8	1.1	1.11	6.42	0.17
224	FS-345	Rice	80	73-0196-00	8/7/13	45.387	-94.6313	0.0	6.85	2.08	2012	14.83	1.1	1.08	6.85	0.16
225	P-34	Anka	26	21-0353-00	9/16/11	46.077	-95.7292	25.9	2.23	0.671	1485	23.57	0.3	0.35	2.23	0.16
226	P-57	Unnamed	50	34-0611-00	9/23/11	45.268	-94.865	74.4	6.42	0.065	1689	12.6	0.9	0.94	6.42	0.15
227	FS-322	Dark	77	69-0790-00	7/10/13	47.639	-92.7781	3.2	175	0.131	2480	1.48	25.5	25.50	175.00	0.15
228	FS-340	Monongalia	46	34-0158-02	7/31/13	45.333	-94.9292	87.9	33.6	0.122	5530	22.1	4.7	4.69	33.60	0.14
229	FS-379	Monongalia	46	34-0158-02	9/13/13	45.333	-94.9292	154.4	34.6	0.242	5436	26.42	3.7	3.66	34.60	0.11
230	FS-79	Lady Slipper	53	42-0020-00	7/27/12	44.572	-95.6216	0.0	330	1.63	3314	1.85	34.1	34.09	330.00	0.10

MPCA Field Data - All MN non-paddy data
CPSC and Sulfate Ratio

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
231	FS-339	Christina	28	21-0375-00	7/31/13	46.073	-95.7567	0.6	14.6	1.93	1741	8.96	1.5	1.50	14.60	0.10
232	FS-176	North Geneva	29	24-0015-00	7/24/12	43.788	-93.271	0.0	15.6	1.54	2212	13.45	1.5	1.46	15.60	0.09
233	FS-78	Lady Slipper	53	42-0020-00	7/27/12	44.57	-95.6275	0.0	335	1.68	2719	1.66	26.5	26.53	335.00	0.08
234	FS-177	South Geneva	30	24-0015-02	7/24/12	43.771	-93.2851	0.0	14.1	3.19	1618	16.71	0.6	0.62	14.10	0.04
235	FS-192	Anka	26	21-0353-00	8/29/12	46.077	-95.7292	2.3	8.44	0.53	1498	22.85	0.4	0.37	8.44	0.04
236	FS-218	Holman	42	31-0227-00	9/13/12	47.301	-93.3445	0.0	24.2	1.01	3035	29.74	1.0	1.04	24.20	0.04
237	FS-353	Holman	42	31-0227-00	8/12/13	47.301	-93.3444	0.0	68	0.583	5094	30.6	2.7	2.71	68.00	0.04
238	FS-85	Bean	8	03-0411-00	8/21/12	46.934	-95.8706	0.0	85	16	1967	11.85	1.4	1.35	85.00	0.02

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Attachment 3

(3 pages)

MPCA Field Data - CPSC Variability within Sites
Sites from MPCA March 2015 Draft, Figure 14

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	LacCore _field_ID	Site_name	Unique site ID	DNR/State ID	Date	Lat	Long	Calculated Wild rice ave stems/m2	surface water SO4 (mg SO4/L)	pore water Total Sulfide (TS, mg S/L)	Sediment Fe (µg/g)	Sediment TOC (%)	potential SO4 standard CPSC120	CPSC120 calculated
2	FS-81	Flowage	1	01-0061-00-204	8/7/12	46.688	-93.337	0.0	0.78	0.134	12470	32.34	14.2	14.19
3	P-51	Flowage	1	01-0061-00-205	9/22/11	46.69	-93.338	160.2	0.56	0.014	5627	20.1	5.4	5.43
4	P-52	Flowage	1	01-0061-00-205	9/22/11	46.69	-93.338	123.1	0.56	0.018	3706	16.52	3.1	3.07
5	P-52	Flowage	1	01-0061-00-206	9/22/11	46.69	-93.338	123.1	0.56	0.018	4641	18.1	4.2	4.25
6	P-52	Flowage	1	01-0061-00-206	9/22/11	46.69	-93.338	123.1	0.56	0.018	4302	21.79	2.9	2.94
7														
8	P-1	Height of Land	5	03-0195-00-209	8/22/11	46.913	-95.6095	62.9	0.24	0.053	1298	1.76	6.0	5.97
9	FS-375	Height of Land	5	03-0195-00-210	9/10/13	46.913	-95.6111	117.5	< 0.5	< 0.011	1795	0.86	26.2	26.23
10	FS-127	Height of Land	5	03-0195-00-210	8/21/12	46.913	-95.6095	111.1	< 0.5	< 0.011	2112	1.32	21.5	21.47
11	FS-338	Height of Land	5	03-0195-00-210	7/30/13	46.913	-95.6116	94.2	< 0.5	0.0554	2641	4.58	7.4	7.44
12	FS-318	Height of Land	5	03-0195-00-210	6/26/13	46.914	-95.6124	43.0	1.21	0.0658	1349	1.13	10.9	10.92
13														
14	FS-374	Little Round	7	03-0302-00-202	9/10/13	46.975	-95.738	37.6	0.12	0.0391	2018	14.8	1.1	1.09
15	P-3	Little Round	7	03-0302-00-202	8/24/11	46.976	-95.7404	57.2	0.46	0.032	1689	20.91	0.5	0.51
16	FS-342	Little Round	7	03-0302-00-203	8/5/13	46.972	-95.7358	58.3	< 0.5	0.0676	4447	25.16	2.6	2.64
17	FS-138	Little Round	7	03-0302-00-203	9/20/12	46.973	-95.735	78.0	< 0.5	0.128	3069	27.48	1.2	1.16
18	FS-319	Little Round	7	03-0302-00-203	6/27/13	46.972	-95.735	17.5	<0.5	0.117	3579	39.84	1.0	1.00
19														
20	FS-56	Rice	19	18-0053-00-203	8/27/12	46.339	-93.8915	19.4	< 0.5	0.0259	83421	31.88	558.1	558.07
21	P-69	Rice	19	18-0053-00-203	9/27/11	46.339	-93.8913	43.0	0.23	0.021	50389	35.55	185.8	185.79
22	FS-376	Rice	19	18-0053-00-203	9/11/13	46.339	-93.8918	46.5	< 0.5	0.0451	65261	33.36	329.7	329.66
23	FS-304	Rice	19	18-0053-00-203	6/10/13	46.339	-93.8906	5.7	< 0.5	0.0236	48287	33.61	183.1	183.07
24	FS-324	Rice	19	18-0053-00-203	7/15/13	46.339	-93.8918	56.7	< 0.5	0.11	44704	33.18	160.3	160.29
25														
26	P-35	Anka	26	21-0353-00-201	9/16/11	46.077	-95.7377	3.0	2.23	0.493	2170	14.84	1.2	1.25

MPCA Field Data - CPSC Variability within Sites
Sites from MPCA March 2015 Draft, Figure 14

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
27	P-34	Anka	26	21-0353-00-201	9/16/11	46.077	-95.7292	25.9	2.23	0.671	1485	23.57	0.3	0.35
28	FS-192	Anka	26	21-0353-00-202	8/29/12	46.077	-95.7292	2.3	8.44	0.53	1498	22.85	0.4	0.37
29														
30	P-42	Monongalia (Middle Fork Crow R)	45.5	34-0158-01-201	9/20/11	45.348	-94.9509	5.7	16.51	0.042	46471	14.76	455.4	455.39
31	FS-313	Monongalia	46	34-0158-01-203	6/23/13	45.333	-94.9293	50.0	34.7	0.0941	6028	19.44	6.4	6.45
32	FS-340	Monongalia	46	34-0158-02-203	7/31/13	45.333	-94.9292	87.9	33.6	0.122	5530	22.1	4.7	4.69
33	FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.333	-94.9292	154.4	34.6	0.242	5436	26.42	3.7	3.66
34	FS-77	Monongalia (near hwy embankment)	46	34-0158-02-204	7/26/12	45.333	-94.9268	121.3	21.7	1.37	4953	18.66	4.6	4.64
35														
36	P-57	Unnamed	50	34-0611-00-201	9/23/11	45.268	-94.865	74.4	6.42	0.286	2311	6.48	3.8	3.80
37	P-57	Unnamed	50	34-0611-00-201	9/23/11	45.268	-94.865	74.4	6.42	0.065	2193	8.1	2.6	2.63
38	FS-183	Unnamed	50	34-0611-00-201	7/30/12	45.268	-94.865	64.9	16.8	0.15	2157	5.61	4.0	3.96
39	P-57	Unnamed	50	34-0611-00-201	9/23/11	45.268	-94.865	74.4	6.42	0.065	1946	13.8	1.1	1.11
40	P-57	Unnamed	50	34-0611-00-201	9/23/11	45.268	-94.865	74.4	6.42	0.065	1689	12.6	0.9	0.94
41														
42	FS-352	Dark	77	69-0790-00-202	8/15/13	47.639	-92.7782	2.9	173	0.136	5120	3.61	35.3	35.35
43	FS-369	Dark	77	69-0790-00-202	9/5/13	47.639	-92.7781	11.8	176	0.052	2037	0.82	35.4	35.41
44	FS-368	Dark	77	69-0790-00-202	9/5/13	47.639	-92.7782	11.1	175	0.305	3354	1.94	33.0	32.96
45	FS-322	Dark	77	69-0790-00-202	7/10/13	47.639	-92.7781	3.2	175	0.131	2480	1.48	25.5	25.50
46														
47	P-28	Raymond	83	73-0285-00-203	9/12/11	45.629	-95.0234	68.6	0.82	0.094	3922	10.06	6.2	6.21
48	FS-53	Raymond	83	73-0285-00-203	8/2/12	45.629	-95.0225	61.1	< 0.5	0.0787	1905	4.79	3.8	3.76
49	FS-343	Raymond	83	73-0285-00-203	8/6/13	45.629	-95.0233	61.4	1.92	0.0903	3270	7.59	6.1	6.13
50														
51	FS-211	Mississippi Pool 4/Robinson Lake	89	79-0005-02-201	8/16/12	44.361	-91.9897	57.6	17.7	0.0714	9265	1.55	304.2	304.23
52	FS-336	Mississippi Pool 4/Robinson Lake	89	79-0005-02-201	7/30/13	44.361	-91.9901	46.5	55.3	0.0602	8193	1.41	269.0	268.98
53	FS-210	Mississippi Pool 4/Robinson Lake	89	79-0005-02-202	8/16/12	44.359	-91.9881	35.3	15.7	0.07	6450	1.16	214.5	214.49
54														

MPCA Field Data - CPSC Variability within Sites
Sites from MPCA March 2015 Draft, Figure 14

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
55	FS-189	Clearwater	96	S002-121	8/28/12	47.937	-95.6906	4.5	23.8	0.117	2856	1.27	40.2	40.17
56	FS-327	Clearwater	96	S002-121	7/17/13	47.937	-95.6906	0.3	23.7	0.117	3521	1.82	39.1	39.06
57	FS-373	Clearwater	96	S002-121	9/9/13	47.937	-95.6909	3.2	34.4	0.0354	5315	3.33	41.8	41.84
58	FS-314	Clearwater	96	S002-121	6/24/13	47.937	-95.6907	0.6	28	0.0664	3946	2.68	30.6	30.60
59														
60	FS-384	Second	117	S007-220	9/19/13	47.52	-92.1925	27.7		0.104	22634	3.42	657.3	657.30
61	FS-310	Second	117	S007-220	6/14/13	47.521	-92.1925	57.6	316	0.0927	31190	4.22	946.8	946.84
62	FS-303	Second	117	S007-220	5/30/13	47.52	-92.1925	0.0	303	0.0991	13086	2.2	388.6	388.62
63	FS-323	Second	117	S007-220	7/11/13	47.52	-92.1925	76.4	405	0.067	10036	2.91	166.9	166.92
64	FS-351	Second	117	S007-220	8/15/13	47.521	-92.1925	66.8	838	0.0447	7088	1.84	148.0	148.03
65														
66	FS-370	Mississippi Pool 8 at Genoa	118	S007-222	9/9/13	43.577	-91.2337	17.8	33.3	0.062	6558	1.43	172.4	172.39
67	FS-208	Mississippi Pool 8 at Genoa	118	S007-222	8/14/12	43.576	-91.2334	41.4	18	0.176	2178	0.41	92.3	92.34
68	FS-334	Mississippi Pool 8 at Genoa	118	S007-222	7/29/13	43.576	-91.2344	52.8	44.2	0.102	1969	0.4	78.3	78.33
69	FS-311	Mississippi Pool 8 at Genoa	118	S007-222	6/20/13	43.577	-91.2341	12.7	29.3	0.107	1544	0.62	29.0	29.04
70														
71	P-13	Partridge	119	S007-443	8/31/11	47.521	-92.1899	65.9	10.39	0.075	11026	1.44	464.3	464.30
72	FS-331	Partridge	119	S007-443	7/24/13	47.521	-92.1904	60.5	14.6	0.112	10082	1.68	325.0	325.02
73	FS-92	Partridge	119	S007-443	9/12/12	47.521	-92.1909	4.1	36.3	0.0741	29463	5.87	571.7	571.67
74	FS-301	Partridge	119	S007-443	5/28/13	47.521	-92.1903	0.0	14.8	0.125	9491	3.94	104.3	104.32
75	FS-366	Partridge	119	S007-443	9/3/13	47.521	-92.19	47.7	34.2	0.057	7671	1.79	178.1	178.11
76	FS-365	Partridge	119	S007-443	9/3/13	47.521	-92.1901	76.7	34.1	0.0393	9179	2.5	168.6	168.62
77														
78	FS-371	Mississippi Pool 5 / Spring	123	S007-660	9/10/13	44.202	-91.8443	39.8	34.4	0.069	3582	0.11	1161.0	1160.97
79	FS-212	Mississippi Pool 5 / Spring	123	S007-660	8/17/12	44.199	-91.8461	29.6	17.2	0.0224	3674	0.22	531.7	531.70
80	FS-335	Mississippi Pool 5 / Spring	123	S007-660	7/30/13	44.195	-91.841	63.0	47.7	0.0342	4362	0.25	634.7	634.70
81	FS-372	Mississippi Pool 5 / Spring	123	S007-660	9/10/13	44.202	-91.8443	26.7	34.8	0.0536	3330	0.33	270.9	270.88
82	FS-312	Mississippi Pool 5 / Spring	123	S007-660	6/21/13	44.202	-91.8444	35.7	28.3	0.0844	3563	0.67	132.2	132.16

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Attachment 4

(4 pages)

MPCA Field Data - CPSC and SEM Ratio
120 ug/L Sulfide

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	LacCore_ field_ID	Site_name	UniqID	DNRStateID	Date	Lat	Long	WR ave stems /M2	WRpre sent	SO4 mg/L	TSmg/L	SedFeµgg	SedTOCpct	CPSC120	CPSC120: probablilis tic formula	CPSC120: determini stic formula	ratio
2	FS-104	Gourd	10	04-0253-00-201	6/27/12	47.8121	-94.965	0.0	NO	0.27		1776	36.87	0.3	0.29	0.08	0.265
3	FS-105	Second	17	15-0091-00-202	6/27/12	47.8258	-95.3637	48.4	YES	0.74	0.119	2527	33.3	0.6	0.64	0.17	0.274
4	FS-218	Holman	42	31-0227-00-202	9/13/12	47.3005	-93.3445	0.0	NO	24.2	1.01	3035	29.74	1.0	1.04	0.29	0.283
5	P-4	Little Flat	6	03-0217-00-201	8/24/11	46.9981	-95.6641	83.1	YES	0.22	0.011	7479	33.13	5.2	5.16	1.47	0.284
6	P-27	Pleasant	13	11-0383-00-206	9/9/11	46.928	-94.4757	28.6	YES	0.49		5331	30.37	3.0	2.99	0.86	0.287
7	FS-93	Turpela	71	69-0427-00-201	9/12/12	47.4613	-92.2371	1.0	YES	3.3	0.115	6979	31.08	4.9	4.87	1.40	0.288
8	FS-139	Welby family farm	93	86-0231-00-202	9/21/12	45.3592	-94.0782	17.2	YES	<0.5	0.118	7267	30.76	5.3	5.33	1.54	0.289
9	P-34	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7292	25.9	YES	2.23	0.671	1485	23.57	0.3	0.35	0.10	0.290
10	FS-214	Bowstring	116	S007-219	9/11/12	47.7024	-94.0608	69.7	YES	1.34	0.256	1974	24.34	0.6	0.58	0.17	0.291
11	FS-180	Lily	90	81-0067-00-202	7/26/12	44.1947	-93.647	38.2	YES	<0.5	0.0295	5095	28.07	3.0	3.01	0.87	0.291
12	FS-207	Kelly Lake	64	66-0015-00-204	8/13/12	44.3542	-93.3743	0.0	NO	1.92	0.0927	4387	27.33	2.3	2.33	0.68	0.291
13	P-17	St. Louis	114	S007-208	9/1/11	47.4668	-91.9355	68.6	YES	1.23	0.04	9654	30.4	9.3	9.34	2.73	0.292
14	FS-134	Bass	43	31-0576-00-207	9/18/12	47.2844	-93.6276	64.0	YES	1.01	0.0664	3740	26.12	1.8	1.81	0.53	0.292
15	FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.3332	-94.9292	154.4	YES	34.6	0.242	5436	26.42	3.7	3.66	1.08	0.295
16	FS-63	Caribou	72	69-0489-00-206	9/3/12	46.8913	-92.3135	0.0	NO	1.21	0.0938	13791	29.44	19.3	19.27	5.74	0.298
17	P-3	Little Round	7	03-0302-00-202	8/24/11	46.9759	-95.7404	57.2	YES	0.46	0.032	1689	20.91	0.5	0.51	0.15	0.299
18	FS-65	Wild Rice	11	09-0023-00-202	9/4/12	46.6712	-92.6055	0.0	NO	0.5	0.083	13650	28.82	19.4	19.38	5.79	0.299
19	FS-250	Little Rice	75	69-0612-00-201	9/20/12	47.7086	-92.4389	29.3	YES	1.03	0.0293	9488	26.45	10.7	10.67	3.21	0.301
20	FS-324	Rice	19	18-0053-00-203	7/15/13	46.3392	-93.8918	56.7	YES	<0.5	0.11	44704	33.18	160.3	160.29	48.35	0.302
21	P-12	Birch	67	69-0003-00-205	8/30/11	47.7357	-91.9428	68.6	YES	3.58	0.104	12431	26.8	17.7	17.66	5.35	0.303
22	P-19	Wolf	69	69-0143-00-202	9/2/11	47.2586	-91.9618	128.8	YES	1.54	0.139	8240	25.1	8.7	8.66	2.62	0.303
23	FS-378	Duck Lake WMA	22	18-0178-00-202	9/12/13	46.7521	-93.8851	113.0	YES	<0.5	0.0251	12151	26.57	17.1	17.08	5.17	0.303
24	FS-126	Bray	58	56-0472-00-202	8/20/12	46.4518	-95.8783	7.6	YES	1.65	0.072	3937	21.95	2.5	2.46	0.75	0.304
25	FS-62	Swan	34	31-0067-02-206	8/30/12	47.289	-93.2124	3.8	YES	14	0.221	4821	22.53	3.5	3.52	1.07	0.304
26	FS-216	Big Sucker	39	31-0124-00-203	9/12/12	47.3919	-93.2658	3.8	YES	7.78	0.145	3559	21.45	2.1	2.08	0.63	0.305
27	FS-181	Rice	66	66-0048-00-203	7/27/12	44.3332	-93.4734	0.0	NO	5.22	0.777	3829	21.67	2.4	2.37	0.72	0.305
28	FS-194	Gilchrist	91	86-0064-00-201	8/31/12	45.2309	-93.824	0.0	NO	6.98	0.355	3117	20.81	1.7	1.67	0.51	0.305

MPCA Field Data - CPSC and SEM Ratio
120 ug/L Sulfide

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
29	P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	YES	0.56	0.018	4302	21.79	2.9	2.94	0.90	0.305
30	FS-346	Westport	63	61-0029-00-205	8/8/13	45.7042	-95.203	6.7	YES	6.3	0.205	3262	19.66	2.0	1.95	0.60	0.309
31	P-25	Lower Rice	107	S006-985	9/8/11	47.3793	-95.4834	114.4	YES	1.02	0.097	2337	17.76	1.2	1.16	0.36	0.313
32	FS-221	Hay Creek Flowage	59	58-0005-00-202	9/17/12	46.0894	-92.4104	97.7	YES	1.95	0.119	9456	22.05	13.2	13.18	4.12	0.313
33	FS-88	Clearwater	98	S004-204	8/24/12	47.5174	-95.3904	148.3	YES	2.04	0.0488	9874	22.17	14.2	14.23	4.45	0.313
34	FS-177	South Geneva	30	24-0015-02-208	7/24/12	43.7709	-93.2851	0.0	NO	14.1	3.19	1618	16.71	0.6	0.62	0.19	0.313
35	FS-179	Rice	84	74-0001-00-201	7/25/12	44.0842	-93.0737	0.0	NO	3.84	0.217	4152	19.07	3.2	3.22	1.01	0.314
36	FS-224	Stone Lake	68	69-0046-00-201	9/19/12	47.5039	-91.8857	21.0	YES	3.26	0.0533	5225	18.87	5.1	5.08	1.61	0.317
37	FS-228	West battle	57	56-0239-00-204	8/15/12	46.2906	-95.6049	144.8	YES	4.03	0.189	3108	17.37	2.1	2.06	0.65	0.317
38	FS-184	Rice	80	73-0196-00-216	7/30/12	45.3864	-94.6309	0.0	NO	2.58	2.97	1523	15.03	0.6	0.62	0.20	0.319
39	FS-349	Sandy-3	76	69-0730-00-205	8/13/13	47.6191	-92.5898	0.0	NO	122	0.0697	14897	20.46	34.6	34.55	11.14	0.322
40	FS-193	Big Mud	79	71-0085-00-201	8/30/12	45.4529	-93.7418	14.3	YES	<0.5	0.0308	12943	18.63	29.5	29.50	9.66	0.327
41	FS-223	Little Sucker	40	31-0126-00-202	9/14/12	47.3765	-93.246	0.0	NO	13.7	0.534	6297	16.56	8.5	8.50	2.79	0.328
42	FS-215	Popple	101	S006-188	9/11/12	47.7254	-94.0817	36.3	YES	<0.5	0.0269	2971	14.42	2.4	2.37	0.78	0.329
43	FS-176	North Geneva	29	24-0015-00-209	7/24/12	43.7876	-93.271	0.0	NO	15.6	1.54	2212	13.45	1.5	1.46	0.48	0.331
44	P-44	Dead Fish	12	09-0051-00-202	9/20/11	46.7451	-92.6863	48.7	YES	0.3	0.056	9685	16.6	19.4	19.39	6.45	0.332
45	P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	YES	6.42	0.065	1689	12.6	0.9	0.94	0.31	0.333
46	FS-87	Bee	60	60-0192-00-202	8/23/12	47.6527	-96.0504	39.8	YES	11	0.67	3054	13.62	2.7	2.67	0.89	0.334
47	FS-125	Tamarac	56	56-0192-00-203	8/19/12	46.3637	-95.5714	0.0	NO	2.33	0.0768	21908	18.41	82.3	82.32	27.50	0.334
48	FS-129	Mink	92	86-0229-00-207	8/23/12	45.2767	-94.0299	0.0	NO	1.22	0.182	4247	13.63	5.0	5.03	1.70	0.337
49	FS-85	Bean	8	03-0411-00-201	8/21/12	46.9337	-95.8706	0.0	NO	85	16	1967	11.85	1.4	1.35	0.46	0.339
50	FS-55	Pelkey	55	49-0030-00-202	8/26/12	45.9962	-94.2273	0.0	NO	3.42	0.0522	30642	17.32	168.8	168.82	57.77	0.342
51	FS-219	Trout	41	31-0216-00-212	9/13/12	47.2592	-93.3942	0.0	NO	38.6	0.117	12535	15	35.9	35.95	12.32	0.343
52	FS-350	Ox Hide	35	31-0106-00-203	8/14/13	47.3351	-93.2132	0.0	NO	25.9	0.119	3889	12.12	4.9	4.89	1.69	0.345
53	FS-199	Rice	102	S006-208	9/5/12	47.6742	-93.6547	75.4	YES	1.57	0.0552	3273	10.88	4.0	3.99	1.40	0.351
54	FS-220	Padua	82	73-0277-00-202	8/7/12	45.623	-95.0186	0.0	NO	0.86	0.23	2291	9.77	2.3	2.29	0.81	0.355
55	FS-75	Mortenson	44	34-0150-02-201	7/24/12	45.3	-94.9062	0.0	NO	<0.5	0.103	9071	12.09	25.0	24.99	8.87	0.355
56	FS-109	Carlos Avery Pool 9	4	02-0504-00-202	7/3/12	45.3192	-93.0611	52.8	YES	<0.5	< 0.011	14736	12.51	61.0	60.98	21.83	0.358
57	FS-339	Christina	28	21-0375-00-315	7/31/13	46.0734	-95.7567	0.6	YES	14.6	1.93	1741	8.96	1.5	1.50	0.54	0.358
58	P-42	Monongalia (Middle Fork Crow R	45.5	34-0158-01-201	9/20/11	45.3481	-94.9509	5.7	YES	16.51	0.042	46471	14.76	455.4	455.39	163.45	0.359

MPCA Field Data - CPSC and SEM Ratio
120 ug/L Sulfide

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
59	FS-82	Rabbit	20	18-0093-02-204	8/8/12	46.5313	-93.9285	0.0	NO	15.3	0.22	10903	11.79	36.7	36.68	13.16	0.359
60	FS-191	Ina	27	21-0355-00-202	8/29/12	46.0715	-95.7281	30.2	YES	7.08	0.274	2216	9.09	2.3	2.34	0.84	0.360
61	FS-52	Blaamyhre	48	34-0345-00-203	8/1/12	45.364	-95.186	102.2	YES	0.62	0.078	3517	9.33	5.5	5.51	2.00	0.363
62	FS-137	Elk	15	15-0010-00-204	9/19/12	47.1952	-95.2249	42.7	YES	<0.5	0.0936	6334	10.07	15.6	15.59	5.68	0.365
63	FS-231	Rice	2	02-0008-00-206	8/17/12	45.1604	-93.121	0.0	NO	3.6	0.145	2159	7.98	2.6	2.60	0.96	0.370
64	P-5	Itasca	16	15-0016-00-208	8/25/11	47.2381	-95.2065	45.8	YES	0.26	0.056	1355	7.4	1.2	1.16	0.43	0.370
65	FS-196	Prairie	115	S007-209	9/3/12	47.2519	-93.4884	44.6	YES	9.63	0.0709	15071	10.51	78.4	78.43	29.16	0.372
66	FS-58	Mississippi River above Clay Bosw	108	S007-163	8/28/12	47.2386	-93.7197	0.0	NO	1.19	0.0806	8636	9.08	32.0	32.02	12.06	0.377
67	FS-200	Louisa	94	86-0282-00-205	8/8/12	45.2998	-94.258	0.0	NO	7.04	0.192	7824	8.76	27.6	27.65	10.46	0.378
68	FS-76	Field	45	34-0151-00-201	7/25/12	45.2964	-94.9058	0.0	NO	<0.5	0.0687	7586	8.68	26.3	26.34	9.97	0.379
69	FS-355	Mississippi River below Clay Bosw	103	S006-923	8/13/13	47.2553	-93.634	78.3	YES	10.2	0.0819	10479	8.98	47.1	47.07	17.88	0.380
70	FS-229	Mill Pond	23	21-0034-00-202	8/16/12	46.0716	-95.2218	102.2	YES	7.16	0.109	5143	7.86	14.0	14.05	5.36	0.382
71	P-26	Lower Rice	109	S007-164	9/8/11	47.3817	-95.4926	120.1	YES	0.55	0.07	2364	6.76	3.8	3.77	1.45	0.384
72	FS-190	Pine	18	15-0149-00-205	8/28/12	47.6841	-95.5414	114.9	YES	14.7	0.368	4477	7.08	12.2	12.19	4.74	0.389
73	FS-54	Little Birch	87	77-0089-00-207	8/3/12	45.7779	-94.7978	70.0	YES	7.4	0.0353	1794	6.02	2.6	2.55	1.00	0.390
74	FS-204	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.742	133.7	YES	5.49	0.0914	1731	5.94	2.4	2.42	0.95	0.391
75	P-31	Cloquet	52	38-0539-00-201	9/14/11	47.4313	-91.4844	74.4	YES	0.81	0.024	4252	6.58	12.1	12.05	4.75	0.394
76	FS-377	Mahnomen	21	18-0126-02-201	9/11/13	46.4986	-93.9956	0.0	NO	21.1	0.0283	16540	7.47	141.1	141.14	56.61	0.401
77	P-20	Gull	9	04-0120-00-203	9/6/11	47.6559	-94.6944	15.6	YES	0.78	0.103	1608	5.08	2.5	2.53	1.02	0.403
78	FS-53	Raymond	83	73-0285-00-203	8/2/12	45.6286	-95.0225	61.1	YES	<0.5	0.0787	1905	4.79	3.8	3.76	1.55	0.411
79	FS-195	Fisher	78	70-0087-00-201	8/31/12	44.7942	-93.4061	20.7	YES	6.85	0.136	11140	5.76	90.1	90.10	37.70	0.418
80	FS-130	Hay	33	31-0037-00-202	9/6/12	47.2874	-93.102	141.0	YES	31.7	0.0738	13154	5.79	123.3	123.26	51.80	0.420
81	FS-131	Hinken	113	S007-207	9/5/12	47.7271	-93.9923	46.8	YES	<0.5	0.0876	2960	4.53	9.4	9.39	3.96	0.422
82	FS-91	Pike	104	S006-927	9/11/12	47.7327	-92.3473	3.5	YES	14.2	0.0656	6565	4.72	41.4	41.36	17.74	0.429
83	FS-80	Mission	95	S001-646	8/6/12	45.8623	-93.0011	87.5	YES	0.62	0.0485	9231	4.83	77.5	77.50	33.45	0.432
84	P-63	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	148.7	YES	1.83	0.01	10269	4.24	111.2	111.17	49.52	0.445
85	P-36	Wild Rice Reservoir	70	69-0371-00-204	9/16/11	46.9098	-92.1636	17.2	YES	1.13	0.023	5555	3.75	39.5	39.51	17.70	0.448
86	FS-86	Eighteen	61	60-0199-00-202	8/22/12	47.6397	-96.0607	40.1	YES	4.29	0.164	1860	3.1	6.1	6.05	2.72	0.450
87	FS-301	Partridge	119	S007-443	5/28/13	47.5213	-92.1903	0.0	NO	14.8	0.125	9491	3.94	104.3	104.32	47.07	0.451
88	FS-83	Mississippi Crow Wing	111	S007-205	8/8/12	46.4386	-94.1251	0.0	NO	3.13	0.127	13451	3.88	207.8	207.75	95.15	0.458

MPCA Field Data - CPSC and SEM Ratio
120 ug/L Sulfide

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
89	FS-67	St. Louis Estuary Pokegama Bay	105	S006-928	9/5/12	46.6859	-92.1606	0.0	NO	9.97	0.112	14015	3.66	241.1	241.10	111.95	0.464
90	FS-202	Long Prairie	110	S007-204	8/9/12	46.0072	-95.2634	13.4	YES	7.71	0.0793	2897	2.85	15.7	15.69	7.30	0.465
91	FS-128	Cromwell	14	14-0103-00-201	8/22/12	46.9651	-96.3171	0.0	NO	41.2	1.22	2948	2.85	16.2	16.23	7.55	0.465
92	FS-188	Stella	54	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	YES	18.1	1.79	1257	2.34	4.0	3.99	1.88	0.472
93	FS-51	Glesne Slough	49	34-0353-00-201	7/31/12	45.3514	-95.1887	99.6	YES	<0.5	0.061	7983	3.01	103.2	103.23	49.05	0.475
94	FS-314	Clearwater	96	S002-121	6/24/13	47.9372	-95.6907	0.6	YES	28	0.0664	3946	2.68	30.6	30.60	14.56	0.476
95	FS-357	Lower Panasa	38	31-0112-00-204	8/15/13	47.3026	-93.2561	0.0	NO	28.5	1.26	2347	2.42	12.7	12.73	6.09	0.478
96	FS-316	Partridge	121	S007-513	6/28/13	47.5137	-92.1899	0.0	NO	24.9	0.098	6291	2.6	77.8	77.80	37.84	0.486
97	P-55	Lady Slipper	53	42-0020-00-204	9/22/11	44.5702	-95.6274	0.0	NO	107.7	14.84	2814	2.09	21.5	21.51	10.67	0.496
98	P-1	Height of Land	5	03-0195-00-209	8/22/11	46.9129	-95.6095	62.9	YES	0.24	0.053	1298	1.76	6.0	5.97	2.99	0.502
99	FS-209	Mississippi Pool 8 at Reno Botton	122	S007-556	8/15/12	43.6025	-91.2686	72.3	YES	18.1	0.0711	9187	2.29	187.6	187.61	94.93	0.506
100	FS-225	Miltona	24	21-0083-00-205	8/13/12	46.0496	-95.4217	0.0	NO	4.11	0.0694	2624	1.77	22.9	22.94	11.77	0.513
101	FS-351	Second	117	S007-220	8/15/13	47.5205	-92.1925	66.8	YES	838	0.0447	7088	1.84	148.0	148.03	77.81	0.526
102	FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.6545	-92.2739	0.0	NO	16	0.0445	6169	1.73	122.0	122.02	64.69	0.530
103	FS-322	Dark	77	69-0790-00-202	7/10/13	47.6389	-92.7781	3.2	YES	175	0.131	2480	1.48	25.5	25.50	13.56	0.532
104	FS-95	Embarrass	73	69-0496-00-203	9/14/12	47.5334	-92.2979	0.0	NO	18.8	0.0298	21847	1.89	1248.9	1248.85	677.38	0.542
105	FS-347	Snowball	36	31-0108-00-202	8/12/13	47.3356	-93.2439	0.0	NO	8.2	0.097	1136	1.19	7.4	7.38	4.00	0.543
106	FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.6518	-92.2372	31.2	YES			4761	1.4	95.5	95.52	52.52	0.550
107	FS-182	Hunt	65	66-0047-00-208	7/27/12	44.3275	-93.4443	0.0	NO	17.1	0.0729	2412	1.21	30.8	30.76	17.06	0.555
108	FS-187	McCormic	81	73-0273-00-203	8/2/12	45.722	-94.9121	8.9	YES	1.54	0.144	1512	1.1	14.0	14.04	7.83	0.557
109	FS-210	Mississippi Pool 4/Robinson Lake	89	79-0005-02-202	8/16/12	44.3593	-91.9881	35.3	YES	15.7	0.07	6450	1.16	214.5	214.49	124.00	0.578
110	FS-226	Louise	25	21-0094-00-202	8/14/12	45.9331	-95.4148	46.5	YES	4.09	0.0746	1833	0.83	28.5	28.49	16.97	0.596
111	FS-185	Hoffs Slough	85	76-0103-00-201	8/1/12	45.3255	-95.7059	0.0	NO	273	0.0343	3512	0.75	112.3	112.32	69.83	0.622
112	FS-311	Mississippi Pool 8 at Genoa	118	S007-222	6/20/13	43.5766	-91.2341	12.7	YES	29.3	0.107	1544	0.62	29.0	29.04	18.30	0.630
113	FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	YES	1.62	0.0659	2505	0.65	69.6	69.60	44.12	0.634
114	FS-312	Mississippi Pool 5 / Spring	123	S007-660	6/21/13	44.2018	-91.8444	35.7	YES	28.3	0.0844	3563	0.67	132.2	132.16	84.21	0.637
115	FS-59	Upper Panasa	37	31-0111-00-202	8/29/12	47.306	-93.2652	0.0	NO	29.6	0.126	895	0.43	15.8	15.77	10.55	0.669
116	P-16	St. Louis	106	S006-929	9/1/11	47.4015	-92.3773	0.0	NO	24.5	0.025	1488	0.1	240.3	240.27	223.05	0.928

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Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*)

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Abstract Iron sulfide plaques have been observed on roots of wild rice (*Zizania palustris*) and other wetland plants grown in sulfur-impacted freshwater ecosystems, but the mechanism of their formation and ramifications for plants have not been investigated. We exposed a model annual wetland plant, *Zizania palustris*, to elevated sulfate concentrations (3.1 mM) and quantified the development of iron oxide and iron sulfide precipitates on root surfaces throughout the plant life cycle. During the onset of seed production, root surfaces amended with sulfate transitioned within 1 week from iron (hydr)oxide plaques to iron sulfide plaques. During the same week, Fe(III) decreased on roots of plants not amended with sulfate but FeS did not accumulate. Prior to FeS accumulation, sulfate-amended plants had taken up the same amount of N as unamended plants. After FeS accumulation, total plant nitrogen did not increase further on sulfate-amended plants, indicating a cessation in nitrogen uptake, whereas total plant N continued to increase in unamended plants. Sulfate-amended plants produced fewer and lighter seeds with less nitrogen than unamended plants. FeS precipitation on roots may be associated with elevated sulfide and inhibited nitrogen uptake before the end of the plant's life cycle, thus affecting the populations of this annual aquatic plant. We propose a mechanism by which a physiologically-induced decline in radial oxygen loss near the end of a plant's life cycle initiates a precipitous decline in redox potential at the root surface and in adjacent porewater, initiating accumulation of iron sulfide plaques. These plaques could be an important locus for iron sulfide accumulation in wetland sediments.

Keywords Root plaques · Radial oxygen loss · Iron–sulfur cycling · *Zizania palustris* · Electron accepting buffer

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Introduction

Introduction of sulfate to low-sulfate freshwater ecosystems and subsequent reduction to sulfide can induce eutrophication, enhance methylmercury production, and decimate populations of sensitive aquatic plant species (Caraco et al. 1989; Gilmour et al. 1992; Smolders et al. 2003). Field observations have correlated elevated sulfide concentrations in sediment with population declines and decreased density of some aquatic plants (Myrbo et al. 2017; Pulido et al. 2012; Smolders et al. 2003). Black iron sulfide (FeS) plaques have been observed on the roots of aquatic plants grown with elevated sulfide in several sulfur addition experiments (Gao et al. 2003; Jacq et al. 1991; Koch and Mendelssohn 1989) including our outdoor mesocosm experiment with self-perpetuating wild rice (*Zizania palustris*) populations (Pastor et al. 2017); however, little is known about conditions conducive to iron sulfide precipitation on roots and the mechanism by which it occurs.

Roots of aquatic plants create redox interfaces that are hot spots for cycling of nitrogen, sulfur, iron, and other metals (Soana et al. 2015; Schmidt et al. 2011; Lee and McNaughton 2004). Many aquatic plants transport oxygen from the atmosphere to the roots through porous tissue called aerenchyma (Armstrong and Armstrong 2005). Radial oxygen loss from roots reacts with ferrous iron in sediment to form iron (hydr)oxide plaques at the interface of the oxidized root surface and the reduced sediment (Christensen and Sand-Jensen 1998; Mendelssohn and Postek 1982; Snowden and Wheeler 1995). Together, radial oxygen loss and iron (hydr)oxide plaques provide a supply of electron accepting compounds at the root surface, hereafter referred to as an electron accepting buffer. This buffer may inhibit sulfide formation and precipitation in several ways. The release of oxygen by plant roots may reoxidize sulfide and inhibit sulfate reduction (Holmer et al. 1998). In addition, Fe(III) can oxidize sulfide, and the reduction of Fe(III) to Fe(II) may outcompete sulfate reduction (Roden and Wetzel 1996; Hansel et al. 2015). Others have observed increased FeS precipitation on roots and in sediments shortly after plant senescence (Jacq et al. 1991; Giblin and Howarth 1984), suggesting a decrease in the strength of the electron accepting buffer. However, the timing of sulfide interactions with iron on root surfaces, particularly in relation to the life cycle of the plants, remains largely unexplored.

To explore these processes, we subjected wild rice, *Zizania palustris*, an annual plant that forms large monotypic stands in the lakes and rivers of Minnesota, Wisconsin, northern Michigan, and Ontario, to enhanced sulfate concentrations. Although radial oxygen loss has not been directly quantified in wild rice, aerenchyma tissue and root surface iron oxides have been studied and documented in this species (Stover 1928; Jorgenson et al. 2012). In a previous mesocosm experiment with wild rice, increasing concentrations of porewater sulfide decreased vegetative biomass production only slightly, but strongly decreased annual seed production, leading to population declines in subsequent years (Pastor et al. 2017). Hydroponics experiments have demonstrated that sulfide reduces nutrient uptake in wetland plants (Joshi et al. 1975; Koch and Mendelssohn 1989) through inhibition of metallo-enzymes in the electron transport chain and subsequent inhibition of ATP production required for nutrient transport (Allam and Hollis 1972; Koch et al. 1990; Martin and Maricle 2015). It is not well understood why the seed production life stage of wild rice is especially vulnerable to sulfide, but decreased seed production may be associated with the timing of favorable conditions for sulfate reduction and concomitant FeS accumulation on roots.

To identify the drivers of FeS formation on the root surfaces, we tested the hypothesis that surface water sulfate loading induces FeS formation on roots. To investigate the implications of FeS root plaques for nitrogen uptake during seed production, we explored the timing of FeS formation on wild rice roots. We exposed wild rice plants to elevated surface water sulfate and quantified the speciation of iron and sulfur on root surfaces and in rooting-zone porewater during reproductive life stages. Throughout the life cycle of the plant, we also monitored growth and seed production.

Methods

Sediment was collected from Rice Portage Lake (MN Lake ID 09003700, 46.703810, − 92.682921) on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota on 5/15/15 and placed in a 400 L polyethylene stock tank (High Country Plastics) where it was homogenized by shovel. Initial total carbon in the sediment was 14.8 ± 1.70% and initial total nitrogen was 1.12 ± 0.13% by dry weight. Eighty 4 L plastic pails were then filled with 3 L of the sediment. Each 4 L pail was placed inside a 20 L bucket that was filled with 12 L of groundwater from an on-site well to provide a 12–15 cm water column. In each pail, two seeds that were harvested in 2014 from Swamp Lake on the Grand Portage Reservation (MN Lake ID 16000900, 47.951856, − 89.856844) were planted on 5/15/15 (Julian day 135).

Forty randomly chosen buckets were amended with sulfate and forty were left unamended. On 6/3/15, the forty amended buckets received an aliquot of stock solution (5.15 g of Na_2SO_4 dissolved in 200 mL of deionized water) to result in 300 mg L^{-1} (3.1 mM) sulfate. We hereafter refer to all porewater, sediment, and plants in these buckets as "amended". This concentration is close to the EPA secondary standard for drinking water, 250 mg L^{-1} (2.6 mM), intended to prevent laxative effects and an unpleasant taste. Although northeastern Minnesota generally has sulfate concentrations less than 10 mg L^{-1} (0.1 mM), concentrations of sulfate higher than 2.6 mM are found in some Minnesota waters, either naturally from geologic sources or from anthropogenic inputs (Myrbo et al. 2017). A sulfate concentration of 3.1 mM caused wild rice populations to go extinct within 5 years in previous mesocosm experiments with the same sediment (Pastor et al. 2017). The overlying water was sampled twice throughout the trial and re-adjusted to 3.1 mM SO_4 by adding additional Na_2SO_4 stock solution on 7/10/15. Unamended buckets had an average surface water sulfate concentration of 0.15 ± 0.01 mM when sampled on 6/23/15, consistent with the concentration of sulfate in groundwater from the on-site well. This is only slightly above observations of Moyle (1944) that wild rice grows best in waters less than 10 mg L^{-1} sulfate. We hereafter refer to all porewater, sediment, and plants in these buckets as "unamended." Shoots were thinned on 6/23/15 to one plant per bucket. Shoot height ranged from 10 to 20 cm and the tallest, most robust shoot in each bucket was left in place.

The annual life cycle of wild rice begins with emergence from the sediment and water column in June, continues with vegetative growth in July, followed by flowering and seed production in August, and ends with the shedding of seeds and death of the plant from late August to late September. Seeds overwinter in the sediment until they germinate in May (Grava and Raisanen 1978; Sims et al. 2012). Four plants were harvested every 2 weeks from randomly chosen amended and unamended buckets beginning at the onset of flowering (7/9/15, day 190) and continuing to the onset of seed production (8/20/15, day 232), after which plants were harvested weekly until senescence (9/22/15, day 265). The first seeds were collected on 8/20/15 (day 232) but were unripe and not yet filled. Mature seeds were not produced until 1 week after the start of seed production (day 239). On the last sample date (day 265) seeds were collected but were unfilled. Stems and leaves were no longer green, indicating that the plants had senesced. Of the four amended replicates sampled on this date, two plants did not produce seeds. Thus, "mature seed production" refers to seeds produced between Julian days 239–253.

Each plant was removed from the sediment and immediately rinsed in buckets of deoxygenated water continuously bubbled with a rapid stream of molecular nitrogen. If seeds were present, they were removed prior to sampling the plant and saved for separate analysis. While submerged in deoxygenated water, the stem was cut just above the root ball so that the shoots could be saved for mass and N analysis. The still submerged roots were then placed in jars full of deoxygenated water, which were immediately placed in a plastic bag flushed with molecular nitrogen and transported to an oxygen-free glove box (Coy Lab Products, 97.5% N_2, 2.5% H_2). In the glove box, the roots were cleaned of sediment and all organic matter except living wild rice roots prior to removing a 1–2 g section of wet root mass for acid volatile sulfide (AVS) and iron analysis.

The plants and seeds were rinsed with deionized water and dried in paper bags for 7 days at 65 °C. The dried plants were weighed, placed in polycarbonate vials with stainless steel balls, and shaken in a SPEX 800 M mixer mill until the samples were in a powdered form. Seeds were counted, weighed, and powdered using the same method. The samples were transferred to glass vials and dried again overnight at 65 °C with caps loosely covering the vials. Samples were quantified for total N on an elemental analyzer coupled to a Finnigan Delta Plus XP isotope ratio monitoring mass spectrometer.

Sediment was collected at the beginning and end of the growing season. Immediately after sediment homogenization (5/15/15), five replicate samples were placed in jars and analyzed for AVS and simultaneously extracted iron. At the end of the growing season (9/22/15), a 7 cm diameter sediment core was collected from the top 10 cm of each bucket prior to root sampling. Jars were filled completely with sediment and placed in a plastic bag filled with nitrogen to prevent oxidation during transport to a glove box. In the glove box, sediment was homogenized and allocated for AVS and iron extraction.

From both sediment and roots, AVS and iron were extracted simultaneously from a 1–3 g wet sample (0.1–0.5 g dry) using 7.5 mL 1 N HCl for 4 h using a modified diffusion method (Brouwer and Murphy 1994). During a room temperature acid incubation with gentle mixing, sulfide was trapped in an inner vial containing 3 mL Sulfide Antioxidant Buffer (SAOB) and subsequently quantified using a ThermoScientific sulfide ion-selective electrode with a detection limit of 0.01 mmol L^{-1}. After the extraction, two aliquots of the 1 N HCl extracts were used for iron quantification. Ferrous iron was immediately quantified colorimetrically using the phenanthroline method on a HACH DR5000 UV–Vis spectrophotometer (Greenberg et al. 1992), and weak acid extractable iron (sum of Fe(II) + Fe(III) concentrations, hereafter referred to as "total extractable iron") was quantified using a Varian fast sequential flame atomic absorption spectrometer with an acetylene torch.

A subset of roots was tested for chromium(II)-reducible sulfur (CRS) to determine whether AVS included all total reduced inorganic sulfur on the roots. A diffusion-based CRS method was used, which can fully extract all amorphous iron sulfide and pyrite and can partially extract elemental sulfur (Burton et al. 2008). The same sampling apparatus was used for extraction of AVS and CRS (see Burton et al. 2008 Fig. 1 for a diagram of the sampling apparatus). Chromic acid for CRS analysis was prepared according to Burton et al. (2008). Inside an oxygen-free glove box, a section of root from a plant previously analyzed for AVS was placed in the analysis bottle. An inner vial containing SAOB was also placed inside the bottle prior to sealing. Bottles were removed from the glove box and injected with chromic acid with no oxygen exposure. CRS was extracted for 48 h and quantified using a ThermoScientific sulfide ion-selective electrode.

One day prior to each root sampling date, the porewater was sampled for sulfide, sulfate, iron, and pH. First, pH was measured in situ with a ThermoScientific Orion pH electrode at a depth of 5 cm below the sediment surface and 2 cm from the stem of the wild rice plant. Porewater was collected using 5 cm length, 2 mm diameter tension lysimeter filters (Seeberg-Elverfeldt et al. 2005) (Rhizons) attached with a hypodermic needle to an evacuated, oxygen-free serum bottle sealed with a 20 mm thick butyl-rubber stopper (Bellco Glass, Inc). The entire filter end of the Rhizon was inserted vertically into the sediment just below the surface. The goal was to draw water from approximately the upper 5 cm of sediment without drawing surface water. The filter was placed with minimal jostling to avoid creating a cavity around the filter that would allow surface water to enter the sediment and contaminate the porewater. The Rhizon was placed approximately 2 cm away from the stem of the wild rice plant and on the opposite side from where pH was measured (Supplementary Fig. S1).

Porewater sulfide samples were drawn into 50-mL serum bottles preloaded with 0.2% 1 M ZnAc and 0.2% 6 M NaOH to preserve sulfide. Sulfide bottles were left to fill overnight, then stored at 4 °C in the sealed serum bottles used for sample collection for approximately 30 days before sulfide was quantified. Samples for porewater sulfate analysis were withdrawn from sulfide sampling bottles and filtered through a Dionex 1 cc metal cartridge and a 0.45 µm polyethersulfone filter approximately 3 months after they were collected. Porewater iron was collected in 8 mL serum bottles preloaded with 40% deionized water, 40% phenanthroline, 20% acetate buffer, and 1% concentrated hydrochloric acid. Iron bottles were filled until the solution turned light red, approximately 10 min. If the solution turned red before 8 mL were collected, samples were diluted with deionized water to bring the total solution to 8 mL. Iron samples were quantified within 2 h of sampling. Iron and sulfide in porewater were quantified colorimetrically using the phenanthroline and methylene blue methods, respectively, on a HACH DR5000 UV–Vis spectrophotometer (Greenberg et al. 1992). Sulfate was quantified using a Dionex ICS-1100 Integrated IC system (AS-DV Autosampler) (Greenberg et al. 1992). The saturation index was calculated to determine if the porewater was saturated with respect to iron sulfide (Eq. 1, $K_{sp} = 10^{-2.95}$)

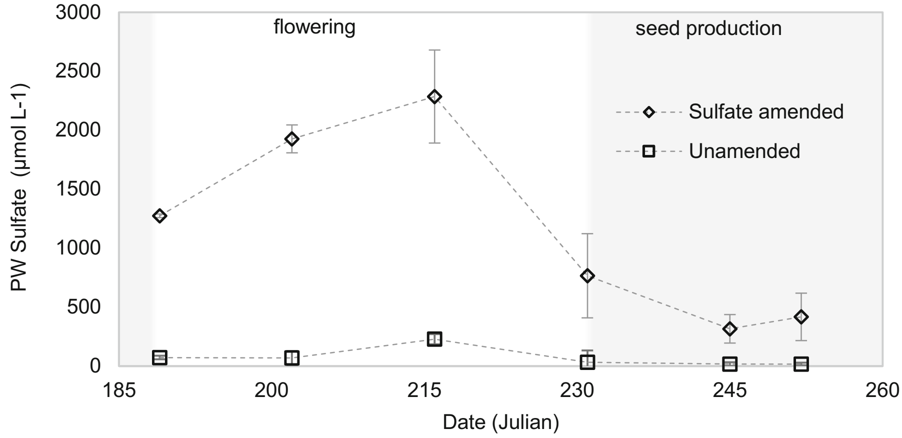

Fig. 1 Seasonal measurements of porewater sulfate concentrations 2 cm from the root surface. Diamonds depict amended plants while squares depict unamended plants. Error bars show one standard deviation around the mean. Shading represents different life stages. Shading on left side of figure represents pre-flowering, unshaded represents flowering, and shading on right represents seed production

(Stumm and Morgan 1996). A positive saturation index indicates oversaturation and a thermodynamic force to drive precipitation, and a negative value indicates undersaturation (and potential dissolution).

$$SI = \log\frac{[IAP]}{K_{sp}} \text{ where } IAP = \frac{[Fe^{2+}][HS^-]}{[H^+]} \quad (1)$$

Geochemical parameters and measured attributes of plants were analyzed using repeated measures analysis of variance to determine differences between amended and unamended treatments over the course of the growing season. Analyses were performed with a repeated measures ANOVA because although individual plants were harvested on each date, each sampling date was not independent of the prior sample dates. A paired *t* test was used to determine differences between AVS and CRS concentrations on subsamples from the same roots. Analyses were performed using the statistical software SAS. Logarithmic transformations were used when data was non-normal. Data are available at the Data Repository for the University of Minnesota (https://doi.org/10.13020/D68W98).

Results

Porewater sulfate and sulfide

Immediately before sulfate was added to amended buckets on Julian day 154, porewater sulfate concentrations were near 40 µmol L^{-1}. By the start of flowering (day 185), sulfate concentrations in amended porewater were over 1200 µmol L^{-1}, 30 times higher than the initial concentration (Fig. 1). Sulfate concentrations continued to rise for the first 30 days of flowering (until day 217), peaking at nearly 2300 µmol L^{-1}. Over a 4 week period (days 217–245) surrounding the onset of seed production, sulfate concentrations in amended porewater decreased by 86% to 315 µmol L^{-1}. Sulfate concentrations in unamended porewater were about 70 µmol L^{-1} at the start of flowering, roughly double the initial concentrations. Sulfate concentrations peaked at 230 µmol L^{-1} in unamended buckets on the same day as in amended buckets. During the same period that sulfate concentrations declined in amended porewater (days 217–245), sulfate concentrations in unamended buckets decreased by a similar proportion, 91%, to 20 µmol L^{-1}. Porewater sulfide did not differ between amended and unamended treatments (Supplementary Fig. S2). Concentrations averaged between 1 to 5 µmol L^{-1} during flowering and increased to an average of 8–17 umol L^{-1} at the start of seed production. Amended rhizospheres had a higher average sulfide concentration than unamended rhizospheres during seed production, but variability was high on the days porewater sulfide was elevated. Porewater sulfide concentrations decreased near the end of seed production and rose slightly at senescence.

Acid volatile sulfur on root surfaces

When grown in sediment with sulfate-amended overlying water (3.1 mM), amended plants developed a black coating on their root surfaces by the beginning of seed production on Julian day 231 (Fig. 2). The black precipitate started just above the root ball and extended along the entire length of the roots in the sediments. Adventitious roots that grew at the surface of the sediment, however, remained white, the natural color of wild rice root tissue. Unamended plants, grown in sediment with low concentrations of sulfate in overlying water (0.15 mM), developed amber coatings characteristic of iron (hydr)oxides over the same time period.

Roots of amended plants began accumulating AVS during the flowering stage (Julian days 190–230) of the life cycle (Fig. 3a). The rate of AVS accumulation abruptly accelerated during the seed production stage (days 231–252) from approximately 2 μmol g^{-1} day^{-1} to over 15 μmol g^{-1} day^{-1}. During the seed production stage, amended roots accumulated up to 100 times more AVS than unamended roots, reaching a maximum mean concentration of 298 ± 74 μmol g^{-1} dw at the end of seed production. In contrast, AVS on unamended roots remained at 3.2 ± 1.7 μmol g^{-1} dw throughout the season (Fig. 3b). Between the end of seed production and final senescence (day 265), AVS concentrations on amended roots remained elevated or decreased slightly.

Fig. 2 Sulfate-amended (left) and unamended (right) roots. Sulfate-amended (3.1 mM sulfate in surface water) root has black color extending from about 0.5 cm above the root ball down to the tips of the roots. Unamended (0.15 mM sulfate in surface water) root has amber color characteristic of iron (hydr)oxides, especially in the 2–3 cm below root ball. The photograph was taken during senescence in October, 2014 from a pilot experiment, but color is typical of roots in this experiment. (Color figure online)

Although AVS concentration in amended sediment increased by one order of magnitude over the life cycle (0.5–5 μmol g^{-1} dw, Supplementary Fig. S3), sediment contained approximately 50 times less AVS per gram than the roots. Concentrations of chromium reducible sulfur on both amended and unamended roots did not differ from AVS concentrations on the same roots during seed production, indicating that crystalline forms of FeS did not make up a significant proportion of reduced sulfur (paired t test, $p = 0.27$, $t = 0.63$, $n = 20$).

Iron speciation on root surfaces

During flowering, concentrations of Fe(III) and Fe(II) were similar between amended and unamended roots (Fig. 3). During seed production, the redox state of iron was altered by the presence of sulfate. Concentrations of Fe(II) were much higher on amended roots compared to unamended roots ($p < 0.001$, $F = 19.1$, $df = 1, 31$), despite no significant difference in concentrations of Fe(III) between treatments. During the first week of seed production (between days 232 and 239), the concentration of ferric iron on amended roots decreased by 86%, from 233 ± 135 to 31.7 ± 30.4 μmol g^{-1} dw while ferric iron on unamended roots decreased by 67%, from 438 ± 208 to 144 ± 131 μmol g^{-1} dw. This abrupt reduction of Fe(III) occurred the same week that the rate of net AVS accumulation increased on amended roots (Fig. 2). Following this transition, Fe(II) concentrations continued to increase (doubled) on amended roots but did not change on unamended roots.

Saturation index in porewater

Although the amended and unamended plants had significant differences in the speciation of solid-phase sulfur and iron on roots, the saturation index of FeS in the sediment porewater 2 cm away from the roots was

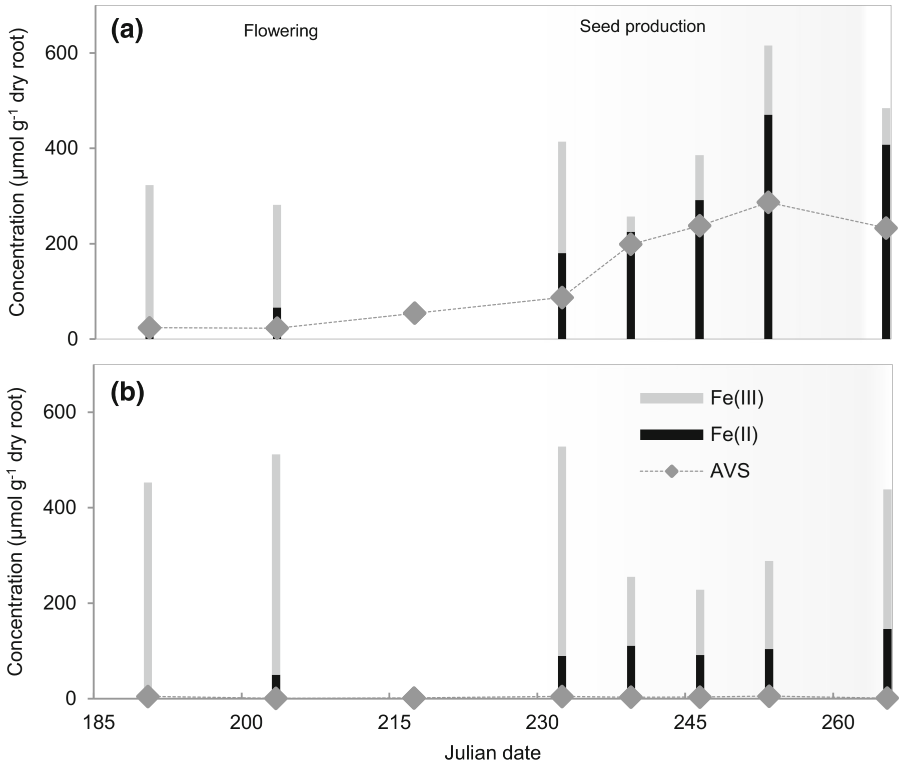

Fig. 3 Seasonal variations in iron speciation and root AVS for **a** sulfate-amended and **b** unamended conditions, and effective E_H on root surfaces. The gray bars in panels **a** and **b** indicate ferric iron and the black bars represents ferrous iron. Root AVS concentrations are shown by gray diamonds. Error bars are omitted for clarity, but standard deviation is on average 33% of the mean

not affected significantly by sulfate amendment ($p = 0.177$, $F = 2.68$, $df = 1,4$) and remained, on average, near zero but mostly negative (− 1.4 ± 0.3 to 0.1 ± 1.0) throughout the life cycle (Supplementary Table S1).

Effects on plants

The transition of plants from the vegetative growth stage to the flowering and seed production stages of the life cycle coincided with the onset of a yellowing and senescence of leaves beginning the third week of August (around day 232). Amended plants, all of which developed FeS plaques on roots, produced fewer seeds ($p = 0.067$, $F = 5.00$, $df = 1,6$, Fig. 4) with less nitrogen ($p = 0.052$, $F = 5.84$, $df = 1,6$) and smaller mass ($p = 0.069$, $F = 4.88$, $df = 1,6$). During flowering, total plant N was similar between amended and unamended plants. But, during the subsequent seed production stage, total plant N continued to increase in the unamended plants, but not in the amended plants ($p = 0.084$, $F = 4.27$, $df = 1,6$).

Discussion

We observed rapid shifts in sulfur and iron speciation at the surface of wild rice roots during the plant life cycle that differed depending on sulfate amendment. At the onset of leaf senescence and seed production, sulfate concentrations in the porewater decreased. This was followed shortly by decreased Fe(III) concentrations on the root surface as well as increased, but highly variable, dissolved sulfide concentrations in porewater. At this stage, solid phase-sulfide sulfide increased clearly and consistently on roots of amended plants, but not on unamended plants. The rapid development of FeS plaques was concomitant with the development of fewer filled seeds with lower nitrogen contents. Total plant nitrogen continued to

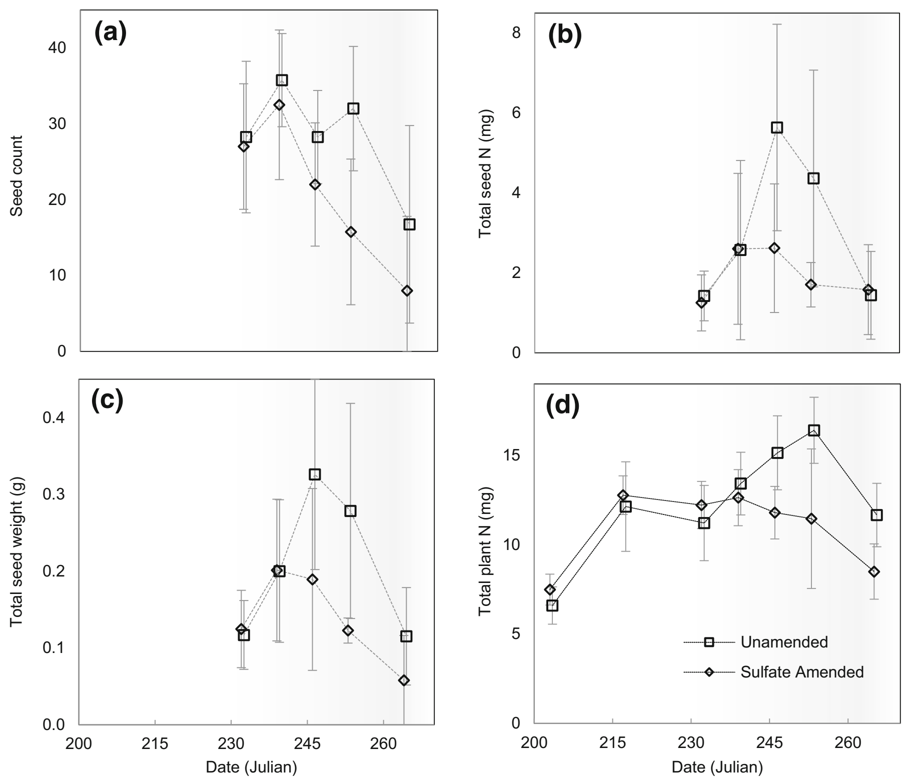

Fig. 4 Plant response in sulfate-amended and unamended conditions; **a** seed count. **b** Total seed mass. **c** Total mass of nitrogen in seeds, **d** total plant nitrogen, calculated by summing nitrogen from seeds, stems, and leaves. Diamonds represent plants grown in surface water with 3.1 mM sulfate and squares represent unamended plants. The shaded background represents the seed production life stage. Error bars represent one standard deviation of four replicates

increase in unamended plants but not in amended plants. The strong divergence between amended and unamended plants in total plant nitrogen and precipitation of FeS suggests a feedback between sulfur biogeochemistry on or near the root surface and plant nutrient uptake.

Sulfate amendments led to more reduced conditions and a more rapid development of iron sulfide precipitate on root surfaces, clearly confirming our hypothesis that surface water sulfate induces FeS accumulation on roots. In the absence of elevated sulfate, unamended plants filled out their seeds even when redox potential declined (Fig. 3, Supplementary Fig S4). In previous experiments with self-sustaining populations of wild rice (Pastor et al. 2017), elevated sulfate had little effect on total vegetative growth of adult plants but was associated with a decrease in the number and weights of seeds produced by mature plants at the late stages of the life cycle. FeS accumulates on roots during the last stages of wild rice's life cycle in which nitrogen taken up by the plant is allocated exclusively to panicles and seeds (Grava and Raisanen 1978; Sims et al. 2012). Porewater sulfide, which is known to decrease nitrogen uptake in plants, increased simultaneously with FeS on roots of amended plants. However, porewater sulfide was variable and increased in both amended and unamended rhizospheres, whereas FeS only increased on amended roots. Nitrogen uptake continued through the seed production phase of unamended plants but not in amended plants, which contained FeS plaques. FeS on roots may be a symptom of elevated porewater sulfide or further exacerbate its effects; our experiment was not able to distinguish between these possibilities. Regardless, the presence of root surface FeS strongly suggests that during seed production, a plant-induced reversal in the flow of electrons occurred: from a net flow of e-accepting capacity away from the root, sustaining Fe(III) in the rhizosphere, to a net flow of e- towards the root, reducing Fe(III) and introducing S(II).

The decline in nitrogen uptake and seed production concomitant with the initiation of FeS plaque precipitation on roots and porewater sulfide accumulation may explain the disproportionate effect of sulfate on seeds compared with its negligible effect on cumulative vegetative biomass prior to flowering and seed production. We suggest that plants are especially vulnerable to sulfide during seed production, because a seasonal decrease in root surface redox potential is compromised by further sulfide-induced depletion of the electron accepting buffer capacity of iron (hydr)oxides. The oxidation states of the amended and unamended root surfaces diverged during the transition from flowering to seed production (Supplementary Fig. S4), suggesting that root surface redox potential is, in part, controlled by a physiological mechanism tied to the plant's life cycle.

We hypothesize a pathway for how the living wild rice roots transition from iron (hydr)oxide plaques to iron sulfide plaques over the growing season (Fig. 5). Initially, conditions in the rooting zone are oxic, likely from radial oxygen loss (Fig. 5, stage [1]), as evidenced by precipitation of iron (hydr)oxides that accumulate equally on both amended and unamended root surfaces. At this initial stage, the root is protected from the electrons contained in sulfide and other reduced species by an ongoing supply of electron accepting inputs, composed of both oxygen from roots and iron (hydr)oxide coatings on roots (Holmer et al. 1998; Roden and Wetzel 1996). Sulfide encountering the iron (hydr)oxide buffer is oxidized or precipitates with iron while the electron accepting buffer is maintained. In amended conditions, some of this electron accepting buffer may be consumed (Fig. 5, stage [2]) during the flowering stage, allowing dissolved sulfide to penetrate nearer to the root surface. A decrease in radial oxygen loss near the onset of seed production, as vegetative growth ceases and leaves senesce, allows dissolved sulfide to reach the root surface. Sulfide exposure may further suppress radial oxygen loss by inducing suberization, the thickening of cell walls that prevents exchange of dissolved gases across the root (Armstrong and Armstrong 2005). After radial oxygen loss is suppressed, the electron accepting buffer capacity of iron (hydr)oxides can no longer be maintained and the remaining quantity of iron (hydr)oxides is then rapidly reduced due to a net decrease in the supply of electron acceptors to the rooting zone. A decrease in radial oxygen is likely tied to the end of the vegetative growth stage of the life cycle because both the amended and the unamended root surfaces simultaneously experience a loss of Fe(III) and a decline in porewater sulfate concentrations. Concentrations of

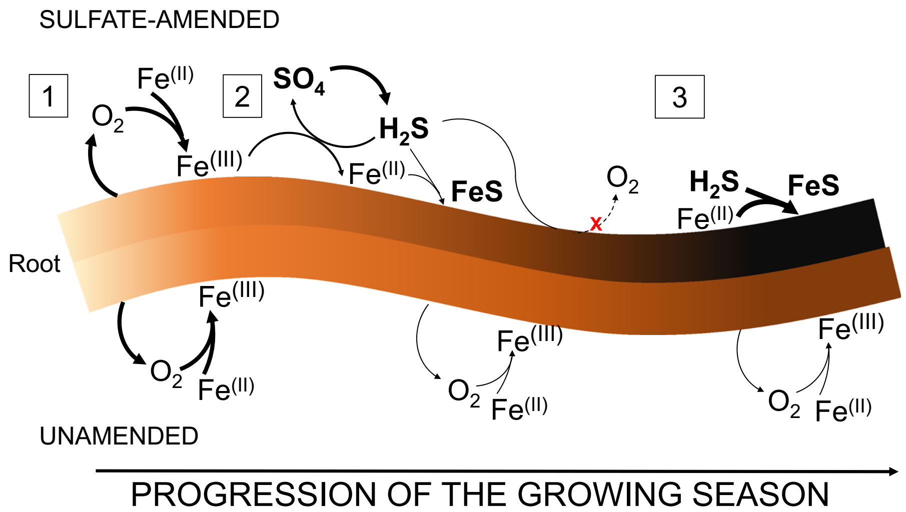

Fig. 5 Proposed mechanism of iron sulfide formation on wild rice roots exposed to elevated sulfate concentrations. Reactions depicted above the root occur on sulfate-amended root surfaces, and reactions depicted below the root occur on unamended root surfaces. Roots are protected by iron (hydr)oxides [1], but these iron (hydr)oxides are reduced by sulfide [2]. Exposure of roots to sulfide may induce suberization, the thickening of root cell walls, which leads to decreased radial oxygen loss. Root surface anoxia accelerates the precipitation of iron sulfides [3]. In unamended roots, radial oxygen loss creates iron (hydr)oxides that remain present the entire growing season but decrease slightly in response to the life-cycle. (Color figure online)

root Fe(III) and porewater sulfate remained low in unamended plants for the rest of the growing season. But, as the amended root surface shifts toward reducing conditions, sulfide almost exclusively precipitates with reduced iron rather than being reoxidized (Fig. 5, stage [3]). In our amended buckets, rapid accumulation of root Fe(II), root AVS, and porewater sulfide occurred within a 1–2 week period during seed production immediately following the precipitous decline of porewater sulfate and root surface Fe(III). In unamended buckets, root Fe(II) and AVS did not accumulate further, and while porewater sulfide increased, it was highly variable.

The most likely explanation for a redox transition at both the unamended and amended roots is a decrease in radial oxygen loss at the end of the vegetative growth stage when the leaves begin to senesce. Many mechanisms of rhizosphere oxidation have been described, including diffusion of atmospheric oxygen (Armstrong 1980), advection induced by temperature and vapor gradients (Dacey 1980) and Venturi-induced convection (Armstrong et al. 1992). Several studies have observed a correlation between light and rhizosphere oxygenation on diurnal time scales (Lee and Dunton 2000; Pedersen et al. 2004; Jensen et al. 2005), suggesting that some, if not most, radial oxygen loss may be photosynthetically derived. It has been previously suggested that accumulation of FeS occurs on white rice (*Oryza sativa*) roots only after plant senescence because dead roots no longer oxidize the rhizosphere (Jacq et al. 1991). However, as the plant approaches senescence, oxygen transport to the roots may decrease due to lower photosynthesis rates, subsequently slowing the regeneration of the electron accepting buffer of the root surface (Biswas and Choudhuri 1980). We observed a decrease in redox around the time that plants started to yellow and show early signs of senescence, consistent with a life-cycle-induced decline in radial oxygen loss.

Despite the rapid accumulation of FeS on roots in amended plants, the saturation index in sediment 2 cm from the roots remained relatively low, suggesting that the most severe decline in redox potential was confined to near the root surface. The Fe(II) in the FeS plaques may have come from the reduction of iron (hydr)oxides previously accumulated on the root surface. On the other hand, the sulfide in FeS plaques must have been supplied from a source external to the root. Although experimental conditions may have impacted the timing of sulfate intrusion to the rooting zone, porewater sulfate concentrations were already well above the half saturation constant for biological sulfate reduction at the start of flowering (Pallud and Van Cappellen 2006), making it unlikely that the redox transition occurred from a delay in sulfate availability and reduction at the root surface. Once leaf senescence began, porewater sulfate concentrations ($\sim$ 2000 μmol L^{-1}) declined by more than 80% followed by rapid accumulation of porewater sulfide (from $\sim$ 2 to 12 μmol L^{-1}) and AVS on the root surfaces ($\sim$ 300 μmol g^{-1}). Adjacent porewater sulfide was relatively low compared to the amount of sulfur in the porewater sulfate and root AVS pools. This suggests that a large amount of sulfur passes through the porewater sulfide pool very quickly, a scenario consistent with our proposed mechanism by which sulfide near the root surface is either oxidized by the electron accepting buffer or precipitated with Fe(II). Sediment AVS (5 μmol g^{-1}) was a larger component of overall solid-phase S accumulation due to its larger mass, but did not, apparently, experience the concentrated introduction of sulfide in the same way as roots. The rapid and concentrated accumulation of iron sulfide on roots in the setting of undersaturated porewater suggests an overwhelmingly plant-dominated geochemical niche very close to the root surface.

Beyond affecting wild rice populations, the mechanism behind the rapid accumulation of FeS on roots has implications for the fate of iron and sulfide in wetland sediments. Vegetated sediment in white rice paddies (Jacq et al. 1991) and in riparian wetlands containing *Phragmites australis* and *Zizania latifolia* (Choi et al. 2006) has higher concentrations of FeS than non-vegetated sediment. Significant accumulation of FeS on white rice roots has been observed after senescence (Jacq et al. 1991), likely because decaying root material stimulates iron and sulfate reduction. When roots coated with FeS decompose, the FeS becomes incorporated into the bulk sediment. Due to the concentrated introduction of both electron donors and acceptors to the subsurface, each generation of an annual plant is effectively a "pump" for the incorporation of FeS precipitate into the sediment. In dense stands of aquatic plants, annual contributions of FeS from roots could significantly alter the geochemistry of the sediment within years to decades. If FeS plaques occur concomitantly with population declines in wetland plants, the plant-induced sulfur pump may only last a few generations but would have implications for changes in species composition in wetland plant communities. Understanding the rates of the distinctly plant-induced sulfur pump and the short- and long- term interactions of near-root processes with bulk sediment could help to predict how the distribution of wetland vegetation and sulfur accumulation change in response to a perturbation in surface water sulfate concentrations.

The results of this study may provide a mechanistic link between observed sulfide toxicity in lab hydroponic experiments (Koch et al. 1990; Koch and Mendelssohn 1989; Pastor et al. 2017) and empirical evidence of sulfur-induced population declines of wetland plants (Lamers et al. 2002; Myrbo et al. 2017; Pastor et al. 2017; Pulido et al. 2012; Smolders et al. 2003). Our observation that sulfur cycling is altered *during* the life cycle rather than after senescence allows for the possibility of rapid feedbacks between sediment and porewater geochemistry on the one hand and annual plant populations on the other. Understanding the timing of when electron accepting buffers are present or absent and how that correlates with the plant life cycle can provide insight into how populations of wild rice and other aquatic plant species will respond to perturbations in sulfur loading to ecosystems.

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Author contributions SL-H and BD collected and analyzed samples. SL-H performed statistical analyses with guidance from JP. All authors contributed to interpreting the data and writing the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

References

Allam A, Hollis J (1972) Sulfide inhibition of oxidases in rice roots. Phytopathology 62:634–639

Armstrong W (1980) Aeration in higher plants. In: Andrews JH, Tommerup IC (eds) *Advances in botanical research*. Academic Press, Boca Raton, pp 225–332

Armstrong J, Armstrong W (2005) Rice: sulfide-induced barriers to root radial oxygen loss, Fe^{2+} and water uptake, and lateral root emergence. Ann Bot 96:625–638. https://doi.org/10.1093/aob/mci215

Armstrong J, Armstrong W, Beckett P (1992) *Phragmites australis*: venturi and humidity induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New Phytol 120(2):197–207

Biswas AK, Choudhuri MA (1980) Mechanism of monocarpic senescence in rice. Plant Physiol 65:340

Brouwer H, Murphy T (1994) Diffusion method for the determination of acid-volatile sulfides (Avs) in sediment. Environ Toxicol Chem 13(8):1273–1275. https://doi.org/10.1897/1552-8618(1994)13[1273:DMFTDO]2.0.CO;2

Burton ED, Sullivan LA, Bush RT, Johnston SG, Keene AF (2008) A simple and inexpensive chromium-reducible sulfur method for acid-sulfate soils. Appl Geochem 23:2759–2766. https://doi.org/10.1016/j.apgeochem.2008.07.007

Caraco N, Cole J, Likens G (1989) Evidence for sulphate-controlled phosphorus release from sediments of aquatic systems. Nature 341(6240):316

Choi J, Park S, Jaffe P (2006) The effect of emergent macrophytes on the dynamics of sulfur species and trace metals in wetland sediments. Environ Pollut 140:286–293. https://doi.org/10.1016/j.envpol.2005.07.009

Christensen K, Sand-Jensen K (1998) Precipitated iron and manganese plaques restrict root uptake of phosphorus in *Lobelia dortmanna*. Can J Bot 76:2158–2163

Dacey J (1980) Internal winds in water lilies: an adaptation for life in anaerobic sediments. Science 210(4473):1017–1019

Gao S, Tanji K, Scardaci S (2003) Incorporating straw may induce sulfide toxicity in paddy rice. Calif Agric 57(2):55–59

Giblin A, Howarth R (1984) Porewater evidence for a dynamic sedimentary iron cycle in salt marshes. Limnol Oceanogr 29(1):47–63

Gilmour C, Henry E, Mitchell R (1992) Sulfate stimulation of mercury methylation in freshwater sediments. Environ Sci Technol 26(11):2281–2287

Grava J, Raisanen K (1978) Growth and nutrient accumulation and distribution in wild rice. Agron J 70:1077–1081

Greenberg AE, Clesceri LS, Eaton AD (1992) Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC

Hansel C, Lentini C, Tang Y, Johnston D, Wankel S, Jardine P (2015) Dominance of sulfur-fueled iron oxide reduction in low-sulfate freshwater sediments. ISME J 9(11):2400

Holmer M, Jensen HS, Christensen KK, Wigand C, Andersen FØ (1998) Sulfate reduction in lake sediments inhabited by the isoetid macrophytes *Littorella uniflora* and *Isoetes lacustris*. Aquat Bot 60:307–324

Jacq VA, Prade K, Ottow JCG (1991) Iron sulphide accumulation in the rhizosphere of wetland rice (*Oryza sativa* L.) as the result of microbial activities. Dev Geochem 6:453–468

Jensen S, Kühl M, Glud R, Jørgensen L, Priemé A (2005) Oxic microzones and radial oxygen loss from roots of Zostera marina. Mar Ecol Prog Ser 293:49–58

Jorgenson K, Lee P, Kanavillil N (2012) Ecological relationships of wild rice, Zizania spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*). Botany 91(3):189–201

Joshi M, Ibrahim I, Hollis J (1975) Hydrogen sulfide: effects on the physiology of rice plants and relation to straighthead disease. Phytopathology 65:1165–1170

Koch MS, Mendelssohn I (1989) Sulphide as a soil phytotoxin: differential responses in two marsh species. J Ecol 77(2):565–578

Koch MS, Mendelssohn IA, McKee KL (1990) Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol Oceanogr 35(2):399–408

Lamers L, Falla S, Samborska E, van Dulken L, van Hengstum G, Roelofs J (2002) Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. Limnol Oceanogr 47(2):585–593

Lee K, Dunton KH (2000) Diurnal changes in pore water sulfide concentrations in the seagrass *Thalassia testudinum* beds: the effects of seagrasses on sulfide dynamics. J Exp Mar Biol Ecol 255:201–214

Lee PF, McNaughton KA (2004) Macrophyte induced microchemical changes in the water column of a northern Boreal Lake. Hydrobiologia 522(1–3):207–220

Martin NM, Maricle BR (2015) Species-specific enzymatic tolerance of sulfide toxicity in plant roots. Plant Physiol Biochem 88:36–41. https://doi.org/10.1016/j.plaphy.2015.01.007

Mendelssohn IA, Postek MT (1982) Elemental analysis of deposits on the roots of *Spartina alterniflora* Loisel. Am J Bot 69(6):904–912

Moyle J (1944) Wild rice in Minnesota. J Wildl Manag 8(3):177–184

Myrbo A, Swain EB, Engstrom DR, Coleman Wasik J, Brenner J, Dykhuizen Shore M, Blaha G (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 122(11):2736–2753

Pallud C, Van Cappellen P (2006) Kinetics of microbial sulfate reduction in estuarine sediments. Geochim Cosmochim Acta 70(5):1148–1162

Pastor J, Dewey B, Johnson NW, Swain EB, Monson P, Peters EB, Myrbo A (2017) Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments. Ecol Appl 27(1):321–336

Pedersen O, Binzer T, Borum J (2004) Sulphide intrusion in eelgrass (*Zostera marina* L.). Plant Cell Environ 27(5):595–602

Pulido C, Keijsers DJ, Lucassen EC, Pedersen O, Roelofs JG (2012) Elevated alkalinity and sulfate adversely affect the aquatic macrophyte *Lobelia dortmanna*. Aquat Ecol 46:283–295

Roden EE, Wetzel RG (1996) Organic carbon oxidation and suppression of methane production by microbial Fe(III) oxide reduction in vegetated and unvegetated freshwater wetland sediments. Limnol Oceanogr 41(8):1733–1748

Schmidt H, Eickhorst T, Tippkoetter R (2011) Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis. Plant Soil 341:221–232. https://doi.org/10.1007/s11104-010-0637-2

Seeberg-Elverfeldt J, Schlüter M, Feseker T, Kölling M (2005) Rhizon sampling of porewaters near the sediment-water interface of aquatic systems. Limnol Oceanogr 3(8):361–371

Sims L, Pastor J, Lee T, Dewey B (2012) Nitrogen, phosphorus, and light effects on growth and allocation of biomass and nutrient in wild rice. Oecologia 170:65–76

Smolders A, Lamers L, den Hartog C, Roelofs J (2003) Mechanisms involved in the decline of *Stratiotes aloides* L. in The Netherlands: sulphate as a key variable. Hydrobiologia 506(1–3):603–610. https://doi.org/10.1023/B:HYDR.0000008551.56661.8e

Snowden R, Wheeler B (1995) Chemical changes in selected wetland plant species with increasing Fe supply, with specific reference to root precipitates and Fe tolerance. New Phytol 131:503–520. https://doi.org/10.1111/j.1469-8137.1995.tb03087.x

Soana E, Naldi M, Bonaglia S, Racchetti E, Castaldelli G, Brüchert V, Viaroli P, Bartoli M (2015) Benthic nitrogen metabolism in a macrophyte meadow (*Vallisneria spiralis* L.) under increasing sedimentary organic matter loads. Biogeochemistry 124(1–3):387–404

Stover E (1928) The roots of wild rice *Zizania Aquatica* L. Ohio J Sci 28(1):43–49

Stumm W, Morgan JJ (1996) Chemical equilibria and rates in natural waters. Aquatic chemistry. Wiley, New Jersey

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Interactions between sulfide and reproductive phenology of an annual aquatic plant, wild rice (*Zizania palustris*)

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ABSTRACT

Aquatic plants live in anoxic sediments that favor formation of hydrogen sulfide, a known phytotoxin. We investigated how the phenology of reproductive life stages of wild rice (*Zizania palustris* Poaceae), an annual aquatic graminoid, is influenced by rooting zone sulfur geochemistry in response to elevated sulfate and sulfide. In addition, we characterized how redox conditions in the rooting zone change throughout reproduction to determine if they are tied to plant life stage. The redox conditions in sediment decreased just prior to flowering, and again just prior to seed production for all plants, allowing sulfide to accumulate at the root surface of sulfate-amended plants. Plants exposed to sulfide initiated seed production later than unamended plants. Sulfide appears to slow plant development in a way that gives the plant less time to allocate nutrients to seeds before senescence. The impact of sulfide in delaying reproductive life stages of wild rice and changing seasonal rooting zone biogeochemistry could extend to other plant species and additional chemical species that change mobility with redox potential, such as phosphate, manganese, mercury, and other metals.

1. Introduction

Many aquatic plants grow in sediments with low redox potential that favors formation of toxic reduced compounds like sulfide. To cope with these conditions, some aquatic plants transport oxygen to the roots through hollow aerenchyma tissue, release it into the rhizosphere, and form iron oxide plaques on root surfaces (Stover, 1928; Mendelssohn et al., 1995; Colmer, 2003; Jorgenson et al., 2012). The released oxygen and iron oxides may protect roots from dissolved sulfide species (Trolldenier, 1988; Van der Welle et al., 2007; Schmidt et al., 2011; Soana and Bartoli, 2013). Many wetland plants, including wild rice, are vulnerable to dissolved sulfide (Koch and Mendelssohn, 1989; Carlson et al., 1994; Lamers et al. 1998; Pastor et al., 2017). Wild rice (*Zizania palustris*, Poaceae), an annual aquatic graminoid which forms large monotypic stands in lakes of the Western Lake Superior region, is especially sensitive during the seedling and seed production life stages, suggesting that the ability to withstand sulfide varies throughout their life cycle (Pastor et al., 2017; LaFond-Hudson et al., 2018).

Plants growing in nutrient-limited conditions sometimes experience ontogenetic drift, a phenomenon in which morphological development through successive life stages is slowed (McConnaughay and Coleman, 1999; Sims et al., 2012). Because the allocation of biomass to different tissues changes throughout a plant's life cycle, delayed development has sometimes been misdiagnosed as morphological plasticity in experiments in which plants are normalized by date or age, rather than size or life stage (Coleman et al., 1994). Nitrogen is the limiting nutrient to wild rice (Sims et al., 2012) and its uptake is tied to specific life stages (Grava and Raisanen, 1978). About 30 % of nitrogen is taken up during early vegetative growth, 50 % is taken up during the growth of the stem until flowering, and 20 % is taken up during seed production (Grava and Raisanen, 1978). Dissolved sulfide inhibits nutrient uptake (Allam and Hollis, 1972; Koch et al., 1990; Martin and Maricle, 2015). If nitrogen uptake in wild rice is inhibited or slowed by sulfide, it may slow the rate at which the plant progresses through subsequent life stages and limit the quantity of N uptake available for seed production.

Near the end of an annual plant's life cycle when plants allocate resources from leaves into flowers and seeds, photosynthesis declines and radial oxygen loss from roots may also decrease, creating favorable conditions for reduction of iron oxides and sulfate (Schmidt et al., 2011). Several mechanisms for maintaining radial oxygen loss from roots have been described, including pressure gradients that actively pump oxygen from new leaves, through roots, to old leaves (Dacey, 1980; Armstrong, 1980; Armstrong et al., 1992); and production and transport as a byproduct of photosynthesis (Marzocchi et al., 2019).

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Table 1
Descriptions of the life stages of wild rice and the range of dates for each life stage in which at least one *Zizania palustris* plant was observed (initially $n = 64$, followed by incrementally smaller sample sizes due to destructive sampling). Ranges are described for plants grown in water amended with 300 mg L^{-1} sodium sulfate or grown in water unamended, with background sulfate concentrations of ~8-14 mg L^{-1}. Life stages 1 through 3 pertain to seedling and early emergent stages that did not develop iron sulfide plaques on roots. Designation and description of life stages from Grava and Raisanen (1978) and Sims et al. (2012).

Lifestage Name	Lifestage Number	Characteristics	Dates Observed	
			Amended	Unamended
Mid tillering	4	Tiller (main stem) grows more than one leaf	210-235	210-222
Jointing	5	Internodes elongate	210-240	210-240
Boot	6	Panicles emerge from stems	235-249	235-245
Early flowering	7	A few flowers bloom, some not yet emerged	235-245	235-245
Mid flowering	8	Most flowers bloom	235-249	235-245
Late flowering	9	Most panicles empty, few flowers still bloom	235-249	235-249
Seed production	10	Seed hull develops, seed filling occurs	240-263	235-263
Seed maturity	11	Filled, ripe seeds present, a few dropped	255-263	255-263
Senescence	12	All seeds dropped, green tissues disappear	280	280

Although the exact mechanism of radial oxygen loss in wild rice is not yet known, the aforementioned mechanisms may be inhibited by the senescence of leaves during reproduction. We previously reported a decline in the redox potential of root surfaces during the seed production life stage (LaFond-Hudson et al., 2018). In plants grown in sediment without sulfur amendment, iron oxide plaques on root surfaces decreased, but in sulfate-amended plants, iron oxide plaques transitioned to iron sulfide, which further accumulated on root surfaces and coincided with production of fewer, smaller seeds with less nitrogen relative to unamended plants. In plants exposed to sulfide, the total uptake of nitrogen ceased during the onset of iron sulfide plaque formation and thickening while unamended plants continued to accumulate nitrogen in seeds (LaFond-Hudson et al., 2018). In this paper, we specifically explore the relationship between sulfur geochemistry and phenology of life stages, as both may control each other through interactions that culminate in the redox potential of root surfaces. We use wild rice (*Zizania palustris,* Poaceae) as our model organism to investigate connections between sulfide and iron geochemistry in the rhizosphere and reproductive phenology and ontogeny. Because wild rice is an annual plant, the ontogeny of development is equivalent to the annual phenology. So in this case, the two words are synonymous, except that ontogeny has the connotation of development whereas phenology has the connotation of seasonality.

Wild rice is a culturally, economically, and ecologically important macrophyte that is harvested for its grain (Fond du Lac Band of Lake Superior Chippewa, 2018). An advantage of using an annual plant is the relatively simple life cycle; root and shoot growth starts over each year, photosynthesis declines and vegetative structures senesce during the transition from vegetative to reproductive life stages, and seeds are produced at the end of the growing season just prior to death. In addition, standard markers of transitions in life cycle stages for wild rice have been established in prior research in the context of nutrient limitation (Grava and Raisanen, 1978; Sims et al., 2012).

Motivated by acute and population-level impacts of sulfide on aquatic plants, we compare the ontogenetic progression of life stages with the development of iron sulfide plaques throughout the life cycle of wild rice. Sulfide may slow ontogenetic development, but plant life stage may in turn control rhizosphere redox conditions and the amount of sulfur present as reactive sulfide. To investigate these geochemical and phenological interactions, we quantify the timing and length of life stages and seed production along with the concurrent accumulation of iron sulfide plaques.

2. Methods

2.1. Experimental design

Individual wild rice plants were grown outside in polyethylene buckets, 32 of which were amended with 300 mg L^{-1} sulfate and 32 of which were left unamended. Although many lakes and rivers in central and northern Minnesota have concentrations of sulfate lower than 10 mg L^{-1}, several current and former wild rice lakes and rivers have sulfate concentrations near or above 300 mg L^{-1}. Additionally, 300 mg L^{-1} is close to the EPA secondary standard for drinking water and is a concentration we have used in several prior sulfate-addition experiments with wild rice. Sediment was collected on 01-Jun-2016 from Rice Portage Lake (MN Lake ID 09003700, 46.7038, -92.6829) on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota. This lake is a productive and unpolluted wild rice lake with little or no settlement along its shores and its sediment is organic-rich mud. The sediment was not sieved, but thoroughly homogenized and loaded into 4 L plastic pails that were set inside 12 L buckets (see LaFond-Hudson et al., 2018) on 25-Jun-2016. Water was added from a nearby well (sulfate concentration ranging from 8 to 14 mg L^{-1}) to provide a 12−15 cm water column. Two wild rice seeds obtained from Rice Portage Lake were planted in each bucket on 26-Jun-2016 (Julian day 177). All buckets had at least one seedling by 28-Jun-2016 (day 179), and the lesser robust plant of the two was removed a week later. Half of the buckets had sodium sulfate added on 28-Jun-2016 and 05-Aug-2016 (days 179, 217) to maintain surface water sulfate concentrations of 300 mg L^{-1}. Plants remained outside for the entire duration of the experiment. Further details on the maintenance of buckets can be found in LaFond-Hudson et al. (2018).

2.2. Sampling methods

To compare changes in pace of progressions through the life cycle, we examined initiation of life stages from a subset of plants that completed their entire life cycle through seed production. We define initiation as the first date a plant was observed to be in a life stage. Life stages were identified visually and nondestructively according to the descriptions codified by Grava and Raisanen (1978) and further subdivided by Sims et al. (2012).

Our observations of the phenology of wild rice began with mid tillering, a life stage in which the main stem, the tiller, grows more than one leaf above the surface of the water (Table 1). Prior life stages include emergence of the seedling from sediment (life stage 0), the floating leaf stage (life stage 1), the first aerial leaf (life stage 2), and the formation of the tiller, the main stem that will eventually produce flowers and seeds (life stage 3). We started observations with mid tillering (life stage 4) because it is the last vegetative growth stage before reproductive life stages. After mid tillering, the internodes of the tiller elongate (jointing, life stage 5) and the panicles emerge (boot, life stage 6) in preparation for flowering (life stages 7–9). Flowering is broken into early (7), mid (8), and late (9) flowering by the proportion of flowers emerged and blooming. Once flowers have finished blooming, a seed hull develops and seed production begins (life stage 10). Filled seeds start to drop once they reach maturity (life stage 11), and senescence is reached once all seeds have dropped and leaves have turned completely yellow (life stage 12). Life stages of each plant and date were recorded eight times during the growing season.

When at least half of the plants were in a specific life stage, four sulfate-amended plants and four unamended plants in that life stage were destructively harvested to determine root surface geochemistry. When the plants entered the seed production life stage, harvests were made on three separate dates, each approximately a week apart, spanning the duration of the seed production life stage. Sampling at a more frequent temporal resolution during seed production enabled us to make detailed observations of the accumulation of iron sulfide (or lack thereof) on the roots during a potentially critical time for sulfide exposure.

2.3. Biological and chemical analysis

On each sampling date, the same eight plants that were harvested were separated into aboveground vegetative tissue, seed tissue, and root tissue according to LaFond-Hudson et al. (2018). Vegetative tissue and seed tissues were dried for seven days at 65 °C and weighed. Total N concentrations were determined with a Thermo Electron Flash EA 1112 CHNS Analyzer. Fresh roots were analyzed for acid volatile sulfide (AVS) and weak acid extractable iron the same day plants were harvested, taking great care to avoid exposure to oxygen (LaFond-Hudson et al., 2018). Iron and acid volatile sulfur (AVS) were simultaneously extracted from entire roots using 1 M deoxygenated HCl for four hours. AVS was volatilized and trapped in a sulfide antioxidant buffer (SAOB) using a modified diffusion method (Brouwer and Murphy, 1994). AVS was quantified using a sulfide-selective electrode. Iron was extracted into the 1 M HCl and analyzed for total extractable iron and Fe(II). Fe (III) was estimated from the difference between total iron and Fe(II). Total iron was quantified using a Varian fast sequential flame atomic absorbance spectrometer with an acetylene torch. Fe(II) was quantified on the day of extraction using the phenanthroline method on the spectrophotometer. After extraction, roots were dried at 38 °C for 24 h to determine dry mass.

2.4. Data analysis

Data are publicly available in the Data Repository of University of Minnesota (DRUM) and can be accessed at https://conservancy.umn.edu/handle/11299/208579. We used a two-sample *t*-test to compare differences between sulfate-amended and unamended conditions for seed measurements and root sulfide. Because we sampled destructively to measure root surface sulfide and iron, dates for the initiation of early reproductive life stages contain a larger sample size relative to the seed production life stage. Conclusions about the initiation of life stages between treatments are based only on the subset of plants that reached seed production in this experiment, and thus are not influenced by changes in sample size. For this subset of plants, we calculate the cumulative distribution of the date on which each life stage was initiated by summing the number of plants that are at or beyond the life stage. We also calculated the duration of seed stage for each plant in this subset using the difference between the first day we observed filled seeds and the first day we observed dropped or missing seeds. In many plants, seed production ended artificially early due to our destructive sampling design. In these cases, we used the harvest date as the end date in our calculation of duration and refer to the resulting value as "experimental duration". We investigated correlations between experimental duration and yield of seed production (seed count, seedhead mass, and seedhead nitrogen mass) to understand progression in seed development within the seed production life stage. We used these linear relationships to infer the seed yield at "true duration", which we define as the probable duration of seed production if plants were not harvested. For true duration, we use the average last date seeds were observed in parallel wild rice experiments. These parallel experiments occurred in the same year, used the same sediment and tested sulfate-addition but did not use destructive sampling (Table S1).

The effective redox potential at the root surface was calculated using a modified Nernst equation (Stumm and Morgan, 2012).

$$p\varepsilon = p\varepsilon^{\circ} + \frac{1}{n} log\frac{\{ox\}}{\{red\}} \quad (1)$$

$$p\varepsilon = 16 - 3pH + log\frac{\{Fe(III)\}}{\{Fe(II)\}} \quad (2)$$

$$E_h{*} = \frac{2.3RTp\varepsilon}{F} \quad (3)$$

While not strictly representative of the activity in solution, we use root surface Fe(III) and Fe(II) as a proxy for the activity of oxidized and reduced Fe in the rooting zone. Because the system is dynamic, root surface (solid-phase) quantities likely mirror the activity of iron in solution enough to draw general conclusions about the direction of the flow of electrons.

3. Results

3.1. Sulfide effects on phenology

When life stage observations began (Julian day 210), unamended plants were ahead by nearly a full life stage (mean life stage of 4.5 ± 0.5 unamended compared with mean life stage of 3.8 ± 0.6 amended, $p < 0.01$, two-sample *t* test), indicating that vegetative growth life stages were delayed by sulfate amendment. Most amended plants initiated jointing later than unamended plants (mean Julian day 217 ± 9 unamended, mean Julian day 226 ± 9 amended, $p = 0.005$, $n = 17$), but both treatments initiated the boot stage at similar times (mean Julian day 237 ± 3 for both, Fig. 1a, b). Because the boot stage occurs quickly, our temporal resolution may not have captured any differences in timing if they existed. From days 220–235, about half of the unamended plants initiated mid flowering, compared to only a quarter of amended plants (Fig. 1c). During the same time frame, one third of unamended plants initiated seed production, compared to no amended plants (Fig. 1d). Eight days later, day 243, a comparable number of amended plants entered seed production. Amended plants entered seed production during a narrower range of time, with ~75 % of plants reaching this life stage between days 240–250 (mean Julian day 247 ± 5), while the initiation of seed production was spread over a 2 week window for unamended plants (244 ± 7 days). Due to the destructive sampling required by our experimental design, we were unable to quantify the end date of seed production in this experiment, but we estimated the end date of seed production from parallel, non-destructive experiments involving sulfate-addition to wild rice mesocosms. The final date of seed collection was consistently close to day 260 for several years and experiments (Table S1). Using day 260 as the final date of seed production, we estimated a 20 % decrease in the true duration of seed production in amended plants compared to the unamended plants.

3.2. Seed production and vegetative biomass

Sulfate amended plants produced 33 % fewer seeds ($p = 0.03$), 50 % less total seedhead mass ($p = 0.01$), and 40 % total seedhead nitrogen ($p = 0.02$) compared to unamended plants (Table 2). Individual seeds were smaller by 33 % ($p = 0.02$), but individual seed N mass did not differ significantly between treatments. Sulfate amended plants had lower vegetative biomass (leaves and stems) during late flowering ($p < 0.01$, $n = 4$), but not prior life stages (Fig. S1). The experimental duration of seed production, calculated from the difference between

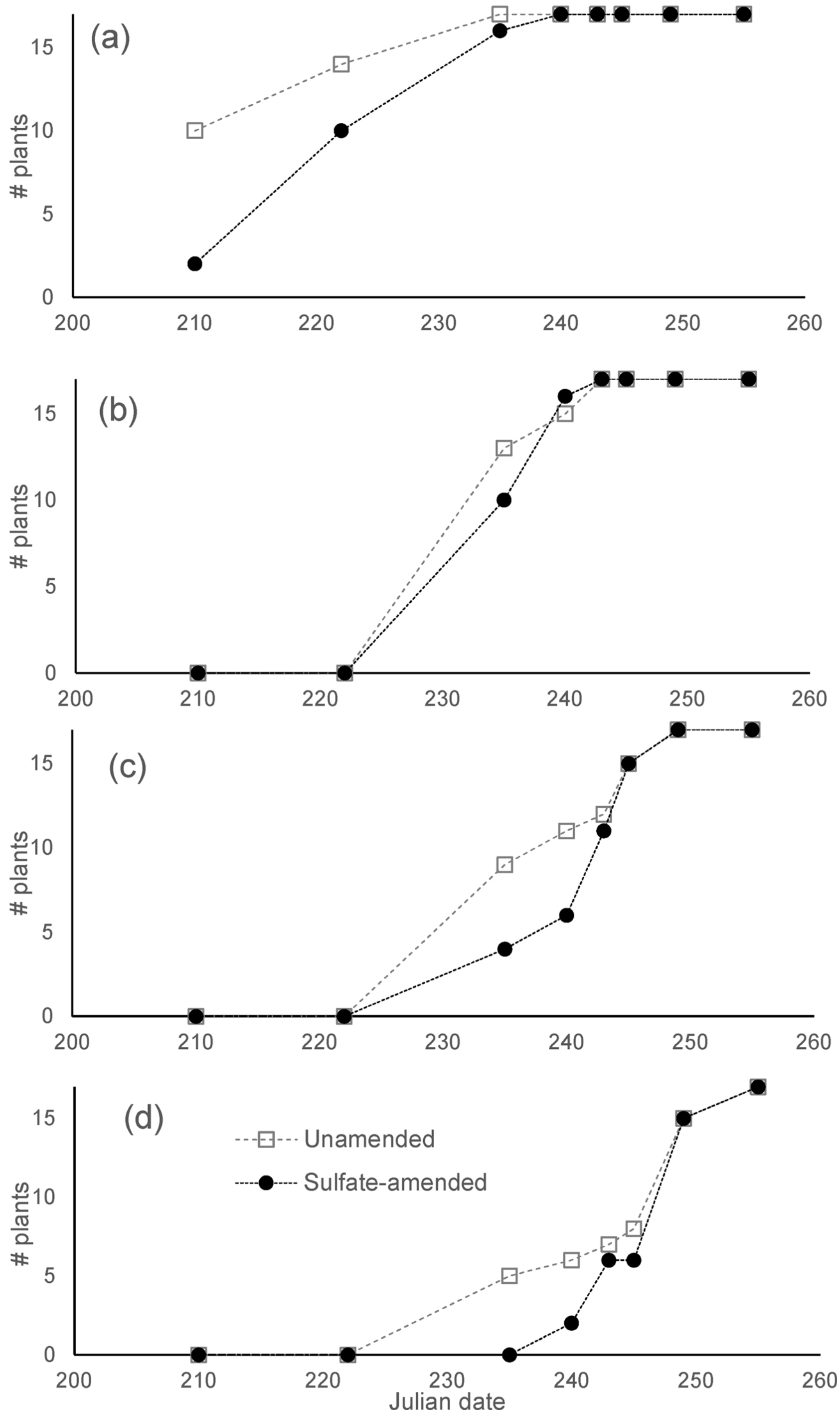

Fig. 1. Cumulative frequency of sulfate-amended (300 mg L^{-1}, filled circles) and unamended plants (open squares) that have initiated a) jointing, life stage 5, when internodes elongate just prior to reproduction, b) boot, life stage 6, when panicles emerge, c) mid flowering, life stage 8, when most flowers bloom, and d) seed production, life stage 10, when seed filling occurs.

first day seeds were observed and the day the plant was destructively sampled, was positively correlated with more filled seeds (p = 0.027), greater seed mass (p = 0.042), and more seed nitrogen (p = 0.012, Fig. 2).

3.3. Root geochemistry

Concentrations of AVS on amended root surfaces were one to two orders of magnitude higher than on unamended root surfaces during jointing, boot, mid flowering, and seed production (Table 2, Fig. S2). Porewater sulfate decreased from mid flowering until senescence, indicating that sulfate-amended plants were likely exposed to sulfide as a consequence of sulfate reduction (Fig. S3). On amended roots, AVS increased from about 10 μmol g^{-1} to about 65 μmol g^{-1} between jointing and boot. Concentrations of root surface sulfide then remained around 65 μmol g^{-1} until seed production. The AVS concentration doubled during seed production (life stages 10–11). On unamended roots, the concentration of sulfide steadily increased from 0.5 to 5 μmol g^{-1}, with the highest concentrations occurring during seed production. However, roots were not visibly black on unamended plants. Decreases

Table 2
Comparisons of acid volatile sulfide (AVS) concentration on root surfaces (μg g^{-1}) and of seed measurements in sulfate-amended (300 mg L^{-1}) and unamended conditions using a two-sample *t* test. AVS concentrations are compared during four reproductive life stages. The average for each treatment is reported with the standard deviation in parentheses ($n = 4$ for AVS during jointing, boot, and flowering; $n = 12$ for AVS during seed production, $n = 10$-12 for seed measurements; not all replicate plants had seeds).

Reproductive life stage	Sulfate-amended	Unamended	P value
Jointing	9.7 (± 3.7)	0.6 (± 0.3)	**$P < 0.01$**
Boot	64.9 (± 39.7)	1.4 (± 0.2)	**$P = 0.05$**
Mid flowering	68.9 (± 42.9)	2.6 (± 0.5)	**$P = 0.03$**
Seed production	144.8 (± 61.6)	3.3 (± 0.8)	**$P < 0.01$**
Seed Measurements			
Filled seed count (# per plant)	10.5 (± 7.3)	16 (± 7.1)	**$P = 0.03$**
Total seedhead mass (g)	0.14 (± 0.07)	0.28 (± 0.16)	**$P = 0.01$**
Total seedhead N mass (mg)	3.05 (± 1.36)	4.93 (± 2.28)	**$P = 0.02$**
Individual seed mass (mg)	11.1 (± 3.27)	15.26 (± 4.75)	**$P = 0.02$**
Individual seed N mass (mg)	0.26 (± 0.15)	0.28 (± 0.08)	$P = 0.38$
Seed N %	2.28 (± 0.63)	1.89 (± 0.48)	$P = 0.06$

in effective redox potential (E_h*), calculated from the ratio of Fe(III) to Fe(II) at root surfaces (Fig. 3, Fig. S4), occurred near both amended and unamended root surfaces between boot and jointing (life stage 5–6) and at the end of flowering (life stage 8–9). During seed production, the effective redox potential decreased more steeply at amended root surfaces.

4. Discussion

The phenology of seed production was delayed in sulfate-amended plants, suggesting ontogenetic drift induced by sulfide. Across both amended and unamended conditions, seedhead mass, seed number, and seedhead N mass correlated with length in the seed production life stage. In the presence of sulfate, delayed seed production and lower seed N uptake both co-occurred with a precipitous drop in redox potential and rapid accumulation of sulfide on roots.

In a natural setting, plants with a delayed start to seed production would have to compensate by either increasing N uptake rate or delaying senescence until a later calendar date. In our experiment, sulfate-amended plants contained less seedhead nitrogen than unamended plants, so the N uptake rate likely did not increase much, if at all. Our experimental design, requiring destructive sampling during seed production, was unable to test the completion of the seed production life stage. To address these limitations, we examined average end dates of seed production in parallel wild rice experiments. The date of last seed collection happened at similar dates or even earlier dates for sulfate-amended plants in these other experiments (Table S1). Thus, it seems likely that sulfate-amended plants do not extend the seed production life stage to compensate for a delay in the initiation of seed production and have a shorter true duration of seed production. Because the seed production yield (number of filled seeds, seedhead mass, seedhead nitrogen) is positively and linearly correlated with experimental duration of seed production (Fig. 2), we suggest that the implications of delayed initiation without delayed completion of seed production are lower reproductive outputs by plants.

The curious timing of iron sulfide precipitation on root surfaces coincident with the beginning of seed production suggests that plants

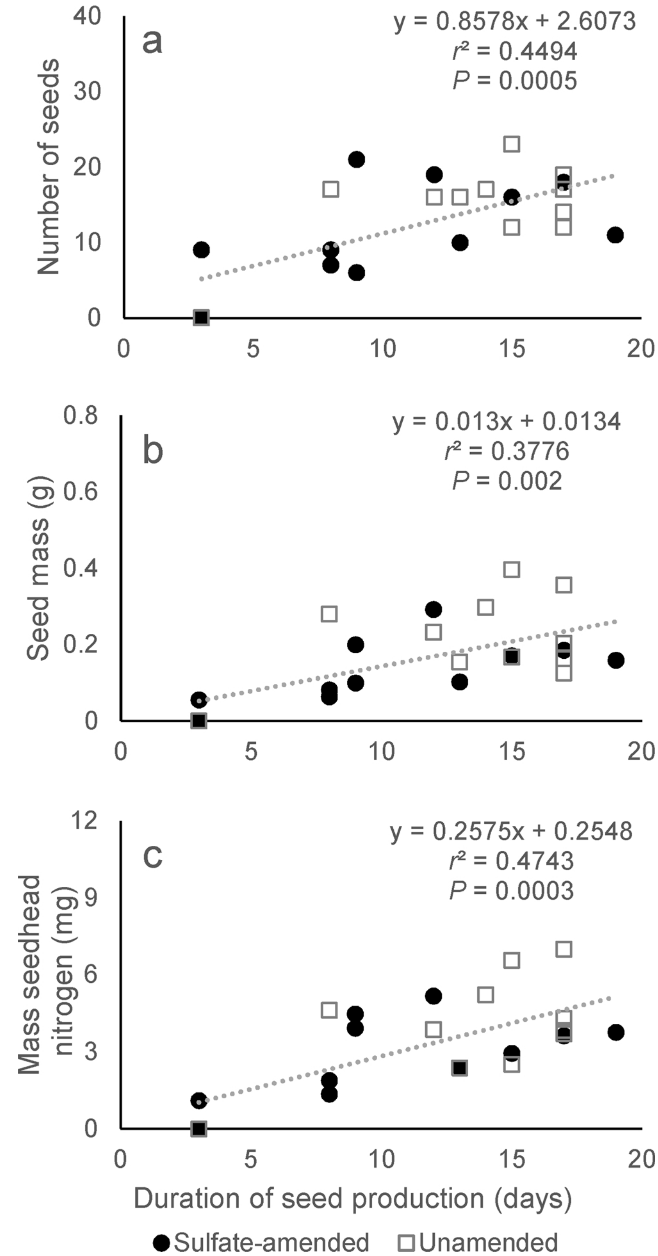

Fig. 2. Relationship between the experimental duration of seed production (days) and a) filled seed count, b) seedhead mass (g), and c) seedhead nitrogen (mg). Filled circles indicate sulfate-amended plants (300 mg L^{-1}) and open squares indicate unamended plants. End dates of duration for each plant were determined either by the date they entered seed maturity or by harvest date if they were harvested before reaching seed maturity.

influence the geochemistry of the sediments and that this influence changes during the plant's life cycle. The redox potential at the root surface, calculated from the ratio of Fe(III):Fe(II), decreased from jointing to boot (life stage 5–6), and again at the end of flowering (life stage 8–9). AVS concentrations increased on amended roots at the same life stages that redox declined. During seed production, the redox potential of amended and unamended plants diverged as the redox potential declined precipitously in amended plants. These decreases in redox potential reflect a net flow of electrons toward the plant root surfaces, suggesting a loss in the oxidizing capacity of the root surface. Transitions into new reproductive life stages are plausible times for plants to reallocate resources from photosynthetic tissues to reproductive tissues (Grava and Raisanen, 1978; McConnaughay and

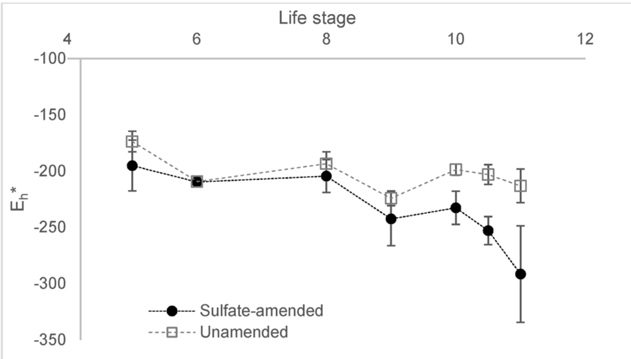

Fig. 3. Effective redox potential calculated from Fe(III) and Fe(II) concentration on roots amended with sulfate (300 mg L^{-1}, filled circles) or left unamended (open squares). Error bars show one standard deviation (n = 4). Life stages were assigned as 10, 10.5, and 11 for Julian dates 252, 257, and 264 respectively to show chronological progression in E_h* during seed production and maturity.

Coleman, 1999; Sims et al., 2012). Experiments with white rice (*Oryza sativa*), a closely related plant, have shown changes from iron oxide to iron sulfide in rhizosphere sediment as the plant entered flowering (Schmidt et al., 2011). We suggest that the change in redox conditions of the root surface at reproductive life stage transitions could be explained by a decrease in radial oxygen loss tied to the life stage of the plant, creating conditions conducive to iron sulfide formation in environments with elevated sulfur.

Plants concomitantly control and are controlled by sulfide. During vegetative growth life stages, plants maintain low sulfide in the rooting zone by releasing O_2 and accumulating Fe(III). However, at key reproductive life stage transitions, excess sulfide appears to overwhelm the plant's ability to oxidize the rhizosphere. The geochemical consequences of both life stage transition and excess sulfide are a precipitous drop in Fe(III):Fe(II) ratio and an accumulation of solid-phase sulfur on roots. The ecological consequences of life stage transitions in the presence of excess sulfide are a delay in reproductive phenology and a decrease in N uptake to seeds. Slower development rates in the presence of sulfide may delay life stage transitions and the geochemical consequences of these life stage transitions for redox potential. Our experimental design was not able to directly determine if redox potential decreased at a later date due to delayed phenology in amended plants. However, our observations do provide evidence that the net effect of sulfide-induced ontogenetic drift is shortened and decreased seed production. This finding hints at a phenological mechanism underlying sulfide-induced inhibition of nitrogen uptake observed in prior work (LaFond-Hudson et al., 2018). Considering that seedlings also experience high mortality when exposed to sulfide, 50 % less total seed mass in the presence of elevated sulfide may lead to rapid population declines, as has been previously observed in a mesocosm experiment (Pastor et al., 2017). Additionally, decreased density of plants in subsequent generations may lead to lower oxygen fluxes into sediment and exacerbate redox conditions that favor production of sulfide.

Sulfide inhibition of nutrient uptake has been demonstrated in other plants (Koch et al., 1990; Martin and Maricle, 2015), as has ontogenetic drift ((McConnaughay and Coleman, 1999; Sims et al., 2012), so other freshwater annual aquatic plant populations may face similar reproductive challenges if exposed to sulfide. Additionally, sulfide and iron interact with nutrients besides nitrogen. Iron plaques can adsorb phosphorus and metals, controlling their availability for uptake (St-Cyr and Campbell, 1996; Christensen and Sand-Jensen, 1998). Reduction of iron plaques in the presence of sulfide may affect uptake of both macro- and micronutrients. Some studies have investigated changes in radial oxygen loss over the growing season in perennial aquatic plants (Soana and Bartoli, 2013, 2014). However, because perennial plants may have different life cycle patterns of radial oxygen loss, the ways sulfide might interact with phenology or reproduction of perennial aquatic plants remains unknown. Clarifying how sulfide interacts with nutrients in rhizospheres of both annual and perennial plants may be important for understanding how wetlands or vegetated littoral zones respond to elevated sulfide conditions on an ecosystem level.

Redox conditions at root surfaces are closely tied to wild rice phenology. Sulfide, through delaying phenology, has the potential to control the timing of changes in redox conditions. By changing the timing and duration of reproductive life stages, sulfide's effects on phenology likely play a role in decreased survival of wild rice populations.

CRediT authorship contribution statement

Sophia LaFond-Hudson: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing - original draft, Visualization. **Nathan W. Johnson:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **John Pastor:** Conceptualization, Supervision, Writing - review & editing, Funding acquisition. **Brad Dewey:** Conceptualization, Methodology, Investigation, Data curation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aquabot.2020.103230.

References

Allam, A.I., Hollis, J.P., 1972. Sulfide inhibition of oxidases in rice roots. Phytopathology 62, 634–639.
Armstrong, W., 1980. Aeration in higher plants. In: Woolhouse, H.W. (Ed.), Advances in Botanical Research. Academic Press, pp. 225–332.
Armstrong, J., Armstrong, W., Beckett, P.M., 1992. Phragmites australis: venturi- and humidity-induced pressure flows enhance rhizome aeration and rhizosphere oxidation. New Phytol. 120, 197–207. https://doi.org/10.1111/j.1469-8137.1992.tb05655.x.
Brouwer, H., Murphy, T.P., 1994. Diffusion method for the determination of acid-volatile sulfides (AVS) in sediment. Environ. Toxicol. Chem. 13, 1273–1275. https://doi.org/10.1002/etc.5620130808.
Carlson, J., Yarbro, L.A., Barber, T.R., 1994. Relationship of sediment sulfide to mortality of Thalassia testudinum in Florida Bay. Bull. Mar. Sci. 54, 733–746.
Christensen, K.K., Sand-Jensen, K., 1998. Precipitated iron and manganese plaques restrict root uptake of phosphorus in Lobelia dortmanna. Can. J. Bot. 76, 2158–2163. https://doi.org/10.1139/b98-181.
Coleman, J.S., McConnaughy, K.D.M., Ackerly, D.D., 1994. Interpreting phenotypic variation in plants. Tree 9, 187–191.
Colmer, T.D., 2003. Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in upland, Paddy and deep-water rice (Oryza sativa L.). Ann. Bot. 91, 301–309. https://doi.org/10.1093/aob/mcf114.
Dacey, J.W.H., 1980. Internal winds in water lilies: an adaptation for life in anaerobic sediments. Science 210, 1017–1019.
Fond du Lac Band of Lake Superior Chippewa, 2018. Expanding the narrative of tribal health: the effects of wild rice water quality rule changes on tribal health. Health Impact Assess. 68.
Grava, J., Raisanen, K.A., 1978. Growth and nutrient accumulation and distribution in

wild rice 1. Agron. J. 70, 1077–1081. https://doi.org/10.2134/agronj1978.00021962007000060044x.
Jorgenson, K.D., Lee, P.F., Kanavillil, N., 2012. Ecological relationships of wild rice, Zizania spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (Zizania palustris). Botany 91, 189–201. https://doi.org/10.1139/cjb-2012-0198.
Koch, M.S., Mendelssohn, I.A., 1989. Sulphide as a soil phytotoxin: differential responses in two marsh species. J. Ecol. 77, 565–578. https://doi.org/10.2307/2260770.
Koch, M.S., Mendelssohn, I.A., McKee, K.L., 1990. Mechanism for the hydrogen sulfide-induced growth limitation in wetland macrophytes. Limnol. Oceanogr. 35, 399–408. https://doi.org/10.4319/lo.1990.35.2.0399.
LaFond-Hudson, S., Johnson, N.W., Pastor, J., Dewey, B., 2018. Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (Zizania palustris). Biogeochemistry 141, 95–106. https://doi.org/10.1007/s10533-018-0491-5.
Lamers, L.P.M., Tomassen, H.B.M., Roelofs, J.G.M., 1998. Sulfate-induced eutrophication and phytotoxicity in freshwater wetlands. Environ. Sci. Technol. 32, 199–205. https://doi.org/10.1021/es970362f.
Martin, N.M., Maricle, B.R., 2015. Species-specific enzymatic tolerance of sulfide toxicity in plant roots. Plant Physiol. Biochem. 88, 36–41. https://doi.org/10.1016/j.plaphy.2015.01.007.
Marzocchi, U., Benelli, S., Larsen, M., et al., 2019. Spatial heterogeneity and short-term oxygen dynamics in the rhizosphere of Vallisneria spiralis: implications for nutrient cycling. Freshw. Biol. 64, 532–543. https://doi.org/10.1111/fwb.13240.
McConnaughay, K.D.M., Coleman, J.S., 1999. Biomass allocation in plants: ontogeny or optimality? A test along three resource gradients. Ecology 80, 2581–2593. https://doi.org/10.1890/0012-9658(1999)080[2581:BAIPOO]2.0.CO;2.
Mendelssohn, I.A., Kleiss, B.A., Wakeley, J.S., 1995. Factors controlling the formation of oxidized root channels: a review. Wetlands 15, 37–46. https://doi.org/10.1007/BF03160678.
Pastor, J., Dewey, B., Johnson, N.W., et al., 2017. Effects of sulfate and sulfide on the life cycle of Zizania palustris in hydroponic and mesocosm experiments. Ecol. Appl. 27, 321–336. https://doi.org/10.1002/eap.1452.
Schmidt, H., Eickhorst, T., Tippkötter, R., 2011. Monitoring of root growth and redox conditions in paddy soil rhizotrons by redox electrodes and image analysis. Plant Soil 341, 221–232. https://doi.org/10.1007/s11104-010-0637-2.
Sims, L., Pastor, J., Lee, T., Dewey, B., 2012. Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. Oecologia 170, 65–76. https://doi.org/10.1007/s00442-012-2296-x.
Soana, E., Bartoli, M., 2013. Seasonal variation of radial oxygen loss in Vallisneria spiralis L.: an adaptive response to sediment redox? Aquat. Bot. 104, 228–232. https://doi.org/10.1016/j.aquabot.2012.07.007.
Soana, E., Bartoli, M., 2014. Seasonal regulation of nitrification in a rooted macrophyte (Vallisneria spiralis L.) meadow under eutrophic conditions. Aquat. Microb. Ecol. 48, 11–21. https://doi.org/10.1007/s10452-013-9462-z.
St-Cyr, L., Campbell, P.G.C., 1996. Metals (Fe, Mn, Zn) in the root plaque of submerged aquatic plants collected in situ: relations with metal concentrations in the adjacent sediments and in the root tissue. Biogeochemistry 33, 45–76.
Stover, E.L., 1928. The roots of wild rice Zizania Aquatica L. Ohio J. Sci. 28 (7).
Stumm, W., Morgan, J.J., 2012. Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters Vol. 126 John Wiley & Sons.
Trolldenier, G., 1988. Visualisation of oxidizing power of rice roots and of possible participation of bacteria in iron deposition. Zeitschrift für Pflanzenernährung und Bodenkunde 151, 117–121. https://doi.org/10.1002/jpln.19881510209.
Van der Welle, M.E.W., Niggebrugge, K., Lamers, L.P.M., Roelofs, J.G.M., 2007. Differential responses of the freshwater wetland species Juncus effusus L. and Caltha palustris L. to iron supply in sulfidic environments. Environ. Pollut. 147, 222–230. https://doi.org/10.1016/j.envpol.2006.08.024.

AGU ADVANCING EARTH AND SPACE SCIENCE

JGR Biogeosciences

RESEARCH ARTICLE
10.1029/2022JG006809

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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Sulfur Geochemistry Destabilizes Population Oscillations of Wild Rice (*Zizania palustris*)

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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;

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

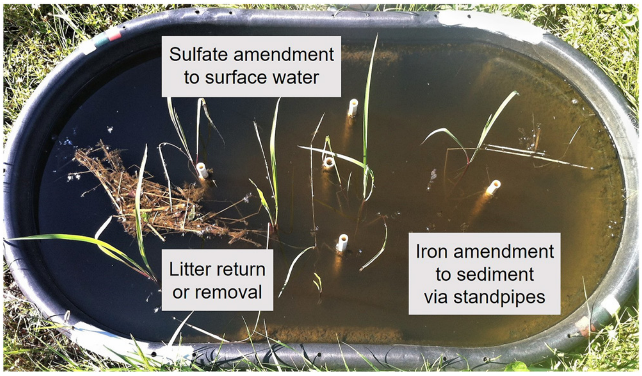

Figure 1. Picture of a mesocom showing the application of sulfate, iron, and litter treatments. The center standpipe was used to moderate the water level after rain events. The other four standpipes connect to a ring of perforated PVC pipes in the sediment to allow release of iron(II) chloride. Sulfate 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 $\Sigma S^{2-}:Fe^{2+}$ 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 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,

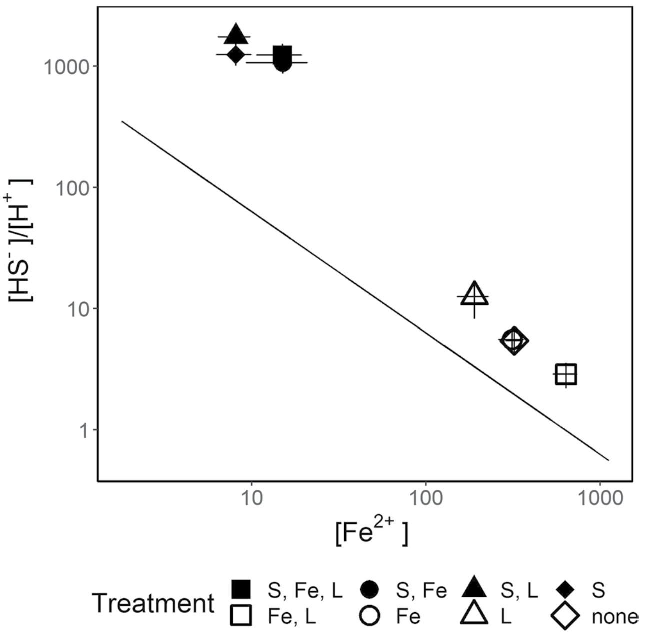

Figure 2. Comparison of ferrous iron (μmol L^{-1}) and sulfide concentrations (both sulfide and H+ in mol L^{-1}) in sediment porewater after 5 years of sulfate amendment (S), iron amendment (Fe), and litter retention (L). Data points represent average porewater measurements for each treatment ($n = 12$–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	$\boldsymbol{p < 0.001}$	$p = 0.53$	$p = 0.64$	S × Fe, Litter × Fe, S × Litter × Fe
Porewater iron	$\boldsymbol{p < 0.001}$	$\boldsymbol{p < 0.001}$	$p = 0.16$	
Porewater pH	$\boldsymbol{p < 0.001}$	$p = 0.64$	$p = 0.57$	S × Litter × Fe
Saturation index of FeS	$\boldsymbol{p < 0.001}$	$p = 0.19$	$p = 0.96$	

Note. Significant interactions (in bold) refer to combinations of sulfate, iron, and/or litter that are significant at $p < 0.05$.

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 $p < 0.05$.

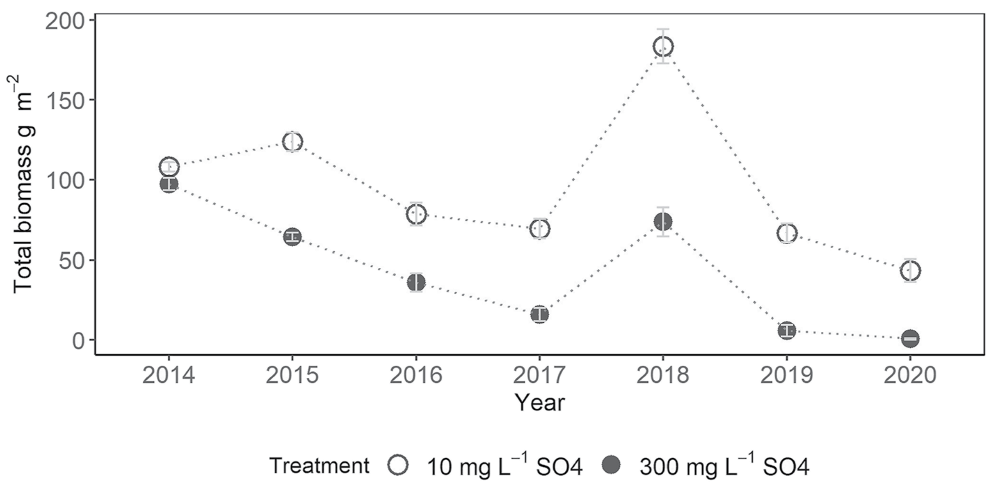

Figure 3. Annual average aboveground biomass (total) density (g m^{-2}) in populations grown in high sulfate (300 mg L^{-1}, filled circles) and low sulfate (10 mg L^{-1}, empty circles) in the overlying water. Error bars depict the standard error around the mean ($n = 20$).

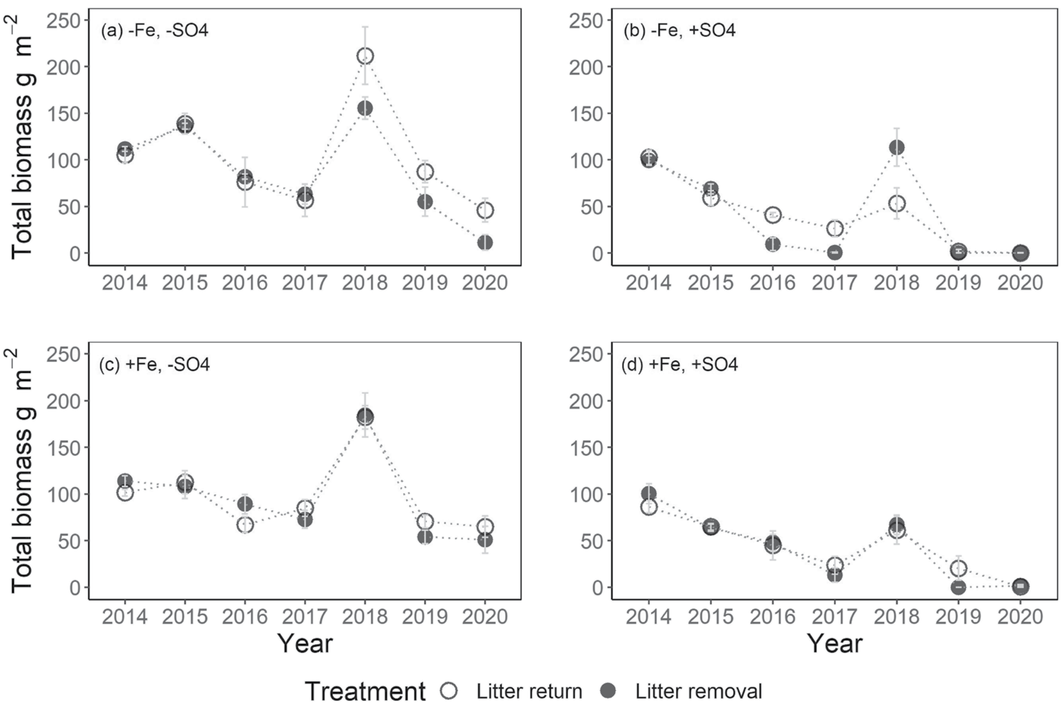

Figure 4. Annual average aboveground biomass (total) density (g m^{-2}) in populations grown with the previous year's aboveground litter returned (empty circles) or removed (filled circles). Populations were treated with combinations of low sulfate ($-SO_4$, 10 mg L^{-1}; a, c) or high sulfate ($+SO_4$, 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}.

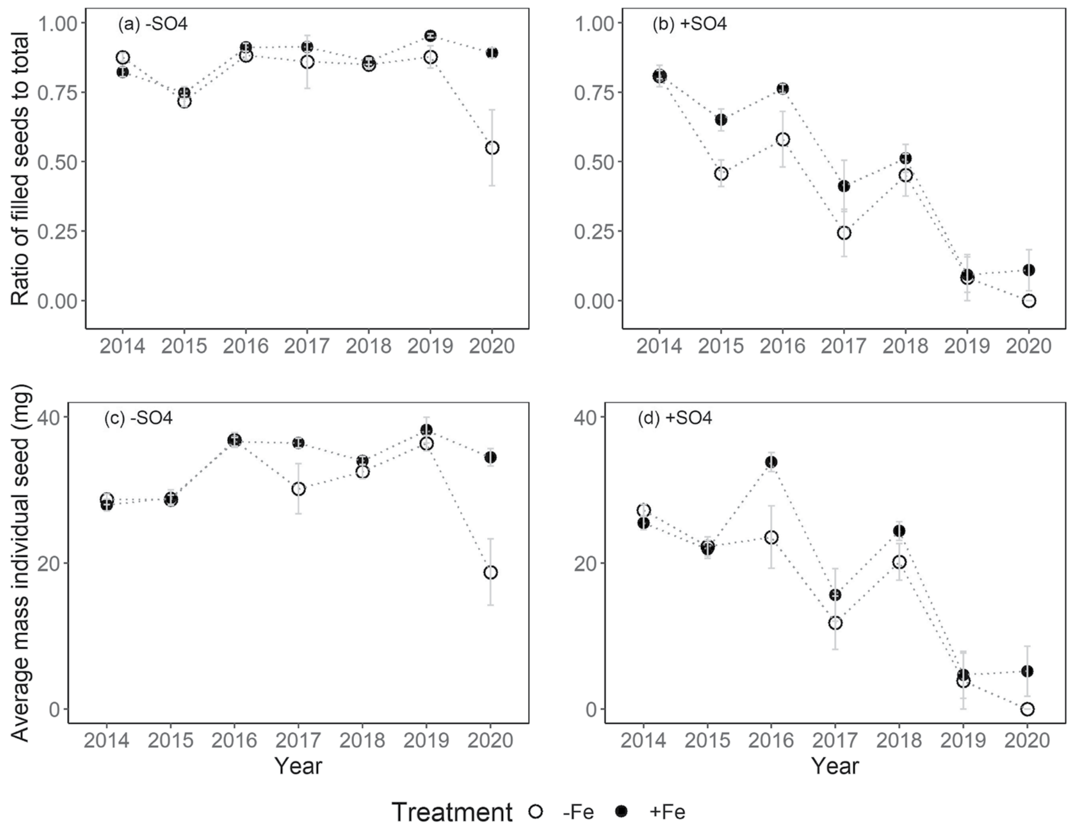

Figure 5. Effects of iron addition (+Fe, 10.9 mg g^{-1}; −Fe, 4.3 mg g^{-1}) on the ratio of filled seeds to total seeds (includes empty husks; a, b) and on average individual seed mass (c), (d) in 10 and 300 mg L^{-1} sulfate. Error bars depict the standard error around the mean ($n = 10$).

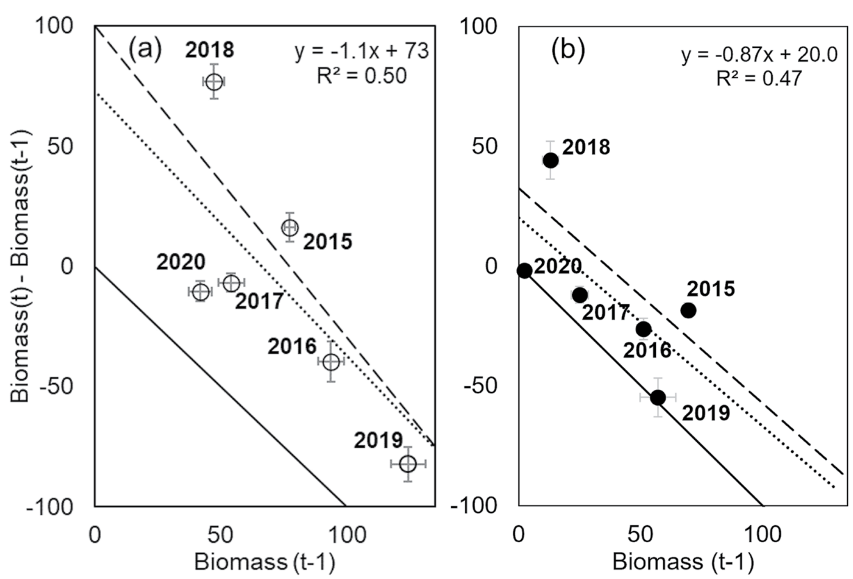

Figure 6. The relationship between average year-on-year change in vegetative biomass (g m^{-2}) to the previous year's vegetative biomass in (a) 10 mg L^{-1} sulfate and (b) 300 mg L^{-1} sulfate. Error bars show the standard error. The slope of the dashed line represents $\partial(dB/dt)/\partial B$ 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.

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

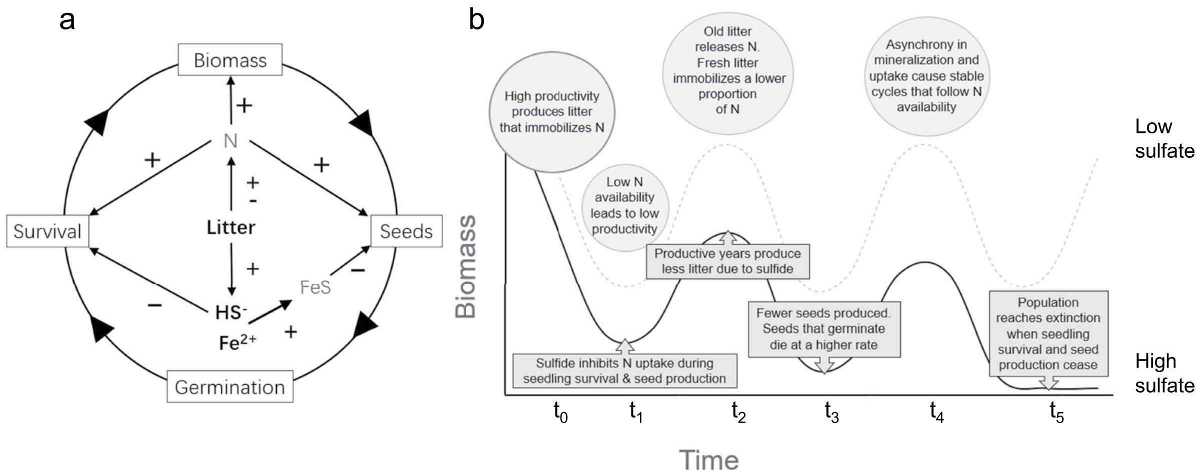

Figure 7. A conceptual model synthesizing the interactions of sulfur, iron, and litter during the life cycle of wild rice (a), and the effect of sulfur on interannual biomass cycles of wild rice (b) based on this study, Walker et al. (2010), and LaFond-Hudson et al. (2018). In (a), the (+) and (−) symbols 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 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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References

Azam, F., Lodhi, A., & Ashraf, M. (1991). Availability of soil and fertilizer nitrogen to wetland rice following wheat straw amendment. *Biology and Fertility of Soils*, *11*, 97–100. https://doi.org/10.1007/BF00336371

Bailey, L. T., Mitchell, C. P. J., Engstrom, D. R., Berndt, M. E., Coleman Wasik, J. K., & Johnson, N. W. (2017). Influence of porewater sulfide on methylmercury production and partitioning in sulfate-impacted lake sediments. *Science of The Total Environment*, *580*, 1197–1204. https://doi.org/10.1016/j.scitotenv.2016.12.078

Blossfeld, S., Gansert, D., Thiele, B., Kuhn, A. J., & Lösch, R. (2011). The dynamics of oxygen concentration, pH value, and organic acids in the rhizosphere of Juncus spp. *Soil Biology and Biochemistry*, *43*, 1186–1197. https://doi.org/10.1016/j.soilbio.2011.02.007

Federation, W. E., & Association, A. P. H. (2005). *Standard methods for the examination of water and wastewater*. American Public Health Association, American Water Works Association, Water Environment Federation.

Fond du Lac Band of Lake Superior Chippewa. (2018). Expanding the narrative of tribal health: The effects of wild rice water quality rule changes on tribal health (Vol. *68*). Health Impact Assessment.

Gao, S., Tanji, K., & Scardaci, S. (2003). Incorporating straw may induce sulfide toxicity in paddy rice. *California Agriculture*, *57*, 55–59. https://doi.org/10.3733/ca.v057n02p55

Gao, S., Tanji, K. K., & Scardaci, S. C. (2004). Impact of rice straw incorporation on soil redox status and sulfide toxicity. *Agronomy Journal*, *96*, 70–76. https://doi.org/10.2134/agronj2004.7000

Grava, J., & Raisanen, K. A. (1978). Growth and nutrient accumulation and distribution in wild rice 1. *Agronomy Journal*, *70*, 1077–1081. https://doi.org/10.2134/agronj1978.00021962007000060044x

Han, C., Ren, J., Wang, Z., Yang, S., Ke, F., Xu, D., & Xie, X. (2018). Characterization of phosphorus availability in response to radial oxygen losses in the rhizosphere of Vallisneria spiralis. *Chemosphere*, *208*, 740–748. https://doi.org/10.1016/j.chemosphere.2018.05.180

Hildebrandt, L. R., Pastor, J., & Dewey, B. (2012). Effects of external and internal nutrient supplies on decomposition of wild rice, *Zizania palustris*. *Aquatic Botany*, *97*, 35–43. https://doi.org/10.1016/j.aquabot.2011.11.002

Johnson, N. W., Pastor, J., & Swain, E. B. (2019). Cumulative sulfate loads shift porewater to sulfidic conditions in freshwater wetland sediment. *Environmental Toxicology and Chemistry*, *38*, 1231–1244. https://doi.org/10.1002/etc.4410

Jorgenson, K. D., Lee, P. F., & Kanavillil, N. (2012). Ecological relationships of wild rice, *Zizania* spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*). *Botany*, *91*, 189–201. https://doi.org/10.1139/cjb-2012-0198

Julian, P., Chambers, R., & Russell, T. (2017). Iron and pyritization in wetland soils of the Florida Coastal Everglades. *Estuaries and Coasts*, *40*, 822–831. https://doi.org/10.1007/s12237-016-0180-3

Kinsman-Costello, L. E., O'Brien, J. M., & Hamilton, S. K. (2015). Natural stressors in uncontaminated sediments of shallow freshwaters: The prevalence of sulfide, ammonia, and reduced iron. *Environmental Toxicology and Chemistry*, *34*, 467–479. https://doi.org/10.1002/etc.2801

LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2018). Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*). *Biogeochemistry*, *141*, 95–106. https://doi.org/10.1007/s10533-018-0491-5

LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2020a). Interactions between sulfide and reproductive phenology of an annual aquatic plant, wild rice (*Zizania palustris*). *Aquatic Botany*, *164*, 103230. https://doi.org/10.1016/j.aquabot.2020.103230

LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2020b). *Supporting data for "Sulfur geochemistry impacts population oscillations of wild rice (Zizania palustris)* (Data Repository). University of Minnesota. https://doi.org/10.13020/cq0g-r486

Lamers, L. P. M., Falla, S.-J., Samborska, E. M., van Dulken, I. A. R., van Hengstum, G., & Roelofs, J. G. M. (2002). Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. *Limnology & Oceanography*, *47*, 585–593. https://doi.org/10.4319/lo.2002.47.2.0585

Lamers, L. P. M., Govers, L. L., Janssen, I. C., Geurts, J. J., Van der Welle, M. E., Van Katwijk, M. M., et al. (2013). Sulfide as a soil phytotoxin—A review. *Frontiers of Plant Science*, *4*, 268. https://doi.org/10.3389/fpls.2013.00268

Lawrence, M. (2016). ez [WWW Document]. Retrieved from https://cran.r-project.org/web/packages/ez/ez.pdf

Lee, P. F. (2002). Ecological relationships of wild rice, *Zizania* spp. 10. Effects of sediment and among-population variations on plant density in *Zizania palustris*. *Canadian Journal of Botany*, *80*, 1283–1294. https://doi.org/10.1139/b02-118

Marzocchi, U., Benelli, S., Larsen, M., Bartoli, M., & Glud, R. N. (2019). Spatial heterogeneity and short-term oxygen dynamics in the rhizosphere of Vallisneria spiralis: Implications for nutrient cycling. *Freshwater Biology*, *64*, 532–543. https://doi.org/10.1111/fwb.13240

Mendelssohn, I. A., Kleiss, B. A., & Wakeley, J. S. (1995). Factors controlling the formation of oxidized root channels: A review. *Wetlands*, *15*, 37–46. https://doi.org/10.1007/BF03160678

Mendelssohn, I. A., & Morris, J. T. (2000). Eco-physiological controls on the productivity of *Spartina alterniflora* Loisel. In M. P. Weinstein, & D. A. Kreeger (Eds.), *Concepts and controversies in tidal marsh ecology* (pp. 59–80). Springer. https://doi.org/10.1007/0-306-47534-0_5

Morse, J. W., Millero, F. J., Cornwell, J. C., & Rickard, D. (1987). The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews*, *24*, 1–42. https://doi.org/10.1016/0012-8252(87)90046-8

Moyle, J. B. (1945). Some chemical factors influencing the distribution of aquatic plants in Minnesota. *The American Midland Naturalist*, *34*, 402–420. https://doi.org/10.2307/2421128

Myrbo, A., Swain, E. B., Engstrom, D. R., Wasik, J. C., Brenner, J., Shore, M. D., et al. (2017a). Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. *Journal of Geophysical Research: Biogeosciences*, *122*, 2736–2753. https://doi.org/10.1002/2017JG003787

Myrbo, A., Swain, E. B., Johnson, N. W., Engstrom, D. R., Pastor, J., Dewey, B., et al. (2017b). Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms: SO_4 reduction mobilizes N, P, C, and mercury. *Journal of Geophysical Research: Biogeosciences*, *122*, 2769–2785. https://doi.org/10.1002/2017JG003788

Pastor, J., Dewey, B., Johnson, N. W., Swain, E. B., Monson, P., Peters, E. B., & Myrbo, A. (2017). Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments. *Ecological Applications*, *27*, 321–336. https://doi.org/10.1002/eap.1452

Pastor, J., & Walker, R. D. (2006). Delays in nutrient cycling and plant population oscillations. *Oikos*, *112*, 698–705. https://doi.org/10.1111/j.0030-1299.2006.14478.x

Pollman, C. D., Swain, E. B., Bael, D., Myrbo, A., Monson, P., & Shore, M. D. (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. *Journal of Geophysical Research: Biogeosciences*, *122*, 2719–2735. https://doi.org/10.1002/2017JG003785

Ruiz-Halpern, S., Macko, S. A., & Fourqurean, J. W. (2008). The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. *Biogeochemistry*, *87*, 113–126. https://doi.org/10.1007/s10533-007-9162-7

Sahrawat, K. L. (2005). Iron toxicity in wetland rice and the role of other nutrients. *Journal of Plant Nutrition*, *27*, 1471–1504. https://doi.org/10.1081/PLN-200025869

Sain, P. (1984). Decomposition of wild rice (*Zizania aquatica*) straw in two natural lakes of northwestern Ontario. *Canadian Journal of Botany*, *62*, 1352–1356. https://doi.org/10.1139/b84-183

Sims, L., Pastor, J., Lee, T., & Dewey, B. (2012). Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. *Oecologia*, *170*, 65–76. https://doi.org/10.1007/s00442-012-2296-x

Sinha, E., & Michalak, A. M. (2016). Precipitation dominates interannual variability of riverine nitrogen loading across the continental United States. *Environmental Science & Technology*, *50*, 12874–12884. https://doi.org/10.1021/acs.est.6b04455

Strogatz, S. H. (1994). *Nonlinear dynamics and chaos: With applications to physics, biology, chemistry, and engineering*. Westview Press.

Sundby, B., Vale, C., Caçador, Z., Catarino, F., Madureira, M.-J., & Caetano, M. (1998). Metal-rich concretions on the roots of salt marsh plants: Mechanism and rate of formation. *Limnology & Oceanography*, *43*, 245–252. https://doi.org/10.4319/lo.1998.43.2.0245

Van der Welle, M. E. W., Niggebrugge, K., Lamers, L. P. M., & Roelofs, J. G. M. (2007). Differential responses of the freshwater wetland species Juncus effusus L. and Caltha palustris L. to iron supply in sulfidic environments. *Environmental Pollution*, *147*, 222–230. https://doi.org/10.1016/j.envpol.2006.08.024

Vogt, D. (2021). *Wild rice monitoring and abundance in the 1854 Ceded Territory (199–2020)* (Tech. Rep. 1854). Treaty Authority.

Walker, R., Pastor, J., & Dewey, B. W. (2006). Effects of wild rice (*Zizania palustris*) straw on biomass and seed production in northern Minnesota. *Canadian Journal of Botany*, *84*, 1019–1024. https://doi.org/10.1139/b06-058

Walker, R. E. D., Pastor, J., & Dewey, B. W. (2010). Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.) in northern Minnesota. *Ecosystems*, *13*, 485–498. https://doi.org/10.1007/s10021-010-9333-6

https://www.epa.gov/sites/default/files/2021-04/documents/2021.4.27_2020_mn_303d_dd_phase_2_.pdf

DECISION DOCUMENT REGARDING THE SULFATE IMPAIRED WATERS EPA IS ADDING TO THE MINNESOTA 2020 CLEAN WATER ACT SECTION 303(d) LIST

The U.S. Environmental Protection Agency partially approved and partially disapproved Minnesota's 2020 Clean Water Act (CWA), 33 U.S.C. § 1251 *et seq.*, Section 303(d) list (Minnesota 2020 Section 303(d) list) on March 26, 2021.[1] EPA concluded that Minnesota's list of water quality limited segments (WQLSs) still requiring total maximum daily loads (TMDLs) was complete (Appendix 1 of the March 26, 2021, Decision Document), with the exception of Minnesota's decision to not identify on the Minnesota 2020 Section 303(d) list any WQLSs for sulfate impairment.[2] In the March 26, 2021 Decision Document, EPA stated that Minnesota's decision to exclude these WQLSs with existing and readily available data and information indicating sulfate impairment was inconsistent with Section 303(d) and EPA's implementing regulations.[3]

Consistent with Section 303(d)(2), this Decision Document includes the details of EPA's identification of 30 WQLSs (Appendix 2 of this Decision Document) still requiring TMDLs under Section 303(d) of the CWA and the implementing regulations pursuant to 40 C.F.R. § 130.7. WQLSs identified in Appendix 2 of this Decision Document are the waters that EPA is adding to Minnesota's 2020 Section 303(d) list. As required by 40 C.F.R. § 130.7(d)(2), EPA will issue a public notice providing for a 30-day public comment period regarding these additions. After considering the comments received, EPA plans to issue another decision document responding to comments and potentially revising the list. Because of the short statutory timeframe given for EPA's action and the complexity of these issues, EPA expects that at that time we will provide further clarification as needed on our action.

EPA is in the process of evaluating extensive additional data and information received through consultation with Tribal Governments and is taking no action on other potential wild rice waters and sulfate impairments at this time. Information received to date, with relevant notes, is found in Appendices 3-5. EPA also recognizes that there may be other information in the possession of stakeholders relevant to identifying those waters subject to the "waters used for production of wild rice" beneficial use and associated water quality data. EPA will complete our analysis of the input received from Tribal Governments, along with any additional information received during this public notice and comment period and will issue a supplemental decision addressing any additional sulfate-impaired wild rice waters, as appropriate. If EPA identifies additional waters used for the production of wild rice for which there are sulfate impaired segments, EPA will provide an additional 30-day period for public review and comment on those WQLSs. EPA will provide the exact schedule and deadlines for receiving

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[1] EPA Decision Document for The Partial Approval of Minnesota's 2020 Clean Water Act Section 303(d) List, March 26, 2021 [hereafter EPA 2020 Decision Document].

[2] MPCA, Responses to the 2020 Draft Impaired Waters List, Public Notice Comments (February 25, 2021), p. 2 of 12 [responses to public comments 5, 6, 8, 10, 11, 13, 15, and 19]; Letter from Tera L. Fong, EPA, to Katrina Kessler, MPCA, March 9, 2021; Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021.

[3] EPA 2020 Decision Document at 19. See also Decision Document for the Approval of Minnesota's 2014 Clean Water Act Section 303(d) List [hereafter EPA 2014 Decision Document], Appendix 1 at 9 (May 29, 2018) ("EPA notes that this state law cannot and does not abrogate the State's obligation to complete a list of impaired waters as required by the Clean Water Act, 33 U.S.C. § 1313(d), a federal law. The State is also required to complete its CWA 303(d) list according to the federal regulations promulgated under the Clean Water Act, including 40 C.F.R. § 130.7.").

public comment at the time EPA publishes our notice, as appropriate. Because of the short statutory timeframe given for EPA's action and the complexity of these issues, EPA expects that at that time we will provide further clarification as needed on our action.

I. Background

A. Sulfate Water Quality Standard

Minnesota Rule (Minn. R.) 7050.0430, states that "All surface waters of the state that are not listed in Minn. R. 7050.0470 and that are not wetlands as defined in part 7050.0186, subpart 1a, are hereby classified as Class 2B, 3C, 4A, 4B, 5, and 6 waters." However, Minn. R. 7050.0224, subpart 1, provides further identification of waters where wild rice is present:

> Wild rice is an aquatic plant resource found in certain waters within the state. The harvest and use of grains from this plant serve as a food source for wildlife and humans. In recognition of the ecological importance of this resource, and in conjunction with Minnesota Indian tribes, selected wild rice waters have been specifically identified [WR] and listed in part 7050.0470, subpart 1. The quality of these waters and the aquatic habitat necessary to support the propagation and maintenance of wild rice plant species must not be materially impaired or degraded.

In addition to the other water quality criteria listed in Minn. R. 7050.0224, the second subpart of this rule states that the Class 4A sulfate criterion of 10 mg/L is "applicable to water used for the production of wild rice during periods when the rice may be susceptible to damage by high sulfate." Minn. R. 7050.0224, subp. 2. Therefore, the State's criterion at Minn. R. 7050.0224, subpart 2 contains a two-part test: The first part requires that waters designated for the use are those "used for the production of wild rice"; while the second part provides that the sulfate criterion of 10 mg/L will be "applicable to water used for the production of wild rice during periods when the rice may be susceptible to damage by high sulfate." Id.

During EPA's review of the State's 2014 list submittal, Tribes asserted to EPA that the sulfate criterion should be applied to all waters of the State. In response, EPA explained:

> EPA agrees that the default designated uses under Minn. R. 7050.0430 (2B, 3C, 4A, 4B, 5, and 6) apply to all waters not specifically designated in Minn. R. 7050.0470 [Minn. R. 7050.0430]. Minn. R. 7050.0430 generally supports the application of the Class 4A criteria to all waters not specified under Minn. R. 7050.0470. However, unlike other water quality criteria listed in Minn. R. 7050.0224, Subp. 2, the applicability of the Class 4A sulfate criterion is further restricted to "water used for the production of wild rice during periods when the rice may be susceptible to damage by high sulfate." Minn. R. 7050.0224 includes the designation of 24 wild rice waters in Minn. R. 7050.0470, subpart 1. EPA understands that the State of Minnesota has the authority to add additional waters to Minn. R. 7050.0470, subpart 1 but the State has never done so. [citation omitted] EPA notes that there has been uncertainty in the State's interpretation and application of the existing sulfate criterion over time, such that there is no clear indication that the criterion applies to waters beyond the 24 waters listed in Minn. R. 7050.0470, subpart 1. . . . However, EPA believes that MPCA has consistently communicated that MPCA considers at least the 24

> state-designated wild rice waters as waters used for the production of wild rice and, thus, subject to the current sulfate criterion (Minn. R. 7050.0224).
>
> Apart from the waters listed in Minn. R. 7050.0470, subpart 1, there are other sources of documentation which contain information on waters where wild rice is found, although EPA has not been able to directly link this information to the State's water quality standards process, as explained. . . . In light of the uncertainty regarding the State's interpretation as to the applicability of its existing sulfate water quality criterion, EPA finds that it was not unreasonable for the State to decline to assess or list waters outside of the 24 state-designated wild rice waters of Minn. R. 7050.0470, subpart 1. EPA continues to expect that the State will develop an appropriate assessment and listing methodology in conjunction with its approved sulfate criterion.[4]

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[4] EPA, 2014 Decision Document, Appendix 2, EPA Response to Tribal Issues Raised During Tribal Consultation on the Final 2014 Minnesota Clean Water Act 303(d) List at 1. In our 2014 Decision Document, Appendix 1, EPA considered the extensive documentation shared by the Tribes and other stakeholders which identified specific waters that these parties believe are in violation of the current numeric sulfate criterion (10 mg/L). There we noted, "While these entities have put forward various approaches to identifying waters they believe are subject to the sulfate criterion, we are unaware of any mechanism for the State to incorporate these considerations other than the State's CWA Section 303(c) process for determining designated uses of water bodies." See also Chamber I , at *9 (noting that "available wild rice records and databases that the Minnesota Department of Natural Resources (MDNR) maintains" are only one aspect of MPCA's determination of whether the sulfate criterion applies to a water and that MDNR's list of waters where wild rice has been identified is not equivalent to "an exhaustive list of waters used for the production of wild rice"). Appendix 1 at note 43, citing *Minn. Chamber of Commerce v. Minn. Pollution Control Agency*, 2012 Minn. Dist. LEXIS 194 (Minn. Dist. Ct., May 10, 2012), *aff'd*, *Minn. Chamber of Commerce v. Minn. Pollution Control Agency*, 2012 Minn. App. Unpub. LEXIS 1199 (Minn. Ct. App., Dec. 17, 2012). See generally EPA's 2014 Decision Document, Appendix 1, for a discussion of EPA's review of available information, including at note 44, discussing EPA's review of the 2008 MDNR Report; and EPA's 2014 Decision Document, Appendix 2.

See also MPCA, Statement of Need and Reasonableness (SONAR), *Amendment of the sulfate water quality standard applicable to wild rice and identification of wild rice waters*, July 2017 [hereafter MPCA 2017 SONAR], at 28 – 30, 35 – 39ff, describing the history of the State's sulfate criterion and noting:

> Minn. R. 7050.0224, subpart 1 currently includes, in addition to general directives about Class 4 waters, a narrative standard that only applies to selected wild rice waters, also referred to as [WR] waters, that are specifically identified in the rule. . . . The current rules apply the wild rice beneficial use to "water used for production of wild rice," but the rules do not specifically identify these waters. Identifying these waters has been a major challenge to the implementation of the existing standard, as identification currently requires a case-by-case evaluation. In 2011, the Minnesota Legislature directed the MPCA to "designate each body of water, or specific portion thereof, to which wild rice water quality standards apply." Legislation also directs the MPCA to establish criteria for waters containing natural beds of wild rice and that the criteria should include (but not be limited to) history of wild rice harvests, minimum acreage and wild rice density.

Id. at 36-38, https://www.pca.state.mn.us/sites/default/files/wq-rule4-15i.pdf (last visited 4/24/2021).

We note that there may be an impression that other, non-State entities can make designations pursuant to the State's standard at Minn. R. 7050.0224, and specifically that the Minnesota ALJ's decision identified the ability of Tribes to make designations of waters. See Email from Sara Van Norman, on behalf of Grand Portage Chair Deschampe, to Cheryl Newton, "Consultation Comments on MN Wild Rice Waters to be Added to the 2020 impaired Waters List," April 8, 2021, enclosing letter from Norman Deschampe, Chairman, and April McCormick, Secretary/Treasurer, Grand Portage Band of Lake Superior Chippewa, to Cheryl Newton, Acting Regional Administrator, April 8, 2021, at 3-4. While EPA agrees that Tribes may make designations of waters within the area for which they have approved programs under CWA Section 303(c), Tribes (or neighboring States) may not make designation decisions for each others' waters. The letter also notes that the ALJ

Between 2012 and 2017, MPCA completed steps toward a rulemaking that would have revised the sulfate standard and would have established a list of some 1300 waters that were to be designated for a "wild rice" use (MPCA's 2017 list of 1300 waters). EPA further notes that MPCA's 2017 rulemaking effort was relevant to State's efforts to interpret the beneficial use as it is described in Minn. R. 7050.0470:

> The wild rice beneficial use was established in 1973 and is not being changed by this rulemaking. This rulemaking provides, for the first time, a specific list of those waters that demonstrate the wild rice beneficial use. . . . The MPCA is clarifying an existing beneficial use, not changing it. The MPCA is not adopting new or revised designated uses, or removing designated uses. Rather, the MPCA is using available information to, via rulemaking, identify which waters demonstrate the beneficial use.[5]

The rulemaking was abandoned after the rule was deemed flawed on several bases in the State's administrative review process.[6] While MPCA ultimately neither revised the sulfate criterion, nor adopted the list of designated waters into rule,[7] during its rule development process, MPCA engaged in an extensive review of available information regarding the location and water quality relating to waterbodies supporting natural stands of wild rice.[8]

In explaining why it chose not to include all waters from the various lists and information reviewed when compiling its list of waters subject to the beneficial use, MPCA noted:

> While the discussion above describes the sources the MPCA used to identify proposed Class 4D wild rice waters, in some instances information was insufficient to make a determination. In

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decision did not limit the interpretation of the State's water quality criterion to one State agency (at id.), but EPA notes, without commenting on this assertion, that there is no dispute between the ALJ decision and MPCA's position that the 2017 list of 1300 waters represents the minimum list to which the State's criterion applies, as further discussed in Part II.A below.

[5] MPCA 2017 SONAR at 41.

[6] Chief Administrative Law Judge's Order on Review of Rules, *In the Matter of the Proposed Rules of the Pollution Control Agency Amending the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Rivers*, April 12, 2018 [hereafter Chief ALJ Order], https://mn.gov/oah/assets/9003-34519-pca-sulfate-water-quality-wild-rice-rules-chief-judge-reconsideration-order_tcm19-335811.pdf (last visited 4/24/21); State of Minnesota Office of Administrative Hearings, *In the In the Matter of the Proposed Rules of the Pollution Control Agency Amending the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Rivers, Minnesota Rules parts 7050.0130, 7050.0220, 7050.0224, 7050.0470, 7050.0471, 7053.0135, 7053.0205, and 7053.0406*, OAH 80-9003-34519, Revisor R-4324, January 11, 2018 [hereafter ALJ Report], https://www.pca.state.mn.us/sites/default/files/wq-rule4-15mm.pdf (last visited 4/24/2021).

[7] MPCA, Environmental Analysis and Outcomes Divisions, *Notice of Withdrawn Rules for Proposed Rules Amending the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Waters; Revisor's ID Number 4324* (April 26, 2018), https://www.pca.state.mn.us/sites/default/files/wq-rule4-15oo.pdf (last visited 4/24/2021).

[8] MPCA 2017 SONAR at 41 - 51. Included in the exhibits reviewed by MPCA were the Minnesota Department of Natural Resources (MDNR) study, *Natural Wild Rice in Minnesota – A Wild Rice Study Report to the Legislature* (2008); MDNR *Wild Rice Harvester Survey Report* (2007); Minnesota Wild Rice Management Workgroup List of 350 Important Wild Rice Waters (2010); 1854 Treaty Authority List of Wild Rice Waters; MDNR Aquatic Plant Management Database; MPCA Biomonitoring Field Sites surveys; University of Minnesota field surveys sites (2011-2013); Minnesota Biological Survey Database; information received in MPCA's 2013 "Call for Data" for locational information on wild rice and sulfate analytical results; MPCA National Pollutant Discharge Elimination System Permit (NPDES) Monitoring Reports; Wild rice waters listed in Minn. R. 7050.0470; Waters identified by MDNR as wild rice waters in 2015; Waters identified through MPCA reviews of other water surveys as listed.

> some cases, the MPCA could not identify the location of the water from the information provided. For example, waters in the MDNR 2007 harvester report were listed on a county-by-county basis. For common lake names, multiple waters within a county with the same names were found (for example, Mud Lake, Round Lake, Deer Lake, etc.), and in some cases, the location of the water could not be precisely identified.
>
> In other cases, the MPCA could not correlate the location of a river or stream with a particular waterbody identifier (WID). Some sources of information listed river and stream locations with only Township and Range data. In these cases, the MPCA reviewed available data (aerial photographs, other sources) to identify the WIDs in that county associated with the river or stream. If multiple WIDs associated with the river or stream were found within the county, and the MPCA was unable to find information to correlate specifically with a single WID where rice was located, the water could not reasonably be included as a proposed wild rice water.[9]

MPCA acknowledged that its list "may not include every water in Minnesota where the wild rice beneficial use has existed since November 28, 1975," and that there "may be wild rice waters but for which there is not yet sufficient information to determine that the beneficial use is demonstrated."[10] A Chief Administrative Law Judge (ALJ) Order ruled that MPCA's list was flawed for using a "weight of evidence" standard to screen information the Agency deemed relevant to making a determination of where its new numeric criterion would apply (i.e., the ALJ determined that MPCA had no legislative mandate to necessarily examine stand density, set criteria for determining minimum acreages of wild rice stands, and other clear standards for screening evidence). Thus, the Chief ALJ Order affirmed the ALJ Report finding that the resulting list does not include all waters previously identified where wild rice may be an existing use,[11] and encouraged MPCA to expand its list to include additional waters that might be subject to the wild rice standards currently in effect.[12]

MPCA, as noted above, did not expand the list, adopt the list into rule, or submit it to EPA for review as a revision to the State's approved water quality standards. In our 2014 Decision Document (issued in 2018), we reviewed this list, among others, and noted

> As part of its efforts to revise the sulfate criterion, MPCA identified a draft list of some 1,300 wild rice waters that may be subject to an anticipated revised sulfate criterion and made that draft list available for public notice and comment [citation omitted]. . . . However, the State has not promulgated a revision to the existing standard nor a revision to the waters that are subject to the standard. Thus, the current standard and the waters to which that standard applies are the only relevant subjects of consideration for the 2014 303(d) list.[13]

Absent further action by the State to identify where the wild use applied, EPA's final action on the 2014, 2016 and 2018 Minnesota Section 303(d) lists reviewed only existing and readily available water quality data for the 24 waters specifically designated as wild rice waters in Minn. R. 7050.0470. While

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[9] MPCA 2017 SONAR at 45.
[10] MPCA 2017 SONAR at 58, cited in Chief ALJ Order at 67, who noted that commenters in MPCA's public comment on the list argued for both the under-inclusiveness and the over-inclusiveness of the list. Id.
[11] ALJ Report at 62-69.
[12] ALJ Report at 69, 79.
[13] EPA 2014 Decision Document, Appendix 1 at 6-7.

consistently acknowledging that it was possible that the criterion applied to more than the 24 waters in Minnesota's rule, EPA declined to apply Minnesota's sulfate criterion beyond these 24 waters.[14]

B. EPA's Review of the February 25, 2021 Submittal from Minnesota

EPA received MPCA's 2020 Section 303(d) List of Impaired Waters still requiring TMDLs, which was submitted as part of Minnesota's 2020 Integrated Report, on February 25, 2021. EPA reviewed Minnesota's submittal, including the listing decisions, the assessment methodology, and supporting data and information to determine whether Minnesota reasonably identified waters to be listed as impaired. MPCA received multiple comments regarding the continued absence of a sulfate/wild rice assessment methodology and Minnesota's failure to assess or list potential sulfate-impaired surface water body segments. The comments submitted during the public comment period cited eight waters as examples of waters used for the production of wild rice and for which readily available data indicated potential impairment for the 10 mg/L sulfate criterion. In response to these comments, MPCA stated that it considered the data and analysis and determined that seven of the eight waters proposed for listing demonstrated sulfate concentrations above 10 mg/L, but Minnesota could not list those waters because Minnesota law bars MPCA from assessing or listing waters against Minnesota's federally approved 10 mg/L standard.[15]

On March 9, 2021, EPA requested clarification on whether MPCA considered eight waters, identified in public comment, as waters used for the production of wild rice and further explanation of how MPCA evaluated data for those eight waters. MPCA's March 15 response affirmed that the eight example waters referenced were, "waters used for the production of wild rice" (although not appearing on the list of 24 waters in the State' rules) and provided MPCA's analysis of the data submitted by Tribes and reviewed by MPCA in concluding that seven of these eight waters are impaired for sulfate.[16]

The letter noted:

> In 2017 the MPCA proposed revisions to the wild rice sulfate standard as part of a rulemaking process [citation omitted]. As articulated in the Statement of Need and Reasonableness (SONAR) for that rule:
>
> > *The MPCA is proposing to eliminate this confusing term ["water used for production of wild rice"] and instead identify specifically where the standard applies, i.e., to a "wild rice water" in order to protect the wild rice beneficial use. The MPCA is not proposing to change the beneficial use of wild rice, but is proposing to modify the phrase in order to more clearly articulate the recognized use. The MPCA is proposing to specifically identify the rivers, streams, lakes, and wetlands demonstrating this beneficial use."* [Citation omitted.]

[14] EPA 2014 Decision Document at 1 and Appendix 1; EPA, Decision Document for the Approval of Minnesota's 2016 and 2018 Clean Water Act Section 303(d) Lists (January 28, 2019) [hereafter EPA 2016-2016 Decision Document] at 1, 30, and Appendix 1, EPA Responses to Tribal Issues Raised during Tribal Consultation on the final 2016 and 2018 Minnesota Clean Water Act 303(d) Lists; see also EPA Decision Document for Minnesota's 2012 Section 303(d) List, July 25, 2013.
[15] MPCA, Responses to the 2020 Draft Impaired Waters List, Public Notice Comments (February 25, 2021), p. 2 of 12 [responses to public comments 5, 6, 8, 10, 11, 13, 15, and 19]; Letter from Tera L. Fong, EPA, to Katrina Kessler, MPCA, March 9, 2021; Letter from Katrina Kessler, MPCA, to Tera L. Fong, March 15, 2021.
[16] Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021.

> The proposed rule revisions included a list of approximately 1300 waters that the MPCA planned to identify as having the wild rice beneficial use designation [citation omitted]. During the rulemaking, many commenters indicated they felt the MPCA's proposed list was too narrow, and the Administrative Law Judge reviewing the rule also found that MPCA inappropriately excluded some waters [citation omitted]. As part of its review of the comments from the Tribal leaders, the MPCA concluded that the eight waters submitted should be considered as "waters used for production of wild rice" for the purpose of evaluating impairment status, because: (1) the eight waters presented in the comments received during the comment period for Minnesota's 2020 Impaired Waters List were on the proposed list in the rulemaking; and (2) based on the fact that the proposed list in the rulemaking could likely be considered the most narrow list of waters that demonstrate the wild rice beneficial use.[17]

The letter detailed MPCA's efforts to specifically identify the eight waters submitted and evaluate available data "from all available databases" including data received during the period 2009 - 2019.[18] The letter concluded:

> The MPCA's analysis is not a complete assessment, and does not represent a final decision on an appropriate assessment methodology. However, the MPCA finds that it demonstrates that under any reasonable assessment methodology consistent with those already developed by MPCA, all of eight waterbodies demonstrate the beneficial use and seven of the eight waters – all except Birch Lake – would be shown to exceed 10 mg/L sulfate.[19]

The letter enclosed the data reviewed by MPCA and described its review in concluding that that seven of the eight waters would be considered impaired for sulfate.[20]

Following our review of MPCA's submittal and supporting information, EPA partially disapproved the State's 2020 list, finding that the State's decision to exclude the seven waters discussed in the State's materials was inconsistent with Section 303(d) and EPA's implementing regulations.[21]

II. EPA's Identification of Water Quality Limited Segments for Inclusion on the Minnesota 2020 Section 303(d) List

Section 303(d)(1) of the CWA directs states to identify those waters within their jurisdiction for which effluent limitations required by Section 301(b)(1)(A) and (B) are not stringent enough to implement any applicable water quality standard (WQS) and to establish a priority ranking for such waters, considering the severity of the pollution and the intended uses of such waters. The Section 303(d) listing requirement

[17] Id.
[18] Id. at 2-3. During consultation held by EPA and Minnesota Tribal Leaders on April 9, 2021, Minnesota Tribal Representatives stated that these eight waters had been provided to MPCA as example waters only. See also Email from April McCormick, Secretary Treasurer, Grand Portage Band of Lake Superior Chippewa, to JoAnn Chase and others, March 17, 2021, and attachments.
[19] Id. at 4.
[20] Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021, Attachments 1 and 2.
[21] EPA 2020 Decision Document at 19.

applies to waters impaired by point sources and/or nonpoint sources, pursuant to EPA's long-standing interpretation of Section 303(d).[22]

EPA regulations provide that states do not need to list waters for which the following controls are adequate to implement applicable standards: (1) technology-based effluent limitations required by the CWA, (2) more stringent effluent limitations required by state or local authority, and (3) other pollution control requirements required by state, local, or federal authority.[23]

A. Rationale for Identifying Waters as Subject to the Wild Rice Beneficial Use

Since 2012, EPA has followed a cautious interpretation of Minn. R. 7050.0224 that has focused our review on the 24 waters listed in Subpart 1,[24] mindful that our review of the State's impaired waters lists was not a review or revision to the State's water quality standards under Section 303(c), 33 U.S.C. § 1313(c).[25] The Agency has consistently stated, however, that our approach could change: "EPA understands that the State of Minnesota has the authority to add additional waters to Minn. R. 7050.0470, subpart 1 but the State has never done so."[26] EPA recognizes that throughout this time there have been various compilations by Tribes, state agencies, and stakeholders that seek to identify the list of waters that are subject to the wild rice beneficial use, and including MPCA's 2017 list of 1300 waters. EPA also recognizes that in 2018, Minnesota's Chief Administrative Law Judge issued an order that affirmed that MPCA's 2017 list of 1300 waters was too narrow based on the legislative charge given to MPCA to develop a new sulfate criterion and a list of associated designated waters.

Since 2012, EPA has received numerous comments from Tribes during our consultations with Tribes on our review of the State's impaired waters lists. We have also received courtesy copies of comments submitted by Tribes and stakeholders providing information regarding potential impairment by sulfate of various waters where wild rice grows.[27]

Since 2012, EPA has also strongly encouraged MPCA to develop an assessment methodology and to engage in a substantive effort to assess and list waters against its current wild rice criterion.[28] The

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[22] U.S. EPA, Office of Water, *Guidance for Water Quality-Based Decisions: The TMDL Process*, EPA 44014-91-001, April 1991 (hereafter, EPA's 1991 Guidance); U.S. EPA, Office of Water, *EPA Guidelines for Preparation of the Comprehensive State Water Quality Assessments (305(b) Reports) and Electronic Updates: Supplement*, EPA-841-B-97-002B, September 1997.
[23] 40 C.F.R. § 130.7(b)(1).
[24] EPA 2014 Decision Document, Appendix 2 ("EPA believes that MPCA has consistently communicated that MPCA considers at least the 24 state-designated wild rice waters as waters used for the production of wild rice and, thus, subject to the current sulfate criterion (Minn. R. 7050.0224)."); see also EPA 2016 – 2018 Decision Document at 30.
[25] See, e.g., EPA's 2014 Decision Document, Appendix 2, p. 3-4 ("EPA believes that it is not appropriate to preempt the State's ongoing and immediate effort of formal revision and promulgation of water quality standards for wild rice, nor is it appropriate to use the assessment process established in CWA § 303(d) to displace the process for establishing and revising water quality standards outlined in CWA § 303(c).").
[26] EPA 2014 Decision Document, Appendix 2, at 3; EPA 2014 Decision Document, Appendix 1 at 10 ("Therefore, although MPCA may designate waters used for the production of wild rice beyond those listed in Minn. R. 7050.0470, subpart 1, EPA does not believe MPCA has done so at this time.").
[27] See, e.g., EPA 2012 Decision Document at 29-31; EPA 2014 Decision Document, Appendices 1 and 2; EPA 2016-2018 Decision Document at 30 and Appendix 1.
[28] See EPA 2012 Decision Document at 29-31; EPA 2014 Decision Document, Appendix 1 at 9-11; EPA 2016 – 2018 Decision Document at 30.

preference that the State take the lead in ascertaining the scope of the wild rice use derived in part from the CWA's recognition of and preference for "the primary responsibilities and rights of States to prevent, reduce, and eliminate pollution" and "to plan the development and use (including restoration, preservation, and enhancement) of land and water resources." Section 101(b), 33 U.S.C. § 1251(b). Unfortunately, EPA's expectations have not been realized. State legislation curtailed MPCA's efforts to list waters against the standard.[29] And although the State made a serious attempt to accomplish a revision to the wild rice criterion in 2017 (including assessing every major list and study of wild rice conducted within State waters, conducting research of its own, holding meetings with Tribes, conducting outreach to stakeholders, and seeking public comment), since the 2018 Chief ALJ Order disapproving the proposed standards revision, there has been no formal revision or clarification of the standard. MPCA withdrew its effort to clarify the wild rice beneficial use and associated criterion,[30] preventing since that date any possible clarification of the wild rice standard or identification of where the beneficial use should apply. This regulatory stasis has prevented the State from carrying out its responsibilities under Section 303(d)(1)(A), 33 U.S.C. § 1313(d)(1)(A), to identify and assess for impairment those waters that may be subject to the wild rice beneficial use, contrary to the purpose of the CWA. We note that this regulatory inaction has prevented the State from meaningfully addressing the calls from Tribes to protect their judicially affirmed rights to gather wild rice in ceded territory within Minnesota.[31] It has also resulted in the State's inaction in responding to comments and information regarding sulfate impairments submitted for each listing cycle by stakeholders.

We recognize that in its 2020 list submittal, MPCA has, for the first time, provided clarification on the applicability of the wild rice beneficial use to a universe greater than the 24 waters listed in Minn. R. 7050.0224, subpart 1, as discussed in Section 1.B above.[32] We also recognize MPCA's statement that it views its 2017 list of 1300 waters (2017 MPCA list) as the minimum universe of waters subject to the wild rice beneficial use and but for state law curtailing the Agency's authority to list waters as impaired, MPCA would have included seven of these waters on its 2020 list of impaired waters.[33] We further note that the 2018 Chief ALJ Order, while faulting MPCA's list as too narrow, did not find that MPCA was mistaken in concluding that MPCA's 2017 list of 1300 waters *were* subject to the beneficial use. Therefore, EPA is revising our previous interpretation of Minn. R. 7050.0224 to be consistent with MPCA's statement that its 2017 list of 1300 waters is the minimum list of waters to which the wild rice beneficial use applies.

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[29] Wild Rice Water Quality Standards, Chapter 4 – S.F. No. 5 (2015, 1st Special Session) (Subsection (a)(2): "the agency shall not list waters containing natural beds of wild rice as impaired for sulfate under section 303(d) of the federal Clean Water Act, United States Code, title 33, section 1313, until the rulemaking described in this paragraph takes effect."); Sulfate Effluent Compliance, Ch. 165, S.F. No. 3376 (2016, Regular Session).
[30] MPCA, Environmental Analysis and Outcomes Divisions, *Notice of Withdrawn Rules for Proposed Rules Amending the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Waters; Revisor's ID Number 4324* (April 26, 2018), https://www.pca.state.mn.us/sites/default/files/wq-rule4-15oo.pdf (last visited 4/24/2021).
[31] See, e.g., President Biden, Memorandum on Tribal Consultation and Strengthening Nation-to-Nation Relationships, January 26, 2021, https://www.whitehouse.gov/briefing-room/presidential-actions/2021/01/26/memorandum-on-tribal-consultation-and-strengthening-nation-to-nation-relationships/ (last visited 4/24/2021); Executive Order 13175 (65 FR 67249, November 9, 2000); Presidential Memorandum: Government-to-Government Relations with Native American Tribal Governments (59 FR 22951, April 29, 1994); EPA Policy for the Administration of Environmental Programs on Indian Reservations (November 8, 1984).
[32] Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021.
[33] Id.

Because we acknowledge that the universe of waters potentially subject to the beneficial use may be greater than MPCA's 2017 list of 1300 waters and requires more time to thoroughly review and analyze the information that we have received on this issue, we are specifically taking no action to approve or disapprove the State's exclusion from the 2020 Section 303(d) list any other potential wild rice waters or sulfate impairments at this time. Information received to date, with relevant notes, is found in Appendices 3-5. EPA also recognizes that there may be other information in the possession of stakeholders relevant to identifying those waters subject to the "waters used for production of wild rice" beneficial use and associated water quality data. EPA will complete our analysis of the input received from Tribal Governments, along with any additional information received during this public notice and comment period and will issue a supplemental decision addressing any additional sulfate-impaired wild rice waters, as appropriate. If EPA identifies additional waters used for the production of wild rice for which there are sulfate impaired segments, EPA will provide an additional 30-day period for public review and comment on those WQLSs. EPA will provide the exact schedule and deadlines for receiving public comment at the time EPA publishes our notice, as appropriate.

B. The Eight Waters Considered by MPCA

As discussed above, EPA views MPCA's affirmation in its March 15, 2021 letter as evidence that MPCA considers those eight waters as waters subject to the beneficial use in Minn. R. 7050.0224, subpart 2; separate from the 24 waters referenced at Minn. R. 7050.0224, subpart 1. These waters are identified in Table 1, below.

Table 1: Eight waters affirmed by MPCA as "waters used for the production of wild rice," in the March 15, 2021 letter

Waterbody Name	Assessment Unit Identifying Number (AUID)
Birch Lake	69-0003-00
Embarrass River	04010201-579
Embarrass River	04010201-A99
Embarrass River	04010201-B00
Little Sandy Lake	69-0729-00
Partridge River	04010201-552
Pike River	09030002-503
Sand River	09030002-501
Sandy Lake	69-0730-00
Second Creek	04010201-952

Tribal Leaders have unequivocally communicated to EPA that these eight waters were given to MPCA "as examples and based upon our access to MPCA data,"[34] (emphasis in original) and that Minnesota Tribal representatives followed their submittal of these eight waters with an analysis of a larger set of "21 (or 50 segments) in May, as examples and based upon our access to MPCA data."[35]

[34] Email from April McCormick, Secretary Treasurer, Grand Portage Band of Lake Superior Chippewa, to JoAnn Chase and others, "Consultation with EPA: 303(d) list MPCA submittal for 2020," March 17, 2021, and attachments. See also letter from Robert Larson, Chair, Minnesota Indian Affairs Council, to Miranda Nichols, MPCA, March 11, 2021.
[35] Id. (noting "that the Excel spreadsheet attached here is part of the Grand Portage letter of May 8, 2020. All signatory tribes in later correspondence confirmed that letter reflected the joint tribal position—namely, that according to MPCA's own data and methodology, there are at least 21 known, impaired wild rice waters that should be listed on the 2020 303(d) List.").

C. Additional Waters Identified for Inclusion on the Section 303(d) List that are on the 2017 MPCA List

As noted above, EPA is revising our previous interpretation of Minn. R. 7050.0224 to be consistent with MPCA's statement that its 2017 list of 1300 waters is the minimum list of waters to which the wild rice beneficial use applies. Through EPA's consultation, Tribes identified additional waters as potential candidates for listing, some of which appear on MPCA's 2017 list. Information regarding these waters is set out in comments EPA received related to our tribal consultation, listed in Appendix 3. These waters were reviewed using EPA's screening analysis (Section III of this Decision Document) and the results of that review are reflected in Appendix 2.

D. Additional Waters that are not on the 2017 MPCA List

Through EPA's consultation with Tribes, additional waters outside the universe of the 2017 MPCA list of 1300 waters were also identified by Tribes (Appendix 3). Because we acknowledge that the universe of waters potentially subject to the beneficial use may be greater than the list of 1300, we are specifically taking no action to approve or disapprove the State's exclusion from its Section 303(d) list any other potential wild rice waters or sulfate impairments at this time. Information received to date, with relevant notes, is found in Appendices 3-5. EPA also recognizes that there may be other information in the possession of stakeholders relevant to identifying those waters subject to the "waters used for production of wild rice" beneficial use and associated water quality data. EPA will complete our analysis of the input received from Tribal Governments, along with any additional information received during this public notice and comment period and will issue a supplemental decision addressing any additional sulfate-impaired wild rice waters, as appropriate. If EPA identifies additional waters used for the production of wild rice for which there are sulfate impaired segments, EPA will provide an additional 30-day period for public review and comment on those WQLSs. EPA will provide the exact schedule and deadlines for receiving public comment at the time EPA publishes our notice, as appropriate.

III. EPA's Screening Analysis for Assessing Waters to Add to the Minnesota 2020 Section 303(d) List

A. Consideration of Existing and Readily Available Water Quality-Related Data and Information

Since our review of Minnesota's 2012 section 303(d) list, EPA has requested that MPCA develop a methodology for assessing sulfate impairments associated with the wild rice beneficial use.[36] MPCA has never developed such a methodology.[37] MPCA has recently stated, however that "Any formalized methodology would include detailing which waters MPCA considers to be waters used for the production of wild rice and the evaluation of data for comparison to the 10 mg/L sulfate criterion."[38]

[36] See EPA 2012 Decision Document at 29-31; EPA 2014 Decision Document, Appendix 1 at 9-11; EPA 2016 – 2018 Decision Document at 30.
[37] Letter from Katrina Kessler to Tera Fong, March 15, 2021.
[38] Id.

Additionally, during consultation with Tribal Leaders, EPA received input regarding recommendations for developing screening methodologies.[39]

EPA considered existing and readily available sulfate data from the following sources:

- EPA's Water Quality Portal (WQP) (https://www.waterqualitydata.us//);
- EPA's How's My Waterway (https://www.epa.gov/waterdata/hows-my-waterway);
- Minnesota's publicly accessible water quality data from MPCA's Surface Water Data Portal (https://webapp.pca.state.mn.us/surface-water/search) and, MPCA's Surface Water Mapping Tool; (https://mpca.maps.arcgis.com/apps/webappviewer/index.html?id=c3ad23220f60416fadcc117f82ba05e3);
- Quality Assured and Quality Controlled (QA/QC'd) data sets from the Metropolitan Council (i.e., Met Council), United States Geological Survey (USGS), and MPCA's TEMPO database (permittee data);
- Water quality data shared by Tribes with supporting documentation of quality assurance/quality control for the data provided to EPA as listed in Appendix 3; and
- Water quality data shared by outside parties, as listed in Appendix 4.

Because of the short statutory timeframe given for EPA's action and the complexity of these issues, EPA expects to provide further clarification and respond to comments, as appropriate, on this approach.

In developing our methodology for screening information related to waters where MPCA has stated that the beneficial use applies (i.e., the MPCA 2017 list of 1300 waters) or other waters submitted to EPA for review, EPA considered the information described below for our analyses of sulfate data[40] for lake and stream segments. We also note that during Tribal consultation, EPA received extensive comments from Tribal representatives regarding recommendations for developing a screening methodology, as noted above. EPA considered this information, together with information from MPCA and others, in developing our screening methodology for today's action. We expect to issue a supplemental decision document to address public comments received during the comment period and will provide further clarification on our screening methodology as needed at that time.

Period of Record:

EPA considered sulfate data collected within the 10-year period (2008-2018), specifically during the time period of October 1, 2008 to September 30, 2018. In circumstances when there were minimal sulfate data available between October 1, 2008 to September 30, 2018, EPA reviewed existing and readily available sulfate data collected in the year preceding (2007-2008) and the year following (2019) the October 2008 to September 2018 time period on a case-by-case basis in order to more completely characterize sulfate conditions in lake and stream segments over the previous 10 year period and to

[39] See, e.g., Email from Sara Van Norman, on behalf of Grand Portage Chair Deschampe, to Cheryl Newton, "Consultation Comments on MN Wild Rice Waters to be Added to the 2020 impaired Waters List," April 8, 2021, enclosing letter from Norman Deschampe, Chairman, and April McCormick, Secretary/Treasurer, Grand Portage Band of Lake Superior Chippewa, to Cheryl Newton, Acting Regional Administrator, April 8, 2021, at 4-6; Email from Nancy Schuldt, Fond du Lac Band, to Cheryl Newton, "Fond du Lac input to EPA impaired wild rice waters draft list," April 19, 2021, at 2.

[40] EPA notes, in Section III and subsequent Sections of this Decision Document, whenever EPA uses the term "sulfate data," this term applies to water quality data that has documented and sufficient quality assurance and quality control.

assess as many waters used for the production of wild rice as possible. The 10-year period (2008-2018) is consistent with the time period which MPCA considered in developing its 2020 list.[41]

EPA also explored historical sulfate data (i.e., sulfate data collected outside of the suggested October 1, 2008 to September 30, 2018 time period) in order to understand the sulfate concentration trends over time. While EPA's assessment determinations generally examined existing and readily available sulfate data of the previous 10 year time period, EPA did account for consistent, historical exceedances of the 10 mg/L sulfate criterion in our overall review of water quality conditions for individual lake and stream segments. Where there were historical data for a particular lake or stream segment, this historical consideration involved looking at all of the data for a segment, regardless of year, to better understand the historical water quality conditions.

Seasonality:

The sulfate criterion to protect wild rice states that the 10 mg/L criterion is, "applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels." However, the scientific evaluation of sulfate conducted by MPCA to support its 2017 rule revisions found that wild rice is vulnerable to elevated sulfate concentrations year-round,[42] and the existing standards does not specify or define a time when wild rice is susceptible to damage by high sulfate levels. Therefore, EPA did not exclude sulfate data from consideration based on the season in which the data were collected.

Data Evaluation for Sulfate:

Typically, chemical specific water quality criteria are based on studies that expose a test organism to a range of concentrations of a toxicant to determine the relationship between the dose and the adverse effect in the organism.[43] Unlike typical chemical-specific criteria, Minnesota's existing 10 mg/L sulfate criterion is based on empirical observations correlating the presence and absence of wild rice stands with low levels of alkali salts, with wild rice tending to be present in waters with sulfate concentrations less than 10 mg/L, and absent in waters with concentrations greater than 10 mg/L.[44]

> In Minnesota, the chemical nature of the water seems to be the principle factor affecting the natural distribution of wild rice. This crop tolerates the entire carbonate (total alkalinity) range of Minnesota waters (5 to 250 p.p.m.), but is intolerant of sulphates [sic]. No large stands of rice occur in waters having a SO_4 content greater than 10 p.p.m., and rice generally is absent from water with more than 50 p.p.m.[45]

Minnesota's 2017 rulemaking described the Minnesota's 10 mg/L criterion this way:

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[41] MPCA, *Guidance Manual for Assessing the Quality of Minnesota Surface Waters for Determination of Impairment: 305(b) Report and 303(d) List, 2020 Assessment and Listing Cycle* (February 2021, at 10 (Period of Record), https://www.pca.state.mn.us/sites/default/files/wq-iw1-04k.pdf (last visited 4/27/2021).

[42] MPCA 2017 SONAR at 81-82.

[43] EPA, *Water Quality Standards Handbook*, Chapter 3, https://www.epa.gov/wqs-tech/water-quality-standards-handbook; (last visited 4/27/2021).

[44] See John B. Moyle, Wild Rice in Minnesota, 8 Journal of Wildlife Management (July 1944): 177-184, https://www.jstor.org/stable/3795695?seq=1#metadata_info_tab_contents (last visited 4/27/2021). MPCA's reviews of the relationship of sulfate to wild rice is publicly available: see, e.g., MPCA "Wild Rice Sulfate Standard Study" with associated links; https://www.pca.state.mn.us/water/wild-rice-sulfate-standard-study (last visited 4/27/2021).

[45] Id.

> The existing 10 mg/L standard was derived based largely on data collected in the 1930s and 1940s, which showed a correlation between areas where wild rice grew and areas with lower levels of sulfate in the water.[46]

As noted in Chapter 3 of EPA's *Water Quality Standards Handbook*, water quality criteria are comprised of three elements: a magnitude (the maximum allowable concentration of the pollutant), a duration (i.e., over what period of time should data be averaged to appropriately evaluate exposure and risk) and a return frequency (i.e., how often can the criterion be exceeded without resulting in an impairment of the use the criterion is intended to protected).[47] Minnesota's existing water quality criterion only specifies the magnitude of the criterion (i.e., 10 mg/L) and does not address either the duration or return frequency. In its proposed rulemaking, Minnesota attempted to establish a magnitude, duration and return frequency, proposing an annual average as the duration and a return frequency of no more than once in ten years:

> In general, numeric water quality standards (also called numeric water quality criteria) include three components: magnitude, duration, and frequency [citation omitted]. The number itself is the magnitude, 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 current wild rice sulfate standard sets a very clear magnitude (10 mg/L). However, it is vague about the duration of the standard ("during periods when the rice may be susceptible to damage by high sulfate levels") and does not speak to the frequency of the standard. The proposed revisions specify a magnitude, define the duration as an annual average, and set a one in ten-year frequency.[48]

In light of the analysis of its criterion contained in MPCA's 2017 SONAR, EPA applied similar principles to evaluating the available toxicity data for waters used for the production of wild rice while also trying to use as much of the existing and readily available data as possible to assess the attainment status of as many wild rice waters as possible. EPA evaluated whether there was an exceedance of the numeric 10 mg/L sulfate criterion generally relying on the following minimum data requirements consistent with a focus on long-term sulfate concentrations and a one in 10 year return frequency:

- EPA generally considered data collected over the time period of October 1, 2008 to September 30, 2018, consistent with Minnesota's 2020 Guidance Manual; and
- EPA identified sites as impaired if the data showed consistent exceedances of the sulfate criterion over time to be generally consistent with Minnesota's recommendation of an annual average, while also being sensitive to limitations on the available data:
  - The sulfate dataset included sulfate data from two separate years, at a minimum, within the time period;
  - The sulfate dataset included five individual sulfate samples; and
  - The sulfate dataset demonstrated consistent exceedance of the numeric 10 mg/L sulfate criterion during the data collection time period (2008-2018) approximating Minnesota's

[46] MPCA 2017 SONAR at 65.
[47] EPA, *Water Quality Standards Handbook*, Chapter 3,https://www.epa.gov/wqs-tech/water-quality-standards-handbook (last visited 4/27/2021).
[48] MPCA 2017 SONAR at 15.

annual average, i.e., more than half of the individual sulfate samples in the total dataset (after averaging multiple samples in a single day) for a lake or a stream segment should be greater than 10 mg/L.

During EPA's review of readily available and existing sulfate data, EPA did consider sulfate data sets with less than five individual sulfate samples. Additionally, EPA considered sulfate data sets which demonstrated less than half of the individual sulfate samples were greater than 10 mg/L. For certain lake and stream segments, EPA considered the historical sulfate data during our review of sulfate data. EPA conducted this historical review on a case-by-case basis in order to best understand the water quality conditions over time. Moreover, EPA, in certain instances, considered water quality conditions in upstream and downstream segments of certain lakes and stream segments. These considerations were intended to aid in EPA's characterization of sulfate water quality conditions in certain hydrologic systems.

Determination of impaired condition:

EPA's analyses of existing and readily available sulfate data for individual lake and stream segments incorporated the following screening analysis:

- The number of total observations per assessment unit (i.e., AUID);
- If there were multiple samples collected on the same day at the same sampling location, then EPA averaged those samples to one sulfate concentration to represent that day/location, consistent with MPCA's approach outlined in its March 15 communication;
- If there were multiple samples collected on the same day, in the same AUID, but at different sampling locations/stations, then EPA used the maximum sample value collected from all of the stations in that AUID to represent the sulfate concentration for that AUID, consistent with MPCA's approach outlined in its March 15 communication; and
- Statistical analyses EPA generated to better understand the duration and frequency components of the existing and readily available sulfate concentration data set for each site included:
  - The number of total observations greater than the numeric 10 mg/L sulfate criterion;
  - A percentage calculation of the number of total observations in that AUID which are greater than the numeric 10 mg/L sulfate criterion;
  - The calculated mean and the standard deviation of the sulfate data set for that AUID; and
  - The minimum and maximum values of the sulfate data set for that AUID.

EPA considered all the above characteristics in our screening analysis of existing and readily available sulfate data. We chose not to focus solely on one component of the screening analysis to determine impairment; rather we took a holistic view of the existing and readily available sulfate concentration data. Where EPA deviated from these procedures, we have so noted in Appendix 2. As noted above, we expect to issue a supplemental decision document to address public comments received during the comment period and will provide further clarification on our screening methodology as needed at that time.

IV. Tribal Consultation

Pursuant to Executive Order 13175, *Consultation and Coordination with Indian Tribal Governments* and with the *EPA Policy on Consultation and Coordination with Indian Tribes (May 2011)*,[49] EPA engaged in tribal consultation on our review of additional waters to be considered for finalizing the 2020 Minnesota Section 303(d) list action.[50] On April 9, 2021, EPA hosted a consultation call with ten federally-recognized Tribes from Minnesota and representatives from two tribal organizations to obtain tribal input on EPA's addition of impaired wild rice waters to Minnesota's 2020 Section 303(d) list following EPA's partial disapproval of the list on March 26, 2021.

Tribes also submitted written comments to EPA.[51] EPA accepted tribal comments and input in the development of our list of waters that EPA added to the Minnesota 2020 Section 303(d) list (Appendix 2 of this Decision Document).

Since at least 2012, Minnesota Tribal Leaders and representatives have raised to EPA their concerns that MPCA has not assessed or listed waters for the nonattainment of the numeric criterion for the sulfate water quality standard. The State's failure to assess and list these waters does not arise from an absence of tribal input into the State's Section 303(d) listing process. On the contrary, Tribal Leaders (as well as some stakeholders) have offered substantive comments to MPCA during each listing cycle on the lack of a methodology for assessing sulfate impairment and the lack of waters proposed for listing. EPA has, as noted above, also consistently urged the State to develop an assessment and listing process for these waters. EPA notes that the concerns of Tribal leaders have unfortunately, with respect to the assessment and listing of wild rice waters, gone virtually unaddressed by MPCA specifically and by Minnesota generally.[52]

Concerns raised by Tribal Leaders have only heightened over the past decade.[53] These concerns include:

- That MPCA's failure to list waters as impaired against its sulfate criterion is contrary to Section 303(d)[54];

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[49] *EPA Policy on Consultation and Coordination with Indian Tribes*, May 4, 2011. https://www.epa.gov/sites/production/files/2013-08/documents/cons-and-coord-with-indian-tribes-policy.pdf (last visited 3/23/21).

[50] EPA letter to Tribal Leaders, February 25, 2021.

[51] See Appendix 3: Compilation of Selected Documents Submitted by Tribes to EPA.

[52] See, e.g., Email from Sara Van Norman to Barbara Wester, "GP/Impaired Waters List: communication," March 18, 2021, including a chronology of the State of Minnesota Governor's office's communications with Minnesota Tribal Leaders regarding the 2020 Minnesota Section 303(d) list.

[53] For a compilation of concerns raised by Minnesota Tribal Leaders, in addition to the voluminous information that has been provided to EPA since 2012, see Appendix 3: Compilation of Selected Documents Submitted by Tribes to EPA.

[54] This position has been articulated in numerous comments. See, e.g., Tribal Leaders letter to Kurt Thiede, Regional Administrator, October 2, 2020; see also Tribal Leaders letter to Governor Tim Walz, October 2, 2020 ("MPCA must keep its promises to tribes and include known, impaired wild rice waters on the 2020 Impaired Waters List."); Tribal Leaders letter to Commissioner Laura Bishop, MPCA, April 27, 2020 ("Minnesota tribes have now made the same comments on four cycles of draft impaired waters lists. MPCA has repeatedly promised to include impaired wild rice waters in the 'next' cycle and has given ever-changing reasons for putting of this date."); EPA, Notes from April 9, 2021 Tribal Consultation Call Regarding Minnesota's 303(d) List.

- The ongoing failure to list waters as impaired against the State's sulfate criterion has harmed and continues to harm Tribal Members in their use of treaty reserved property rights[55];
- The protection of wild rice is a primary environmental justice issue for Native American citizens of Minnesota[56];
- Wild rice is a sacred food to the Chippewa/Ojibwe peoples and the State's current regulatory inaction threatens to further diminish an already impaired resource[57];
- EPA's federal trust responsibility supports EPA's efforts to list wild rice waters as impaired against the sulfate criterion to safeguard this treaty reserved resource[58];
- Any assessment and listing effort should take into account all available information, including the large body of information compiled by Tribes since at least 2012[59];
- MPCA should not be precluded from listing waters because of the lack of a formal assessment methodology[60]; and
- Any assessment and listing effort must include a concerted effort to increase MPCA's sampling effort to collect meaningful data regarding assessment of the universe of wild rice waters of interest to the Tribes.[61]

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[55] Tribal Leaders raised these issues in multiple communications. See, e.g., Tribal Leaders letter to Kurt Thiede, Regional Administrator, October 2, 2020; Tribal Leaders letter to Cheryl Newton, Acting Regional Administrator, March 3, 2021 and attachments; Letter from Benjamin Benoit, Leech Lake Band of Ojibwe, Environmental Director, to Tera Fong, et al., April 16, 2021, and attachments.

[56] Tribal Leaders raised these issues in multiple communications. See, e.g., Tribal Leaders letter to Cheryl Newton, Acting Regional Administrator, March 3, 2021 and attachments.

[57] Tribal Leaders raised these issues in multiple communications. See, e.g., Email from Sara Van Norman, on behalf of Grand Portage Chair Deschampe, to Cheryl Newton, "Consultation Comments on MN Wild Rice Waters to be Added to the 2020 impaired Waters List," April 8, 2021, enclosing letter from Norman Deschampe, Chairman, and April McCormick, Secretary/Treasurer, Grand Portage Band of Lake Superior Chippewa, to Cheryl Newton, Acting Regional Administrator, April 8, 2021, with attachments.

[58] Tribal Leaders raised these issues in multiple communications. See, e.g., id., and Email from Sara Van Norman to Cheryl Newton, Acting Regional Administrator, enclosing attachments to the Tribal Leaders' letter to EPA of March 3, 2020. ("The EPA has a trust responsibility to tribes and their members. We urge you to protect clean water and manoomin (in Ojibwe)—psin (in Dakota)—wild rice for future generations of our tribal citizens, and for all Minnesotans.").

[59] Tribal Leaders raised these issues in multiple communications. See, e.g., Letter from Shelley Buck, President, Prairie Island Indian Community, to Tera Fong, R5 Water Division Director, April 16, 2021; Email from April McCormick, Secretary Treasurer, Grand Portage Band of Lake Superior Chippewa, to JoAnn Chase and others, "Consultation with EPA: 303(d) list MPCA submittal for 2020," March 17, 2021, and attachments; Email from Sara Van Norman, on behalf of Grand Portage Chair Deschampe, to Cheryl Newton, "Consultation Comments on MN Wild Rice Waters to be Added to the 2020 impaired Waters List," April 8, 2021, enclosing letter from Norman Deschampe, Chairman, and April McCormick, Secretary/Treasurer, Grand Portage Band of Lake Superior Chippewa, to Cheryl Newton, Acting Regional Administrator, April 8, 2021, with attachments.

[60] Tribal Leaders raised these issues in multiple communications. See, e.g., Letter from J. Catherine Chavers, President, Minnesota Chippewa Tribe, to Miranda Nichols, MPCA, January 8, 2020; Tribal Leaders letter to Cheryl Newton, Acting Regional Administrator, March 3, 2021.

[61] Tribal Leaders raised these issues in multiple communications. See, e.g., Letter from Nancy Schuldt, Water Projects Coordinator, Fond du Lac Environmental Program, to Cheryl Newton, Acting Regional Administrator, April 15, 2021.

V. Information Provided by Other Parties

On April 14, 2021, EPA received a letter from WaterLegacy that included water quality information related to waters potentially impaired for sulfate.[62] These waters are catalogued in Appendix 4.

VI. Conclusions

Waters assessed and determined to be impaired are found in Appendix 2.

EPA also recognizes that there may be other information in the possession of stakeholders relevant to identifying those waters subject to the "waters used for production of wild rice" beneficial use and associated water quality data. EPA will complete our analysis of the input received from Tribal Governments, along with any additional information received during this public notice and comment period and will issue a supplemental decision addressing any additional sulfate-impaired wild rice waters, as appropriate. If EPA identifies additional waters used for the production of wild rice for which there are sulfate impaired segments, EPA will provide an additional 30-day period for public review and comment on those WQLSs. EPA will provide the exact schedule and deadlines for receiving public comment at the time EPA publishes our notice, as appropriate. Because of the short statutory timeframe given for EPA's action and the complexity of these issues, EPA expects that at that time we will provide further clarification as needed on our action.

VII. Public Notice

EPA considered tribal input received by EPA on or before April 19, 2021 in developing the list of waters that we are publishing today.

Pursuant to 40 C.F.R. § 130.7(d)(2), EPA will issue a public notice providing for a 30-day public comment period regarding the addition of the 30 sulfate-impaired waters to Minnesota's Section 303(d) List in Appendix 2. The public is invited to provide comment on the details included in this Decision Document and to present any additional information which may be relevant to this topic and EPA's action.

Commenters interested in sharing comments about individual waters should include the following information in their comment to EPA:

- Clear identification of the assessment unit identification (or AUID), for the stream segment or lake to be added or considered by EPA;
- Explanation of and evidence for whether waters are or are not "waters used for the production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels" as that designation is used in Minnesota's existing water quality standards at Minn. R. 7050.0224;

[62] Email from Paula Maccabee, WaterLegacy, "Data Pertaining to EPA Listing of 2020 Minnesota Wild Rice Waters," with attachments.

- What water quality data supports that specific waters are or are not in exceedance of the 10 mg/L sulfate criteria (e.g., Minnesota data from its Surface Water Data portal or other data sources); and
- Any documentation of quality assurance/quality control for sulfate data provided to EPA.

Following the close of the 30-day public comment period, EPA will consider all the input received, make any appropriate revisions, and transmit any final additions to Minnesota. EPA will respond to input received from Tribes through tribal consultation and any public comments received during the public comment period.

For access to documents included in Appendices 3-5, interested parties should contact Paul Proto (proto.paul@epa.gov) and EPA will provide a link to a FTP website where documents referenced in Appendices 3-5 can be downloaded.

Appendices

Appendix 1: MPCA's List of approximately 1,300 proposed wild rice waters (updated April 2021)

Appendix 2: Waters EPA is adding to the Minnesota 2020 Section 303(d) List (April 26, 2021)

Appendix 3: Compilation of Selected Documents Submitted by Tribes to EPA

Appendix 4: Compilation of Information Submitted by Outside Parties

Appendix 5: Other Information Relevant to this Action

Appendix 3: Compilation of Selected Documents Submitted by Tribes to EPA

1. EPA Decision Document for Minnesota's 2012 Section 303(d) List, July 25, 2013 – Response to Tribal Comments and Tribal Comments received.
2. EPA, *Decision Document for the Approval of Minnesota's 2014 Clean Water Act Section 303(d) List* (May 29, 2018) – Response to Tribal Comments and Tribal Comments received.
3. EPA, Decision Document for the Approval of Minnesota's 2016 and 2018 Clean Water Act Section 303(d) Lists (January 28, 2019) - Response to Tribal Comments and Tribal Comments received.
4. Letter from Catherine J. Chavers, President, Minnesota Chippewa Tribe, to Miranda Nichols, MPCA, January 8, 2020.
5. Tribal Leaders letter to Kurt Thiede, Regional Administrator, October 2, 2020.
6. Letter from Kurt Thiede, Regional Administrator to Tribal Leaders, October 30, 2020.
7. Tribal Leaders letter to Cheryl Newton, Acting Regional Administrator, March 3, 2021.
8. Email from Sara Van Norman to Cheryl Newton, Acting Regional Administrator, enclosing attachments to the Tribal Leaders' letter to EPA of March 3, 2020.
9. Letter from Melanie Benjamin, Chief Executive, Mille Lacs Band of Ojibwe, to Cheryl Newton, Acting Regional Administrator, March 12, 2021.
10. Email from April McCormick, Secretary Treasurer, Grand Portage Band of Lake Superior Chippewa, to JoAnn Chase and others, "Consultation with EPA: 303(d) list MPCA submittal for 2020," March 17, 2021, and attachments.
11. Email from Sara Van Norman to Barbara Wester, "GP/Impaired Waters List: communication," March 18, 2021.
12. EPA, *Decision Document for The Partial Approval of Minnesota's 2020 Clean Water Act Section 303(d) List*, March 26, 2021.
13. Letter from Tera L. Fong, EPA, to Katrina Kessler, MPCA, March 9, 2021.
14. Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021.
15. Email from April McCormick, Secretary Treasurer, Grand Portage Band of Lake Superior Chippewa, to JoAnn Chase and others, March 17, 2021, and attachments.
16. Email from Sara Van Norman, on behalf of Grand Portage Chair Deschampe, to Cheryl Newton, "Consultation Comments on MN Wild Rice Waters to be Added to the 2020 impaired Waters List," April 8, 2021, enclosing letter from Norman Deschampe, Chairman, and April McCormick, Secretary/Treasurer, Grand Portage Band of Lake Superior Chippewa, to Cheryl Newton, Acting Regional Administrator, April 8, 2021, with attachments.
17. EPA Notes from Tribal Consultation Call Regarding Minnesota's 2020 CWA 303(d) List.
18. Letter from Nancy Schuldt, Water Projects Coordinator, Fond du Lac Environmental Program, to Cheryl Newton, Acting Regional Administrator, April 15, 2021, and attachments.
19. Letter from Benjamin Benoit, Leech Lake Band of Ojibwe, Environmental Director, to Tera Fong, et al., April 16, 2021, and attachments.
20. Letter from Shelley Buck, President, Prairie Island Indian Community, to Tera Fong, R5 Water Division Director, April 16, 2021.

21. Email from Nancy Schuldt, Fond du Lac Band, to Cheryl Newton, “Fond du Lac input to EPA impaired wild rice waters draft list,” April 19, 2021, with attachments.
22. Email from Eric Krumm, Leech Lake Band of Ojibwe, “Leech Lake Band of Ojibwe Comment Letter on the Partial Rejection of Minnesota’s 303(d) List and Lists of Wild Rice Waters for Sulfate Review, April 19, 2021, with attachments.
23. Email from Melanie Benjamin, Chief Executive, Mille Lacs Band of Ojibwe, to Cheryl Newton, “Mille Lacs Band of Ojibwe – attachment,” April 20, 2021.

Appendix 4: Compilation of Information Submitted by Outside Parties

April 9, 2021 Email from WaterLegacy

Attachments to Email:

- Sulfate data summaries-All WIDs Spreadsheet
- Potential Additional WR Waters SulfateDataSummary Spreadsheet

April 14, 2021 Email from WaterLegacy

Attachments to Email:

- Process of Analysis (Word document)
- WL Lakes Spreadsheet (MPCA Sulfate Data Summaries, WRW Lists)
- WL Rivers Spreadsheet (MPCA Sulfate Data Summaries, WRW Lists)
- WL Additional Lakes Spreadsheet (Notes on MPCA Spreadsheet)
- Attachment A MPCA 2017 Draft WRW List (Advisory Committee)
- Attachment B MPCA 2013 Draft WRW Impaired Waters List (Advisory Committee)
- Attachment C MPCA Surface Water Data Download (Specific Lakes and Streams)
- MPCA Spreadsheets Received April 9, 2021

Appendix 5: Other Information Relevant to this Action

1. MPCA, Statement of Need and Reasonableness (SONAR), *Amendment of the sulfate water quality standard applicable to wild rice and identification of wild rice waters*, July 2017. (https://www.pca.state.mn.us/water/protecting-wild-rice-waters)

2. MPCA, Environmental Analysis and Outcomes Divisions, *Notice of Withdrawn Rules for Proposed Rules Amending the Sulfate Water Quality Standard Applicable to Wild Rice and Identification of Wild Rice Waters; Revisor's ID Number 4324* (April 26, 2018). (https://www.pca.state.mn.us/sites/default/files/wq-rule4-15oo.pdf)

3. EPA Decision Document for Minnesota's 2012 Section 303(d) List (July 25, 2013).

4. EPA, Decision Document for the Approval of Minnesota's 2014 Clean Water Act Section 303(d) List (May 29, 2018).

5. EPA, Decision Document for the Approval of Minnesota's 2016 and 2018 Clean Water Act Section 303(d) Lists (January 28, 2019).

6. Letter from Tera L. Fong, EPA, to Katrina Kessler, MPCA, March 9, 2021.

7. Letter from Katrina Kessler, MPCA, to Tera L. Fong, EPA, March 15, 2021, with Attachments 1 and 2.

8. EPA Decision Document for The Partial Approval of Minnesota's 2020 Clean Water Act Section 303(d) List, (March 26, 2021).