



## RESEARCH ARTICLE

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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

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

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

**Plain Language Summary** In the water-saturated soils of wetlands, which are usually anoxic, decomposition of dead plants and other organic matter is greatly retarded by the absence of oxygen. However, the addition of sulfate can allow bacteria that respire sulfate, instead of oxygen, to decompose organic matter that would not otherwise decay. The accelerated decay has multiple consequences that are concerning. The bacteria that respire sulfate “breathe out” hydrogen sulfide (also called sulfide), analogous to the conversion or respiration of oxygen to CO<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.

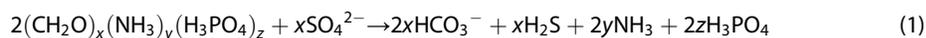
### 1. Introduction

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 Gemerden, 2000). In addition, phosphorus is mobilized when oxidized Fe compounds with significant capacity to bind phosphate are converted to FeS compounds, which are incapable of binding phosphate (Lamers et al., 1998; Maynard et al., 2011). Thus, MSR mobilizes P both by mineralization of P-containing OM and by changing the form of Fe in sediment.

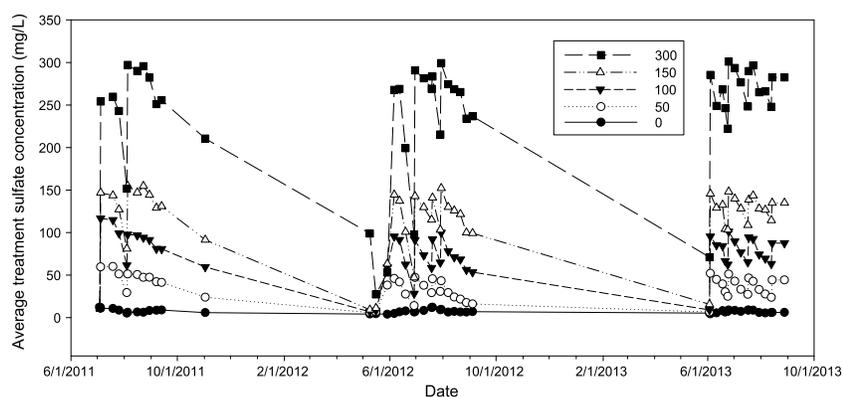
In addition to releasing C, N, and P, producing potentially toxic concentrations of sulfide, and reducing the solubility of metals, MSR is a primary process leading to the formation of MeHg, the bioaccumulative form of Hg (Gilmour et al., 1992; Hsu-Kim et al., 2013), although other microbial groups can also methylate Hg (Podar et al., 2015). In some cases, MSR can lead to toxic levels of MeHg higher in the food chain. The relationship between  $\text{SO}_4$  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>Depl</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>Depl</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 DOC + DIC, are therefore used as indicators of OM mineralization in interpreting the mobilization of N, P, and Hg to surface waters (Figure 2 and Tables 2 and 3).

In contrast to many marine systems, it is likely that  $\text{SO}_4$  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  $\text{SO}_4$  Concentrations on  $\text{SO}_4$  Reduction (Quantified as  $\text{SO}_4$  Depletion), Organic Matter Mineralization, and Mercury Methylation

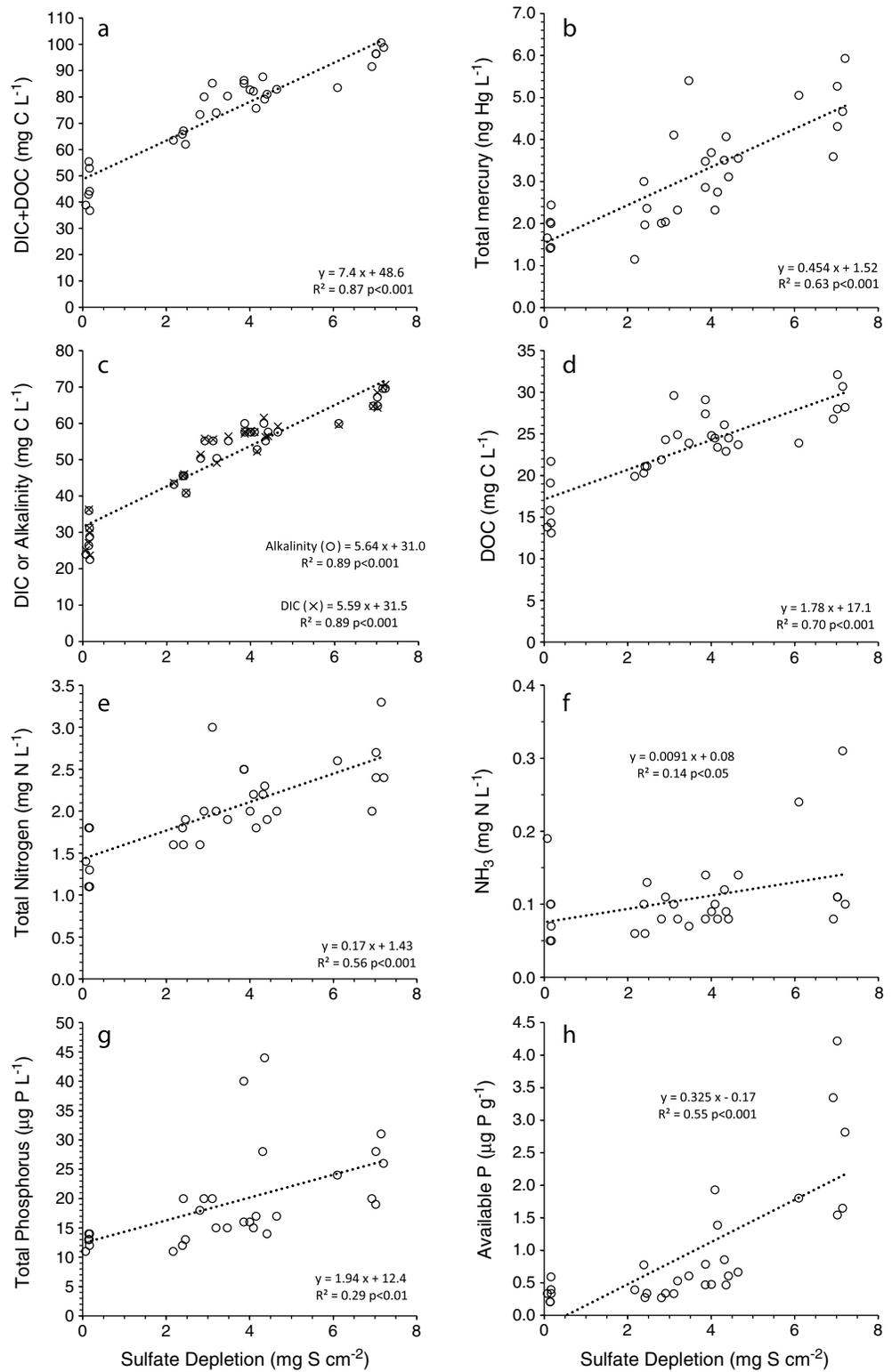
Variable	Matrix	Average of each sulfate treatment ( $n = 6$ for each treatment)						Correlation with $\text{SO}_4$ depletion (Spearman)	
		Control	50	100	150	300	Max/Min	Rho	$p$ value
<i>Variables mainly associated with <math>\text{SO}_4</math> reduction</i>									
$\text{SO}_4$ (T-W mean $\text{mg SO}_4 \text{ L}^{-1}$ )	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
$\text{SO}_4$ depletion ( $\text{mg S cm}^{-2}$ )	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 ( $\mu\text{g S L}^{-1}$ )	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 ( $\mu\text{g L}^{-1}$ )	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 ( $\text{mg S kg}^{-1}$ )	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
$\text{H}^+$ ion ( $\mu\text{mol L}^{-1}$ )	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 ( $\text{mg C L}^{-1}$ )	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 ( $\text{mg C L}^{-1}$ )	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 ( $\text{mg N L}^{-1}$ )	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 ( $\text{mg N L}^{-1}$ )	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 ( $\mu\text{g P L}^{-1}$ )	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 ( $\mu\text{g P g}^{-1}$ 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 ( $\text{ng L}^{-1}$ )	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 ( $\text{ng Hg L}^{-1}$ )	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 ( $\text{ng L}^{-1}$ )	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  $\text{SO}_4$  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  $(\text{SO}_4)_{\text{Depl}}$  is an estimate of the increase of that variable in mesocosm surface waters per unit  $\text{SO}_4$  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  $(\text{SO}_4)_{\text{Depl}}$  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 ○ and ×, respectively; the two regressions are superimposed); (d) surface water DOC; (e) surface water total nitrogen; (f) surface water ammonia; (g) surface water total phosphorus; (h) available phosphate in the sediment, as quantified on ion-exchange resin.

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

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

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

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

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

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

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

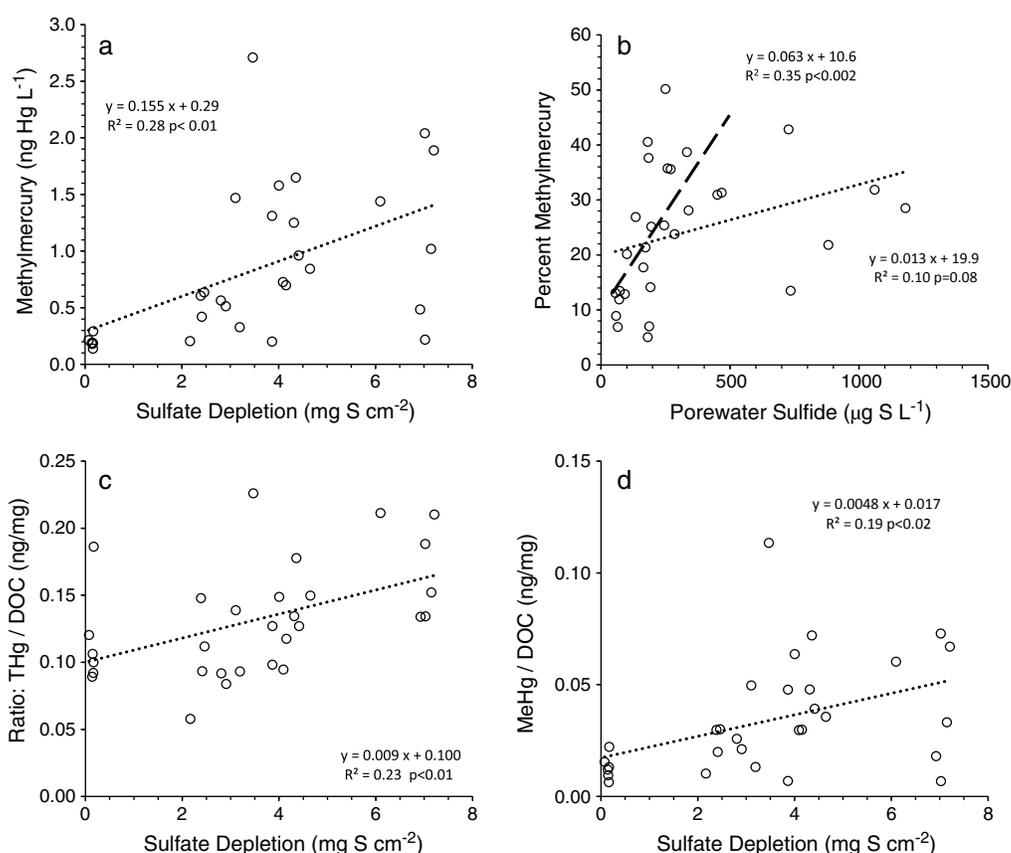
#### 4.2. Effects of $SO_4$ Reduction on Mercury and Methylmercury in Surface Water

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

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

Molar ratio in sediment <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>	19	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).

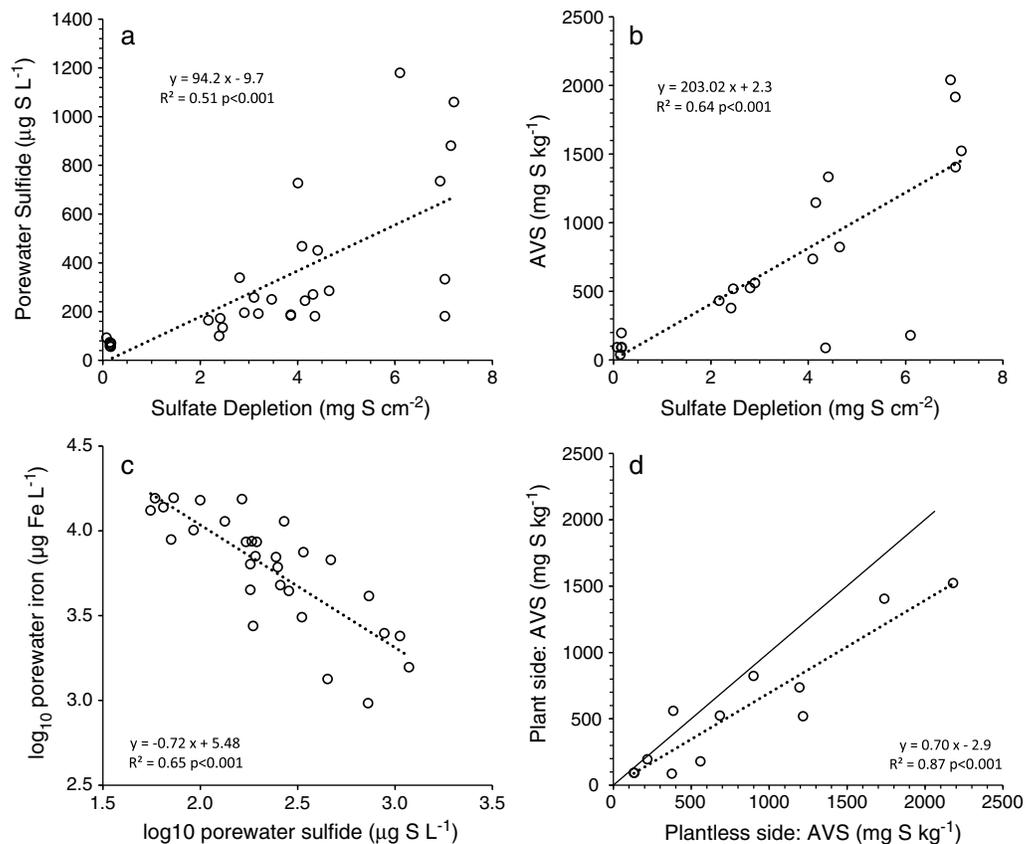
If movement of DOC from sediment to surface water were the sole mechanism for the Hg increase in surface water, a constant Hg:DOC ratio would be expected on the  $(\text{SO}_4)_{\text{Depl}}$  gradient. However, THg:DOC, iHg:DOC, and MeHg:DOC ratios in surface water are all significantly correlated with  $\text{SO}_4$  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 10–15  $\text{mg L}^{-1}$ . 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.

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