

Friday, January 12, 2024

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**RE: Minnesota 2024 Clean Water Act Section 303(d) List of Impaired/Total Maximum Daily Load Waters**

Dear Miranda Nichols:

On behalf of Northeastern Minnesotans for Wilderness (“NMW”), thank you for the opportunity to comment on Minnesota’s 2024 draft 303(d) list. NMW is a 501(c)(3) non-profit corporation founded in Ely by residents of northeastern Minnesota. Since 1996, NMW has worked to protect the Boundary Waters Canoe Area Wilderness (“Boundary Waters”), its watershed, the Rainy River-Headwaters, and Voyageurs National Park, as well as to foster appreciation and support for preservation of wilderness and wild places.

NMW supports and incorporates by reference the 303(d) comments of WaterLegacy submitted to Minnesota Pollution Control Agency (“MPCA”) today. MPCA is right to identify a much larger set of wild rice waters, and to recognize 20 more wild rice waters as impaired for sulfate. Birch Lake (WID 69-0003-00) and the lowest reach of the Dunka River (WID 09030001-987) are at last and necessarily on the list. All should be on the final 303(d) list MPCA sends to the U.S. Environmental Protection Agency (“EPA”) for review and approval. NMW thanks MPCA staff for their work on the above.

NMW is disappointed, however, that MPCA intends to exclude Birch Lake and other wild rice waters impaired for sulfate from point sources from its priority list for Total Maximum Daily Load (TMDL) development in the 2024-2026 biennium. MPCA put a “2” in Column N of the [draft 2024 303\(d\)](#) list for each of the 55 sulfate-impaired wild rice waters (35 previously listed and the 20 now added). Only a “1” in Column N indicates an MPCA priority for TMDL development and waste load allocation.

MPCA should see that after having avoided enforcement of the wild rice sulfate standard for decades it is not sufficient now to list Birch Lake, but must expedite next steps in the Clean Water Act’s impaired waters restoration framework. NMW urges MPCA to send EPA a 2024 303(d) list that re-prioritizes Birch Lake and other sulfate-impaired wild rice waters downstream from industrial point sources for TMDL development this biennium.

Doing so is necessary and will yield important improvements in water quality not only with respect to wild rice, but also will reduce methyl-mercury production and mercury in

fish tissue because, as research and the MPCA's own findings confirm, sulfate plays a key role in methylation of mercury in aquatic environments where the oxygen availability is low.<sup>1,2,3,4</sup> MPCA must not make the mistake of asserting that dissolved organic carbon better alone better explains methylmercury data than does dissolved organic carbon and sulfate; it does not. MPCA knows well that Birch Lake and other waters downstream, including White Iron Lake, Farm Lake, Garden Lake, and Fall Lake, when last assessed, all had fish-tissue mercury loads greater than 0.572 mg Hg/kg fish tissue,<sup>5</sup> and thus require more action to be taken than implementation of the Statewide Mercury TMDL to achieve compliance. Prioritizing Birch Lake and other sulfate-impaired wild rice waters downstream from industrial point sources for TMDL development this biennium would help. and MPCA should seize the opportunity in 2024.

Thank you very much for the opportunity to comment. Note that PDFs of all footnoted documents are included/submitted with this comment.



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Donna Keclik, [donna.keclik@epa.gov](mailto:donna.keclik@epa.gov)

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<sup>1</sup> MPCA. 2006-10-19. MPCA Strategy to Address Indirect Effects of Elevated Sulfate on Methylmercury Production and Phosphorus Availability.

<sup>2</sup> Myrbo, A., E.B. Swain, N.W. Johnson, D.R. Engstrom, J. Pastor, B. Dewey, P. Monson, J. Brenner, M. Dykhuizen Shore, and E.B. Peters. 2017. Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. *Journal of Geophysical Research: Biogeosciences*, 122, 2769–2785.

<sup>3</sup> Coleman Wasik, J.K., D.R. Engstrom, C.P.J. Mitchell, E.B. Swain, B.A. Monson, S.J. Balogh, J.D. Jeremiason, B.A. Branfireun, R.K. Kolka, and J.E. Almendinger. (2015). The effects of hydrologic fluctuation and sulfate regeneration on mercury cycling in an experimental peatland. *J. Geophys. Res. Biogeosci.*, 120, 1697—1715.

<sup>4</sup> Jeremiason, J.D., Engstrom, D.R., Swain, E.B., Nater, E.A., Johnson, B.M., Almendinger, J.S., Monson, B.A., & Kolka, R.K. (2006). Sulfate Addition Increases Methylmercury Production in an Experimental Wetland. *Environmental Science & Technology*. (40) 3800-3806.

<sup>5</sup> MPCA. June 2022. Rainy River-Headwaters Watershed Restoration and Protection Strategy Report. See p. 13, Figure 4. Aquatic consumption impairments in the Rainy River-Headwaters Watershed.

# Footnoted Document #1

## **MPCA Strategy to Address Indirect Effects of Elevated Sulfate on Methylmercury Production and Phosphorus Availability**

**Summary:** Although there is evidence that elevated sulfate loading can increase methylmercury production and phosphorus mobilization, it is premature to develop specific sulfate concentration limits or other regulatory responses based on these effects. The deleterious effects of sulfate may be restricted to certain areas of the state, certain background sulfate concentrations, or other environmental controlling factors. These factors will be explored in a multi-year data collection effort combined with ongoing data analysis. It is anticipated that sensitive areas of the state will be identified and appropriate controls on sulfate discharges will be developed if necessary. The primary focus of the strategy is to pursue research to further understand impacts from sulfate on methylmercury production and phosphorus mobilization and to use the research to guide the future need for additional requirements or controls in environmental review and NPDES permits. This strategy was approved by the MPCA Risk Managers on August 28, 2006 and the MPCA WQ Policy Forum on October 19, 2006.

**Problem Statement:** Research indicates a correlation between sulfate loading and methylmercury (MeHg) production and phosphorus (P) mobilization under certain conditions. Many waters of the state are impaired as a result of MeHg in fish tissues and excess nutrients. MPCA staff need to better understand the relationship between sulfate concentration and MeHg production/P mobilization so that appropriate responses, if necessary, can be developed. Sulfate is a common constituent in domestic and industrial wastewaters. Additional information is needed so that the MPCA can develop a permitting strategy for existing, expanding and new domestic and industrial process wastewater discharges. The strategy must reflect varying MeHg production and P availability under differing environmental conditions.

### **MPCA Actions to Monitor & Evaluate Sulfate Impacts**

MPCA staff will evaluate the following hypotheses over three to five years.

- 1) Elevated sulfate discharge into low-sulfate receiving waters significantly increases MeHg concentrations (as percent of total mercury) and P concentrations.
- 2) Elevated sulfate discharge into high-sulfate receiving waters has no significant effect on MeHg concentrations (as percent of total mercury) and P concentrations.
- 3) Elevated sulfate discharge into low-sulfate waters has greater effect on P concentrations when the iron to P ratio is low in the sediments of the receiving water.

Environmental Analysis and Outcomes Division will coordinate the following activities to evaluate the above hypotheses and support eventual changes in the environmental review and permitting practices:

- 1) Continued research at Wetland 6 in the Marcell Experimental Forest north of Grand Rapids;
- 2) Milestone Monitoring – permanently add sulfate, TOC, total mercury, and MeHg to the MPCA’s ambient water quality monitoring sites; (In FY07 Milestones did include THg, MeHg, sulfate, and TOC, through use of the Mercury Trends allotment).

- 3) Continue to track and participate in the research of national / international work groups;
- 4) Compile and map existing surface water sulfate concentration data in Minnesota;
- 5) Compile and map existing effluent sulfate concentration data in Minnesota;
- 6) Compile and map existing stormwater sulfate concentration data in Minnesota (if few data have been collected, consider obtaining representative data);
- 7) Fish Consumption Advisory Monitoring - Work with DNR and MDH to collect fish for mercury analysis of fish tissue at a subset of sites where environmental data is being collected on water or sediments;
- 8) Implement the Environmental Review and NPDES Permitting actions (below) Regional, Municipal and Industrial Divisions will lead as appropriate; and
- 9) Compile data from the above activities and complete an evaluation of the hypotheses.

### **Environmental Review and NPDES Permitting**

While research shows a relationship between sulfate concentration and MeHg production/P mobilization, there is currently insufficient information to reach firm conclusions on whether specific point source (non-stormwater) discharges containing sulfate may impact water quality or cause/contribute to water quality impairments. The following information will guide the development of programmatic direction and procedures to address sulfate discharges. This approach includes 1) further characterization of the problem, 2) development of interim permitting and environmental review procedures, 3) research of sulfate impacts from point source dischargers, and 4) annual incorporation of new knowledge into the permitting and environmental review procedures. Prior to development of the interim procedures, NPDES permit writers and environmental review staff will need to manage projects on a case-by-case basis. They will use the current knowledge (as outlined below and in Appendix A) and work with the program supervisor and Ed Swain to assess and respond to the environmental risk from sulfate discharges.

### **Environmental Review**

If a new or expanding domestic or industrial process wastewater discharge triggers environmental review for a wastewater-related threshold (not a non-wastewater related threshold) or if wet air controls that contribute sulfate to a wastewater stream are proposed the impact from sulfate must be evaluated in the environmental review document. The environmental review should include available data on projected effluent design flow rate, sulfate concentration, and sulfate load as well as best estimates of receiving water flow rate (7Q10 and other statistics) and concentrations of sulfate, mercury, MeHg, iron, ortho-P, total P, and, as a measure of organic matter in the water, TOC and/or DOC. If receiving water flow was measured concurrently with water sampling, flow data should also be included. The environmental review must also include available data on the organic matter, mercury, iron, and P content of the sediments of receiving waters and lakes or impoundments downstream. It is understood that available data may be limited. To the extent possible, qualitative discussion of downstream conditions and mitigative options should also be included.

## NPDES Permitting

If a new, expanding or existing domestic or industrial wastewater discharge for “high risk” situations is encountered, 1) the need for effluent and/or receiving water monitoring for sulfate, mercury, MeHg, iron, ortho-P and/or total P should be considered; and 2) if research or other information supports a likely impact from sulfate in a specific situation an evaluation of the treatment technologies and pollution prevention opportunities should be included with the permit application. Existing discharges will be addressed at the time of reissuance. A guidance for project proposers and NPDES permit writers will be developed by June 2007 to explain the procedures for addressing sulfate discharges. In the interim, permit writers will work with the program supervisor and Ed Swain to assess and respond to the environmental risk from sulfate discharges.

Currently, high-risk situations may include:

- Discharge of elevated sulfate concentrations into high-organic aquatic environments (e.g., wetlands that drain to fisheries, lakes with organic sediment, rivers with slow-moving back waters, ponds where rising water might inundate vegetation).
- Discharge of elevated sulfate into low-sulfate waters (< 40 ppm or so) where sulfate may be a limiting factor in the activity of sulfate-reducing bacteria (SRB).
- Discharge of elevated sulfate into streams with fluctuating water levels and bordering wetlands. Rising water levels would introduce sulfate into the high-organic wetland matrix, followed by falling water levels that hydraulically deliver elevated MeHg and/or phosphate to the stream.
- Discharge of elevated sulfate to waters that flow to a lake or impoundment downstream that may thermally stratify even temporarily in the summer or be cut off from the atmosphere from ice cover in the winter. Either stratification or ice cover can produce anoxic water, in which sulfate can be converted to sulfide, potentially enhancing both mercury methylation and phosphate release.

Conditions that decrease the risk that elevated sulfate loading may enhance mercury methylation:

- Discharge of elevated sulfate to waters with high background sulfate (>100 ppm or so), including downstream waters.
- Discharge of elevated sulfate to highly oxygenated, turbulent waters with low-organic sediment and no adjacent riparian or lacustrine wetlands, and none downstream.

## Research Impacts of Sulfate from Domestic and Industrial Process Wastewater Discharges

MPCA staff will pursue funding to study specific impacts from domestic and industrial process wastewater discharges of sulfate on MeHg production and P availability in receiving waters. The study (or series of smaller studies) will include site-specific evaluations at facilities representing the various high risk situations identified in “Environmental Review and NPDES Permitting” above. This work may include effluent and receiving water monitoring for sulfate, mercury, MeHg, iron, ortho-P, total P, and supporting parameters that may reveal biogeochemical mechanisms, such as DOC, pH, oxygen, nitrate, and potassium. The work will include an evaluation of the data to determine whether domestic and industrial process wastewater discharges are impacting receiving waters during any time of the year with a particular focus on the summer months. Some of the study work may need to be contracted out to a research entity

(i.e. UMD, NRRI, U of M St. Anthony, U of Toronto). Funding sources may include Legislative Initiative, CW Legacy Act, GLNPO, salary savings, or other related project savings.

#### Action Items / Resource Needs

- 1) Risk Managers need to select an EAO Division representative to coordinate the overall Sulfate Strategy **by August 28, 2006**. Action Complete: Marvin Hora will be overall coordinator.
- 2) Sulfate Strategy Coordinator (Marvin Hora) will work with the appropriate managers to recommend staff team members to develop guidance documents described in the Environmental Review and NPDES Permitting action items below **by September 25, 2006**. Recommendation: Team should include Ed Swain, Jeff Stollenwerk, Deb Lindlief, Dana Vanderbosch, Bruce Wilson and a GIS specialist (see MPCA Actions 4 & 5 above).
- 3) Water Policy Team reviews and approves the Sulfate Strategy including staff assignments **by October 31, 2006**. Jeff Stollenwerk will coordinate.
- 4) EAO staff should develop funding requests, detailed plans and funding applications, RFPs and conduct study oversight necessary to complete research on impacts of sulfate from domestic and industrial process wastewater discharges. **Ed Swain - Ongoing**.
- 5) The Sulfate ER/NPDES Permitting staff team (from item 2 above) further defines and characterizes high-risk situations/criteria and develops interim procedures for environmental review and NPDES permitting activities. This action should be completed **by February 28, 2007**. Estimated time commitment – 40 to 80 hours for each team member.
- 6) The Sulfate ER/NPDES Permitting staff team (from item 2 above) develops brief guidance for project proposers and MPCA staff that provides background on the sulfate issue and factors that will need to be evaluated as part of the environmental review and/or permit process. Guidance should also address permitting projects that do not require environmental review. The team should develop procedure documents that will be included in the program manual for the environmental review and the NPDES Permit Writers' Manual. This document will provide background on the sulfate issue and issues that will need to be evaluated as part of the environmental review and/or permit process. These actions should be completed and presented to the WQ Policy Forum for review and approval **by June 29, 2007**. Estimated time commitment – 30 to 40 hours for each team member.
- 7) If necessary, revise the Illuminated EAW document and NPDES permit application to include background on the sulfate issue and issues that will need to be evaluated as part of the environmental review and NPDES permitting. These actions should be completed **by July 31, 2007**. ER Staff, Permit Staff and EAO staff – 10 hours each.
- 8) Complete technical review of environmental review submittals and NPDES permit applications. Develop responses to comments on specific projects. **Timeline is project-specific**. Environmental Review, Municipal/Industrial engineers and permit writers lead, and EAO staff support – workload could vary greatly.
- 9) Review research findings and if necessary incorporate into permitting and environmental review procedures. Sulfate ER/NPDES Permitting staff team (from item 2 above) 10 to 20 hours – **Annually**.

- 10) Provide technical assistance to permit writers regarding high-risk case-specific monitoring requirements and information protocols for targeted facilities or facility types. – EAO staff **as needed** – 40 to 80 hours per year.
- 11) Update agency managers on policy development needs, including needs to revise the sulfate standard - Strategy Coordinator – **Annually**.



## **Attachment A**

### **MPCA Strategy to Address Indirect Effects of Elevated Sulfate on Methylmercury Production and Phosphorus Availability**

#### **Technical Background**

Sulfur naturally cycles in aquatic systems between sulfate and sulfide, depending on multiple factors, including oxygen availability, hydrologic fluctuations, and organic matter degradation. Sulfate is a relatively inert chemical species, but its conversion to sulfide has a number of undesirable indirect effects that this strategy ultimately seeks to minimize. Under certain as-yet undefined environmental conditions, additional sulfate may enhance MeHg production and the availability of P for algal growth. The mechanisms associated with enhanced MeHg production and P availability are different, but are both associated with the tendency during decay of organic matter for natural bacteria to convert sulfate to sulfide after oxygen is depleted. This group of bacteria is called sulfate-reducing bacteria (SRB).

The initial tasks of the strategy involve collecting and interpreting data so that defensible quantitative permitting limits on sulfate discharge can be established. For instance, aquatic systems that are naturally elevated in sulfate due to local geological sources may not be sensitive to moderate increases in sulfate concentration. Other environmental attributes may make some systems more or less sensitive to added sulfate, including existence of wetlands and background dissolved iron concentrations.

Elevated sulfate can enhance MeHg production because SRBs are known to convert inorganic mercury (which is widely available due to atmospheric pollution) to MeHg, the only form that accumulates in fish. When the availability of sulfate controls the activity of SRBs, then additional sulfate may cause additional fish contamination. Recent research (Jeremiason et al. 2006) has documented increased MeHg production through increased sulfate concentrations in a wetland environment. SRBs produce MeHg when certain environmental factors coincide: low oxygen and adequate levels of bioavailable inorganic mercury, sulfate, and decaying organic matter. High organic matter can, of course, cause low oxygen because other bacteria will consume available oxygen in the first phases of organic matter degradation. SRBs are most active in aquatic systems because water decreases atmospheric oxygen availability and maintains a moist environment in which bacteria can thrive. SRB production of MeHg can be constrained by low mercury, low sulfate, low organic matter, or high oxygen. There is also a hypothesis that continued production of sulfide by SRBs can produce negative feedback by reducing mercury availability through the formation of sulfide-mercury chemical bonds. However, it is not clear how to model such negative feedback, and the production of sulfide is not necessarily permanent, as sulfide can oxidize back to sulfate. So, at this point, trying to maintain high sulfide does not seem like a viable strategy. However, data collection will provide empirical information on this hypothesis.

Elevated sulfate can enhance P availability because of an indirect effect of sulfide production. When aquatic systems become anoxic (common in both hypolimnia and wetlands) there is a tendency for enhanced P release from sediment to the water. While anoxic, iron oxides become soluble, which causes the dissolution of phosphate that had co-precipitated with the iron during an oxygenated phase. The phosphate will largely re-precipitate with the iron when the water is

oxygenated, unless the iron to phosphate ratio is too low. During anoxia, sulfide may be produced, which has the unfortunate ability to form a precipitate with the dissolved iron—unfortunate because elevated levels of sulfide can decrease the amount of iron that is available to co-precipitate the P. If the P is not precipitated upon oxygenation (either turnover of a lake or hydraulic movement in a wetland), then the additional P will likely stimulate algal growth above the historical range for that waterbody (Caraco et al. 1993).

Both of these indirect effects of elevated sulfate are difficult to model in a quantitative manner. One impediment is that the conversion to sulfide may be downstream from the site of sulfate discharge because the required combination of low oxygen and elevated organic matter may not occur immediately below the discharge. Sulfate conversion may occur when water flows laterally into adjacent wetlands or when the water reaches an impoundment or lake deep enough to have a hypolimnion. Enhanced loading of P and MeHg would occur when the anoxic water mixes back into surface water. This mixing would occur in a lake when the hypolimnion mixes with the epilimnion, and in rivers with lateral wetlands during a falling hydrograph.

Sulfate comes from a variety of sources. Generally, natural background sources result from marine rock and glacial till containing some marine rock such as limestone or shale. Surface water and ground water in the granitic Canadian Shield area is expected to have relatively low sulfate concentrations while waters in other parts of the state are expected to have relatively higher sulfate concentrations. Anthropogenic sources include air deposition (typically less than 1 mg/l) and domestic and industrial wastewater discharges. Wastewater sulfate concentrations can be elevated above surface water concentrations simply because of use of high-sulfate groundwater. In addition, sulfate may be elevated in wastewater by concentration through evaporation, capture of sulfur compounds by air pollution control equipment, or various industrial processes (e.g. lime addition in taconite production).

It is important to minimize the effect of sulfate on MeHg and P because Minnesota's water quality is threatened by these chemicals state-wide. Federal NPDES permitting regulations prohibit the authorization of wastewater discharges that may cause or contribute to water quality impairments. Numerous water bodies in the state are listed as impaired because the MeHg concentrations in fish tissues make the fish unsuitable for frequent human consumption. Similarly, numerous water bodies are impaired because of excess P concentrations.

Treatment technologies for sulfate removal from wastewaters are limited. Reverse osmosis and evaporation are energy intensive and generally considered infeasible. A new treatment technology, submerged packed bed, has shown potential but there is an unevaluated risk of MeHg production within the treatment system. Land application or rapid infiltration basins may be effective but must be evaluated on a case-by-case basis.

While research indicates a strong correlation between sulfate loading and MeHg production in a sulfate-poor wetland, the factors that control MeHg production and P release in other surface waters are not documented. The research results do not, however, tell us how aquatic systems higher in sulfate react to increased sulfate loading. We have not reached a sufficient level of confidence with our understanding of the controlling factors such that firm effluent limitations based on these phenomena can be established. Therefore, a permitting strategy will need regulatory and study/monitoring components to reflect our varying levels of understanding of MeHg production under differing environmental scenarios. MeHg study and control is further complicated by the lack of a standard EPA analytical method and limited commercial laboratories that are prepared to conduct MeHg analyses. EPA has developed Draft Method 1630 (January 2001) for MeHg analyses. The draft method can be found at:

<http://www.epa.gov/nerleerd/108Complete.pdf#search=%22mercury%20method%20methyl%201630%20site%3Aepa.gov%22>

and

<http://www.brooksrand.com/FileLib/1630.pdf>

MPCA staff have used Frontier Geosciences in Seattle, WA for recent analyses. It is anticipated that the MDH lab, and possibly other labs in Minnesota, would gear-up to run Draft Method 1630 if demand for this work increased.

**Notes: [since this note does not seem to be referred to anywhere, perhaps it should be moved up into the text.—otherwise, it is not contributing to the appendix]**

- 1) As a general rule, the order of depletion of electron acceptors during bacterial metabolism in aquatic systems is O<sub>2</sub>, NO<sub>3</sub>, Fe<sub>2</sub>O<sub>3</sub>, MnO<sub>2</sub>, then SO<sub>4</sub>. SRBs are known to produce MeHg and it is thought that iron-reducing bacteria may also methylate mercury under certain conditions. In any given environmental setting, it is not easy to determine which bacteria are dominating degradation of organic matter. To achieve an understanding of biogeochemical mechanisms of the effects of elevated sulfate, it may be desirable to measure a number of parameters, including sulfate, total mercury, MeHg, iron, ortho-P, total P, and supporting parameters such as DOC, pH, oxygen, nitrate, and potassium (for an example of the utility of measuring this suite of parameters, see Balogh et al. 2004). For instance, elevated nitrate or oxidized iron could negate the effect of elevated sulfate because the bacterial community likely finds it energetically advantageous to consume either of those two chemicals as electron acceptors before consuming sulfate. Without information on nitrate and iron, the effect of elevated sulfate may appear to be inexplicably unpredictable. Potassium data may be useful in a different way—elevated potassium can be an indicator of a hydraulic source area in decaying organic matter such as a wetland. When potassium is correlated over time with DOC, MeHg, and P, then the weight of evidence tends toward wetlands as the source area for all of the materials.

#### **Literature Cited:**

Balogh, S.J., Y. Nollet, and E.B. Swain. 2004. Redox Chemistry in Minnesota Streams during Episodes of Increased Methylmercury Discharge. *Environmental Science & Technology*. 38:4921-4927.

Caraco, N.F., J. J. Cole, and G. E. Likens. Sulfate control of phosphorus availability in lakes. *Hydrobiologia*. 253:275-280.

Jeremiason et al. 2006. Sulfate addition increases methylmercury production in an experimental wetland. *Environmental Science & Technology*. 40:3800-3806.

# Footnoted Document #2



## RESEARCH ARTICLE

10.1002/2017JG003788

This article is a companion to Myrbo et al. (2017), <https://doi.org/10.1002/2017JG003787> and Pollman et al. (2017), <https://doi.org/10.1002/2017JG003785>.

## Key Points:

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

## Supporting Information:

- Supporting Information S1
- Figure S1
- Data Set S1

## Correspondence to:

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## Citation:

Myrbo, A., Swain, E. B., Johnson, N. W., Engstrom, D. R., Pastor, J., Dewey, B., ... Peters, E. B. (2017). Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms. *Journal of Geophysical Research: Biogeosciences*, 122. <https://doi.org/10.1002/2017JG003788>

Received 25 JAN 2017

Accepted 6 SEP 2017

Accepted article online 25 SEP 2017

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

A. Myrbo<sup>1</sup> , E. B. Swain<sup>2</sup> , N. W. Johnson<sup>3</sup>, D. R. Engstrom<sup>4</sup>, J. Pastor<sup>5</sup>, B. Dewey<sup>5</sup>, P. Monson<sup>2</sup>, J. Brenner<sup>6</sup>, M. Dykhuizen Shore<sup>2,7</sup>, and E. B. Peters<sup>2,8</sup>

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

**Plain Language Summary** In the water-saturated soils of wetlands, which are usually anoxic, decomposition of dead plants and other organic matter is greatly retarded by the absence of oxygen. However, the addition of sulfate can allow bacteria that respire sulfate, instead of oxygen, to decompose organic matter that would not otherwise decay. The accelerated decay has multiple consequences that are concerning. The bacteria that respire sulfate “breathe out” hydrogen sulfide (also called sulfide), analogous to the conversion or respiration of oxygen to CO<sub>2</sub>. Sulfide is very reactive with metals, which makes it toxic at higher concentrations. In addition to the release of sulfide, the sulfate-accelerated decomposition of plants releases phosphorus and nitrogen, fertilizing the waterbody. Decomposition also mobilizes mercury (which is everywhere, thanks to atmospheric transport) into the surface water. The microbes that convert sulfate to sulfide also methylate mercury, producing methylmercury, the only form of mercury that contaminates fish. This study demonstrates that adding sulfate to a wetland can not only produce toxic levels of sulfide but also increase the surface water concentrations of nitrogen, phosphorus, mercury, and methylmercury.

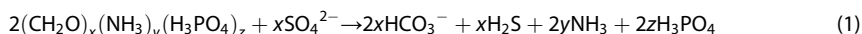
### 1. Introduction

Organic matter (OM) accumulates in the sediments of aquatic systems when sediment concentrations of terminal electron acceptors (TEAs) are too low for microbes to completely decompose OM, especially when the supply of the most energy-efficient TEA, oxygen, is low. In water-saturated, organic-rich sediment, microbial sulfate reduction (MSR) can be a dominant pathway for the respiration of OM because oxygen is depleted in the uppermost sediment (Boye et al., 2017). Dissolved sulfate (SO<sub>4</sub>) concentrations in continental surface

waters are often low (less than  $50 \text{ mg L}^{-1}$  or  $0.5 \text{ mmol L}^{-1}$ ) (e.g., Gorham et al., 1983) compared to ocean concentrations ( $2,800 \text{ mg L}^{-1}$  or  $29 \text{ mmol L}^{-1}$ ). Because of lower  $\text{SO}_4$  concentrations, and because MSR rates can be limited by  $\text{SO}_4$  concentrations (Holmer & Storkholm, 2001), the biogeochemical significance of MSR is often considered minimal in freshwater and low-salinity systems (e.g., Capone & Kiene, 1988; Nielsen et al., 2003; Stagg et al., 2017). However, absolute rates of MSR are not clearly lower in freshwater systems than in marine systems (Pallud & Van Cappellen, 2006), and in some cases, rapid cycling between oxidized and reduced forms of S can occur (Hansel et al., 2015).

In this study, we investigated the cascade of biogeochemical effects associated with increased MSR that result from increased surface water  $\text{SO}_4$ . We simultaneously quantified three different categories of biogeochemical responses related to MSR: (1) mineralization of organic matter and associated release of dissolved C, N, P, and Hg; (2) methylation of Hg; and (3) production of sulfide.

The stoichiometric release of the constituents of OM during MSR, notably C, N, and P, is a phenomenon long recognized by marine scientists. For instance, Boudreau and Westrich (1984) constructed a model of the MSR-mediated decomposition of marine sediment. They showed that  $\text{SO}_4$  is reduced to sulfide ( $\text{H}_2\text{S}$ ) in stoichiometric proportion to the mineralization of C, N, and P according to the reaction



C is released as both dissolved inorganic carbon (DIC, from complete oxidation, produced as bicarbonate alkalinity in stoichiometric proportion to sulfide (reaction (1); Boudreau & Westrich, 1984)) and dissolved organic carbon (DOC, from partial oxidation). The nutrients N and P are released in forms that are readily taken up by plants; N is released as ammonia, and P as phosphate. The mineralization of sediment organic matter associated with MSR releases sulfide ( $\text{S}^{2-}$ ) into sediment pore water, which speciates, depending on the pH, into hydrogen sulfide ( $\text{H}_2\text{S}$ ) and bisulfide ( $\text{HS}^-$ ), henceforth collectively termed sulfide. If reduced S compounds accumulate in the sediment, there may be additional consequences to an aquatic system, such as toxic concentrations of sulfide in pore water (Lamers et al., 2013; Pastor et al., 2017; Myrbo et al., 2017) or conversion of sediment Fe(III) to FeS compounds, which enhances the mobilization of P (Curtis, 1989; Maynard et al., 2011).

The multiple biogeochemical consequences of MSR in freshwater systems have been investigated and documented in more than two dozen publications (Table S1 in the supporting information), which typically address a single issue, such as the production of alkalinity that neutralizes atmospherically deposited  $\text{H}_2\text{SO}_4$  (Baker et al., 1986; Cook et al., 1986; and others) or the methylation of Hg (Gilmour et al., 1992; Branfireun et al., 1999, 2001; and others). Experimental studies addressing  $\text{SO}_4$  reduction, sulfide production, associated OM mineralization, and release of nutrients have been broader (Lamers et al., 2001, 2002; Weston et al., 2006, 2011; and others), but aside from the results reported in this paper, only the experiments of Gilmour, Krabbenhoft, et al. (2007) and Gilmour, Orem, et al. (2007) have investigated all three categories of biogeochemical consequences of  $\text{SO}_4$  reduction: OM mineralization, Hg methylation, and sulfide accumulation (Table S1). We also investigated the potential for Hg to be released by mineralization, a phenomenon proposed by Regnell and Hammar (2004).

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

The release of sulfide into sediment pore water has multiple biological and geochemical consequences, several of which are related to the reactivity of sulfide with metals. If dissolved sulfide accumulates in pore water, it can negatively affect multicellular organisms inhabiting the sediment because sulfide can denature a range of metal-containing biomolecules, including cytochrome C oxidase, which is essential for respiration by both animals and plants (Bagarinao, 1992). Because aquatic sediment is a primary site of sulfide production, plants that root in sediment are vulnerable to toxic sulfide concentrations (Lamers et al., 2013; Pastor et al., 2017). However, if the watershed supplies sufficiently high loading of reactive Fe or other metals to the sediment, pore water sulfide concentrations may stay below toxic levels even while MSR proceeds as an important mineralization process (Pollman et al., 2017). The formation of FeS compounds effectively detoxifies sulfide (e.g., Marbà et al., 2007; Van der Welle et al., 2007). When Fe availability exceeds the production of sulfide, the accumulation of FeS is a measure of cumulative  $\text{SO}_4$  reduction, which can be quantified as acid-volatile sulfide (AVS) (Heijs & van Gernerden, 2000). In addition, phosphorus is mobilized when oxidized Fe compounds with significant capacity to bind phosphate are converted to FeS compounds, which are incapable of binding phosphate (Lamers et al., 1998; Maynard et al., 2011). Thus, MSR mobilizes P both by mineralization of P-containing OM and by changing the form of Fe in sediment.

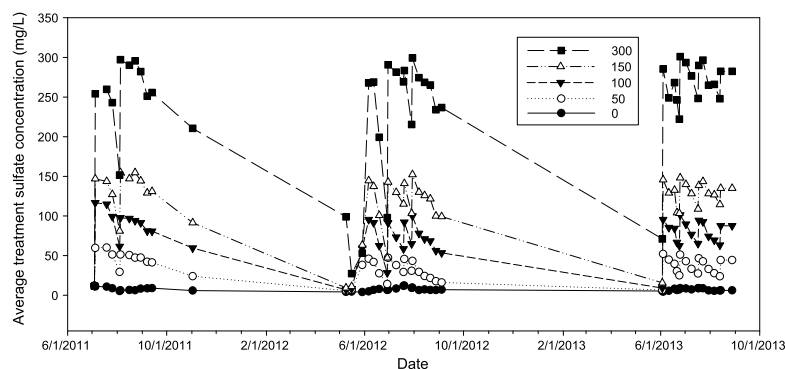
In addition to releasing C, N, and P, producing potentially toxic concentrations of sulfide, and reducing the solubility of metals, MSR is a primary process leading to the formation of MeHg, the bioaccumulative form of Hg (Gilmour et al., 1992; Hsu-Kim et al., 2013), although other microbial groups can also methylate Hg (Podar et al., 2015). In some cases, MSR can lead to toxic levels of MeHg higher in the food chain. The relationship between  $\text{SO}_4$  concentrations and MeHg production is complex, however, and both field and laboratory studies in freshwater and saline ecosystems suggest that there is a dual effect of S on Hg methylation. At low  $\text{SO}_4$  concentrations, the addition of  $\text{SO}_4$  can stimulate MSR and Hg methylation (Jeremiason et al., 2006). At higher  $\text{SO}_4$  concentrations, a greater abundance of inorganic sulfide appears to decrease the availability of inorganic Hg for Hg methylation (Hsu-Kim et al., 2013; Johnson et al., 2016). Because it has been observed that low  $\text{SO}_4$  additions often increase Hg methylation and higher  $\text{SO}_4$  concentrations decrease methylation, it has been proposed that there is a range of  $\text{SO}_4$  and sulfide concentrations are optimal for Hg methylation, above which methylation is inhibited (Hsu-Kim et al., 2013). There is some debate regarding the underlying mechanism, but there is substantial evidence suggesting that dissolved inorganic sulfide above concentrations of 300–3,000  $\mu\text{g L}^{-1}$  has an inhibitory effect on Hg methylation (Bailey et al., 2017).

This study presents results from 30 wetland mesocosms in which the surface waters were treated to maintain a wide range of  $\text{SO}_4$  concentrations over the course of 5 years (2011–2015) to assess the impact on wild rice, *Zizania palustris* (Pastor et al., 2017). We took advantage of this experiment to analyze the geochemical conditions in surface and pore water in the mesocosms during late summer 2013, 3 years into the experiment. Pastor et al. (2017) specifically examined the effect of increased  $\text{SO}_4$  loading on wild rice, whereas this paper examines the broader biogeochemical impact of augmenting  $\text{SO}_4$  to a low- $\text{SO}_4$  system.

## 2. Materials and Methods

### 2.1. Experimental Design

The experimental setup (Figure S1 in the supporting information), described in detail by Pastor et al. (2017), consisted of thirty 375 L polyethylene stock tanks containing sediment from a wild rice lake (Rice Portage Lake; +46.6987°, -92.6886°) in which wild rice was grown in self-perpetuating populations at five  $\text{SO}_4$  treatment levels (control, 50, 100, 150, and 300  $\text{mg L}^{-1}$ ).  $\text{SO}_4$  concentrations in six replicate mesocosms were routinely monitored, and amendments of  $\text{SO}_4$  were added as  $\text{Na}_2\text{SO}_4$  during the growing season as  $\text{SO}_4$  was removed by MSR (Figure 1). Due to MSR, the mesocosm surface waters actually had time-weighted average concentrations of 7, 27, 59, 93, and 207  $\text{mg L}^{-1}$ , respectively. Local well water containing an average of 10.6  $\text{mg L}^{-1}$   $\text{SO}_4$  was added as needed to compensate for evapotranspiration. Precipitation in the region contains an average of 2.1  $\text{mg L}^{-1}$   $\text{SO}_4$ , and Rice Portage Lake has an average  $\text{SO}_4$  concentration of 2.2  $\text{mg L}^{-1}$  (Fond du Lac Band, 2016), so the control was slightly elevated above the ambient  $\text{SO}_4$  concentration of the sediment source for the experiment. During the ice-free period (generally May through October), the surface water temperature ( $T$ ) measured in the morning was correlated with the previous day's mean air temperature (mesocosm  $T = 0.72$  air  $T + 4.4$  °C;  $R^2 = 0.65$ ). Peak air temperature is reached in July, when the average



**Figure 1.**  $\text{SO}_4$  concentrations in surface waters of each treatment, showing repetitive depletion and periodic amendment with  $\text{Na}_2\text{SO}_4$  (average of six mesocosms per treatment on each sampling date).

temperature is  $18.8^\circ\text{C}$  (based on 1981–2010 air temperatures measured at the Duluth, Minnesota, airport, 10 km from the experimental site).

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

## 2.2. Methods

### 2.2.1. Sample Collection

Rhizon™ samplers with a 10 cm long, 2.5 mm diameter, cylindrical porous tip (hydrophilic membrane pore size  $0.12\text{--}0.18\ \mu\text{m}$  (Rhizosphere.com, Netherlands; Shotbolt, 2010)), were connected by Teflon-taped Luer-Lok connectors and silicone tubing to a syringe needle. The sampler was inserted into the sediment, and the needle was then inserted through the 20 mm thick butyl rubber septum of an evacuated serum bottle (Bellco Glass) to initiate pore water draw through the tubing and displace air. After water was observed entering the serum bottle, the needle was removed from the first sacrificial bottle and inserted through the septum of a second evacuated serum bottle to collect the sample. One Rhizon and bottle were used to collect a sample for dissolved iron, preserved with 20% nitric acid. A second Rhizon and evacuated,  $\text{N}_2$  gas-flushed sealed bottle, preloaded with 0.2 mL 2 N zinc acetate, 0.5 mL 15 M NaOH, and a stir bar, was used to collect a sample for dissolved sulfide analysis. Each Rhizon was positioned to sample pore water from the top 10 cm of sediment and to avoid collecting water from above the sediment surface. However, it is conceivable that some surface water was able to follow the path of the Rhizon into the sediment and dilute or partially oxidize the pore water sample.

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

Pore water P availability was measured with three mixed bed ion exchange bags (Fisher Rexyn 300 resin) placed in the sediment of each tank in spring and harvested at the end of the growing season in 2013. A 3.8 cm diameter piston corer was used to obtain 10 cm long sediment samples for various analyses. Sediment samples for the analysis of AVS were taken monthly from June to October 2013 from replicate mesocosms of four  $\text{SO}_4$  treatments (control, 50, 150, and  $300\ \text{mg L}^{-1}$ ; no mesocosm was sampled more



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

### 2.2.2. Laboratory Analyses

Surface water and pore water analyses were conducted by the Minnesota Department of Health Environmental Laboratory (MDHEL). Total P was measured by in-line ultraviolet/persulfate digestion and flow injection (APHA, 2005, 4500 P-I), DOC by persulfate-ultraviolet oxidation and IR CO<sub>2</sub> detection (APHA, 2005, 5310-C), and alkalinity by automated titration (APHA, 2005, 2320-B). Pore water sulfide samples were prepared for inline distillation and flow injection colorimetric analysis using procedures that avoided exposure to oxygen. The sulfide serum bottle was weighed to determine the amount of sample collected and to adjust for the slight dilution factor of an alkaline antioxidant that was added by injection through the stoppers. The sealed samples were then placed on a stir plate for at least 1 h and subsamples withdrawn for analysis through a needle. Reanalysis of sealed, processed samples 12 months later shows no significant difference in sulfide concentrations, indicating that the sulfide samples were stable prior to analysis (data not shown). SO<sub>4</sub> concentration was measured using a Lachat QuikChem 8000 Autoanalyzer (Lachat Method 10-116-10-1-A). The resin was eluted using a KCl solution and analyzed for PO<sub>4</sub> using a Lachat Autoanalyzer, following the methods of Walker et al. (2006).

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

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

## 3. Data Analysis

### 3.1. Sulfate Depletion as the Independent Variable

Because SO<sub>4</sub> is relatively unreactive under oxidized conditions, its loss is attributable to diffusion or transpiration-driven advection (Bachand et al., 2014) into sediment and conversion to sulfide by bacteria. Surface water SO<sub>4</sub> concentrations decreased partly due to dilution by precipitation but largely from loss after movement into the sediment and reduction to sulfide. Sulfide would largely be retained in the sediment as FeS compounds, although some could be lost to the atmosphere as H<sub>2</sub>S gas (Bagarinao, 1992) or as volatile organic sulfur compounds (Lomans et al., 2002). The cumulative SO<sub>4</sub> lost from surface water was calculated from a mass balance for each mesocosm from the inception of the experiment in spring 2011 through fall 2013; this quantity, termed here SO<sub>4</sub> depletion, (SO<sub>4</sub>)<sub>DepI</sub>, is used as a proxy for net MSR, following Weston et al. (2006). The surface water remained frozen from approximately 1 December to 1 April each winter, and the mesocosms were covered with plastic from November to late April each year and not amended with SO<sub>4</sub>. SO<sub>4</sub> reduction was the major biogeochemical process altered by the experimental treatments, and therefore, (SO<sub>4</sub>)<sub>DepI</sub> is the independent variable used in subsequent data analyses. It was only possible to perform a complete mass balance for SO<sub>4</sub>, the only parameter consistently quantified in source water, precipitation, and overflow water.

### 3.2. Calculation of DIC From Measured Alkalinity

Dissolved inorganic carbon (DIC ≡ [CO<sub>3</sub><sup>2-</sup>] + [HCO<sub>3</sub><sup>-</sup>] + [CO<sub>2</sub>\*], where [CO<sub>2</sub>\*] = [CO<sub>2(g)</sub>] + [H<sub>2</sub>CO<sub>3</sub>]) was calculated from measured alkalinity and speciated using pH, temperature, and specific conductance of the surface water. At the pH range of the mesocosms (7.60–8.84), 95–98% of DIC is in the form of HCO<sub>3</sub><sup>-</sup>, so DIC concentration on a molar basis is nearly the same as alkalinity (ALK) on an equivalent basis (DIC = 0.988 ALK + 0.077, R<sup>2</sup> = 0.995). In studies of freshwater, most inorganic carbon data are presented in terms of alkalinity because

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

### 3.3. Statistical Analysis

Statistical analysis was conducted with R version 3.2.3 and STATA (StataCorp, 2015). The effect of increased sulfate availability was assessed through both categorical analysis of the sulfate treatments (Kruskal-Wallis ANOVA test, followed by Dunn's test for multiple comparisons with Holm-Sidak corrections) and through linear regression and nonparametric Spearman rank correlations. We rely primarily on regressions against  $\text{SO}_4$  depletion to detect the effects of enhanced sulfate-reduction driven mineralization, rather than categorical analysis of the sulfate treatment results, because (a) biogeochemical changes are not driven directly by  $\text{SO}_4$  concentration, but rather by MSR, quantified as  $\text{SO}_4$  depletion; (b) although  $\text{SO}_4$  depletion may be highly correlated to  $\text{SO}_4$  concentration, deviations between experimental mesocosms develop over time, so cumulative  $\text{SO}_4$  depletion values eventually no longer align exactly with treatment categories, but rather become continuous variables; and (c) regression provides more statistical power than ANOVA and builds models that allowed us to describe the relationships between  $\text{SO}_4$  depletion and response variables (Cottingham et al., 2005). However, when the relationship is not linear, ANOVA and comparison of treatments through Dunn's analysis can help describe the nature of a relationship.

## 4. Results and Discussion

### 4.1. The Impact of $\text{SO}_4$ Reduction on Mineralization of Sediment Organic Matter

Increased concentrations of surface water  $\text{SO}_4$  resulted in increased sulfate reduction, which necessarily increased the mineralization of organic carbon, as described by reaction (1). Concentrations of surface water DOC and DIC increased in proportion to sulfate reduction, as measured by  $(\text{SO}_4)_{\text{Depl}}$  (Table 1 and Figure 2). The marine literature generally assumes complete mineralization of particulate organic carbon (POC) to DIC in the water column (e.g., Boudreau & Westrich, 1984) (reaction 1), but in freshwater systems and especially wetlands, not all carbon is completely oxidized during decomposition, and a portion of POC may be mobilized as DOC (Howes et al., 1985; Selvendiran et al., 2008). In principle, the constituents of organic matter, such as the nutrients N and P, are mobilized in proportion to the mass of carbon mineralized as a result of MSR-driven decomposition. Surface water DOC and DIC, and the sum  $\text{DOC} + \text{DIC}$ , are therefore used as indicators of OM mineralization in interpreting the mobilization of N, P, and Hg to surface waters (Figure 2 and Tables 2 and 3).

In contrast to many marine systems, it is likely that  $\text{SO}_4$  reduction in these sediments was limited more by  $\text{SO}_4$  than by organic carbon, given that  $(\text{SO}_4)_{\text{Depl}}$  was linearly proportional to the average  $\text{SO}_4$  concentration (Figure S2a;  $R^2 = 0.87$ ), without any obvious curvature to the relationship that would indicate saturation of MSR.

Regressions of surface water DOC and DIC against  $\text{SO}_4$  depletion demonstrate that, on a net basis, about 60% more DIC than DOC was mobilized to the surface water as a result of MSR-driven mineralization (slope of 0.235 mM C per unit  $\text{SO}_4$  depletion compared to 0.148; Table 2). The significantly positive slope of the DIC:DOC ratio against  $\text{SO}_4$  depletion (Table 2) indicates that increasingly more DIC than DOC was observed in the surface water as sulfate depletion increased. Some mineralization of DOC to DIC likely occurs in the surface water as a result of exposure to oxygen, aerobic bacteria, and sunlight, processes that could have a larger effect as DOC increases.

Not only did surface water DIC and DOC increase in concert with sulfate reduction, but parallel increases occurred in surface water concentrations of constituents of organic matter: N, P, and Hg (Table 1 and Figure 2). DIC, DOC, total P, total N, ammonia, and total Hg in surface water all had increases from the control to the highest  $\text{SO}_4$  addition of about twofold, (2.3, 1.7, 1.9, 1.8, 1.7, and 2.6-fold, respectively, Table 1). However, available phosphate in the sediment, an estimate of P availability in pore water, had a larger increase (7.5-fold). MSR consumes acidity as the DIC-based alkalinity is produced (Baker et al., 1986), which increased the average pH from 7.57 to 7.81, a 44% decrease in hydrogen ion concentration (Table 1). If the sulfide subsequently oxidizes (which could happen in a natural system during drought (Laudon et al., 2004) or intentional dewatering), a proportional quantity of alkalinity is consumed as acid is produced

**Table 1**

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

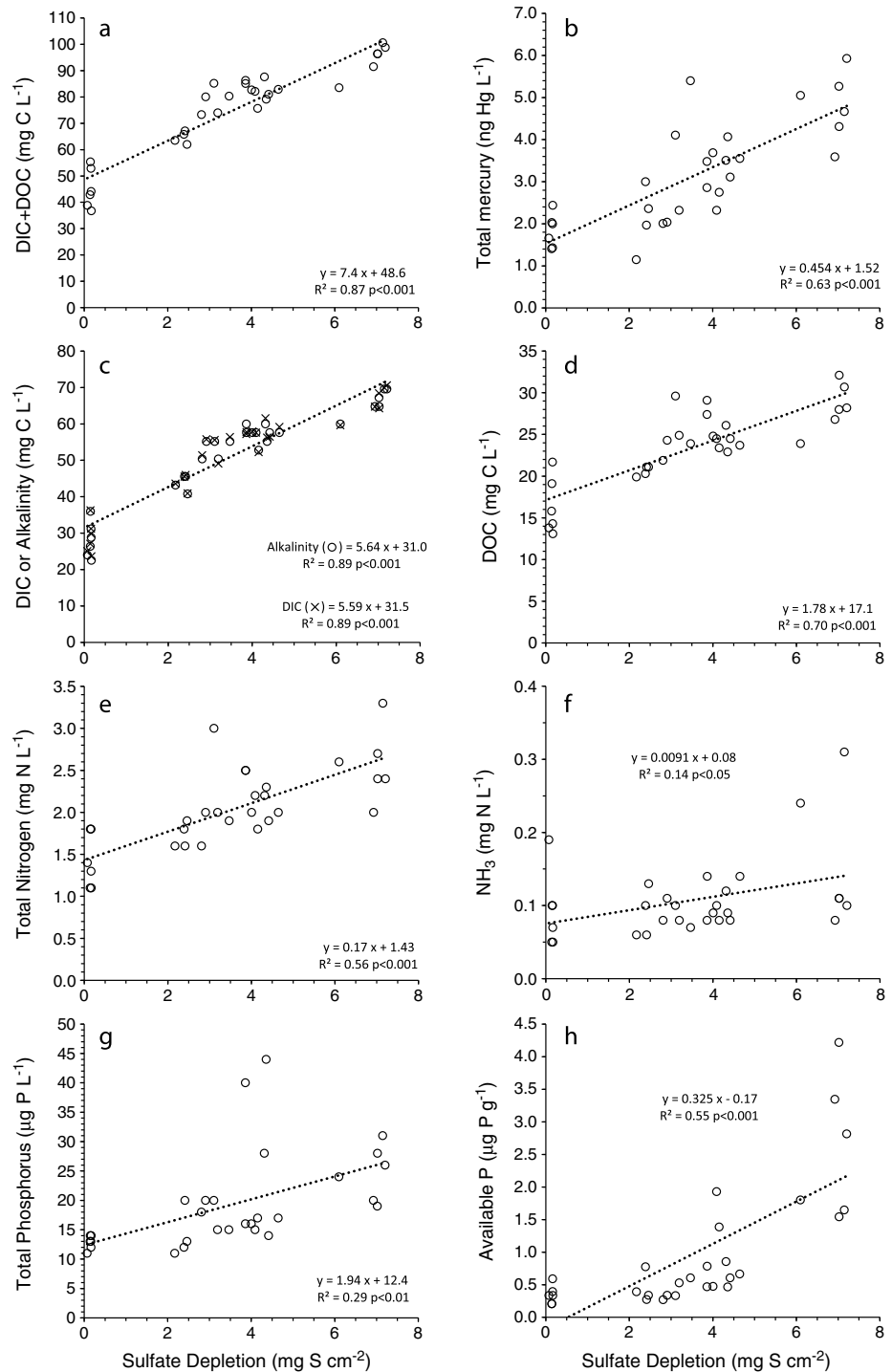
Variable	Matrix	Average of each sulfate treatment ( $n = 6$ for each treatment)					Correlation with $SO_4$ depletion (Spearman)		
		Control	50	100	150	300	Max/Min	Rho	$p$ value
<i>Variables mainly associated with <math>SO_4</math> reduction</i>									
$SO_4$ (T-W mean $mg\ SO_4\ L^{-1}$ )	sw	6.7 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

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

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

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

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



**Figure 2.** The release of constituents of sedimentary organic matter as a function of SO<sub>4</sub> depletion, showing linear regressions (dotted lines). (a) Sum of surface water DIC and DOC; (b) surface water total mercury; (c) surface water alkalinity and DIC (symbols  $\circ$  and  $\times$ , respectively; the two regressions are superimposed); (d) surface water DOC; (e) surface water total nitrogen; (f) surface water ammonia; (g) surface water total phosphorus; (h) available phosphate in the sediment, as quantified on ion-exchange resin.

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

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

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

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

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

significantly above saturation with respect to the atmosphere and the observation of increasing DIC and DOC with increasing  $(SO_4)_{Depl}$  (Table 1) provide strong evidence of sulfate-induced increases in net carbon mineralization in the mesocosms.

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

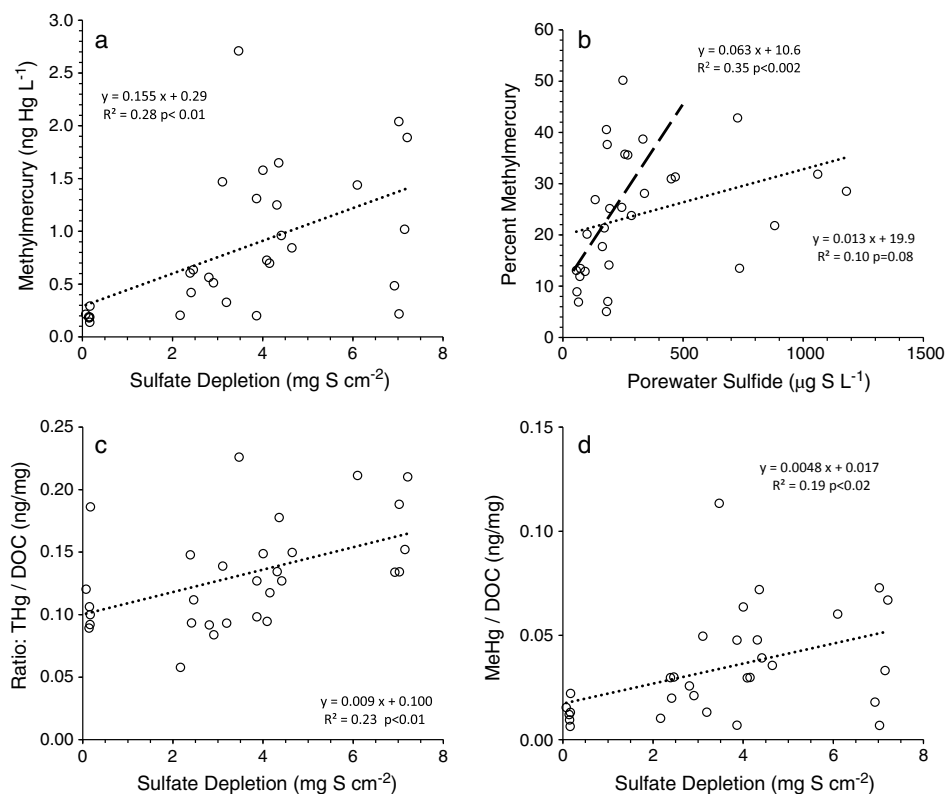
#### 4.2. Effects of $SO_4$ Reduction on Mercury and Methylmercury in Surface Water

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

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

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

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



**Figure 3.** The response of surface water Hg variables to  $\text{SO}_4$  depletion and the production of pore water sulfide, showing linear regressions. (a) MeHg as a function of  $\text{SO}_4$  depletion; (b) percent MeHg as a function of pore water sulfide, showing regressions for all data (dotted line) and for the subset of data extending only to a pore water sulfide concentration of  $468 \mu\text{g S L}^{-1}$  (dashed line); (c) ratio of THg to DOC as a function of  $\text{SO}_4$  depletion; (d) ratio of MeHg to DOC as a function of  $\text{SO}_4$  depletion.

THg, inorganic Hg (iHg), and MeHg all increased significantly with increased  $(\text{SO}_4)_{\text{Depl}}$  (Table 1 and Figures 2b and 3a,  $p < 0.0001$ ) and were greater in the highest sulfate amendment by factors of 2.6, 2.2, and 5.9, respectively (Table 1). The relative increase in THg (2.6-fold) is greater than that for DIC, DOC, TN, and TP, which range from 1.7 to 2.3-fold (Table 1). DOC enhances the solubility of both iHg and MeHg and can facilitate the movement of Hg from sediment into surface water (Ravichandran, 2004). The 5.9-fold increase in MeHg indicates that MeHg flux to surface waters was enhanced by sulfate loading disproportionately more than sedimentary release of THg (2.6-fold) and the increase in surface water DOC (1.7-fold).

The genes required to methylate Hg have been found in a wide variety of anaerobic bacteria, including  $\text{SO}_4$ -reducing bacteria, iron-reducing bacteria, and methanogens (Podar et al., 2015). Though some pure culture and experimental evidence exist for mercury methylation by other bacteria, extensive pure culture, experimental, and landscape-scale observations suggest  $\text{SO}_4$ -reducing bacteria dominate Hg methylation in many freshwater and marine environments. The relatively large increase in surface water MeHg in response to increased  $(\text{SO}_4)_{\text{Depl}}$  in this experiment supports the assumption that MSR was responsible for most of the observed production of MeHg. It is likely that increased  $\text{SO}_4$  loading to low- $\text{SO}_4$  aquatic systems with organic sediment will result in increased Hg methylation even though the relative importance of Hg methylation in the environment by different groups of bacteria is still a subject of debate (Paranjape & Hall, 2017).

If movement of DOC from sediment to surface water were the sole mechanism for the Hg increase in surface water, a constant Hg:DOC ratio would be expected on the  $(\text{SO}_4)_{\text{Depl}}$  gradient. However, THg:DOC, iHg:DOC, and MeHg:DOC ratios in surface water are all significantly correlated with  $\text{SO}_4$  depletion (Table S2 and Figures 3c and 3d). Therefore, all forms of Hg (THg, iHg, and MeHg) increase in surface waters more than

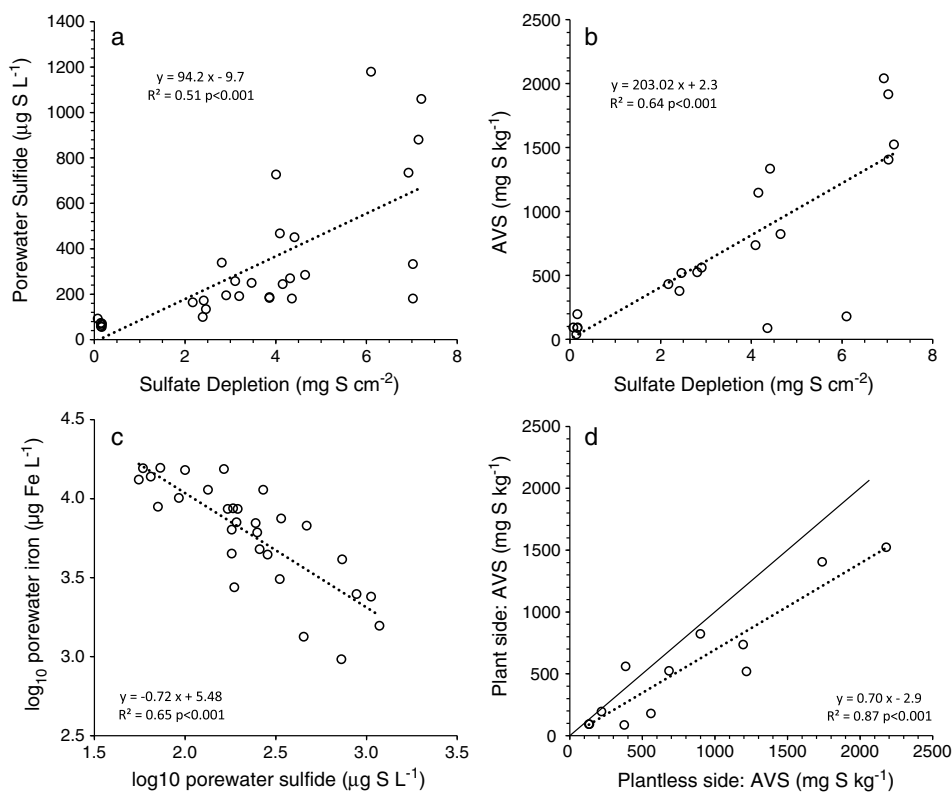


does DOC, indicating that a sulfate-induced enhancement of carbon mineralization may act in combination with either enhanced methylation or an enhanced capacity of DOC to carry Hg. Changes to the binding strength of the DOC in heavily S-impacted mesocosm sediment are possible, as thiol groups on DOC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

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

Recent research has shown that in many ecosystems, higher concentrations of pore water sulfide may inhibit MeHg production through either thermodynamically or kinetically controlled reactions with inorganic Hg (Benoit et al., 2003; Hsu-Kim et al., 2013). We plotted %MeHg, rather than the MeHg concentration, against pore water sulfide because we are interested in identifying the pore water sulfide zone of greatest efficiency for the methylation and mobilization of mercury. In this experiment the MSR-driven mineralization of OM released THg to surface water in addition to producing pore water sulfide. Accordingly, because THg is not constant, plotting %MeHg is the most accurate way to identify peak methylation efficiency. In principle, the restricted bioavailability of Hg to methylating bacteria results in a maximum in MeHg production at intermediate concentrations of pore water sulfide. Consistent with previous research in sulfate-impacted freshwater ecosystems (Gilmour et al., 1998; Gilmour, Krabbenhoft, et al., 2007; Gilmour, Orem, et al., 2007; Bailey et al., 2017), MeHg production was most efficient at intermediate sulfide concentrations. In the control, where average sulfide was  $69 \mu\text{g S L}^{-1}$ , MeHg averaged only 11% of THg in surface waters. In the intermediate  $\text{SO}_4$  treatments, which had average sulfide concentrations of 224 and  $393 \mu\text{g S L}^{-1}$ , MeHg production efficiency peaked significantly higher, at averages of 30% and 32%, respectively (Table 1). %MeHg declined to an average of 23% in the highest  $\text{SO}_4$  treatment, which had an average sulfide concentration of  $728 \mu\text{g S L}^{-1}$ . Given the relatively great scatter in the relationship between %MeHg and sulfide (Figure 3b), it would be most defensible to conclude that the decrease in %MeHg began to occur somewhere between 300 and  $700 \mu\text{g S L}^{-1}$ . There is a strong positive relationship ( $p < 0.001$ ) between sulfide and %MeHg if the five sulfide concentrations greater than  $727 \mu\text{g S L}^{-1}$  are excluded from the regression (which leaves only sulfide concentrations less than  $468 \mu\text{g S L}^{-1}$ , since there is a gap in sulfide concentrations; Figure 3b). Other studies have identified sulfide zones of peak methylation roughly comparable to that found here. In South Florida, Orem et al. (2011) found that sulfide ranging from 5 to  $150 \mu\text{g S L}^{-1}$  did not inhibit methylation but that sulfide concentrations greater than  $1,000 \mu\text{g S L}^{-1}$  did. In a subboreal Minnesota wetland enriched in  $\text{SO}_4$  from mining discharge, Bailey et al. (2017) found that sulfide concentrations above  $\sim 650 \mu\text{g S L}^{-1}$  inhibited methylation.

The relationship between surface water  $\text{SO}_4$  and Hg methylation can be strongly affected by site-specific conditions. Because of the variable conversion of  $\text{SO}_4$  in surface water to sulfide in pore water—primarily due to differences in OM and Fe availability (Pollman et al., 2017)—researchers have found a broad range in the  $\text{SO}_4$  concentration associated with maximum efficiency of Hg methylation. For example, Orem et al. (2014) observed that two different areas in the Everglades Protection Area had peak surface water MeHg concentrations at  $\text{SO}_4$  concentrations of 2 and  $10\text{--}15 \text{ mg L}^{-1}$ . In the mesocosms presented here peak surface water %MeHg was observed in the two sulfate treatments that averaged 59 and  $93 \text{ mg L}^{-1}$  (Table 1).



**Figure 4.** AVS and pore water sulfide, as related to  $\text{SO}_4$  depletion, pore water iron, and presence of rooted plants. (a) Pore water sulfide as a function of  $\text{SO}_4$  depletion; (b) AVS from the vegetated side of the mesocosms as a function of  $\text{SO}_4$  depletion; (c) pore water iron as a function of pore water sulfide; (d) AVS compared between the vegetated side and nonvegetated side. The solid 1:1 line shows that in almost all mesocosms more AVS is found in the side without plants.

### 4.3. Effects of $\text{SO}_4$ Reduction on Pore Water and Sediment Sulfide

Pore water sulfide increased at higher  $(\text{SO}_4)_{\text{Depl}}$ , although with greater variance at higher  $(\text{SO}_4)_{\text{Depl}}$  (Figure 4a), possibly as a result of variable oxidation of sulfide that may depend on the proximity of the Rhizon sampler to plant roots (Schmidt et al., 2011) or of variable bioturbation by invertebrates (Lawrence et al., 1982). When  $\text{SO}_4$  is reduced through MSR, the sulfide produced has a number of nonexclusive potential fates: the sulfide could (1) be oxidized within the sediment; (2) remain in the sediment pore water as free sulfide; (3) diffuse into oxygenated surface water, to be oxidized; (4) react with metals in the sediment, forming insoluble precipitates (dominated by iron-sulfide compounds); or (5) be lost to the atmosphere as  $\text{H}_2\text{S}$  gas or as volatile organic sulfur compounds. Because precipitation reactions are fast relative to redox reactions and diffusion, most of the sulfide probably forms metal precipitates if metals are available. When precipitation dominates the fate of sulfide produced from MSR, the continuous reduction of  $\text{SO}_4$  and precipitation of iron sulfides form quasi-steady states between surface water  $\text{SO}_4$  and pore water sulfide (Figure S2b) and between pore water sulfide and pore water iron (Figures 3 and 4c). The overall mass of sulfide in the mesocosm sediment, quantified through analysis of AVS (from sediment in the vegetated area), is closely correlated with  $\text{SO}_4$  depletion (Figure 4b) even though AVS may not include all the reduced sulfide in sediments. It is likely that most of the AVS in these sediments is present as an FeS precipitate because other metals are at low concentrations in these sediments, which came from a relatively pristine (unpolluted) lake (Fond du Lac Band, 2016; Pastor et al., 2017). Note that there are two mesocosms with especially low AVS concentrations (Figure 4b). It is possible that the AVS in the specific location in these mesocosms where sediment core samples were collected was influenced by



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

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

#### 4.4. Mesocosms as Models for Ecosystem-Scale Effects of $\text{SO}_4$ Reduction

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

1. Relatively long surface water residence times: the increased loading of N, P, C, Hg, and MeHg to the surface water of the mesocosms was readily detected because the lack of hydraulic loading from a watershed minimized dilution and loss through the outflow. The impact of an increase in  $\text{SO}_4$  loading on surface water concentrations of N, P, C, Hg, DIC, and DOC would be lower in waters with shorter residence times. For instance, Baker and Brezonik (1988), in modeling increases in alkalinity from atmospheric  $\text{SO}_4$  loading, noted that net increases in alkalinity would be most important in waters with long residence times (>5 years) and that there would be little increase in alkalinity in waters with much shorter residence times (<1 year). However, the measured concentrations may not represent the maximum impact of MSR-driven mineralization because the mesocosm wall may enhance removal from the surface water (point number 2, below).
2. Presence of the mesocosm wall: the mesocosms have a relatively high ratio of wall and sediment surfaces to the volume of overlying water, enhancing the removal of surface water nutrients and Hg to periphyton or inorganic sinks such as iron oxyhydroxides. Natural aquatic systems have less proportional loss to surfaces. The quantitative estimates of internal loading of N, P, and Hg in response to MSR-induced carbon mineralization may have been underestimated by the measured surface water concentrations, given that significant loss of these constituents to periphyton may have occurred. In addition, THg was filtered prior to analysis, which would have removed any Hg associated with phytoplankton or other suspended particles.
3. Lack of either overland or groundwater loading of particulate and dissolved material, specifically iron: the availability of iron in sediment is a primary controller of the fate of MSR-produced sulfide (Pollman et al., 2017). In natural aquatic systems, iron would be supplied at a relatively constant rate from the system's watershed over the long term, although varying in magnitude from watershed to watershed (Maranger et al., 2006; Winter, 2001). This experiment was not an accurate long-term mimic of pore water sulfide concentrations because the external supply of iron was cut off at the inception of the experiment. With no loading of iron, but continued loading of  $\text{SO}_4$ , the continued production of sulfide would be expected to eventually consume all available Fe, allowing pore water sulfide levels to exceed those expected in a natural system at equivalent surface water  $\text{SO}_4$  concentrations. This mesocosm experiment provides

evidence for just such a result. The experiment continued for 2 years after the 2013 sampling presented here. In the fifth year (August 2015) pore water sulfide was much greater than had been observed in 2013, and disproportionately so in the highest  $\text{SO}_4$  treatment, which was most likely to consume available Fe. Between the 2013 and 2015, pore water sulfide increased in the control  $\text{SO}_4$  treatment (about  $7 \text{ mg SO}_4 \text{ L}^{-1}$ ) from an average value of  $69 \mu\text{g L}^{-1}$  in 2013 to  $116 \mu\text{g L}^{-1}$  in 2015, a 68% increase. Pore water sulfide in the highest treatment (nominally  $300 \text{ mg SO}_4 \text{ L}^{-1}$ , Table 1) increased from an average value of  $728 \mu\text{g L}^{-1}$  in 2013 to  $9,350 \mu\text{g L}^{-1}$  in 2015, a 1,184% increase (Pastor et al., 2017). In a survey of 108 Minnesota waterbodies with a wide range of surface water sulfate, only two exceeded a pore water sulfide level of  $3,200 \mu\text{g L}^{-1}$  (Myrbo et al., 2017).

## 5. Conclusions

This study demonstrates that increased  $\text{SO}_4$  loading to inland waters with organic-rich sediments can significantly increase the decomposition of sedimentary organic matter, which increases internal loading to surface water of the chemical constituents of organic matter, including DIC, DOC, P, N, and Hg. Associated changes include increased production of sulfide and methylmercury and increased alkalinity and pH. Any one of these changes could alone cause significant secondary changes in the structure of an aquatic ecosystem but, taken together, could cause a cascade of primary and secondary environmental changes: increased availability of nutrients (N and P), which can alter dominant plant species, organic carbon production, oxygen consumption, and redox; increased pore water sulfide, which can be toxic to benthic animals and plants; increased MeHg production, which can affect fish and other consumers in the aquatic food web; increased DOC, which can alter light transmission, thermal stratification, and aquatic chemistry; and increased DIC production, which increases alkalinity and pH, affecting aquatic chemistry and biota. Each of these changes resulting from higher surface water  $\text{SO}_4$  and consequent increases in MSR has been documented in the literature, but the entire suite of associated changes in aquatic chemistry has not heretofore been demonstrated in an integrated fashion. The degree to which an increase in  $\text{SO}_4$  loading affects the ecological structure of the receiving water will depend on the relative increases in N, P, DIC, DOC, Hg, MeHg, pH, and sulfide, which will be a function of background geochemistry and hydrology of the specific system. In this experiment, the changes in these parameters were linearly proportional to  $\text{SO}_4$  reduction, which, in turn, was linearly proportional to the time-weighted average  $\text{SO}_4$  concentration. The linear responses of the parameters to  $\text{SO}_4$  additions suggest that ecologically significant changes may occur even when  $\text{SO}_4$  concentrations are elevated only modestly and that dramatic changes may occur with higher sulfate loading.

## Acknowledgments

This work was supported by the Clean Water Fund, created by the Clean Water, Land and Legacy Amendment to Minnesota's constitution; by the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa with band funds and water quality funds provided by the Environmental Protection Agency; by Minnesota Sea Grant; by NSF 0715808 to Pastor and others; and by NSF 0949962 to Myrbo and others. A partial data set is available in the EarthChem database: <https://doi.org/10.1594/IEDA/100701>. The full data set is available in the Data Repository for U of M (DRUM): <https://doi.org/10.13020/D6595Z>.

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# Footnoted Document #3



## RESEARCH ARTICLE

10.1002/2015JG002993

## Key Points:

- Examined effects of variable hydrology and sulfate inputs on sulfur and mercury cycling
- Sulfate inputs to peatlands create more effective methylating environments
- Drought enhances methylmercury production within and release from peatlands

## Supporting Information:

- Supporting Information S1

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## Citation:

Coleman Wasik, J. K., D. R. Engstrom, C. P. J. Mitchell, E. B. Swain, B. A. Monson, S. J. Balogh, J. D. Jeremiason, B. A. Branfireun, R. K. Kolka, and J. E. Almendinger (2015), The effects of hydrologic fluctuation and sulfate regeneration on mercury cycling in an experimental peatland, *J. Geophys. Res. Biogeosci.*, 120, 1697–1715, doi:10.1002/2015JG002993.

Received 18 MAR 2015

Accepted 20 JUL 2015

Accepted article online 23 JUL 2015

Published online 4 SEP 2015

## The effects of hydrologic fluctuation and sulfate regeneration on mercury cycling in an experimental peatland

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**Abstract** A series of severe droughts during the course of a long-term, atmospheric sulfate-deposition experiment in a boreal peatland in northern Minnesota created a unique opportunity to study how methylmercury (MeHg) production responds to drying and rewetting events in peatlands under variable levels of sulfate loading. Peat oxidation during extended dry periods mobilized sulfate, MeHg, and total mercury (Hg<sub>T</sub>) to peatland pore waters during rewetting events. Pore water sulfate concentrations were inversely related to antecedent moisture conditions and proportional to past and current levels of atmospheric sulfate deposition. Severe drying events caused oxidative release of MeHg to pore waters and resulted in increased net MeHg production likely because available sulfate stimulated the activity of sulfate-reducing bacteria, an important group of Hg-methylating bacteria in peatlands. Rewetting events led to increased MeHg concentrations across the peatland, but concentrations were highest in peat receiving elevated atmospheric sulfate deposition. Dissolved Hg<sub>T</sub> concentrations also increased in peatland pore waters following drought but were not affected by sulfate loading and did not appear to be directly controlled by dissolved organic carbon mobilization to peatland pore waters. Peatlands are often considered to be sinks for sulfate and Hg<sub>T</sub> in the landscape and sources of MeHg. Hydrologic fluctuations not only serve to release previously sequestered sulfate and Hg<sub>T</sub> from peatlands but may also increase the strength of peatlands as sources of MeHg to downstream aquatic systems, particularly in regions that have experienced elevated levels of atmospheric sulfate deposition.

### 1. Introduction

Peatlands are sites of active biogeochemical cycling for many elements, including sulfur and mercury, because they provide a gradient in oxidation-reduction potentials that are exploited by different microbial communities to gain metabolic energy from chemical transformations [Blodau *et al.*, 2007; Bottrell *et al.*, 2007; Deppe *et al.*, 2010]. Peatlands, and wetlands in general, are considered to be sinks for atmospherically deposited sulfate, in part because sulfate-reducing bacterial (SRB) communities consume incoming sulfate [Pester *et al.*, 2012; Spratt *et al.*, 1987; Urban *et al.*, 1989]. However, there is a significant body of literature showing that drought cycles can alter this function and make peatlands sources of sulfate to downstream aquatic environments [Bailey *et al.*, 1986; Dillon *et al.*, 2007; Eimers *et al.*, 2004; Mitchell and Likens, 2011]. Therefore, predicted changes in climate that promote drought conditions [Sheffield and Wood, 2008] could have the secondary effect of recycling sulfate previously sequestered in peatlands and increasing sulfate inputs to downstream aquatic systems.

While sulfate release from peatlands following drought has been widely investigated, little research has been conducted on the response of mercury biogeochemistry to drought and drought-induced sulfate release. Mercury is a contaminant of great concern in many freshwater systems, particularly because the most common organic form of mercury, methylmercury (MeHg), is biomagnified in aquatic food chains, putting humans and piscivorous wildlife at risk to its neurotoxic effects [Mergler *et al.*, 2007; Munthe *et al.*, 2007]. Peatlands are generally considered to be sinks for total mercury inputs (Hg<sub>T</sub>) from atmospheric deposition

and upland runoff but sources of MeHg to downstream aquatic systems [Branfireun *et al.*, 1998; St. Louis *et al.*, 1994]. The transformation of inorganic mercury to MeHg in the environment is predominantly an anaerobic process carried out by bacterial groups that carry the *hgcAB* gene cluster [Parks *et al.*, 2013], particularly SRB [Gilmour *et al.*, 2013]. Because mercury methylation can be stimulated by sulfate addition to sulfur-limited aquatic systems [Branfireun *et al.*, 1999; Gilmour *et al.*, 1992; Jeremiason *et al.*, 2006] drought-induced sulfate release represents a potential mechanism whereby peatlands could become even larger sources of MeHg in the landscape.

Most research investigating the effect of hydrology on mercury cycling has focused on reservoir creation (i.e., inundation/flooding) [Bodaly *et al.*, 1997; Hall *et al.*, 2005; St. Louis *et al.*, 2004], export from watersheds [Babiarz *et al.*, 1998; Balogh *et al.*, 2006; Bushey *et al.*, 2008; Mitchell *et al.*, 2008b], and cascading effects on biota [Bodaly and Fudge, 1999; Bodaly *et al.*, 1984; Evers *et al.*, 2007] but not the direct effects of drought on MeHg production/release within peatlands. Gilmour *et al.* [2004] performed rewetting incubations on dried Everglades sediment in the laboratory and observed both sulfate release and a consequent rise in mercury methylation, while Feng *et al.* [2014] found that drying and rewetting cycles within wetlands constructed for water treatment resulted in sulfate release and downstream increases in MeHg production. A recent series of studies in California, USA, also highlighted the importance of drying and rewetting to MeHg production within and export from agricultural and wetland systems managed for rice production and waterfowl habitat [Alpers *et al.*, 2014; Marvin-DiPasquale *et al.*, 2014; Windham-Myers *et al.*, 2014]. A few studies have specifically addressed the issue of drought influence on mercury bioaccumulation. Snodgrass *et al.* [2000] found that a drying period was important in explaining higher fish mercury levels in South Carolina depressional wetlands, and George and Batzer [2008] invoked drought conditions to explain elevated invertebrate mercury levels in the Okefenokee Swamp. Ackerman and Eagles-Smith [2010] and Feng *et al.* [2014] likewise cite drying and rewetting patterns as an important control on Hg<sub>T</sub> body burdens in caged fish experiments in agricultural and constructed wetland systems receiving elevated sulfur inputs.

The study reported here was part of an 8 year whole-ecosystem experiment on the effects of elevated sulfate deposition on MeHg production in a boreal peatland [Coleman Wasik *et al.*, 2012; Jeremiason *et al.*, 2006]. Two severe droughts occurred during the course of that study, effectively overlaying a drying and rewetting manipulation onto the sulfate addition experiment. The purpose of this paper is to describe the effects of these drought events on mercury cycling in the context of the depositional history of sulfate. The experimental peatland was divided into treatments that received differing sulfate loads, and intensive pore water sampling was used to monitor dissolved sulfate, Hg<sub>T</sub>, and MeHg concentrations before, during, and after drought. Solid phase (peat) samples were also collected over the course of the experiment and are discussed in greater detail elsewhere [Coleman Wasik *et al.*, 2012]. In addition, water levels were experimentally manipulated in mesocosm enclosures to simulate natural drought-induced changes in sulfur and mercury cycling. Our main objectives were to (1) determine whether differential atmospheric sulfate loading affected sulfate release following drought, (2) understand how the oxidizing effects of drought affected mercury cycling, and (3) explore the interaction between drought-induced sulfate release and MeHg production.

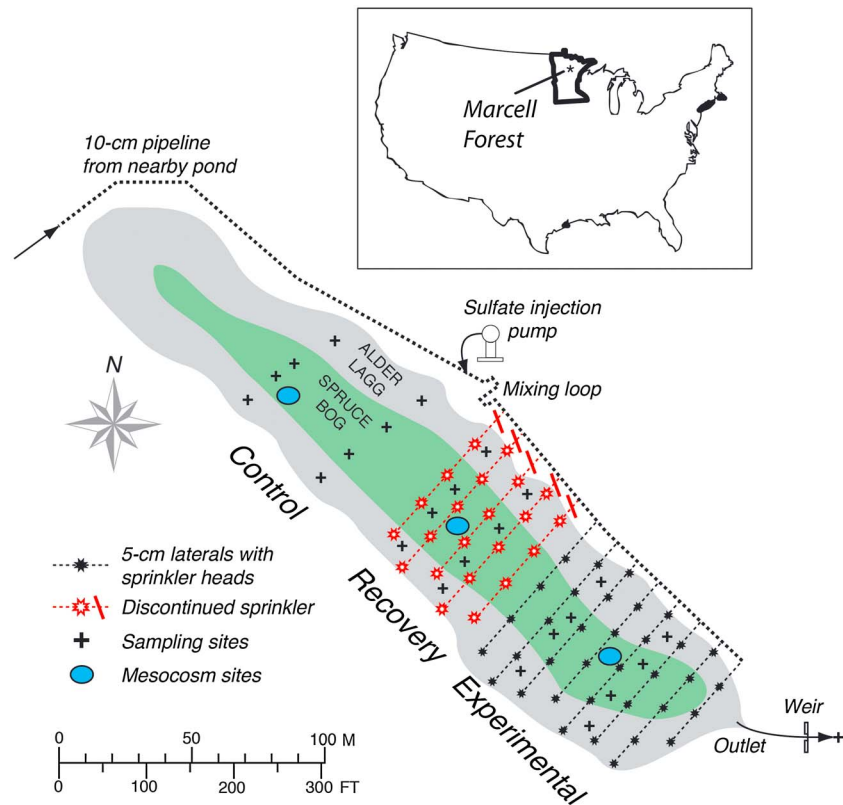
## 2. Materials and Methods

### 2.1. Field Site Description

The study was conducted in the S6 peatland located within the Marcell Experimental Forest (MEF), a unit of the Chippewa National Forest in northern Minnesota (Figure 1). The 2.0 ha S6 peatland occupies an elongate, ice-block depression common in the glacial landscape surrounding the MEF [Sebestyen *et al.*, 2011]. The raised ombrotrophic center of the S6 peatland is dominated by an overstory of mature black spruce (*Picea mariana*) and tamarack (*Larix laricina*) and an understory of ericaceous shrubs (e.g., *Chamaedaphne calyculata* and *Ledum groenlandicum*), herbaceous forbs (e.g., *Cypripedium acaula* and *Menyanthes trifoliata*), and *Sphagnum* spp. [Marcell Experimental Forest (MEF), 2013]. Alder (*Alnus rugosa*) along the peatland margin delineates the minerotrophic lagg, which receives runoff from a 6.9 ha white spruce (*Pinus glauca*) and red pine (*Pinus resinosa*) upland [MEF, 2013].

The regional climate at the MEF is continental, with annual precipitation averaging 710 mm between 2000 and 2008 (Table 1). A significant portion of the precipitation is received during the winter months, and because hydrology in the S6 peatland is driven by precipitation, spring snowmelt is typically the largest





**Figure 1.** A schematic of the experimental design within the S6 peatland illustrating the PVC rainfall simulator, location of sampling sites, and experimental mesocosm locations. See text for details. The inset map shows the location of the Marcell Experimental Forest in Minnesota.

hydrologic event of the year [Nichols and Verry, 2001]. The S6 peatland is hydrologically perched above the regional groundwater table, and therefore, its water table elevation (WTE) and outflow are heavily dependent on precipitation. The lagg margin represents the dominant flow path for both the central bog and upland catchment, with the central bog generally contributing most of the total outflow [Rauneker, 2010]. WTE and outflow are monitored continuously by the United States Forest Service Northern Research Station at a centrally located bog well and a 120° V notch weir, respectively. Upland near-surface flow and interflow collectors are used to estimate hydrologic and chemical inputs from the uplands.

**2.2. Sulfate Deposition Experiment**

Results reported here were obtained during a long-term study (2001–2008) of the effects of elevated atmospheric sulfate deposition on MeHg production in a sulfur-limited peatland. Ambient sulfate deposition, recorded since 1977 at the MEF (National Atmospheric Deposition Program site MN16) [National Atmospheric Deposition Program, 2014] decreased by 50% from 11 kg ha<sup>-1</sup> yr<sup>-1</sup> in the early 1980s to approximately 5.5 kg ha<sup>-1</sup> yr<sup>-1</sup> in the mid-2000s. Sulfate deposition to the experimental treatment in this study was increased by 32 kg ha<sup>-1</sup> yr<sup>-1</sup> (~4X the ambient 1990s rate) to simulate late twentieth century sulfate deposition rates experienced across much of eastern North America.

**Table 1.** Average Annual Precipitation, Outflow, and Water Table Elevation (WTE) in the S6 Peatland for the Periods 1964–2008, 2000–2008, and 2005–2007<sup>a</sup>

	Precipitation mm	Outflow mm (m <sup>3</sup> )	WTE Mean (m asl)	WTE Min (mm)	WTE Max (mm)	Median Δ WTE (mm)	Max Δ WTE (mm)
1964–2008	773	155 (13,832)	422.675	−475	+442	360	660
2000–2008	710	110 (9,766)	422.696	−414	+421	400	620
2005–2007	660	91 (8,128)	422.634	−352	+95	550	620

<sup>a</sup>WTE Mean represents the mean for the period of record indicated. WTE Min and WTE Max are the difference in mm between the WTE mean and the WTE Min or Max. Δ WTE is the difference between the annual maxima and minima WTE in the peatland.

The experimental design of the overall study has been described previously [Coleman Wasik *et al.*, 2012; Jeremiason *et al.*, 2006]. Briefly, in 2001 the S6 peatland was divided roughly in half into control and experimental treatments, and a PVC rainfall simulator was constructed across the experimental portion. This system consisted of a 10 cm main pipeline that ran along the northern edge of the peatland and 13 5 cm lateral lines extending across the original experimental treatment, each with rotating sprinkler heads mounted on 1 m vertical risers spaced at regular intervals. Dilute surface water (specific conductivity =  $20 \mu\text{S cm}^{-1}$ ) was drawn from a nearby pond, and a concentrated sodium-sulfate solution was injected into the main pipeline at a point downgradient of the control treatment. A mixing loop in the main pipeline ensured that the concentrated sulfate was thoroughly mixed with the source water. Sulfate was added in three simulated rainfall events each year (spring, summer, and fall). Each sulfate addition was followed by a rinsing period to wash sulfate off the vegetation, resulting in a total of 6–8 mm of simulated rainfall. In the spring of 2006 a new recovery treatment was established by discontinuing sulfate addition to the upgradient, one third of the original experimental treatment. A bromide tracer was added during each application to monitor movement of application water. However, bromide was not conservative in the peat and so served instead as a presence/absence indicator rather than a quantitative tracer.

### 2.3. Water Table Mesocosm Experiment

Twelve water table mesocosms were installed across the peatland in July of 2007 to experimentally manipulate hydrologic fluctuations and measure the effects on sulfur and mercury cycling. Four 75 cm lengths of 30.5 cm (ID) PVC pipe were driven into homogeneous lawn areas of the central bog within each treatment (control, recovery, and experimental). Each mesocosm was allowed to equilibrate overnight, and pore waters were sampled the next day to capture mercury and sulfate concentrations prior to water table manipulation. Deionized water was then added to each mesocosm until the water table was approximately 1 cm above the peat surface. Not all mesocosm installations were successful in maintaining experimental water levels above the peatland water table. If water levels in mesocosms fell by more than 5 cm overnight (owing to leakage out the bottom), the PVC pipe was repositioned and again monitored for leaks. Mesocosms were reset up to 2 times before abandoning the effort at that location. Once mesocosms were successfully installed, pore waters were sampled on days +1, +2, +3, +7, +9, +11, and +13 after raising the water table. Deionized water was added periodically to maintain water levels at the peat surface as sampling and evaporative losses caused declines. The mesocosm experiments were initiated 1 week prior to the summer 2007 sulfate addition. Mesocosms located in the experimental treatment were covered during the sulfate application, following which 130 mg of  $\text{Na}_2\text{SO}_4$  was added directly to each in a dilute, deionized water solution. This application rate was comparable to the amount added to the S6 peatland during the summer sulfate addition.

### 2.4. Pore Water Sampling

The short-term effects of sulfate addition were monitored through intensive sampling of peatland pore waters before and after each addition. Initially, two transects were established in the control and experimental treatments, and four 1 m<sup>2</sup> sampling plots were evenly distributed among the central bog and lagg margins along each transect. Sampling plots were preferentially located in lawn areas [Branfireun, 2004]. In 2006 two transects were established in the newly created recovery treatment, and the original experimental treatment transects were relocated further downgradient to ensure that sampling occurred within the treated area. At the same time instrument sites housing a pressure transducer and nested temperature and oxidation-reduction potential electrodes (at 10, 20, and 30 cm depths) interfaced to a Campbell data logger were also installed in the central bog along the southernmost transect in each treatment. These sites were used to monitor the interaction between local water table elevation and redox conditions in the peat. Pore water samples were collected in triplicate from bog plots located next to the instrument sites in order to increase sample numbers and assess the local heterogeneity in pore water chemistry. Pore waters were collected from each plot on days –1, +1, +3, and +7 relative to each sulfate addition as well as on day +14 for every spring and fall addition. Beginning in 2006 pore waters were sampled with greater frequency in the spring, either starting with snowmelt or beginning soon thereafter, and an additional sampling day was added 1 week prior to the fall sulfate additions. In the fall of 2007 pore waters from each plot were also sampled on days +2, +4, +9, +14, +18, and +27 after a large rainfall event on 6 September.

Pore waters were collected using a portable peristaltic pump and a 1.9 cm ID, Teflon probe with a 5 cm perforated tip. The probe was inserted into the peat 5–10 cm below the water table surface [Branfireun *et al.*, 2001; Branfireun *et al.*, 1999]. Pore waters were then drawn through 0.64 cm ID Teflon tubing by a peristaltic GeoPump and passed through acid-washed 47 mm Teflon filter holders (Savillex Co.) prefitted with ashed, 0.7  $\mu\text{m}$ , glass-fiber filters into sample bottles. Samples for dissolved  $\text{Hg}_T$ , MeHg, and major anions were collected from each plot on every sampling day. Samples for dissolved organic carbon (DOC) were collected from each plot 1 day prior to sulfate additions in 2005 and 2006 and on each sampling day in 2007 and 2008. All mercury samples were collected directly into new, 125 mL PETG bottles using accepted, clean sampling techniques [Bloom and Fitzgerald, 1988] and preserved by acidifying to 0.5% (vol/vol) with high-purity HCl. Field duplicates and equipment blanks accounted for 10% of all samples.

## 2.5. Analytical Methods

### 2.5.1. Anions

Pore water samples were analyzed for anions ( $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ , and  $\text{Br}^-$ ) by ion chromatography on a Dionex DX-500 according to standard methods. Each run included 10% deionized water blanks, 10% sample duplicates, and check standards. Check standards and duplicates were within 10%, and detection limits for each anion were 0.01  $\text{mg L}^{-1}$  in each year.

### 2.5.2. Dissolved Organic Carbon

Pore water samples were analyzed for DOC according to standard methods by either a UV-persulfate oxidation method on a Tekmar-Dohrmann Phoenix 8000 or by catalytic combustion on a Shimadzu carbon analyzer. All samples were analyzed in duplicate. Check standards and equipment blanks accounted for 10% of analyzed samples. Sample replicates and check standards were within 10%, and equipment blanks were generally less than 1  $\text{mg L}^{-1}$  DOC each year.

### 2.5.3. Mercury

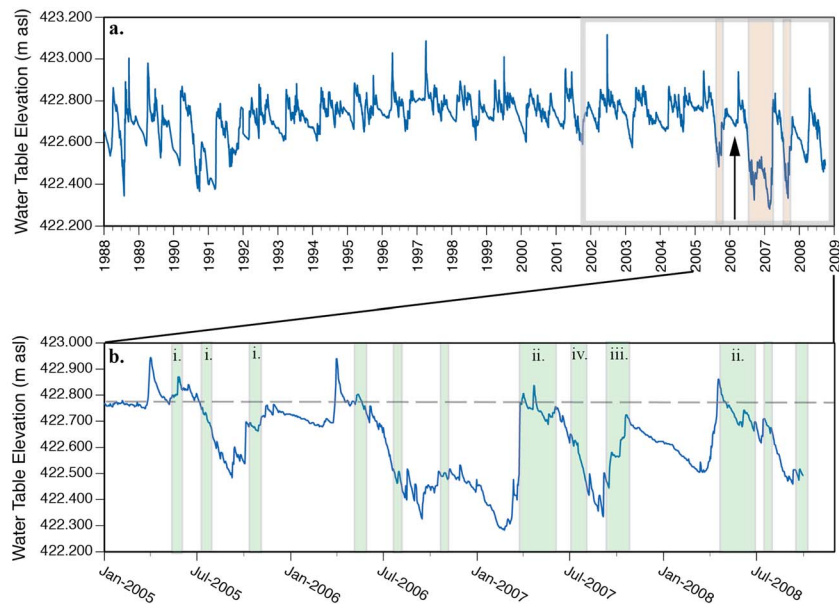
Dissolved  $\text{Hg}_T$  was analyzed according to Environmental Protection Agency (EPA) method 1631, Revision E on a Tekran 2600 Automated Total Mercury Analyzer. Samples were allowed to oxidize overnight with bromine monochloride to convert all mercury species to  $\text{Hg}^{2+}$  and then neutralized with hydroxylamine prior to analysis. Mercury was converted to  $\text{Hg}^0$  using stannous chloride reduction, purged from solution, and trapped on gold traps. Mercury was then thermally desorbed in a stream of argon and analyzed by cold vapor atomic fluorescence spectroscopy (CVAFS). The instrument was calibrated daily, and each analytical run included 20% deionized water blanks, 10% sample duplicates, and 5% matrix spikes. In all years spike recoveries were between 78 and 114%, relative percent differences between duplicates were less than 10%, and method blanks were below 1  $\text{ng L}^{-1}$ .

Dissolved MeHg was analyzed according to methods described in Bloom [1989] and Liang *et al.* [1994]. Samples were first distilled with 8 M  $\text{H}_2\text{SO}_4$  and 20% KCl (wt/vol) in an extraction manifold using acid-cleaned Teflon vessels. Distillates were refrigerated and analyzed within 48 h. All mercury species in solution were ethylated using sodium tetraethylborate, purged from solution in a stream of nitrogen, and trapped on Tenax traps. The trapped mercury species were thermally desorbed in a stream of argon or helium and separated during passage through a chromatographic column. The separated mercury species were then converted to  $\text{Hg}^0$  in a pyrolytic trap and analyzed by CVAFS on a Tekran 2500 or Brooks Rand Model III spectrometer. The instruments were calibrated daily, and each analytical run included 5% deionized water blanks, 10% sample duplicates, and 5% matrix spikes. In all years spike recoveries were between 98 and 103%, relative percent differences between duplicates were less than 12%, and method blanks were below 0.15  $\text{ng L}^{-1}$ .

Poor calibration curve linearity, high blanks, or quality control samples more than 15% deviation from expected concentrations in any  $\text{Hg}_T$  or MeHg analysis precluded sample analysis until the analytical issue was resolved. Quality assurance and control results for total and methyl-mercury analyses for each year can be found in Tables S1 and S2 in the supporting information.

## 2.6. Numerical Analyses

All statistical analyses were performed using the statistical software R [R-Development-Core-Team, 2011]. The Wilcoxon rank-sum analysis was used to compare mean sulfate and mercury concentrations between two treatments at a time on each day and within each treatment before and after sulfate additions or storm events. Kruskal-Wallis analyses were used to (1) assess differences in sulfate and mercury concentrations in



**Figure 2.** (a) Twenty-year record of water table elevation in the S6 peatland (1988–2008). The gray box bounds the study period. The arrow indicates the start of the recovery treatment. The brown-shaded bands denote the severe droughts that occurred during the course of the sulfate addition experiment. (b) This period of recent variability is magnified, and sulfate additions and sampling periods in each year are indicated by the green-shaded bands. The average elevation of the peat surface is indicated by the dashed horizontal line. Roman numerals denote sampling periods highlighted in Figure 4 (i), Figure 5 (ii), Figure 6 (iii), and Figure 7 (iv).

the spring of 2007 and the spring of 2008 that resulted from very different antecedent moisture conditions and (2) assess the effect of water-level manipulations in the experimental water table mesocosms. Statistical analyses of mesocosm data were conducted separately for  $Hg_T$ , MeHg, %MeHg,  $SO_4^{2-}$ , and DOC within each treatment. The day relative to experimental water table rise was considered as a nominal variable in the analysis. A  $P$  value less than or equal to 0.05 was considered significant.

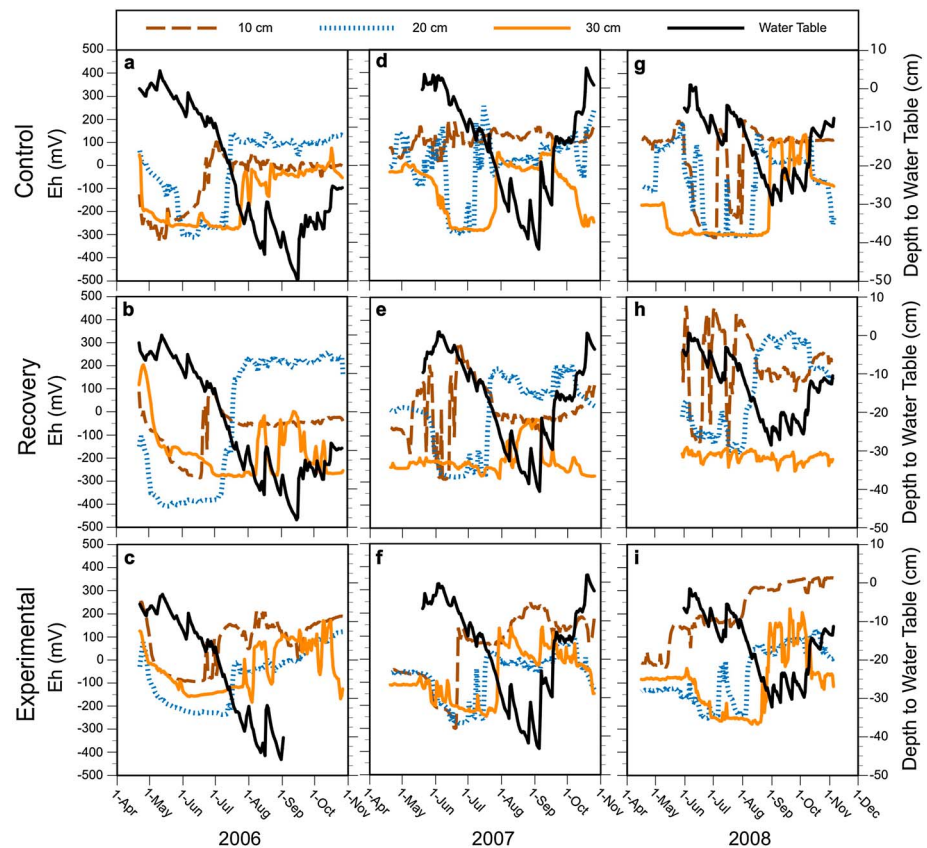
A series of multiple regression analyses was performed to ascertain correlation between the magnitude and duration of fluctuations in WTE and sulfate concentrations within each treatment. Sulfate data were averaged by treatment for each sampling day and then natural log transformed prior to regression analyses to normalize residual error. Sulfate values from the experimental treatment that were collected within 1 week of sulfate addition were not included in the analysis to avoid bias in the data set. To isolate the influence on observed pore water chemistry of the magnitude and duration of the water table fluctuations, we calculated the maximum change in the water table and the duration of that change for each of five different periods (10, 20, 30, 60, and 90 days) preceding each pore water sampling date.

### 3. Results

#### 3.1. Drought in the S6 Peatland

##### 3.1.1. Effect on Water Table Elevation

The S6 peatland is considered to be a poor fen with little or no connection to the regional groundwater table [Sebestyen *et al.*, 2011]. The center of the peatland is raised relative to its margins creating an ombrotrophic system that relies predominantly on atmospheric precipitation for water and nutrient inputs. The lack of a moderating, regional hydrologic influence results in relatively large interannual and intraannual variations in water table elevations (WTEs) and outflow (Table 1). Water levels and outflow generally reach peak values during and after spring snowmelt, decline over midsummer to late summer, and usually rebound during the fall after vegetation senescence (Figure 2). This general pattern varies from year to year. For example, during abnormally wet years there may be no summer decline, while during abnormally dry years there may be no fall rebound (e.g., 1999 and 2006, respectively; Figure 2a). Severe droughts have occurred at the MEF several



**Figure 3.** Eh profiles at 10, 20, and 30 cm depths and depth to water from the peat surface in the control, recovery, and experimental treatments in 2006, 2007, and 2008.

times over the nearly 50 years of data collection (1967–1968, 1976–1977, 1990–1991, and 2006–2007) and were initiated by a year in which the area received less than 600 mm of precipitation.

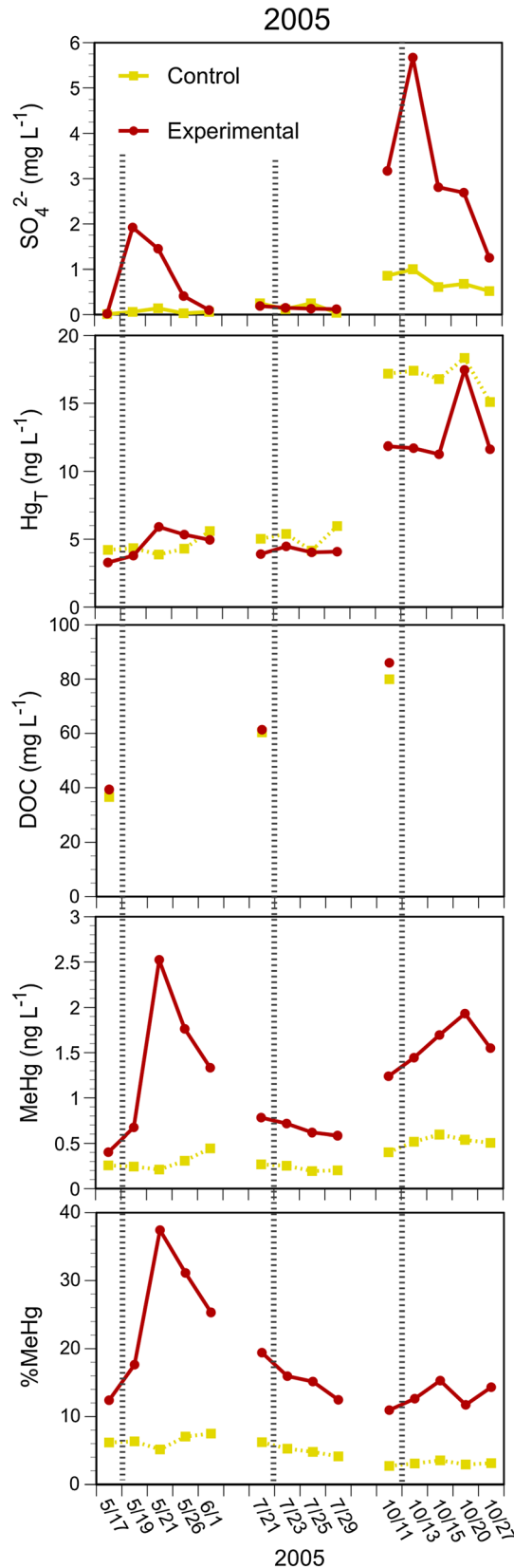
The most recent drought occurred during the course of the 8 year sulfate addition experiment in S6 (Figure 2b). In 2006 the MEF received 561 mm of precipitation. The WTE reached its annual maximum of 422.94 m above sea level (asl) on 31 March during the spring snowmelt and then declined to a minimum of 422.33 m asl on 15 September. The water table rebounded slightly in late September/early October but then resumed a slow decline until snowmelt the following spring.

In February 2007 the WTE in S6 reached 422.28 m asl—the lowest level measured in 30 years—and then rebounded more than 550 mm during the snowmelt period in late April, resaturating peat that had been dry for nearly 9 months. The S6 WTE remained relatively stable throughout May and then began a decline through the summer, similar to that seen the previous year. In September several large rain events over the MEF raised the WTE 390 mm over the course of 6 weeks (6 September to 19 October). The water table began another decline in late October that lasted through the winter. However, the wetland froze in a saturated condition as opposed to the very desiccated state of the previous year. In 2008 the WTE resumed a more historically typical pattern.

### 3.1.2. Effect on Oxidation-Reduction Potential

The oxidation-reduction potentials measured within each treatment at three different depths in 2006, 2007, and 2008 provided insight on the depth of oxygen penetration into the peat as water tables rose and fell (Figure 3). Generally, redox conditions were moderately elevated in the early spring of each year and then became more negative as the peatland thawed and warmed. As the water table fell past each probe depth during the summer the corresponding redox potentials jumped to very positive values indicating the intrusion of oxygen. When the water table rebounded in the fall redox potentials declined slowly toward their previous levels, presumably as oxygen was consumed.





The peat at 10 cm in each treatment was often subject to oxidizing conditions regardless of whether the peatland was experiencing drought or not (Figure 3). Strongly negative redox values were primarily observed at 10 cm depth during the spring when the water table was at or near the peat surface. The peat at 20 cm depth experienced larger changes in redox conditions over the course of each year in response to declining water tables and large rainfall events. Strongly negative values prevailed during the spring and early summer periods while the late summer and fall were characterized by positive redox values. Large rainfall events on 1 July 2007 and 13 July 2008 caused transient increases in redox values at 20 cm depth (Figures 3d–3i), possibly owing to downward percolation of oxygenated rainwater. Shortly thereafter the WTE continued its steep summer decline, and redox potentials spiked upward and remained there well into the fall of both years. Redox conditions were most consistent at 30 cm depth among treatments and years, declining to low steady values in spring or early summer and then spiking upward as WTE fell below the probe depth in midsummer. Because water tables fell particularly low in 2006 and 2007, oxygen was able to penetrate to 30 cm depth for extended periods of time (Figures 3a–3f).

**3.2. Response of Pore Water Sulfate and Mercury to Drying Events**  
**3.2.1. Water Table Elevation and Sulfate Addition**

Sulfate was added to the experimental treatment 3 times during each field season between 2002 and 2008 to observe possible stimulation of mercury methylation. The effectiveness of each sulfate addition was influenced by the position of the water table, as

**Figure 4.** Pore water chemistry in the S6 peatland in 2005 (May–October). Dashed lines indicate experimental sulfate additions. DOC samples were collected only on the day prior to each sulfate addition in 2005.

exemplified by trends in pore water %MeHg and sulfate concentrations in 2005 (Figure 4). Over the sulfate addition and sampling period in spring of 2005 the WTE was high, averaging 422.825 m asl. Sulfate concentrations in experimental treatment pore waters increased nearly 2 orders of magnitude from near detection before the sulfate addition to  $1.92 \pm 0.03 \text{ mg L}^{-1}$  after the addition. Three days after the sulfate addition, as sulfate concentrations were declining, %MeHg rose by 3X (from  $12 \pm 9\%$  to  $39 \pm 18\%$ ). Because concurrent  $\text{Hg}_T$  concentrations remained constant in the experimental treatment, this MeHg rise is attributed to increased production. Sulfate and  $\text{Hg}_T$  concentrations and %MeHg in the control treatment were stable ( $<0.14 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ,  $<5 \text{ ng Hg}_T \text{ L}^{-1}$ , and 5–8% MeHg) throughout the spring period.

By the time of the summer sulfate addition water tables had fallen 130 mm since the spring addition. In contrast to the spring period sulfate concentrations did not increase in the experimental treatment, but instead remained similar to control treatment levels, likely because added sulfate did not reach the water table. Experimental treatment %MeHg levels also remained stable over the period but were elevated 2–3 times above control treatment levels. Again,  $\text{Hg}_T$  concentrations in the control and experimental treatments were stable and consistently low over the monitoring period ( $4\text{--}5 \text{ ng L}^{-1}$ ; Figure 4). DOC levels during the summer application period were 50% higher than spring concentrations.

In the fall of 2005 sulfate concentrations were already elevated in both control and experimental treatment pore waters ( $0.86 \pm 1.39$  and  $3.17 \pm 1.83 \text{ mg L}^{-1}$ , respectively; Figure 4) prior to the sulfate addition, which itself was preceded by an 80 mm rain event. Sulfate concentrations in experimental treatment pore waters increased to  $5.67 \pm 1.83 \text{ mg L}^{-1}$  following the addition, while %MeHg increased only modestly (from  $11 \pm 6\%$  to  $15 \pm 4\%$ ), despite sulfate concentrations that were nearly 3 times those that were associated with a 3X increase in %MeHg after the spring addition. Moreover, %MeHg levels in the control treatment were stable over the sampling period and lower than during either the spring or summer sulfate additions (3–4%).  $\text{Hg}_T$  concentrations in both treatments were 3–4X higher than at any time during the previous spring or summer (Figure 4), and DOC concentrations were 2X spring concentrations.

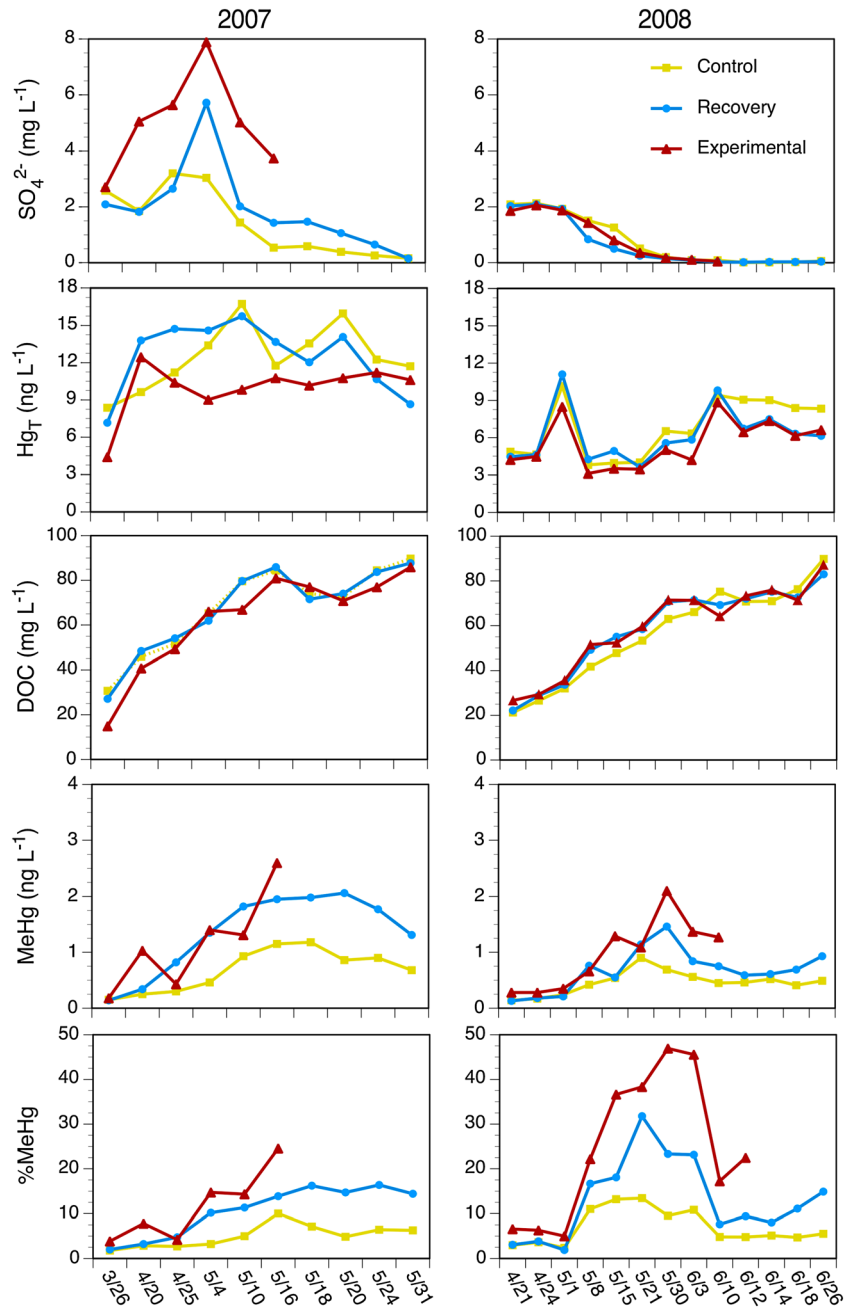
### 3.2.2. Rewetting Events

The severe droughts in 2006 and 2007 and the rewetting events that followed caused large swings in WTE and highlighted the effects of hydrologic fluctuations on sulfur and mercury biogeochemistry in the S6 peatland.

#### 3.2.2.1. Spring Thaw Period

The 2006 drought persisted into the winter causing the upper 30–40 cm of the acrotelm in the S6 peatland to freeze in an oxidized state. Therefore, an extensive sampling campaign was undertaken in the spring of 2007 to monitor sulfur and mercury cycling as the peatland resaturated. On 26 March pooled snowmelt was sampled from the frozen peat surface, and water chemistries were found to be uniform among treatments ( $2\text{--}3 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ,  $4\text{--}8 \text{ ng Hg}_T \text{ L}^{-1}$ ,  $0.14\text{--}0.18 \text{ ng MeHg L}^{-1}$ , and 1.7–3.9% MeHg; Figure 5). As the peat slowly thawed over the next 6 weeks a “natural” sulfate addition ensued. Sulfate concentrations peaked at very high levels for this peatland ( $3.20 \pm 3.54$ ,  $5.72 \pm 8.54$ , and  $7.89 \pm 2.58 \text{ mg SO}_4^{2-} \text{ L}^{-1}$  in the control, recovery, and experimental treatments, respectively). As sulfate concentrations declined MeHg concentrations and %MeHg reached peak levels that were significantly higher than early season lows ( $P < 0.05$ ; Wilcoxon rank sum) and were significantly different among treatments ( $P < 0.05$ ; control =  $1.18 \pm 1.53 \text{ ng MeHg L}^{-1}$ ,  $10 \pm 10\%$ ; recovery =  $2.06 \pm 2.34 \text{ ng MeHg L}^{-1}$ ,  $16 \pm 6\%$ ; and experimental =  $2.60 \pm 2.06 \text{ ng MeHg L}^{-1}$ ,  $25 \pm 14\%$ ).  $\text{Hg}_T$  concentrations increased significantly in the control and recovery treatments to 12 and  $16 \text{ ng L}^{-1}$ , respectively ( $P < 0.05$ ; Wilcoxon rank sum), and more than doubled relative to levels observed during the first sampling in each treatment. However,  $\text{Hg}_T$  concentrations did not show any systematic differences among treatments over the monitoring period. DOC concentrations rose steadily over the entire spring thaw period and were not significantly different among treatments ( $P > 0.05$ ; Wilcoxon rank sum).

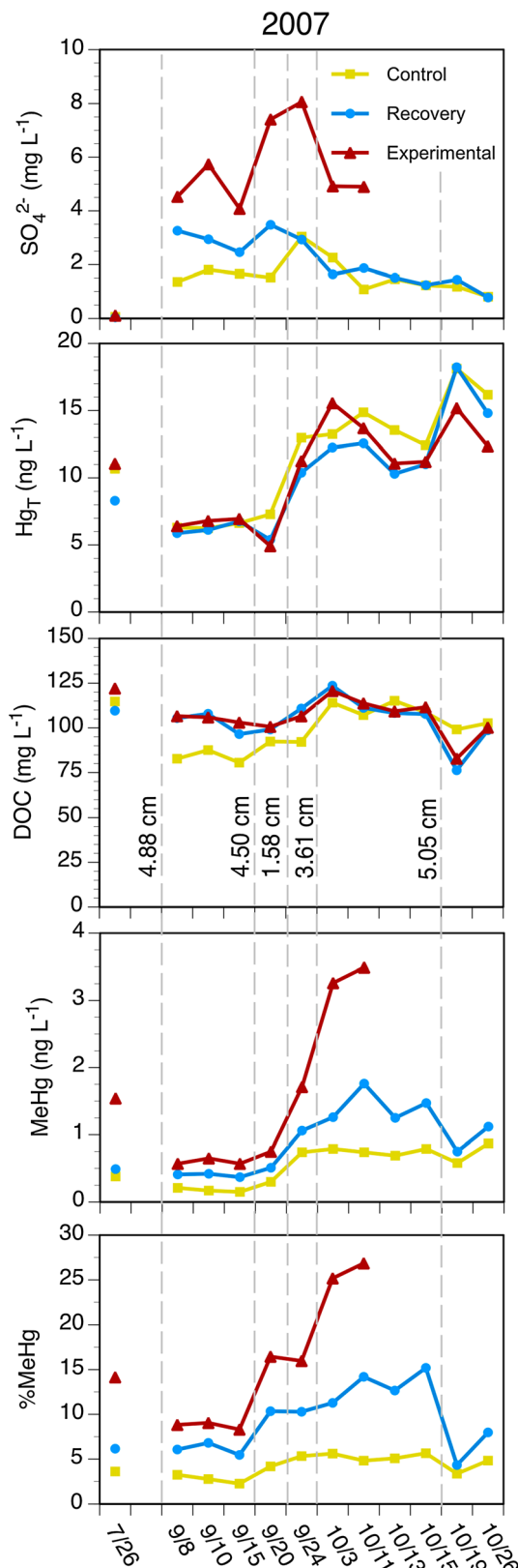
The sampling schedule developed for the spring of 2007 was followed in the spring of 2008 because antecedent moisture conditions prior to the spring of 2008 (described above) were opposite those in the spring of 2007 and provided a natural, experimental comparison (Figure 5). Sulfate concentrations were again near  $2 \text{ mg L}^{-1}$  in snowmelt water pooled on the frozen peat surface in all three treatments. However, in 2008 sulfate concentrations remained nearly identical among treatments over the entire sampling period and declined steadily over the thaw period to near-detection limits just prior to the spring 2008 sulfate addition. Despite much lower sulfate concentrations during the spring thaw period, MeHg concentrations



**Figure 5.** Pore water chemistries in each treatment of the S6 peatland over the spring-thaw and sulfate addition periods in 2007 and 2008. Only preaddition data are shown for sulfate, MeHg, and %MeHg levels in the experimental treatment.

followed a similar pattern to that observed in 2007 (Figure 5). Peak MeHg concentrations were somewhat lower than those seen in 2007 ( $0.90 \pm 0.80$ ,  $1.46 \pm 1.51$ , and  $2.10 \pm 0.72$   $\text{ng L}^{-1}$  in the control, recovery, and experimental treatments, respectively), but %MeHg levels appeared to be higher in 2008 ( $13 \pm 8$ ,  $32 \pm 6$ , and  $47 \pm 22\%$  in the control, recovery, and experimental treatments, respectively), and the difference between the control treatment and the recovery and experimental treatments was more pronounced than in 2007 (Figure 5).  $\text{Hg}_T$  concentrations were generally lower than in 2007; again, there were no significant differences in  $\text{Hg}_T$  concentrations among treatments ( $P > 0.05$ ; Wilcoxon rank sum). Dissolved organic carbon concentrations rose steadily again over the entire 2008 spring thaw period and were not significantly different among treatments ( $P > 0.05$ ; Wilcoxon rank sum).





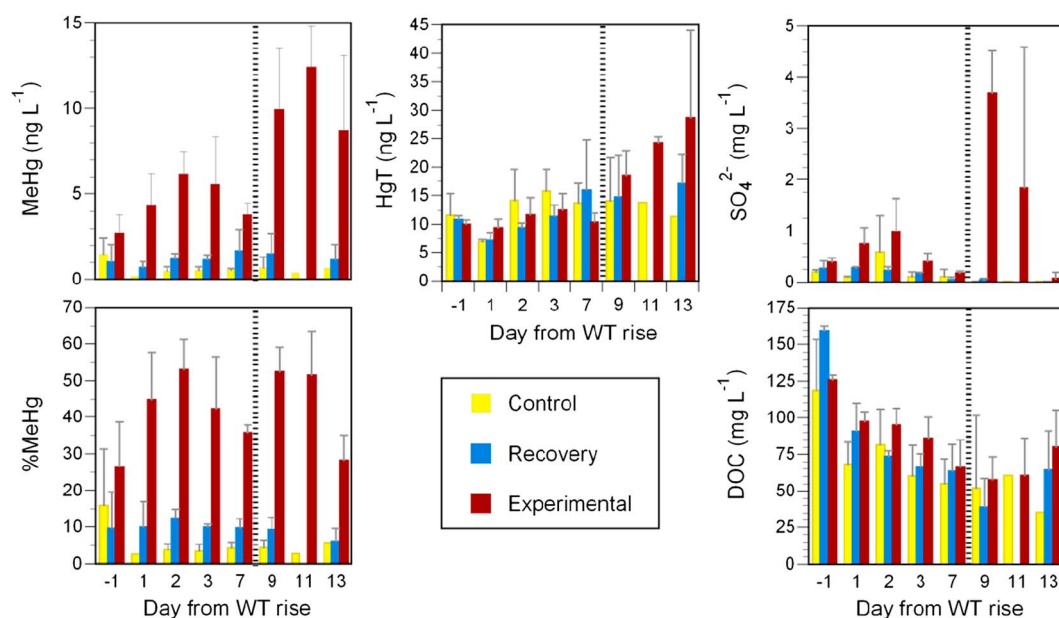
### 3.2.2.2. Fall Water Table Rise

In September 2007 a series of large rainfall events drove a relatively rapid water table rise and relieved the severe summer drought. As was seen during the rewetting event in the spring of 2007, sulfate concentrations rose significantly from late July values as the peat resaturated (Figure 6;  $P < 0.05$ ; Wilcoxon rank sum), and significant differences existed in peak sulfate concentrations among the treatments ( $P < 0.05$ ; Wilcoxon rank sum;  $3.04 \pm 3.58 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ ,  $3.48 \pm 2.58 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ , and  $8.06 \pm 4.86 \text{ mg SO}_4^{2-} \text{ L}^{-1}$ , in the control, recovery, and experimental treatments, respectively). In early September following the first rainfall event, average MeHg concentrations, and %MeHg in the control and recovery treatments were comparable with late July values (Figure 6), while in the experimental treatment MeHg concentrations were significantly lower ( $P < 0.05$ ; Wilcoxon rank sum). Subsequently and over the course of three additional rain events, MeHg concentrations and %MeHg rose significantly ( $P < 0.05$ ; Wilcoxon rank sum), reaching peak levels by early October (control =  $0.87 \pm 0.63 \text{ ng MeHg L}^{-1}$  and  $6 \pm 4\%$  MeHg; recovery =  $1.76 \pm 0.90 \text{ ng MeHg L}^{-1}$  and  $15 \pm 6\%$  MeHg; experimental  $3.49 \pm 1.69 \text{ ng MeHg L}^{-1}$  and  $27 \pm 15\%$  MeHg). Hg<sub>T</sub> concentrations were also significantly lower in early September relative to late July ( $P < 0.05$ ; Wilcoxon rank sum), then rose significantly by late September ( $10\text{--}13 \text{ ng L}^{-1}$ ;  $P < 0.05$ ; Wilcoxon rank sum) and were similar among treatments throughout the entire water table rise. Fall DOC concentrations were comparable to late July levels and remained relatively constant over the entire monitoring period.

### 3.3. Experimental Water Table Manipulation

A series of water table mesocosms was used to experimentally simulate a water table rise after a period of low WTE in mid-July 2007. Hg<sub>T</sub>, MeHg, sulfate, and DOC concentrations in pore waters were measured 1 day prior to the experimental WTE rise and for up to 2 weeks thereafter. The effects of the water table experiments varied by

**Figure 6.** Pore water chemistries in each treatment of the S6 peatland over the fall water table rise in 2007. Only preaddition data are shown for sulfate, MeHg concentrations, and %MeHg levels in the experimental treatment. Major rainfall events are indicated by dashed lines and depths (cm).



**Figure 7.** Pore water chemistries in the water table mesocosms in each treatment. The dashed lines indicate experimental sulfate additions to the experimental treatment.

treatment and by chemical constituent (Figure 7). In the control and recovery treatments the water table rise did not have a significant effect on any of the chemical constituents measured ( $P > 0.05$ ; Kruskal-Wallis), whereas in the experimental treatment the water table rise significantly affected all measured constituents ( $P \leq 0.05$ ; Kruskal-Wallis).  $Hg_T$  concentrations rose over the duration of the experiment while DOC concentrations fell. Sulfate, MeHg, and %MeHg each peaked 2 days after the water table rise and then declined until day 8. Because summer sulfate additions in 2005 (Figure 4) and 2006 did not cause any measurable changes to sulfate or mercury in experimental treatment pore waters, the experimental treatment water table mesocosms were also used to determine whether sulfate applied during high WTE in the summer would cause similar MeHg responses to those measured during the spring. Sulfate was applied to the water table mesocosms in the experimental treatment 1 week following the WTE manipulation. In contrast to trends observed during the summer sulfate additions in 2005–2007, sulfate concentrations peaked in the experimental treatment mesocosms 1 day following the sulfate addition (day 9 after the water table rise), while MeHg and %MeHg peaked 3 days after sulfate addition (day 11).

## 4. Discussion

### 4.1. Sulfate Release After Drought

#### 4.1.1. Sulfate and Antecedent Moisture Conditions

The sulfate concentrations measured in S6 pore waters were similar to those reported for other boreal peatlands [Mitchell *et al.*, 2008a; St. Louis *et al.*, 1994] as well as for peatland mesocosms experimentally amended with sulfate [Bergman *et al.*, 2012; Branfireun *et al.*, 1999]. However, the sulfate concentrations in this study tended to be much lower than those measured in areas that are currently, or were historically, impacted by high levels of atmospheric sulfate deposition, such as the northeastern United States [Mitchell and Likens, 2011; Selvendiran *et al.*, 2008] and eastern Canada [Eimers and Dillon, 2002; Eimers *et al.*, 2007; Warren *et al.*, 2001]. Sulfate concentrations in S6 pore waters rose following each extended dry period in this 8 year study, which is consistent with observations in other peatland, temperate wetland, and stream systems [Bayley *et al.*, 1986; Bayley *et al.*, 1992; Devito and Hill, 1999; Eimers and Dillon, 2002; Eimers *et al.*, 2007; Kerr *et al.*, 2012; Mitchell and Likens, 2011; Warren *et al.*, 2001].

In this study the average sulfate concentration for each sampling date (excluding experimental treatment values immediately following sulfate addition) appeared to be inversely related to antecedent moisture

**Table 2.** Regression Statistics for the Sulfate Concentrations in the Control, Recovery, and Experimental Treatments Against the Maximum Change in WTE ( $\Delta WTE$ ) Over the Preceding 10, 20, 30, 60, and 90 Day Periods and the Duration of That Change ( $\Delta t$ )<sup>a</sup>

Treatment	Preceding Period	Max $\Delta WTE$ Coefficient	Max $\Delta t$ Coefficient	Max $\Delta WTE$ * Max $\Delta t$ Coefficient	Model RSE (d.f.)	$r^2$	F Statistic P value
Control	10 day	17.29	0.20	-0.83	2.28 (113)	0.09	0.01
	20 day	28.57***	0.16***	-1.43***	2.15 (113)	0.19	<0.01
	30 day	7.99*	0.04	-0.19	2.31 (113)	0.06	0.07
	60 day	5.71*	0.08***	0.003	2.03 (113)	0.28	<0.01
	90 day	2.74	0.05***	0.007	2.18 (113)	0.16	<0.01
Recovery	10 day	10.81	0.09	-0.61	1.59 (51)	0.10	0.14
	20 day	12.03	0.08	-0.41	1.54 (51)	0.17	0.02
	30 day	8.88	0.04	-0.18	1.49 (51)	0.22	<0.01
	60 day	2.51	0.03	0.03	1.40 (51)	0.32	<0.01
	90 day	-8.37*	0.004	0.15**	1.57 (51)	0.14	0.05
Experimental	10 day	21.72*	0.10	-2.02	1.87 (44)	0.20	0.02
	20 day	19.26*	0.11	-0.83	1.87 (44)	0.20	0.02
	30 day	5.87	0.01	-0.06	1.93 (44)	0.15	0.07
	60 day	3.95	0.06*	0.01	1.79 (44)	0.27	<0.01
	90 day	4.09	0.08***	-0.01	1.63 (44)	0.40	<0.01

<sup>a</sup>Multiple regression equation:  $\log [SO_4^{2-}] = \text{max } \Delta WTE * \text{max } \Delta t + b + e$ . Significance codes.

\*\*\*Pr(>|t|) < 0.001.

\*\*Pr(>|t|) < 0.01.

\*Pr(>|t|) < 0.05.

conditions. Pore water sulfate concentrations were lowest when the water table had been high over the preceding time period and were highest when the water table had been low (Figure S1 in the supporting information). Furthermore, the relationship between pore water sulfate and changes in WTE became stronger with greater amplitude of fluctuation and increasing length of the drawdown period as indicated by the higher  $r^2$  and lower  $P$  values for the 60 and 90 day WTE regressions as compared to the 10 day WTE regressions (Table 2). Longer drought appears to result in greater mineralization of the peat. The increasing strength of the relationship between sulfate concentrations and the length of the drawdown period is not surprising given that other studies have found that the sulfate that appears during a rewetting event comes from the oxidation of organic sulfur compounds stored in the peat [Eimers *et al.*, 2003; Mandernack *et al.*, 2000; Mörth *et al.*, 1999]. Isotopic studies of sulfur cycling in peat have found that sulfate added to peatland mesocosms is predominantly incorporated into the organic-sulfur fraction of the peat matrix through bacterial sulfate reduction and plant uptake [Bartlett *et al.*, 2009; Chapman and Davidson, 2001] and that the sulfate released during rewetting events has a light isotopic signature relative to atmospheric deposition, suggesting reoxidation of sulfur from the “lighter” carbon-bound sulfur pool [Mandernack *et al.*, 2000; Mörth *et al.*, 1999].

The precipitation-driven hydrology of the S6 peatland allowed water tables to decline as much as 50 cm in particularly dry years, causing desiccation and oxidation of deep peat layers that normally experience strongly reducing conditions (Figure 3). Dramatic hydrologic fluctuations coupled with the high organic content of the peat make it likely that the sulfate released during rewetting events in this peatland comes from the carbon-bound sulfur pool. Furthermore, inorganic sulfur concentrations were low across the peatland ( $3 \pm 2\%$ ), making readily oxidized sulfur compounds like acid-volatile sulfides an unlikely source of recycled sulfate.

#### 4.1.2. Sulfate Release After Elevated Sulfate Deposition

For any given drying event more sulfate was mobilized into pore waters in the experimental treatment than in either the control or recovery treatments. Following rewetting events in the spring and fall of 2007, sulfate concentrations in experimental-treatment pore waters were more than twice that in the control treatment, while sulfate concentrations in the recovery treatment were intermediate between the control and experimental treatments (Figures 5 and 6). Because sulfate disappeared from pore waters following sulfate additions and rewetting events, and because no significant differences were found in the solid total-sulfur pool among the treatments [Coleman Wasik *et al.*, 2012], it appears that a greater fraction of the organic sulfur pool was available for release in peat that had recently experienced elevated sulfate loading. Furthermore, the

finding that sulfate release was greater in the recovery treatment than in the control treatment 2 years after sulfate additions had ended indicates that this more labile organic sulfur pool persisted for some time after elevated sulfate deposition had ceased. These observations provide support for our previous hypothesis [Coleman Wasik *et al.*, 2012] that newly added sulfate gradually becomes incorporated into more recalcitrant forms of organic sulfur over time.

The water table mesocosm experiments confirmed both the differential remobilization of sulfate among treatments and the importance of the duration of WTE drawdown and peat oxidation. Mesocosms in the experimental treatment experienced a significant increase in sulfate concentrations following the water table manipulation (increased WTE). No such sulfate rise was detected in the control or recovery treatments, and the rise that did occur in the experimental treatment was much lower than that observed following the 2006 and 2007 droughts. Average peak sulfate concentrations in the experimental treatment following each drought were roughly  $8 \text{ mg L}^{-1}$  as compared to  $1.0 \text{ mg L}^{-1}$  in experimental-treatment mesocosms following the WTE manipulation. The muted release in the mesocosms was likely a result of the short oxidation period experienced by the peat prior to mesocosm installation. The peat was not as desiccated as it had been during the 2006 and 2007 droughts—only the top 10–15 cm of peat experienced oxidizing conditions for approximately 3–4 weeks. Shorter-duration drawdowns likely affect loosely bound sulfate and labile organic sulfur compounds, whereas during extended droughts microbial communities and physical processes may begin to break down more recalcitrant pools of organic sulfur leading to greater sulfate remobilization.

The finding that sulfate is remobilized from wetlands following drought is not unique to this study. However, most previous research has involved ecosystems that were experiencing concurrent changes in ambient sulfate deposition and regional hydrology (drought cycles). The experimental design of the study presented here elucidates the additive effect of past and current sulfate deposition levels on the naturally occurring release of sulfate caused by drought cycles and provides insight into the mechanisms whereby sulfate release from historically impacted peatlands may decline.

## 4.2. Effect of Drought on Mercury Cycling

### 4.2.1. Total Mercury

Total mercury ( $\text{Hg}_T$ ) concentrations in S6 pore waters averaged between 3 and  $12 \text{ ng L}^{-1}$  during most sampling periods, which is similar to values reported for other peatlands [Heyes *et al.*, 2000; Mitchell *et al.*, 2008a; Regnell and Hammar, 2004; Selvendiran *et al.*, 2008]. However, during the fall of 2005 and the spring and fall of 2007, average  $\text{Hg}_T$  concentrations in pore waters jumped to  $12\text{--}20 \text{ ng L}^{-1}$  (Figures 4–6). These three sampling periods coincided with rewetting events in S6, likely indicating oxidative release of  $\text{Hg}_T$  from peat. The spring of 2007 and 2008 present a natural experimental contrast between dry and wet antecedent moisture conditions and its effect on  $\text{Hg}_T$  release. Over the entire spring thaw period in 2007 (26 March to 16 May) average  $\text{Hg}_T$  concentrations were 109–142% higher than the hydrologically similar period in 2008 (21 April to 30 May). It is interesting to note that  $\text{Hg}_T$  and sulfate release were very different following water table rise in the fall of 2007. Whereas sulfate concentrations 2 days after the initial fall water table rise were an order of magnitude higher than they had been on the last sampling day of the summer addition,  $\text{Hg}_T$  concentrations were 20–50% lower than they had been on the last sampling day of the summer addition. Furthermore,  $\text{Hg}_T$  concentrations remained stable for more than a week after the first major rain event that initiated the water table rise. Once  $\text{Hg}_T$  concentrations did start to rise, they more than tripled over the following 4 weeks. These observations suggest that peatlands have the potential to become large, short-term sources of mercury to downstream systems if mercury binding within the peat is disrupted by drought-induced oxidation.

The observed  $\text{Hg}_T$  releases were not necessarily controlled by DOC mobility. Given the close association between mercury and organic matter [Dittman and Driscoll, 2009; Driscoll *et al.*, 1995; Kolka *et al.*, 2001], it might be expected that the amount of  $\text{Hg}_T$  released would remain stable relative to DOC during peat oxidation and resaturation following a drought. However, we found that  $\text{Hg}_T$  concentrations in pore waters were substantially elevated relative to DOC 1 month following rewetting events in the fall of 2005 and 2007, indicating that short-term release of  $\text{Hg}_T$  following drought is more pronounced than for DOC (Figure S2 in the supporting information). Furthermore, whereas pore water  $\text{Hg}_T$  concentrations in the early spring of 2007 were 2–3X those measured in the early spring of 2008, the trend in DOC concentrations was consistent from year to year, rising slowly from  $\sim 20 \text{ mg L}^{-1}$  to  $\sim 80 \text{ mg L}^{-1}$  over the 2 month period following snowmelt.

The finding that  $Hg_T$  concentrations can vary independently of DOC concentrations in peatland pore waters warrants further investigation in light of the proposed use of continuous, in situ DOC measurements as a proximal indicator of mercury export from watersheds [Dittman *et al.*, 2009; Burns *et al.*, 2013].

Sulfate additions did not appear to affect pore water or solid-phase  $Hg_T$  concentrations during wet or dry periods, contrary to observations of Åkerblom *et al.* [2013], who found that long-term sulfate addition (10–20 kg ha<sup>-1</sup> yr<sup>-1</sup> for 14 years) to peatland mesocosms caused declines in solid phase  $Hg_T$ . In our study the inventory of  $Hg_T$  in the top 8 cm of peat in the experimental treatment was generally lower than that in the control treatment each year (with the exception of 2005), although the differences were not significant, nor was there a trend in the experimental treatment over the course of the 8 year study [Coleman Wasik *et al.*, 2012]. Sulfate addition might have been expected to mobilize mercury from the peat if that mercury was released from the carbon utilized by bacterial communities or if sulfides generated by the activity of sulfate-reducing bacteria (SRB) caused mercury to be stripped from the solid phase [Skjyllberg, 2008]. There was no evidence of this, as  $Hg_T$  concentrations in the control treatment pore waters were generally higher than those in the experimental and recovery treatments on a given sampling day, and there was no systematic trend in pore water  $Hg_T$  in the recovery treatment that would otherwise indicate a lingering effect of previous sulfate additions. Perhaps no effect was observed because the large pool of mercury present on the solid phase was a more important control on pore water  $Hg_T$  concentrations than the enhancement of microbial activity due to sulfate addition [Coleman Wasik *et al.*, 2012].

#### 4.2.2. Methylmercury

MeHg concentrations and %MeHg observed in this study (0.1–4.0 ng L<sup>-1</sup> and 2–50%, respectively) fall within the ranges reported in other boreal peatland studies [Bergman *et al.*, 2012; Branfireun *et al.*, 1999; Heyes *et al.*, 2000; Mitchell *et al.*, 2008a]. The MeHg present in peatland pore waters can come either from physical release (desorption) from the solid phase (where >99% of MeHg is found) or from net methylation. To support the elevated SRB-mediated production of MeHg observed in this system, sources of carbon, sulfate, and inorganic mercury must be available to microbes [Benoit *et al.*, 2002]. Because MeHg and the substrates required for SRB-mediated mercury methylation can all be released from the solid phase through peat oxidation, it is difficult to know whether simple oxidation or sulfate-stimulated methylation is more important in controlling MeHg flux from wetlands following drought. In this study both mechanisms (release and production) were observed to occur.

As described above, sulfate concentrations rose dramatically in all treatments in the spring of 2007 as the S6 peatland resaturated after a 9 month drought. Given that spring sulfate additions during the entire 8 year study consistently induced large methylation events in the experimental treatment [Coleman Wasik *et al.*, 2012; Jeremiason *et al.*, 2006], we expected that this large drought-induced pulse of sulfate in peatland pore waters would have a similar effect on MeHg production across treatments. Indeed, average pore water MeHg concentrations were significantly higher (29%, 146%, and 80% in the control, recovery, and experimental treatments, respectively;  $P < 0.01$ ; Kruskal-Wallis) during the snowmelt period in 2007 (26 March to 25 April) than in the hydrologically similar period in 2008 (21 April to 1 May). On the other hand, %MeHg levels during snowmelt were statistically the same between the 2 years ( $P = 0.54$ ; Kruskal-Wallis), suggesting that release of MeHg (and  $Hg_T$ ) from the solid phase occurred as the peat was resaturated following drought. However, as sulfate concentrations began to decline, MeHg concentrations and %MeHg levels rose further, while  $Hg_T$  concentrations remained relatively stable, likely indicating new MeHg production as a result of SRB activity. The same trend was observed when the WTE was manipulated in the water table mesocosm experiments providing further confirmation that a pulse of sulfate to pore waters during rewetting events can stimulate mercury methylation (Figure 7).

Despite significantly higher MeHg concentrations in the spring of 2007 as compared with 2008 ( $P < 0.01$ ; Kruskal-Wallis), %MeHg levels in 2007 were significantly lower than in 2008 ( $P < 0.005$ ; Kruskal-Wallis). That is, a larger fraction of pore water  $Hg_T$  was methylated in 2008 relative to 2007. This difference may be a function of the stable hydrologic conditions (consistently high WTE) present during the spring of 2008 as opposed to the spring of 2007 (initially low WTE). Because SRB activity requires anoxia, sulfate reduction and Hg methylation may have been inhibited for a period of time in 2007 by elevated oxygen in the peat profile. This idea is supported by the observation that sulfate concentrations continued to increase beyond the initial mercury release in late April of 2007. It is less likely that this delayed effect was a result of temperature



because in each spring sulfate concentrations began to decline well before the peat had thawed completely (Coleman Wasik, St. Croix Watershed Research Station, unpublished data, 2008).

The fall rewetting event in 2007 provided further confirmation that drought can cause not only MeHg release but also stimulate MeHg production. The largest rise in  $Hg_T$  concentrations occurred between 20 September and 24 September, and thereafter  $Hg_T$  concentrations stabilized. On the other hand, MeHg concentrations and %MeHg levels in the recovery and experimental treatments continued to increase beyond 24 September coincident with declining sulfate concentrations. These sustained increases likely represent new MeHg production caused by the drought-induced sulfate pulse.

This study allowed us to observe the effect of different atmospheric sulfate deposition rates on MeHg release and production in the context of hydrologic variability. More MeHg was produced and released in experimental and recovery treatments than in the control treatment following each drought. We previously reported [Coleman Wasik *et al.*, 2012] much higher MeHg concentrations in the solid phase within the experimental and recovery treatments relative to the control treatment and suggest here that a larger pool of MeHg is available for drought-induced release in peat that has experienced elevated rates of sulfate deposition. Furthermore, because the organic sulfur pool formed from recent sulfate deposition is more susceptible to oxidation and mobilization following drought, the potential exists for greater MeHg production from the activity of SRB as peat is resaturated. Finally, it appears that recent exposure to elevated sulfate deposition may have “primed” SRB communities in the experimental and recovery treatments because of either changes in the bacterial community itself or changes in their geochemical environment. In the spring of 2008 sulfate concentrations in peatland pore waters were the same among treatments after snowmelt and over the entire spring thaw period. However, MeHg concentrations and %MeHg levels increased to a much greater degree in experimental and recovery treatments relative to the control treatment. The observation that greater methylation ensued in treatments exposed to elevated rates of sulfate deposition—despite having, for a period of time, similar concentrations of pore water sulfate—may indicate that the bacterial community in treated peat was more able to efficiently reduce added sulfate and as a result methylate more mercury.

## 5. Conclusions

This study provides important insights on the effects of drought and subsequent water table fluctuations on sulfur and mercury cycling in a boreal peatland. Because two severe droughts occurred during the course of an experimental manipulation of atmospheric sulfate deposition, we were able to examine the in situ interaction of hydrologic fluctuations with varying sulfate loads on sulfur and mercury biogeochemistry. Sulfate concentrations in peatland pore waters were a function of antecedent moisture conditions in combination with experimental manipulations. Because the sulfate that reappeared in pore waters during rewetting events likely came from the large pool of organic sulfur in the peatland, prolonged water table drawdowns lead to greater sulfate release in all treatments. However, sulfate mobilization was highest and most responsive to drying conditions in the experimental treatment where recently added sulfate had become incorporated into the organic sulfur pool, yet was still relatively labile compared with organic sulfur in the control treatment.

The effect of antecedent moisture conditions on mercury biogeochemistry was more complicated. Although  $Hg_T$  concentrations increased significantly in peatland pore waters during rewetting events following drought,  $Hg_T$  release was not always immediate. Despite the common finding that peatlands are sinks for  $Hg_T$  in the landscape, the large release of mercury from the peat following drought provides evidence that peatlands can also be short-term sources of inorganic mercury to downstream aquatic systems under these specific hydrologic conditions.

In contrast, wetlands are well-known sources of MeHg to downstream aquatic systems [Babiarz *et al.*, 1998; Bushey *et al.*, 2008; St. Louis *et al.*, 1994], and sulfate stimulation of in situ methylation has almost certainly contributed to the flux of MeHg from the S6 peatland [Jeremiason *et al.*, 2006]. Based on findings from the full 8 years of sulfate addition [Coleman Wasik *et al.*, 2012], it was expected that the high pore water sulfate observed following the 2006 and 2007 droughts would significantly stimulate mercury methylation in peatland pore waters. Although there was evidence of increased MeHg production as the drought-induced sulfate was consumed, our results also demonstrate the potential for drought to further elevate MeHg flux from peatlands because of oxidation and desorption of MeHg from the solid phase.

This study was equally revealing regarding the effects of elevated sulfate deposition on mercury biogeochemistry beyond stimulation of mercury methylation. Although mercury export from the S6 peatland was not examined in this study, peatland pore waters represent an important component of outflow from this system under the right hydrological conditions [Mitchell *et al.*, 2008b]. In our experimental treatment, sulfate release following drought was greater than that in the control treatment. Not only was that sulfate then available to drive SRB activity and Hg methylation but it was also available for export to downstream aquatic systems (e.g., lakes and other wetlands) that could be equally susceptible to in situ net methylation. Drought-induced MeHg release in the experimental treatment was also greater relative to the control treatment during rewetting events because a larger pool of MeHg had built up in the solid phase of the experimental treatment as a result of chronically elevated sulfate loading [Coleman Wasik *et al.*, 2012]. This observation implies the potential for greater MeHg export from sulfate-impacted peatlands to downstream aquatic systems relative to unimpacted peatlands. Finally, the observation in the spring of 2008 that net methylation (as inferred from changes in % MeHg) was greater in the experimental treatment relative to the control—despite similar concentrations of pore water sulfate—indicates that chronically elevated sulfate deposition had increased the methylation efficiency of the SRB community. The cumulative effect of elevated sulfate deposition to peatlands is to create more effective conditions for methylation and stronger sources of MeHg within a landscape. Furthermore, the fact that changes in sulfate, MeHg, and %MeHg in recovery-treatment pore waters were always intermediate between those in the control and experimental treatments demonstrates that the effect of elevated sulfate deposition on peatlands persists for some period of time after sulfate deposition has declined.

#### Acknowledgments

Funding for this long-term project came from the U.S. EPA–Science to Achieve Results Program grant R827630, the Great Lakes Commission, Great Lakes Air Deposition program, and the Minnesota Pollution Control Agency. The USDA Forest Service's Northern Research Station provided access to the study site as well as substantial in-kind support. Many individuals assisted with sulfate additions and sample collection including P. Hoff and D. Helwig (Minnesota Pollution Control Agency); A. Stephens, A. Baczynski, and W. Daniels (St. Croix Watershed Research Station); and J. Heissel, C. Green, and J. Westlake (Northern Research Station). We also gratefully acknowledge the support of the analysts and technicians including Y. Nollet (Metropolitan Council Environmental Services), D. Nelson and J. Larson (Northern Research Station), and C. Eckley and M. Collins (University of Toronto, Mississauga). Special thanks go to R. Kyllander and C. Dorrance at the Northern Research Station for the administrative and field assistance throughout the course of the project. The data presented in this paper may be obtained by sending a written request to the corresponding author.

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# Footnoted Document #4

# Sulfate Addition Increases Methylmercury Production in an Experimental Wetland

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Atmospheric mercury is the dominant Hg source to fish in northern Minnesota and elsewhere. However, atmospherically derived Hg must be methylated prior to accumulating in fish. Sulfate-reducing bacteria are thought to be the primary methylators of Hg in the environment. Previous laboratory and field mesocosm studies have demonstrated an increase in methylmercury (MeHg) levels in sediment and peatland porewaters following additions of sulfate. In the current ecosystem-scale study, sulfate was added to half of an experimental wetland at the Marcell Experimental Forest located in northeastern Minnesota, increasing annual sulfate load by approximately four times relative to the control half of the wetland. Sulfate was added on four separate occasions during 2002 and delivered via a sprinkler system constructed on the southeast half (1.0 ha) of the S6 experimental wetland. MeHg levels were monitored in porewater and in outflow from the wetland. Prior to the first sulfate addition, MeHg concentrations (filtered, 0.7  $\mu\text{m}$ ) were not statistically different between the control ( $0.47 \pm 0.10 \text{ ng L}^{-1}$ ,  $n = 12$ ; mean  $\pm$  one standard error) and experimental  $0.52 \pm 0.05 \text{ ng L}^{-1}$ ,  $n = 18$ ) halves. Following the first addition in May 2002, MeHg porewater concentrations increased to  $1.63 \pm 0.27 \text{ ng L}^{-1}$  two weeks after the addition, a 3-fold increase. Subsequent additions in July and September 2002 did not raise porewater MeHg, but the applied sulfate was not observed in porewaters 24 h after addition. MeHg concentrations in outflow from the wetland also increased leading to an estimated 2.4 $\times$  increase of MeHg flux from the wetland.

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Our results demonstrate enhanced methylation and increased MeHg concentrations within the wetland and in outflow from the wetland suggesting that decreasing sulfate deposition rates would lower MeHg export from wetlands.

## Introduction

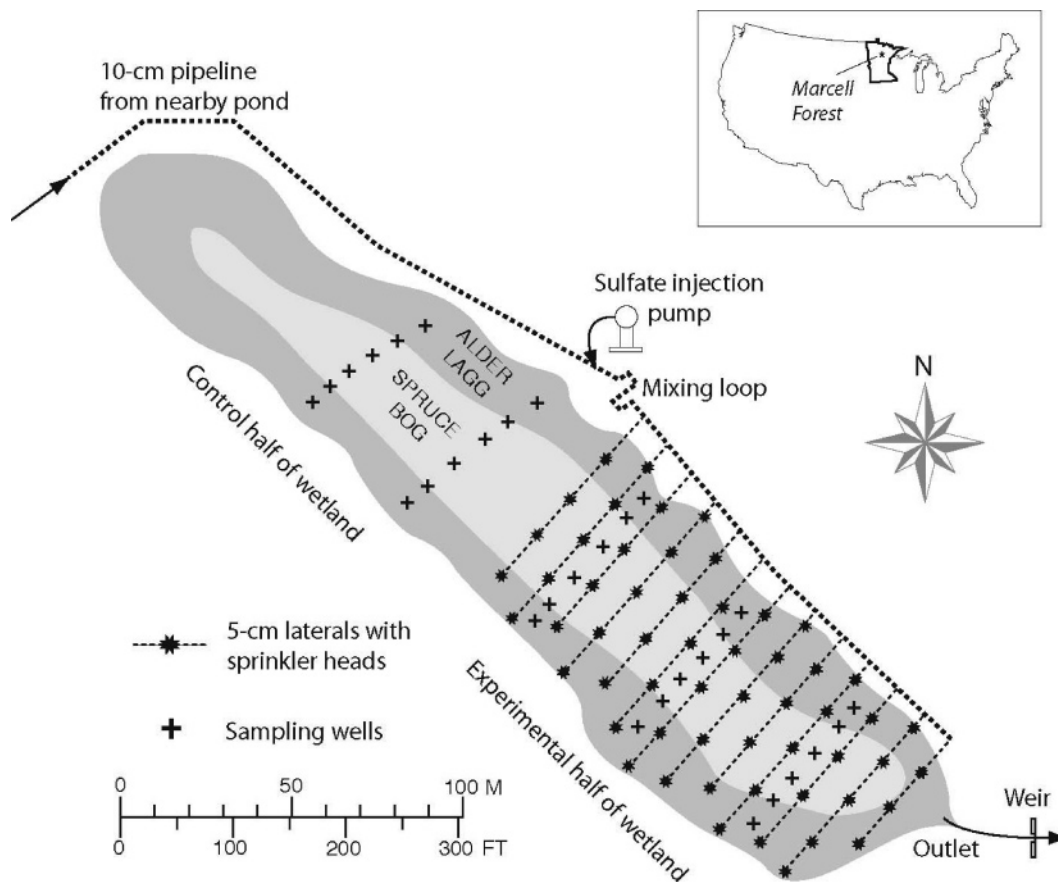
Efforts to reduce mercury (Hg) emissions in Minnesota and throughout the rest of the world assume change in atmospheric deposition of Hg will ultimately result in a proportional change of methylmercury (MeHg) concentrations in fish, all other things being constant. Accordingly, it is thought that fish now have mercury concentrations that are 3–4 times greater than natural (preindustrial) levels, because there is strong evidence that atmospheric Hg deposition is currently 3–4 times greater than natural rates (1–6). However, the proportion of Hg that is methylated and bioaccumulated in fish may not have been constant in some aquatic systems over that time period. Higher than expected Hg concentrations in fish may be the result of increased sulfate deposition to sulfate-poor ecosystems, where sulfate availability controls the activity of the bacteria that methylate Hg. A comparison of museum fish from the 1930s collected from low alkalinity lakes in northern Minnesota and fish collected from the same lakes in the 1980s indicated a 10-fold increase in Hg concentrations (7), consistent with the sulfate-enhancement hypothesis.

Hg methylation in natural systems is primarily by sulfate-reducing bacteria in sediments (8–11) and in wetlands (12–16), but has also been observed in floating macrophytes and periphyton (17). Wetlands, being a major source of MeHg to waters where fish exist (18–21), represent a critical link between atmospheric Hg deposition and accumulation of MeHg in aquatic food chains. The objective of this study is to determine if enhanced sulfate loads elevate MeHg levels in a sub-boreal *Sphagnum*/conifer wetland. Previous studies conducted in the laboratory and in field microcosms demonstrate a link between increased sulfate reduction rates and enhanced Hg methylation (8, 12). In this study, we artificially increased sulfate loads to an experimental wetland to examine the impact of increased sulfate deposition on Hg methylation at the watershed scale.

## Material and Methods

**Site Description.** The United States Department of Agriculture Forest Service Marcell Experimental Forest (MEF; Figure 1) is an 890 ha tract of land located 40 km north of Grand Rapids, Minnesota (47°32'N, 93°28'W). The experimental site, wetland S6, is one of seven small watersheds that have been used for long-term study of forest hydrology and Hg cycling at the MEF (22–26). Climatic and hydrologic data have been collected continuously at monitoring stations since 1959. Two peatland/upland forest watersheds have been instrumented and studied in detail, including hydrology (27, 28), nutrient cycling and behavior (29, 30), and release of organic carbon and acidity (31). A National Atmospheric Deposition Program (NADP) site has been operating at Marcell since 1978 and the first Mercury Deposition Network (MDN) station began operation at the MEF in 1992 (32, 33). Hydrologic monitoring and other related research continues at the MEF.

The landscape of the MEF is typical of morainic landscapes in the western Great Lakes region. The S6 watershed contains an elongate 2.0 ha mature black spruce (*Picea mariana*) and



**FIGURE 1.** The S6 wetland in the Marcell Experimental Forest, northern Minnesota. The irrigation system consists of ~360 m of 10-cm diameter PVC pipe running adjacent to the north side of the S6 wetland. From this main line, thirteen 5-cm diameter laterals, spaced 14 m apart, extend across the experimental half of the wetland. Adjustable sprinkler heads spaced at 16-m intervals along each lateral operate with a spray radius of approximately 8–9 m and rotate on 0.6-m vertical risers. Wells for sampling peat pore waters are arrayed along five transects, each consisting of two lagg wells, two bog wells, and two “transition” wells between the bog and the lagg.

tamarack (*Larix laricina*) wetland. The S6 wetland (Figure 1) is characterized by an alder (*Alnus rugosa*) lagg (a zone of higher pH at the contact with mineral-soil uplands) encircling the slightly raised spruce/*Sphagnum* bog. Outflow from the S6 watershed (pH = 4.9 ± 0.7) has been monitored with a 120° V-notch weir since 1964 (34). The 6.9 ha upland was clear-cut in 1980 to convert the upland from predominantly aspen (*Populus tremula*) to white spruce (*Picea glauca*) and red pine (*Pinus resinosa*).

**Sulfate Additions.** Sulfate was added to the experimental half of the S6 wetland in five simulated rainfall events (6–10 mm) from November 2001 through October 2002 by means of a PVC irrigation system (35) constructed in 2001 (Figure 1). The system consists of ~360 m of 10-cm diameter PVC pipe running adjacent to the north side of the wetland. From this main line, thirteen 5-cm diameter laterals, spaced 14 m apart, extend across the experimental half of the wetland. Adjustable sprinkler heads spaced at 16 m intervals along each lateral operate with a spray radius of approximately 8–9 m and rotate on 0.6 m risers. Valves installed on each lateral allowed flow rates to be maintained to operate sprinkler heads at the desired radius. The PVC pipes were glued together at most joints, but flexible hosing at several joints allows for temperature contraction and expansion. Source water for the system was drawn from a dilute (conductivity ~10  $\mu\text{S cm}^{-1}$ ), low mercury (<1 ng L<sup>-1</sup>), rain-fed pond, and a concentrated sodium sulfate solution was injected into the main line resulting in sulfate concentrations in the irrigation water of ~200 mg L<sup>-1</sup>. A mixing loop after the injection point ensured a homogeneous sulfate solution. When the desired amount of sulfate had been added, a 1-mm

rainfall equivalent cleared the lines and “washed” the sulfate off plant surfaces and into the peat porewaters. The 2002 sulfate load delivered by the irrigation system was 32 kg ha<sup>-1</sup>, equivalent to approximately four times current annual atmospheric deposition and similar to atmospheric sulfate deposition in the northeastern United States (32, 33). The sulfate load was seasonally distributed based on historical sulfate deposition rates. Lithium bromide was used as a hydrologic tracer, but it appears to be nonconservative, and was not as useful as hoped.

**Field Sampling.** Filtered water samples were collected from 30 peat wells 1 day prior to, and 1, 3, 5, 7, 14, 28, and 56 days following, each sulfate addition. The wells were situated along 5 transects designated as experimental (ET1, ET2, and ET3) or control (CT2 and CT3). Each transect consisted of 6 wells: 2 lagg wells (one each in the N and S lags), 2 bog wells, and 2 transition wells. The bog wells were located in the raised black spruce area of the wetland, the lagg wells were in the alder lagg, and the transition wells were located between the lagg and raised bog portions of the wetland. Unfiltered samples were collected at the S6 and nearby S7a outlet weirs every two weeks and whenever peat well sampling occurred. All mercury samples were collected in acid-cleaned 125 mL Teflon bottles using established protocols (24). Peat wells were designed to integrate peat porewater from the surface of the water table down to about 25 cm and by design collected porewater from depths corresponding to greatest hydraulic conductivity. Peat wells consisted of acid-cleaned 5-cm diameter PVC pipes cut to a length of 45 cm and driven approximately 35 cm into the peat. Approximately 40 holes (0.65-cm diameter) were drilled

into the wells to allow porewater to flow freely. A 2.5-cm diameter, finely slotted, acid-cleaned PVC Geoprobe screen, capped on the bottom, was inserted into each well and wells were capped between samplings. Samples were drawn from inside the Geoprobe screen with a hand pump and filtered through 0.7  $\mu\text{m}$  ashed glass fiber filters. Field duplicates and blanks constituted approximately 20% of all samples collected. Experimental results from the November 2001 and October 2002 additions are not presented in this paper because many of the sample wells froze shortly after sulfate additions. Outflows from sampled watersheds were measured at 120° V-notch weirs with individually calibrated stage–discharge relations and hourly stage readings (S7a) or a continuous strip-chart recorder (S6).

**Laboratory Methods.** Accepted clean methods were utilized throughout the collection and analysis of mercury and methylmercury samples. Samples analyzed for total mercury were first oxidized with 0.2 N bromine monochloride, neutralized with hydroxylamine, and then analyzed using the stannous chloride/cold vapor atomic fluorescence spectroscopic (CVAFS) method (24, 36). Analysis of MeHg was performed using the aqueous distillation/CVAFS method (37, 38). Briefly, following distillation, water samples were ethylated with sodium tetraethylborate, purged with nitrogen and collected on Tenax TA (Alltech 60–80 mesh) traps. Hg species were thermally desorbed from the Tenax in an argon stream and separated on an OV-1 chromatographic column, converted to elemental mercury in a pyrolytic column, and analyzed on a Tekran 2400 CVAFS. Lab duplicates and performance standards were routinely analyzed as part of the quality assurance plan. Sulfate and other anions were measured by ion chromatography (Dionex ICS 2000), while cations were measured with ICP-MS (ThermoFinnigan PQ ExCell).

## Results and Discussion

**Porewater MeHg Concentrations.** Dramatic increases in porewater MeHg concentrations were observed following the May 22, 2002 sulfate addition (Figure 2a). One day prior to the addition (Day –1), MeHg levels in the peat porewaters were not significantly different ( $p = 0.62$ ) in the control ( $0.47 \pm 0.10 \text{ ng L}^{-1}$ ,  $n = 12$ ; mean  $\pm$  one standard error) versus the experimental ( $0.52 \pm 0.05 \text{ ng L}^{-1}$ ,  $n = 18$ ) half of the wetland (Figure 2a). In the period between the May and July additions, MeHg porewater levels in the experimental half increased and remained elevated, while the control half exhibited no statistically significant change relative to Day –1. All MeHg concentrations in the experimental half were statistically higher than those of Day –1 at  $p < 0.05$  except for Day 56 ( $p = 0.13$ ). Porewater MeHg levels in the experimental half were also higher than the control half at  $p < 0.05$  except for Day 1 ( $p = 0.06$ ), demonstrating that the sulfate addition elevated MeHg levels after the May addition and, relative to the control half, maintained them for an extended period of time. Total Hg levels were similar between the experimental and control halves at this time; however, the fraction of total Hg occurring as MeHg increased after the May sulfate addition and remained elevated (Figure 2b). In addition, other water chemistry parameters (cations, anions, pH, and DOC) unimpacted by the sulfate addition behaved similarly between the experimental and control halves.

Changes in MeHg levels in the experimental half were inversely related to sulfate concentration in the peat porewaters in the first four sampling dates following the May addition (Figure 2a). Sulfate levels were undetectable at Day –1 in both the control and experimental halves. Following the May addition the average sulfate concentration increased to  $1.09 \pm 0.33 \text{ mg L}^{-1}$  ( $n = 18$ ) at Day 1 in the experimental half of the wetland and remained undetectable in the control half. As the sulfate reducing bacteria utilized the added sulfate,

levels began to drop gradually, until sulfate was undetectable again on June 5 (Day 14) and porewater MeHg concentrations were at a local maximum,  $1.63 \pm 0.27 \text{ ng L}^{-1}$  ( $n = 18$ ). Following June 5 and prior to the July addition, sulfate levels across the wetland were detectable, but lower in the control half, although not statistically ( $p > 0.05$ ). The average sulfate concentration in the control during 2002 was  $0.02 \pm 0.01 \text{ mg L}^{-1}$ .

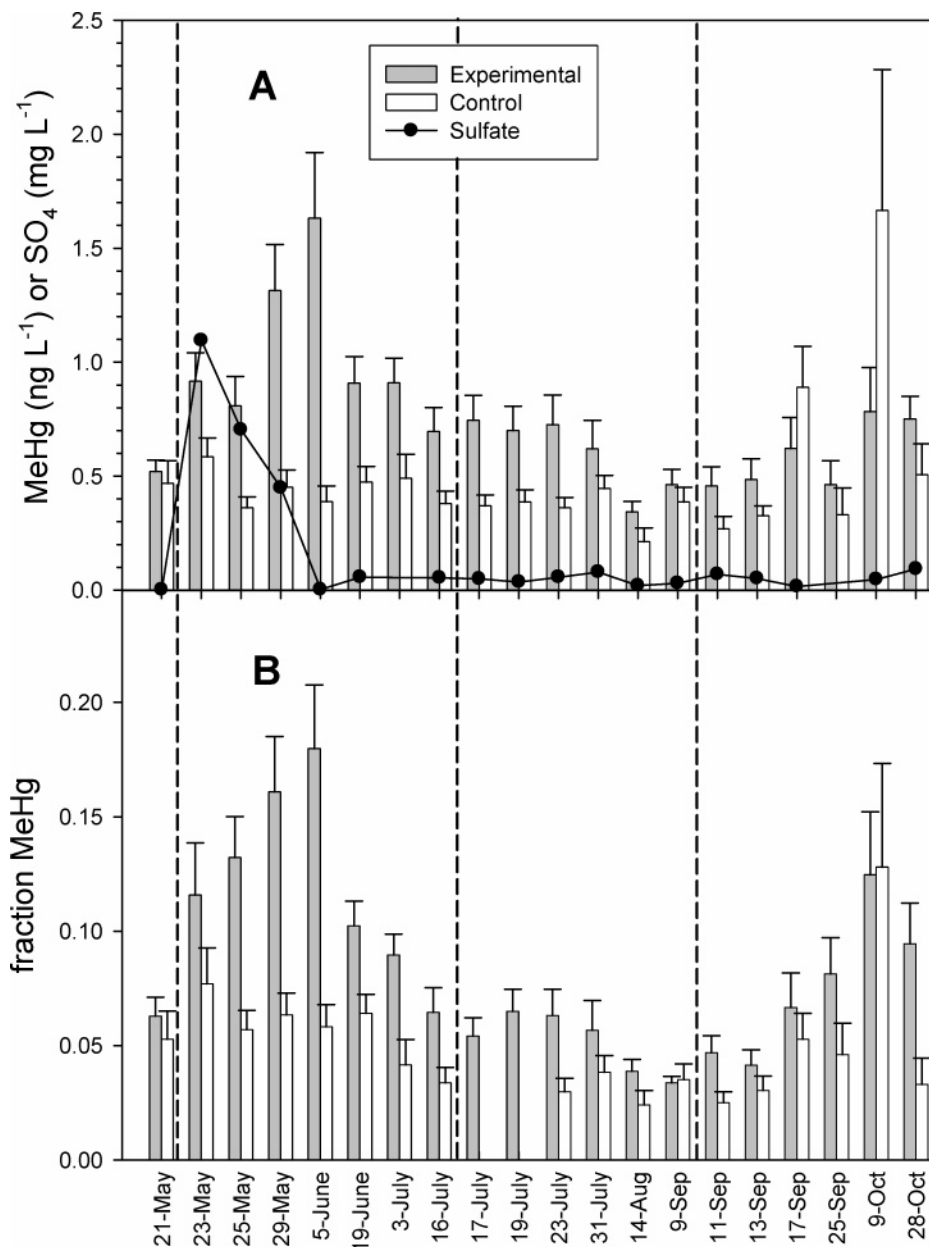
MeHg levels decreased after the June 5 maximum, but not back to the pre-addition levels. Net methylation (methylation – demethylation) was apparently enhanced in the experimental half of the wetland by the addition of sulfate. Two possible mechanisms for sustaining the elevated MeHg concentrations include the creation of a larger biologically available sulfur pool (14, 39, 40) or an increase in sulfate-reducing bacteria that methylate mercury.

The current study employed a large number of sampling wells collecting depth-integrated porewaters dispersed over a large area (2.0 ha). The large scale and experimental design makes it difficult to compare to other studies. However, similar studies done at smaller scales and at specific depth intervals were conducted in the Experimental Lakes Area (ELA), Canada (12) and in Degero Stomyr in northern Sweden (14). In the current study, MeHg porewater concentrations increased by a factor of 3 (from  $0.52 \pm 0.05 \text{ ng L}^{-1}$  to  $1.63 \pm 0.27 \text{ ng L}^{-1}$ ) two weeks after a 4 $\times$  increase in sulfate load (Figure 2a). Branfireun et al. (12) reported MeHg increases of up to 10 $\times$  following a 20 $\times$  increase in sulfate load to an experimental mesocosm ( $0.16 \text{ m}^2$ ) in a poor fen peatland at ELA. A 2 $\times$  increase in sulfate load at the ELA study site resulted in a 3–4-fold increase in MeHg levels (12). The ELA study was conducted over 5 days and in most cases MeHg in the porewaters returned to pre-addition levels. The study in Sweden (14) examined MeHg in porewaters from sedge peatland microcosms ( $4 \text{ m}^2$ ) dosed with sulfate for three years. A MeHg increase of approximately 5 $\times$  was reported in the mesocosm receiving an  $\sim 7\times$  increase in sulfate load.

Rain events influence MeHg levels in S6 not only by supplying sulfate, nutrients, and mercury, but also by transporting added sulfate within the wetland or flushing it from the wetland. The first rainfall after the spring addition—12 mm on May 28 and 17 mm on May 29—was not substantial enough to flush the added sulfate from the wetland. Indeed, the estimated sulfate load transported from the wetland was only 0.36 kg from May 21–June 5 compared to the added sulfate of 14.3 kg. An extremely large rain event (208 mm) occurred on June 22–24, preceded by a smaller event (36 mm) on June 18–19, resulting in record flows from S6 (Figure 3b). The amount of sulfate transported from the wetland at this time was 4.3 kg, still a relatively small amount compared to what was added. Despite this extreme hydrologic event, MeHg in the porewaters of the experimental half of the wetland exceeded those in the controls.

Contrary to expectations from the May sulfate application, MeHg concentrations did not increase in peat porewaters following the July and initially after the September sulfate additions (Figure 2). Moreover, there was no observed increase in porewater sulfate in the experimental peat wells, even 1 day after the applications. However, MeHg concentrations remained elevated in the experimental half relative to the control until late September. The most likely explanation for this seasonal contrast is temperature, which plays a key role in controlling sulfate reduction and methylation/demethylation rates. At the time of the May addition peat temperatures (as measured at the nearby S2 wetland, 0.4 km away), were still quite cool ( $4.5 \text{ }^\circ\text{C}$  at 5 cm), the bog having thawed only weeks before, and the added sulfate persisted for two weeks and changes in MeHg were observed. Peat temperatures increased slowly to above  $16 \text{ }^\circ\text{C}$  by the time of the July addition and were still at  $15 \text{ }^\circ\text{C}$  for the third addition



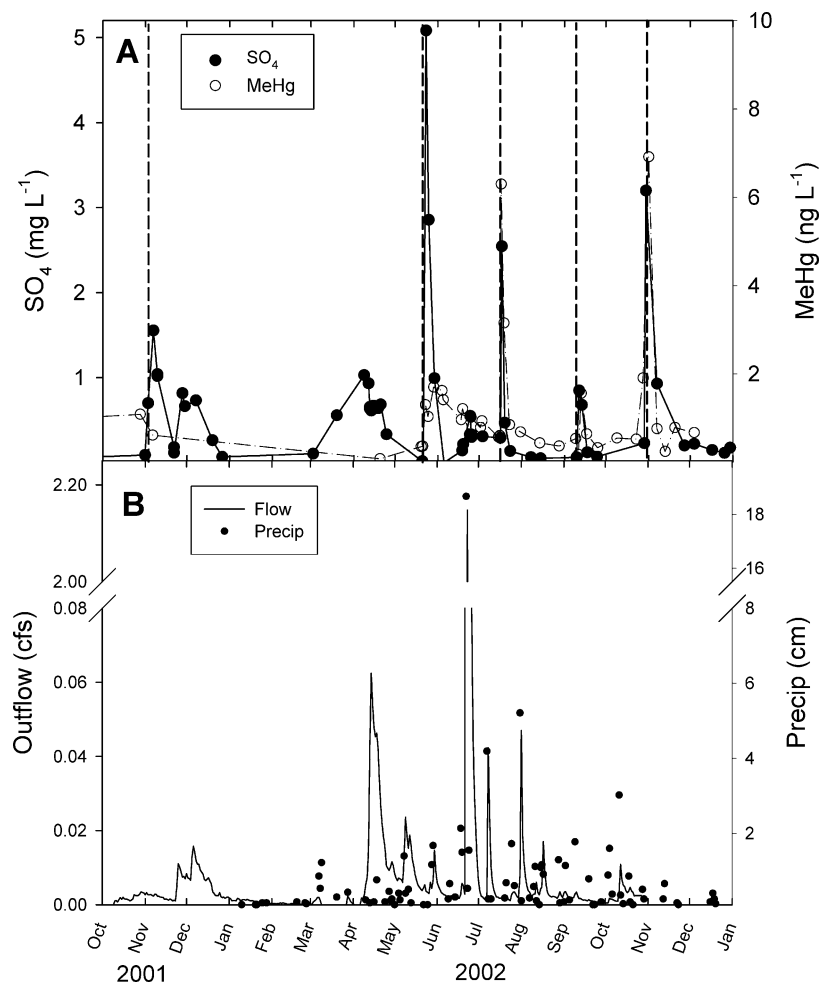


**FIGURE 2. (A) MeHg concentrations ( $\pm 1$  standard error) in pore waters from control and experimental peat wells and sulfate concentrations in experimental peat wells only; sulfate was generally below detection ( $< 0.01 \text{ mg L}^{-1}$ ) in the control wells. Each dotted line represents a sulfate application. (B) The fraction of total Hg existing as MeHg in control and experimental peat wells.**

in early September. The warm late-summer peat temperatures likely led to very high sulfate reduction rates such that much of the added sulfate may have been consumed within 24 h (the first sampling day) following the July and September applications. Some of the sulfate may have also been entrained in the more abundant vegetation during the summer additions.

A subsequent decrease in peat temperature and outflow in late September/early October coincided with more variable MeHg concentrations and the control half actually exceeding MeHg levels in the experimental half on a few days, but these differences are not statistically significant (Figure 2). Currently, we cannot explain these observations, but they appear independent of the sulfate addition. The limited MeHg results from after the October 2002 addition (not presented because of extensive well freeze-up) were also highly variable and

may be related to decreases in temperature. A few of these samples had MeHg concentrations exceeding  $10 \text{ ng L}^{-1}$ , however they could not be independently verified by additional late season field collections. Decreased temperatures might have contributed to the increase in MeHg concentrations, but other factors including Hg deposition through litterfall or possibly organic matter oxidation owing to late-season water-level fluctuations could have played a role. Litterfall, which begins in mid-September, is an important component of the total Hg flux to the Marcell wetlands, contributing nearly twice the Hg delivered by wet deposition alone (41, 42). Water level in the wetland was decreasing at this time creating relatively stagnant conditions. Flow from S6 decreased substantially in September 2002 with only a few small rain events (Figure 3b). With the decline in water level, labile organic matter in the surface peat may have been oxidized releasing bound mercury as well as sulfate to the dissolved phase.



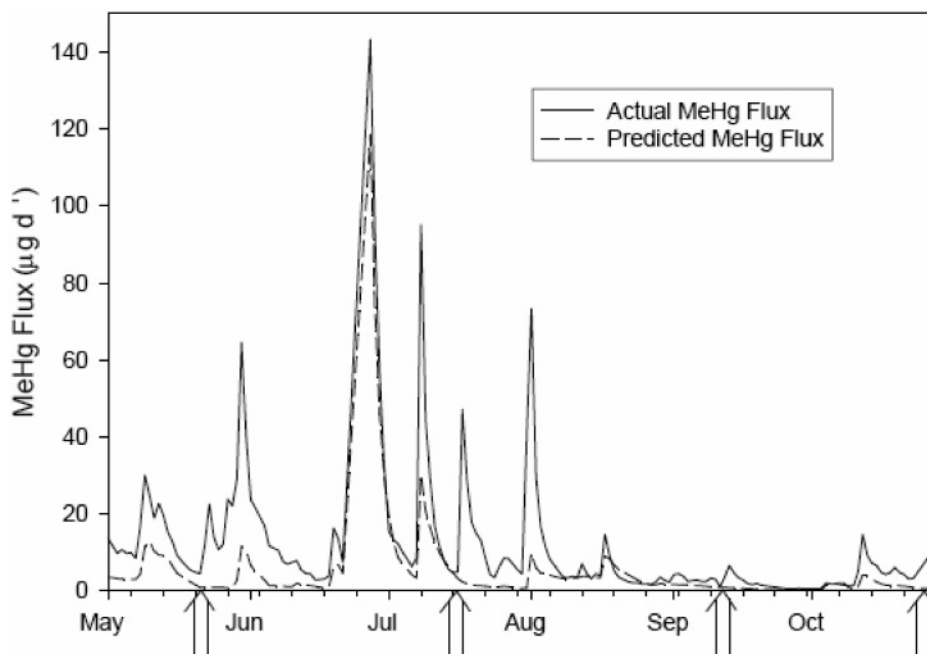
**FIGURE 3. (A) MeHg and sulfate concentrations in the outflow from the S6 wetland. (B) Hydrologic outflow and precipitation events at S6. Flows were measured by chart recorder at the S6 weir (in operation since 1964), and precipitation was measured with a rain gauge located near the west end of the S6 wetland.**

**MeHg Export from S6.** MeHg and sulfate concentrations increased at the S6 weir following each sulfate addition (Figure 3a), although the timing of the increases varied over the course of the experiment. Elevated concentrations observed at the weir after the July and September additions are in contrast to the peat wells where increases in sulfate or MeHg were not observed (but MeHg remained elevated relative to the control). Higher sulfate concentrations persisted at the weir following the May and late October additions, consistent with the peat well trends. A small pool impounded behind the weir likely contributed to these trends. Although sulfate was not added directly to the pool, some sulfate flowed into it within hours of each addition, increasing sulfate concentrations. Sulfate levels at the weir then declined over time as the pool was flushed by additional sulfate-depleted water from the wetland. For example, in May the flushing rate,  $k_f$ , of the weir pool was  $1.37 \text{ d}^{-1}$ , ( $k_f = \text{flow}/\text{volume}$ ). The observed first-order loss of sulfate from the pool,  $k_{\text{obs}}$  ( $0.27 \text{ d}^{-1}$ ), from Day 1 to Day 7 was significantly less than  $k_f$  indicating a substantial flow of sulfate from the wetland to the weir pool. Sulfate levels in the peat porewaters were elevated at this time (Figure 3). In contrast, pool flushing rates following the July ( $0.48 \text{ d}^{-1}$ ) and September ( $0.33 \text{ d}^{-1}$ ) additions, were similar to  $k_{\text{obs}}$  for July ( $0.59 \text{ d}^{-1}$ ) and September ( $0.37 \text{ d}^{-1}$ ) suggesting that a pulse of sulfate was introduced to the weir pool within hours after these additions and then simply flushed out. Presumably due to high sulfate reduction rates or the sulfate never reaching the water table, sulfate in peat

porewaters was insignificant during July and September and thus outflow of sulfate from the wetland to the pool was insignificant at this time. Water chemistry samples were not taken frequently enough following the October 2002 addition to calculate  $k_{\text{obs}}$  accurately.

MeHg trends at the weir closely track those for sulfate (Figure 3a). Following the May addition, MeHg concentration gradually increased at the weir, similar to the peat porewaters (Figure 2). The concentrations at the weir and in the peat porewaters were also similar at this time indicating that the peat porewaters were supplying the MeHg flowing over the weir. However, following the July and September additions, MeHg concentrations at the weir spiked immediately after each addition and the weir concentrations exceeded peat porewater concentrations. It is not clear if these spikes were due to high levels of MeHg flowing from the wetland or MeHg formation in the weir pool itself. However, based on the flushing rate of the pool, it appears that the dominant loss process for sulfate was flushing and that sulfate reduction in the weir pool was negligible.

Empirically modeled MeHg export from S6 without sulfate addition was compared to measured MeHg export in 2002. The observed daily MeHg export exceeded the predicted MeHg export during periods immediately following sulfate additions. To model MeHg export from S6 in the absence of sulfate additions, data from 2001 (prior to the 2002 sulfate additions to S6) showed a strong correlation between flows at the S6 weir and a nearby wetland weir, S7a ( $r^2 = 0.71$ ).



**FIGURE 4.** Actual and predicted fluxes of MeHg from the S6 wetland for 2002. The predicted flux is that which would have occurred in the absence of sulfate addition and is based on a correlation of 2001 (pretreatment) MeHg fluxes from S6 with those from a nearby reference wetland, S7a (see text). Arrows indicate experimental sulfate applications.

Furthermore, MeHg export from S7a was correlated to MeHg export from S6 in 2001

$$\log \text{Flux}_{S6} = 1.23 \times \log \text{Flux}_{S7a} - 1.62 \quad (r^2 = 0.77 \text{ in } 2001) \quad (1)$$

where  $\text{Flux}_{S6}$  ( $\mu\text{g d}^{-1}$ ) is the measured MeHg flux out of wetland S6 and  $\text{Flux}_{S7a}$  ( $\mu\text{g d}^{-1}$ ) is the measured flux out of wetland S7a.  $\text{Flux}_{S6}$  and  $\text{Flux}_{S7a}$  are daily fluxes determined from average daily flows measured at the weirs and MeHg concentrations interpolated between sampling dates (see Supporting Information). In 2001, the weirs were sampled biweekly and in 2002 additional samples were collected from the weir at S6 corresponding to each porewater sampling date. Using eq 1, the MeHg flux for May through October 2002 that would have come from S6 in the absence of sulfate addition was estimated and compared to the actual flux (Figure 4). Excluding the high flow values from the June 22–24 storm event and the unusually high MeHg concentration observed the day after the October 2002 addition (including these values yields an even greater enhancement), the MeHg flux observed in 2002 (1780  $\mu\text{g MeHg}$ ) was more than two times greater (144%) than would have occurred without sulfate addition (730  $\mu\text{g MeHg}$ ).

In this study, enhanced MeHg concentrations were observed in the experimental peat porewaters and in the flow from the S6 wetland following sulfate addition. Enhanced MeHg concentrations were not observed in peat porewaters following the July and September additions, but the added sulfate did not increase porewater sulfate concentrations due to either rapid sulfate utilization or entrainment in overlying vegetation. Not all MeHg and sulfate trends observed can be readily explained in this initial year of sulfate addition, but sulfate addition enhanced MeHg concentrations in most cases, despite the fact that our addition of sulfur was negligible relative to the sulfur pool in the upper 30 cm of peat. At no point in the study were there any indications that the sulfate load decreased methylation as has been observed in the past in lake enclosures (43). The most likely explanation for these observations is that biologically available sulfur is a limiting factor in this system for the methylating bacteria. The addition

of the limiting factor, sulfate, increased MeHg levels and may have increased the biologically active sulfur pool in S6. One possible implication of this study is that historic increases in atmospheric sulfate deposition (now on the decline) may have enhanced contemporary MeHg production and export from wetlands, contributing to widespread mercury contamination of aquatic food chains. It follows that decreases in sulfate deposition could result in less export of MeHg from wetlands and possibly result in lower MeHg levels in fish.

### Acknowledgments

This research was funded by the U.S. EPA Science to Achieve Results (STAR) Program, Grant R827630. We gratefully acknowledge the assistance of Deacon Kyllander and Art Elling of the U.S. Forest Service for assistance with sample collection and weir-flow monitoring. We thank Daniel Helwig for experimental design assistance, planning, and support. We also thank the Minnesota Department of Natural Resources for equipment usage and those who helped construct the irrigation system: Neal Hines, Kelly O'Hara, Paul Hoff, Howard Markus, and Harold Wiegner.

### Supporting Information Available

Additional plots and further information on methods related to eq 1 and Figure 4 used to estimate enhanced export of MeHg from the S6 wetland. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Received for review November 30, 2005. Revised manuscript received March 29, 2006. Accepted April 6, 2006.

ES0524144

# Footnoted Document #5

June 2022

# Rainy River - Headwaters Watershed Restoration and Protection Strategy Report





Figure 4. Aquatic consumption impairments in the Rainy River–Headwaters Watershed

