



## RESEARCH ARTICLE

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

## Key Points:

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

## Supporting Information:

- Table S1
- Table S2
- Table S3
- Data Set S1
- Figure S1

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

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

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

### 1. Introduction

Minnesota is unique among U.S. states and Canadian provinces in having a water quality standard that regulates sulfate ( $\text{SO}_4$ ) to protect wild rice, *Zizania palustris* and *Zizania aquatica*. The more common wild rice species in Minnesota, *Z. palustris* (northern wild rice), is an annual emergent aquatic grass that forms monocultures in shallow freshwaters (wetlands, lakes, and rivers) in the area of the Laurentian Great Lakes (Minnesota, Wisconsin, Michigan, Ontario, and Manitoba). Wild rice is culturally important to multiple groups in Minnesota, especially Ojibwe, Dakota, and other Native Americans, and also provides habitat and food for waterfowl and other wildlife (Vennum, 1988). In 1977, the Minnesota legislature voted to make wild rice the Minnesota state grain. Minnesota Rule 7050.0224, promulgated in 1973, seeks to limit the exposure of wild rice to  $\text{SO}_4$  concentrations exceeding  $10 \text{ mg L}^{-1}$  ( $0.1 \text{ mmol L}^{-1}$ ). This value was based on empirical research that correlated water chemistry to aquatic plant assemblages and included the observations that no large populations of *Z. palustris* occur in waters exceeding  $10 \text{ mg L}^{-1}$   $\text{SO}_4$  and that stands are uncommon where  $\text{SO}_4$  concentrations exceed  $50 \text{ mg L}^{-1}$  (Moyle, 1944, 1945). In addition, Moyle (1956) noted that plantings of wild rice seed in high- $\text{SO}_4$  regions generally failed. A larger unpublished Minnesota Department of Natural Resources data set also shows that sites with reported wild rice

presence (DNR, 2008) are generally correlated with surface water  $\text{SO}_4$  below  $10 \text{ mg L}^{-1}$  (Figure S1 in the supporting information).

We report here on a multiyear field survey that was part of a larger study (Myrbo et al., 2017; Pastor et al., 2017; Pollman et al., 2017) designed to reevaluate the  $10 \text{ mg L}^{-1}$   $\text{SO}_4$  standard by testing potential mechanisms by which  $\text{SO}_4$  might be harmful to wild rice.  $\text{SO}_4$  is a relatively nontoxic and unreactive compound under aerobic conditions. Pastor et al. (2017) and Fort et al. (2014) have shown that  $\text{SO}_4$  is not directly toxic to wild rice at concentrations up to  $1,600 \text{ mg L}^{-1}$ , which exceeds concentrations in virtually all natural surface waters of the upper Midwest (Gorham, Dean, & Sanger, 1983). The U.S. Environmental Protection Agency's nonmandatory drinking water standard of  $250 \text{ mg L}^{-1}$   $\text{SO}_4$  ( $2.6 \text{ mmol L}^{-1}$ ) is based on taste rather than toxicity (EPA, 2010).

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

$\text{SO}_4$  concentrations in most freshwaters are less than a few percent of the mean concentration in seawater ( $2,800 \text{ mg L}^{-1}$  ( $29.1 \text{ mmol L}^{-1}$ )). We surveyed 108 different lakes, streams, and wetlands across Minnesota, where the median  $\text{SO}_4$  concentration in lakes is  $10 \text{ mg L}^{-1}$  (10th and 90th percentiles of 0.2 and  $285 \text{ mg L}^{-1}$  (MPCA, 2016)). In the much higher  $\text{SO}_4$  concentrations of marine waters, it is well established that  $\text{SO}_4$  can diffuse into sediment and be converted by microbial sulfate reduction (MSR) to potentially toxic sulfide that influences the presence and absence of rooted macrophytes, such as seagrasses (Borum et al., 2005; Ingold & Havill, 1984; Koch & Erskine, 2001; Lamers et al., 2013).

Despite a long-standing assumption that  $\text{SO}_4$  is benign (Pester et al., 2012; Schindler, 1986; Urban et al., 1994) and plays a negligible role in freshwater biogeochemistry (e.g., Capone & Kiene, 1988), there is evidence that  $\text{SO}_4$  availability in freshwaters can control the concentration and therefore the toxicity of hydrogen sulfide ( $\text{H}_2\text{S}$ ) in sediment pore water to plants and animals (Bagarinao, 1992; Kinsman-Costello, O'Brien, & Hamilton, 2015; Lamers et al., 2013; Wang & Chapman, 1999). The chemical species of  $\text{H}_2\text{S}$  varies with pH; below pH 7,  $\text{H}_2\text{S}$  dominates, and above pH 7, the bisulfide ion ( $\text{HS}^-$ ) dominates. For simplicity in this discussion we refer to the sum of the two species as sulfide.

#### 1.1.1. Sulfide Toxicity to Freshwater Plants

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

The toxicity of elevated sulfide to freshwater plants was first recognized in paddy-grown white rice (*Oryza sativa*) in the 1950s (Lamers et al., 2013; Pearsall, 1950). Rice paddies and other water-saturated soils present a profound challenge for rooted plants because of the chemical changes caused by the absence of oxygen and resulting potential toxicity of the pore water (Ponnamperuma, 1972). Anaerobic decomposition of organic matter results in elevated pore water concentrations of ammonia, organic acids, and variable concentrations of sulfide and ferrous Fe, depending on the availability of  $\text{SO}_4$  and Fe. The interaction of Fe and the S cycle is complicated (Hansel et al., 2015), but because under anoxic conditions sulfide forms an insoluble precipitate with Fe, elevated pore water sulfide concentrations occur when Fe availability is relatively low (Ponnamperuma, 1972; van der Welle et al., 2006). It is thought that the iron sulfide precipitates are relatively inert and that only sulfide dissolved in pore water is potentially toxic. The concentration of sulfide in pore water is the balance between production and competing fates of sulfide, including precipitation with metals such as Fe, oxidation by oxygen introduced by bioturbation or by release from the roots of macrophytes (Armstrong & Armstrong, 2005), and by downward advection of surface water due to groundwater movement or transpiration by dense macrophyte stands (Bachand et al., 2014).

### 1.1.2. Geochemical Consequences of Enhanced Microbial Sulfate Reduction

Increased  $\text{SO}_4$  availability can allow increased anaerobic decomposition of organic matter, releasing the inorganic nutrients that generally limit growth of higher plants (N, P, and K) (Lamers, Tomassen, & Roelofs, 1998; Myrbo et al., 2017; Weston et al., 2006, 2011). Enhanced decomposition breaks down particulate and dissolved organic carbon (DOC) in the sediment, which can increase DOC and dissolved inorganic carbon (DIC) in the overlying water (Myrbo et al., 2017).

In addition to supporting organic matter mineralization, MSR production of sulfide causes a cascade of reactions that can alter ecosystem functioning independent of any toxicity to plants and animals. First, sulfide can participate in redox reactions, chemically reducing Fe, which converts Fe from solid phase Fe(III) oxyhydroxides to water-soluble Fe(II) (Hansel et al., 2015). The dissolution of the Fe oxyhydroxides releases sorbed ions into solution, including phosphate and trace metals (Caraco, Cole, & Likens, 1993; Davranche & Bollinger, 2000; Søndergaard, Jensen, & Jeppesen, 2003). Second, sulfide can precipitate dissolved metals, including essential plant nutrients Fe, Cu, and Zn, decreasing their bioavailability and leading to nutrient deficiency (Kirk, 2004; Lamers et al., 1998; Neue & Bloom, 1989; Neue & Lantin, 1994). In systems unpolluted with heavy metals, the sulfide precipitate is overwhelmingly dominated by iron sulfide compounds, consisting of a range of stoichiometries and minerals (Schoonen, 2004), which we here term “FeS compounds.” Third, the conversion of  $\text{SO}_4$  to sulfide entails the production of DIC, or alkalinity, an effect not fully appreciated in freshwater systems until the mechanistic consequences of acid rain were investigated in the 1980s (Baker, Brezonik, & Pollman, 1986; Cook et al., 1986; Schindler, 1986). Alkalinity is thought to be a major factor influencing the distribution of aquatic species, including macrophyte species (Moyle, 1945; Vestergaard & Sand-Jensen, 2000). In addition, elevated alkalinity may further enhance decomposition, producing a positive feedback to the effects of  $\text{SO}_4$ -driven mineralization (Geurts et al., 2009; Roelofs, 1991).

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

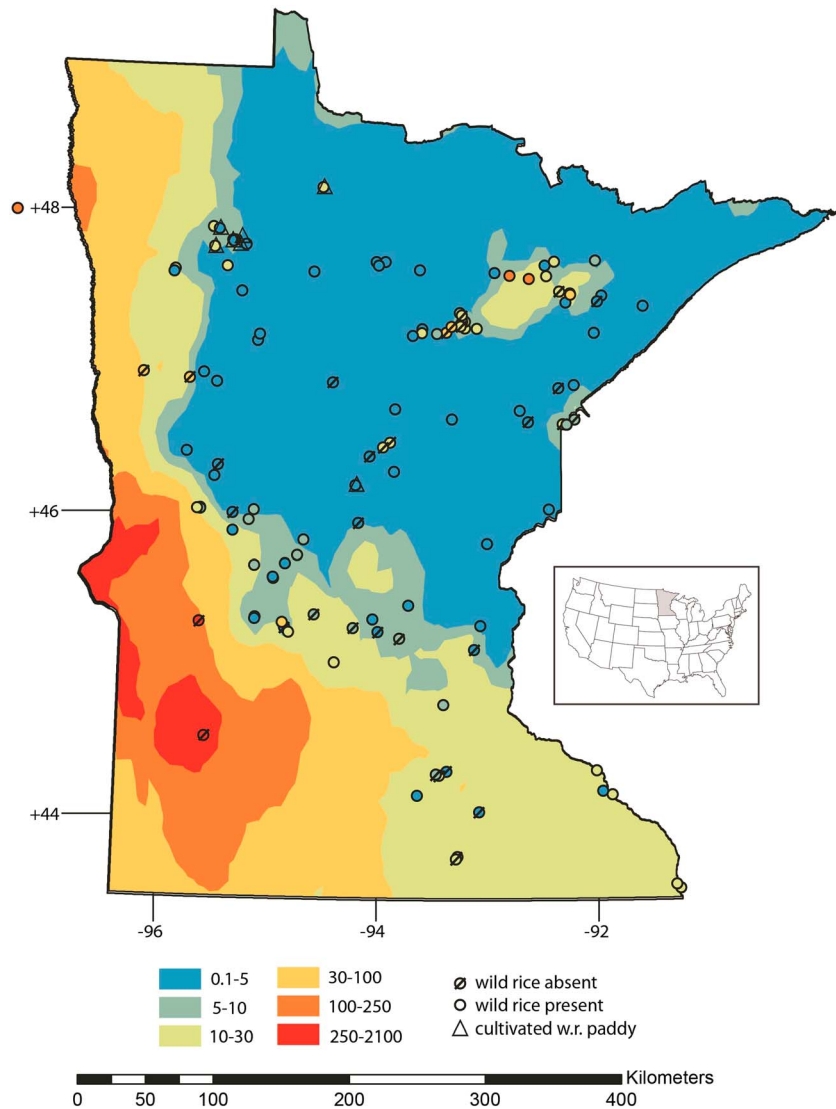
The purpose of this study was to examine the multiple ways that increases in  $\text{SO}_4$  concentration and sulfide production can change the biogeochemical functioning of freshwater ecosystems and potentially negatively affect the growth and reproduction of wild rice populations. Before any field data were collected for this study, alternative hypotheses regarding the most likely negative effects were identified (MPCA, 2011), so that appropriate data would be available to test them. The primary hypotheses of how increases in surface water concentrations of  $\text{SO}_4$  could harm wild rice populations include direct toxicity by elevated pore water sulfide; reduced bioavailability of Fe, Cu, or Zn; and increased P bioavailability promoting the growth of phytoplankton and macrophytes that compete for light and space. Additional variables were quantified so that exploratory data analysis could be pursued in addition to evaluation of the primary hypotheses. Surface water, pore water, and sediment physical and chemical properties were collected from 108 different sites, both with and without wild rice present, to inform evaluation of these multiple interrelated hypotheses.

## 2. Methods

### 2.1. Study Area

#### 2.1.1. Physical Environment

$\text{SO}_4$  concentrations in surface water vary with geology and climate (Gorham et al., 1983) in a northeast-southwest gradient across Minnesota. Bedrock and bedrock-derived glacial deposits of northeastern Minnesota (the “arrowhead”) comprise slowly weathered crystalline materials, generally low in S, and surface waters are dilute (specific conductance of  $<140$  and often  $<30 \mu\text{mho cm}^{-1}$ ; Gorham et al., 1983). Within this area of naturally low  $\text{SO}_4$ , iron-mining activities in the “Iron Range” district of northeastern Minnesota have created an “island” of lakes and streams elevated in  $\text{SO}_4$  (Figure 1), a result of the weathering of sulfide minerals in waste rock piles and tailing basins. The surficial geology of southwestern Minnesota, in contrast, is derived from marine shales and carbonates that are relatively S-rich and readily weathered: surface waters often exceed  $500 \mu\text{mho cm}^{-1}$  and may exceed  $7,000 \mu\text{mho cm}^{-1}$  (Gorham et al., 1983).  $\text{SO}_4$  concentration is positively correlated with conductivity above about  $200 \mu\text{mho cm}^{-1}$  and  $10 \text{ mg L}^{-1}$  or  $0.1 \text{ mmol L}^{-1}$   $\text{SO}_4$  (Figure 7B in Gorham et al., 1983). Overprinting this geological pattern is a strong climatic gradient with warmer, drier conditions toward the southwest (toward the northern Great Plains) and colder, more humid conditions to the northeast (toward the Laurentian Great Lakes), which enhances the conductivity gradient: more evaporative conditions in the southwest serve to concentrate surface waters and further increase ionic



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

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

**2.1.2. Habitat Preferences of Wild Rice**

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

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

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

### 2.1.3. Site Selection

The goal of the field survey was to identify and sample potential wild rice habitat across a gradient of  $\text{SO}_4$  concentrations. Potential wild rice habitat was defined simply as shallow water (30–120 cm deep, though some sampled sites had water as shallow as 5 cm) that was sufficiently transparent to support rooted aquatic macrophytes. Turbidity from dense phytoplankton blooms or suspended solids may effectively exclude rooted macrophytes due to light limitation (Scheffer, 1998).

Most Minnesota lakes that host wild rice populations have low  $\text{SO}_4$  concentrations; the median concentration of wild rice lakes is  $1.8 \text{ mg L}^{-1}$ , and the 75th percentile is  $3.6 \text{ mg L}^{-1}$  (MPCA, 2014). If only those waters hosting abundant wild rice were sampled, most of those would be low in  $\text{SO}_4$ , and little would be learned about how elevated  $\text{SO}_4$  concentrations (or other important variables) might affect wild rice. Conservation biologists commonly identify important habitat variables through binary (i.e., is the species present or absent at a given site?) logistic regression, which requires the sampling of sites that do not support the species of interest (e.g., Carroll, Zielinski, & Noss, 1999; Peeters & Gardeniers, 1998; van der Heide et al., 2009). Consequently, potential wild rice habitats with a range in  $\text{SO}_4$  concentrations were sampled without regard to whether the waterbody hosted or was known to host wild rice. An effort was made to sample waterbodies that covered the range of  $\text{SO}_4$  concentrations across Minnesota and especially to sample sites in high- $\text{SO}_4$  regions that had reports of recent or historical presence of wild rice. This strategy also resulted in the sampling of a high- $\text{SO}_4$  wild rice site in the North Branch of the Turtle River ( $198 \text{ mg SO}_4 \text{ L}^{-1}$ ), 40 km into North Dakota from northwestern Minnesota (Figure 1). A gradient of  $\text{SO}_4$  concentrations was sampled by identifying potential wild rice habitat in two areas of elevated  $\text{SO}_4$ , the “island” of elevated  $\text{SO}_4$  in the Iron Range region of northeastern Minnesota and waters naturally elevated in  $\text{SO}_4$  to the west and south of the known range of wild rice (Figures 1 and S1). Nutrient (N and P) availability (reflecting natural soil fertility as well as agricultural runoff) also increases to the west and south, potentially supporting phytoplankton growth sufficient to exclude macrophytes, including wild rice. Consequently, in western and southern Minnesota, DNR lake databases were screened for the presence of water lilies (*Nuphar variegata* or *Nymphaea odorata*), the occurrence of which indicates transparency sufficient to support rooted macrophytes that also often cooccur with wild rice. In an analysis of DNR aquatic plant surveys from 1,753 shallow lakes, we found that the odds of finding wild rice where there are water lilies are 26 times the odds of finding wild rice where there are no water lilies, with a 95% confidence interval of 20–36 times (neither wild rice nor water lilies = 968; wild rice but not water lilies = 60; no wild rice but water lilies = 272; both wild rice and water lilies = 453).

With these considerations, water bodies were selected for sampling based on preliminary data including average water depth, presence of water lilies, conductivity or  $\text{SO}_4$  concentration, and geographic distribution (i.e., to sample widely across the state). Both rivers and lakes were sampled, as were seven different cultivated wild rice paddies. Within a given water body, the field team chose a location for sampling based on a decision tree (Table S1 in the supporting information).

## 2.2. Field Methods

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

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

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

Pore water samples were obtained from cores processed on shore using 10 cm Rhizon™ filters (pore size 0.12–0.18  $\mu\text{m}$ ) (Rhizosphere.com, Netherlands; Shotbolt, 2010) inserted vertically into the core tops, following extrusion of overlying water, and connected to evacuated serum bottles with PVC/polyethylene tubing and a stainless steel needle. Three separate cores were sampled, one for nutrients (nitrate + nitrite, TP, and TN) and DOC (70 mL), a second for metals (50 mL), and a third for dissolved silica, Cl, and  $\text{SO}_4$  (30 mL). A fourth core was sampled for pore water sulfide, but in this case, the serum bottle was preloaded with 0.2 mL of 2.0 N zinc acetate, 0.5 mL of 15 M sodium hydroxide, and a stir bar, flushed with a nitrogen atmosphere, evacuated, and preweighed. Air was flushed from the Rhizon-tubing assembly with sample pore water using a second evacuated bottle before the needle was inserted into the sulfide sample bottle. In 2011 only pore water samples for sulfide and metals were collected. Pore water pH was measured on a fifth core by inserting the probe of a Hach model HQ40d pH meter into the sediment to a depth of 5 cm and allowing the reading to stabilize.

A composite sediment sample was collected from the uppermost 10 cm of the sixth, seventh, and eighth cores, placed into a stainless steel bowl, and stirred under nitrogen atmosphere to homogenize. A 50-mL subsample was placed in a polypropylene sample bottle along with 1.0 mL of 1.0 N zinc acetate for analysis of acid-volatile sulfide (AVS). The headspace of the AVS sample bottle was flushed with  $\text{N}_2$  and the bottle capped; that bottle was placed in a larger glass jar and that jar flushed with  $\text{N}_2$  and tightly sealed. The sample was immediately placed in a cooler with dry ice to freeze. The remaining composited sediment was placed into a polycarbonate container and stored on ice for later analysis.

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

## 2.3. Laboratory Methods

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

Surface and pore water samples were analyzed for anions (Cl and SO<sub>4</sub>) by ion chromatography on a Dionex ICS-3000 (MDHEL), Fe, Ca, Na, Mg, and K by inductively coupled optical emission spectrometry on a Varian 715-ES (MDHEL), Mn, Cu, Zn, Co, Ni, Al, As, and Se by inductively coupled mass spectrometry on a Perkin Elmer Elan DRCE (MDHEL), and DOC by UV-persulfate oxidation on a Tekmar-Dohrmann Phoenix 8000 (SCWRS and MDHEL). N and P were measured by colorimetric methods (on a Lachat Quikchem Flow-Injection Autoanalyzer) at SCWRS and MDHEL following cadmium-reduction (nitrate and ammonia) or dual alkaline-persulfate digestions (TP and TN). Silica was measured colorimetrically at SCWRS on the Lachat Autoanalyzer and at MDHEL on a Beckman Coulter DU 800 UV/VIS spectrophotometer. Pore water sulfide was analyzed colorimetrically on each lab's Lachat Autoanalyzer following in-line acid distillation and NaOH trapping (SM 4500-S2). Alkalinity was measured by potentiometric acid titration (MDHEL) or as DIC by acid digestion and IR detection (SCWRS). DIC was converted to alkalinity using pH, temperature, and specific conductance of the surface water.

Sediment samples from 2011 were analyzed by combustion for total carbon (TC), total nitrogen (TN), and total sulfur (TS) using, respectively, a Tekmar Phoenix 8000 CO<sub>2</sub> analyzer, an Elementar Vario Max N analyzer, and a LECO sulfur analyzer (UMNSL). Samples from 2012 and 2013 were analyzed for CHN on a Costech 4010 Elemental Analyzer (UMN Stable Isotope Laboratory). Total inorganic carbon (carbonate) was analyzed by coulometric titration on a UIC CM5015 CO<sub>2</sub> coulometer, while water and organic matter content were determined by loss-on-ignition methods (Heiri, Lotter, & Lemcke, 2001) in the UMN LacCore facilities. Sediment AVS was analyzed colorimetrically, as above for pore water sulfide, following acid-distillation and in-line alkaline trapping (SM 4500-S2; Hsieh & Shieh, 1997).

Sediment phosphorus was extracted from freeze-dried sediments following methods of Engstrom and Wright (1984) for total-P and Hietjes and Lijklema (1980) for P fractions (NH<sub>4</sub>Cl-extractable, NaOH-extractable, HCl-extractable, and residual (organic)-P). The P extracts were measured colorimetrically by flow-injection autoanalyzer (SCWRS). Extractable iron and trace metals were quantified from a 0.25 g homogenized freeze-dried sediment subsample incubated in 0.5 M HCl for 30 min at 80°C. The samples were centrifuged, decanted, and analyzed by ICP-MS at GAC. This extraction releases metal oxyhydroxides, sulfides, and loosely bound phases from the sediment without appreciably attacking the silicate matrix (Balogh et al., 2009).

#### 2.4. Data Subsets and Statistical Analysis

From 2011 to 2013, 260 site visits were conducted in 108 different natural waterbodies, including lakes, small streams, backwaters of the Mississippi River, and wetlands, plus 7 different cultivated wild rice paddies. For a variety of logistical reasons, the full suite of samples could not be collected on some site visits. Three subsets of the field data were identified for the analyses reported here. A subset that consists of all of the samples from natural waterbodies (excluding the cultivated paddies) with virtually complete analyses (surface water, pore water, and bulk sediment;  $n = 194$ ) was termed for internal purposes Class D. Pollman et al. (2017) used Class D to develop a structural equation model to elucidate key variables that govern the concentration of sulfide in sediment pore water. A second subset, Class S, of 51 samples from 7 stream and 8 lake sites was each sampled 3 to 5 times from 27 May to 19 September 2013, to provide a data set to assess seasonality in variables. A third subset, Class B, was used for conducting probabilistic analyses to identify the most likely parameters controlling the presence and absence of wild rice and to examine Spearman nonparametric correlations among field variables. Class B consists of one sample from each site ( $n = 108$ ) and excludes samples collected in 2011, which were analyzed with slightly different lab methods. Although Class B was not created as a random sample of Minnesota waterbodies, the frequency distribution of SO<sub>4</sub> concentrations is intermediate between a probability-based survey conducted by USEPA (MPCA, 2016) and a list of known wild rice water bodies, which are overwhelmingly low-SO<sub>4</sub> lakes (MPCA, 2014). Therefore, the Class B data set is reasonably representative of potential wild rice habitat, and binary logistic regressions can be used to approximate the probability of wild rice occurrence as a function of field variables such as pore water sulfide. The Class B data set is used in all analyses presented here, with two exceptions: (1) Figures 3a–3c present the full Class D data set, plus for comparison, data from the cultivated wild rice paddies; (2) Statistical analysis was performed with the software package R version 3.2.3.

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

**Table 1**

Field Variable Correlation With the Presence-Absence of Wild Rice, Assessed Through Binary Logistic Regression (BLR), Plus Spearman Correlations Between Variables<sup>a</sup>

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

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

\* $p < 0.05$ . \*\* $p < 0.01$ . \*\*\* $p < 0.001$ .

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

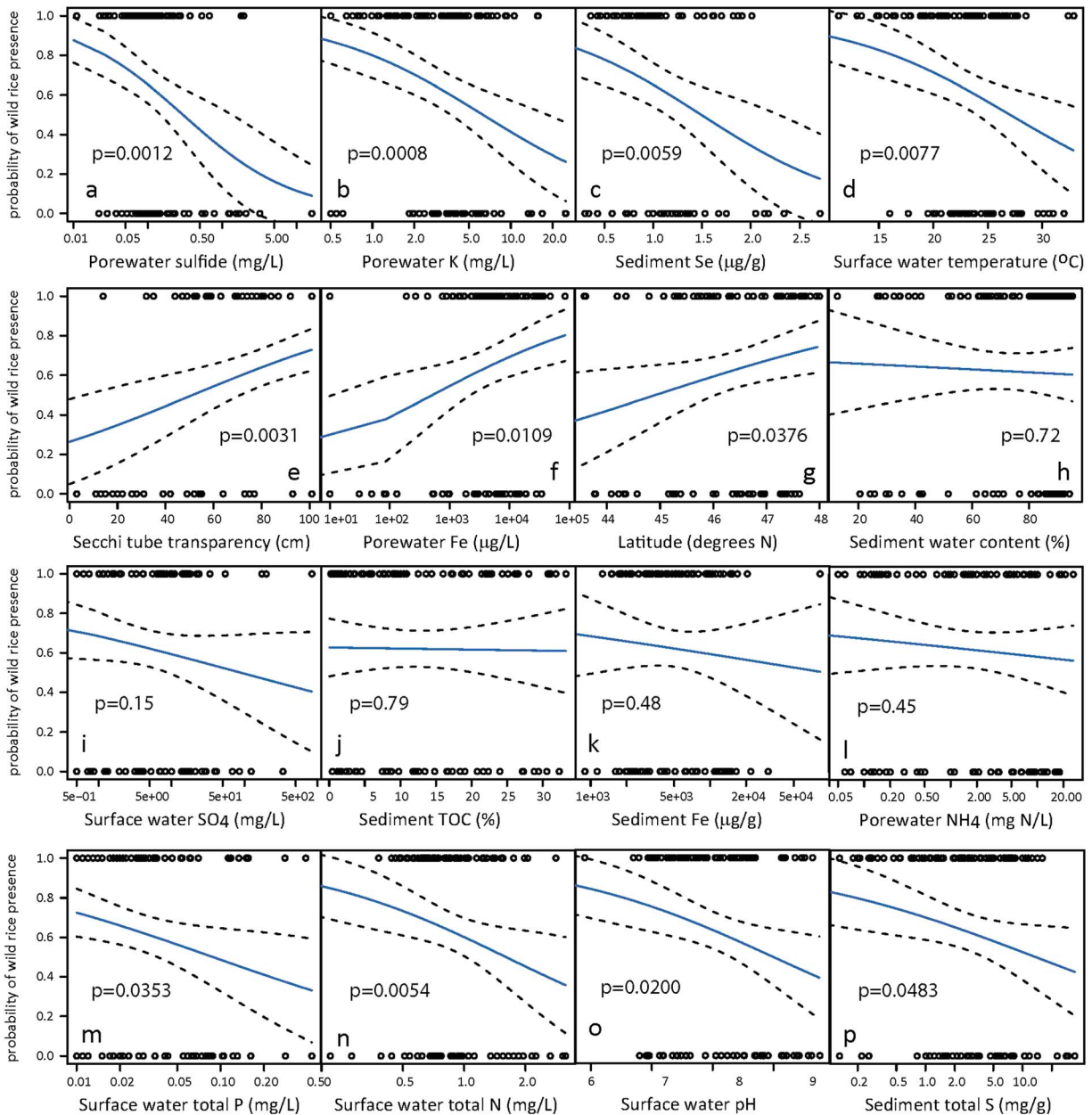
$$y = A + B \sin(2\pi \text{ time}) + C \cos(2\pi \text{ time}) \tag{1}$$

### 3. Results and Discussion

#### 3.1. Field Variables Associated With Wild Rice Presence and Absence

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

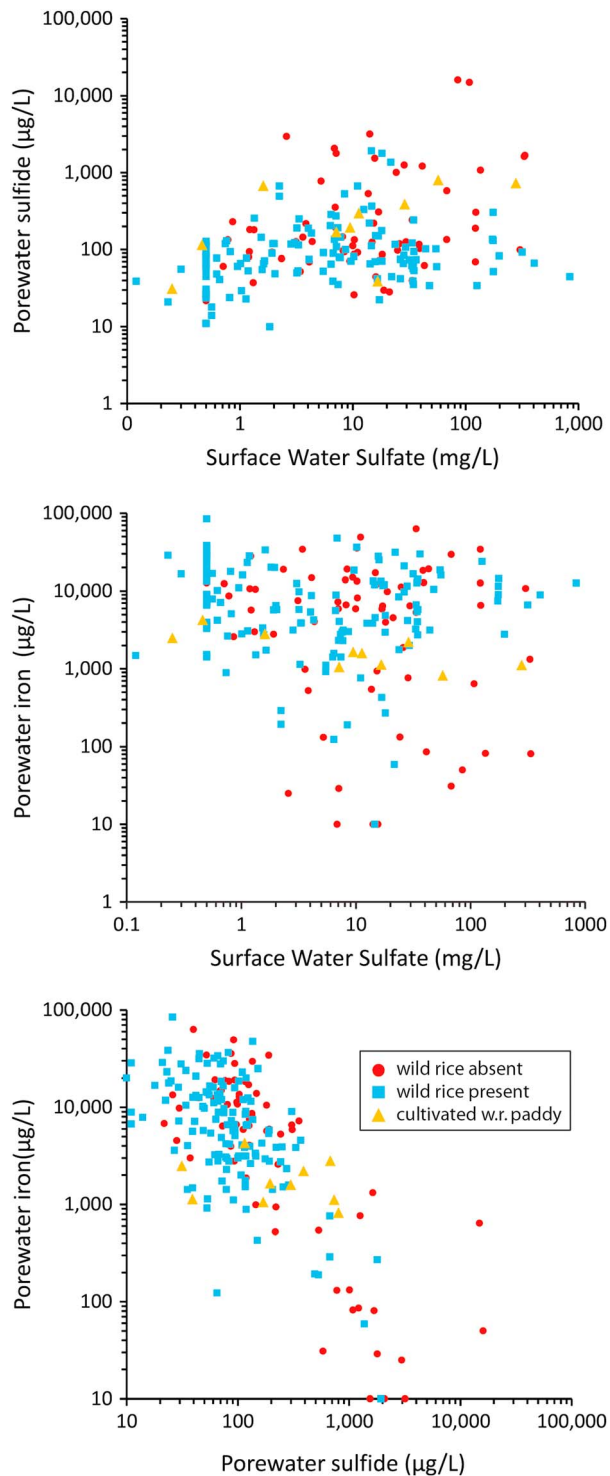




**Figure 2.** Binary logistic regressions for 16 variables found to be statistically significant in this study or indicated in the literature as important for wild rice habitat. The open circles indicate the value for a given parameter of each site used in the analysis; the circles at the top of a plot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

correlations to help understand the relationships between environmental factors of interest. Wild rice density is negatively correlated with pore water sulfide, potassium (K), and  $\text{NH}_4$  and positively correlated with surface water alkalinity (Table 1). There are likely factors controlling the density of wild rice at any particular location in addition to the variables measured, such as herbivory and hydrological disturbances.

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



**Figure 3.** Class D data plus cultivated wild rice paddies (yellow triangles) showing sites with wild rice present (blue squares) and absent (red circles). Note that (a) pore water sulfide is not linearly related to surface water  $\text{SO}_4$ , indicating that additional factors besides  $\text{SO}_4$  concentration contribute to the sulfide concentration in pore water; (b) wild rice can occur in sites over a wide range of surface water  $\text{SO}_4$  values as long as pore water Fe is relatively high; and (c) pore water Fe and sulfide have a strong negative relationship, due to the energetically favorable formation of FeS compounds, and that wild rice mainly occurs where pore water sulfide is  $< 1,000 \mu\text{g L}^{-1}$ .

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

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

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

Our primary a priori hypothesis was that elevated surface water  $\text{SO}_4$  can produce elevated pore water sulfide concentrations which, in turn, negatively affect potential wild rice habitat. A plot of pore water sulfide against  $\text{SO}_4$ , with wild rice presence noted (Figure 3a), suggests that wild rice is generally not found at sites with elevated sulfide. Of the sites with pore water sulfide greater than  $1,000 \mu\text{g L}^{-1}$ , wild rice was present in 23% (3 of 13, with only a few plants found at 2 of those 3 sites). In contrast, wild rice was present at 74% of the sites where sulfide was less than  $1,000 \mu\text{g L}^{-1}$ . Despite historical reports of *Zizania aquatica* in Minnesota, inspection of voucher specimens, following the taxonomy of Terrell et al. (1997), found only *Z. palustris* at all sites sampled. *Zizania aquatica* (Southern wild rice) is rare in Minnesota, but its range extends east through Wisconsin and the southern part of the Great Lakes region to New England and occurs in freshwater tidal marshes south to Florida and west to Louisiana (Terrell et al., 1997). The findings of this study, especially those related to temperature, may not apply to *Z. aquatica*.

Despite a strong relationship between sulfide and wild rice, the relationship between  $\text{SO}_4$  and pore water sulfide among the 108 field sites was not a simple linear function. High surface water  $\text{SO}_4$  did not always result in high pore water sulfide (above  $1,000 \mu\text{g L}^{-1}$ ), and some samples with surface water  $\text{SO}_4$  less than  $10 \text{ mg L}^{-1}$  had pore water sulfide greater

than 1,000  $\mu\text{g L}^{-1}$  (Figure 3a). The 33 samples with surface water  $\text{SO}_4$  below 1.0  $\text{mg L}^{-1}$  all had pore water sulfide less than 200  $\mu\text{g L}^{-1}$ , suggesting that surface water  $\text{SO}_4$  might limit maximum sulfide production.

Wild rice was present at high and low  $\text{SO}_4$  concentrations as long as pore water Fe was high and sulfide was low (Figures 3b and 3c): pore water Fe and sulfide each exert antipathetic control over the concentration of the other (Figure 3c; Pollman et al., 2017), and wild rice mainly persists in sites where pore waters are low in sulfide and high in Fe. Pollman et al. (2017) developed a structural equation model showing that variations in three variables ( $\text{SO}_4$ , sediment TOC, and sediment extractable Fe) contribute nearly equally to the observed variations in pore water sulfide among the sites in this study. Sulfate-reducing bacteria are simultaneously limited in their production of pore water sulfide by surface water  $\text{SO}_4$  and sediment TOC (which are roughly proportional to available  $\text{SO}_4$  and labile organic matter, respectively). Concentrations of sulfide in pore water are then constrained by the availability of pore water Fe, which is in turn controlled by the overall supply of Fe in the sediment (Pollman et al., 2017).

If pore water sulfide concentration is a causative factor controlling the presence of wild rice, as hypothesized, then other variables may be statistically significant because they either vary with the process of  $\text{SO}_4$  reduction or with the sulfide itself. Pastor et al. (2017) showed that wild rice growth declines in proportion to sulfide concentrations in both hydroponic and outdoor mesocosm experiments. In a study of the Pastor et al. mesocosms, Myrbo et al. (2017) showed that the enhanced mineralization of plant litter associated with  $\text{SO}_4$  reduction produced increases in surface water P and N, and increases in pore water sulfide caused decreases in pore water Fe. These effects from the controlled mesocosm experiment are consistent with correlations observed among sites in the field data between pore water sulfide and surface water N and pore water Fe (Table 1). Surface water P is not significantly correlated with pore water sulfide in the field data, despite dual mechanisms of P mobilization from sediment, (1) the interaction between sulfide and Fe (Caraco et al., 1993; Maynard, Dahlgren, & O'Geen, 2011; Smolders & Roelofs, 1993) and (2) mineralization of organic matter. The lack of correlation of surface water P with pore water sulfide in this field study is not surprising because given the wide variety of landscapes sampled, there is no reason that pore water sulfide would be proportional to the mass of Fe that has reacted with sulfide and released sorbed P. In  $\text{SO}_4$  addition experiments where the P and Fe content of the sediment is held constant, there is often a significant correlation between pore water sulfide and mobilized P (Myrbo et al., 2017). In contrast to the lack of correlation of pore water sulfide with surface water P in this field study, AVS is significantly correlated with surface water P ( $\rho = 0.30$ ,  $p < 0.01$ , Table 1), presumably because AVS is proportional to the iron that has reacted with sulfide.

Elevated pore water sulfide is a product of  $\text{SO}_4$  reduction-driven mineralization of sediment organic matter, which also releases the constituents of the decaying plant material, N, P, K, silica, and C (either as DIC, which increases alkalinity, or DOC) into sediment pore water and the overlying surface water (Myrbo et al., 2017). Consequently, the occurrence of wild rice is negatively associated not only with pore water sulfide ( $p = 0.001$ ) but also with elevated pore water K ( $p = 0.0008$ ) and surface water TN ( $p = 0.005$ , Table 1). Median concentrations of these variables are lower in wild rice waters compared to waters with no observed wild rice (88 versus 126  $\mu\text{g L}^{-1}$  sulfide, 2.4 versus 4.3  $\text{mg L}^{-1}$  pore water K, and 0.74 versus 0.95  $\text{mg L}^{-1}$  TN, Table S3). Pore water sulfide is itself positively correlated with pore water K, TN,  $\text{NH}_4$ , and silica, surface water K, alkalinity, and pH, and sediment TS and AVS (Tables 1 and S4).

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

AVS largely consists of solid-phase sulfide, which is not available to plants and therefore is not significantly associated with wild rice presence/absence ( $p > 0.10$ , Table 1). AVS is a measure of cumulative sulfide production, which is proportional to past mineralization of organic matter, consistent with significant positive correlations between AVS and pore water TP and  $\text{NH}_4$ , and surface water TP and alkalinity ( $p < 0.01$ , 0.05, 0.01, and 0.01, respectively, Table 1).

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

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

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

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

### 3.4. Temperature as a Causal Variable and Associated Correlated Variables

Wild rice germinates at higher rates following longer exposure at temperatures closer to freezing (Kovach & Bradford, 1992), consistent with the significant association of wild rice absence with lower latitudes (BLR  $p = 0.04$ ) and warmer summer water temperature (BLR  $p = 0.008$ ; medians of  $21.9^\circ$  and  $23.7^\circ\text{C}$  with and without wild rice, respectively). Sites with warmer summer water temperatures generally also have warmer winter temperatures. Warmer and shorter winters would cause lower germination rates, ultimately reducing the probability that a population could persist over the long term. For this reason, the Minnesota Department of Natural Resources hypothesizes that climate change may push the range of wild rice farther north (DNR, 2008, 2016). However, the temperature control of wild rice germination has not been adequately investigated to rigorously assess this hypothesis. The observed association of wild rice with lower pH surface waters (BLR  $p = 0.04$ ; median pH values of 7.8 and 8.5 at sites with and without wild rice, respectively) may be due simply to the correlation of wild rice with cooler surface waters: the solubility of  $\text{CO}_2$  is higher in colder water, leading to lower pH (Spearman correlation between temperature and pH = 0.35,  $p < 0.0001$ ).

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

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

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

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

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

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

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

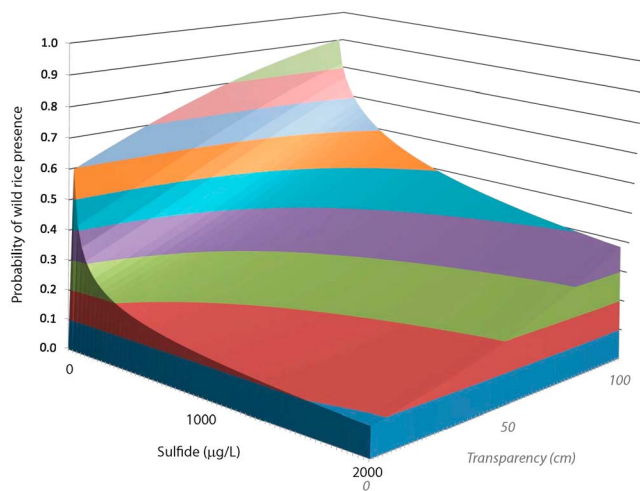
$$\text{Logit (WR presence)} = 0.532 + 0.0183 (\text{trans}) - 1.169 (\log_{10} \text{pw sulfide}) - 0.107 (\text{Temp}) \quad (2)$$

where odds =  $\exp(\text{logit})$  and probability =  $(\text{odds}/(1 + \text{odds}))$ .

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

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

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



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

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

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

### 3.7. Seasonality in Field Variables

Fifteen natural wild rice sites were sampled 3 to 5 times in 2013 to determine which field variables exhibit statistically significant seasonal trends that might be important to consider in the overall analysis, given that different sites were sampled in different months. Some variables conformed to expected seasonal trends, such as water temperature ( $p = 0.001$ ), which increased in early summer, peaked about 1 August, and then declined. Some of the surface water variables exhibited monotonic increased concentrations over the summer (alkalinity, Na, Mg, and SO<sub>4</sub>;  $p = 0.0009, 0.008, 0.021, \text{ and } 0.05$ , respectively). One hypothesis that explains this observation is that waterbodies in Minnesota, after being diluted by spring snowmelt, in general become more concentrated as the summer progresses due to evaporation and, for waterbodies with shorter residence times, greater influence of groundwater flux. Cl and Ca had nonsignificant positive slopes ( $p = 0.29$  and  $0.37$ , respectively), whereas K exhibited no trend ( $p = 0.48$ ). As expected, none of the solid-phase variables of the homogenized 10 cm long sediment cores showed any significant seasonal trends (e.g., sediment extractable Fe  $p = 0.93$ , sediment TOC  $p = 0.96$ ). Among the pore water variables, only K and pH exhibited significant trends. Pore water K values decreased over the summer ( $p = 0.006$ ), likely as a result of diffusion into surface water or uptake by growing rooted macrophytes. Pore water pH generally increased over the summer ( $p = 0.003$ ), which may reflect loss of CO<sub>2</sub> as the sediment warmed over the summer, reducing the solubility of gases. The lack of seasonality in pore water sulfide ( $p = 0.62$ ) indicates that sulfide concentrations were in steady state with the variables that exert primary control over its concentration, surface water SO<sub>4</sub>, extractable Fe, and sediment TOC (Pollman et al., 2017). Of the variables controlling pore water sulfide, only SO<sub>4</sub> exhibited even marginally significant seasonal trends ( $p = 0.05, 0.93, \text{ and } 0.97$ , for SO<sub>4</sub>, extractable Fe, and sediment TOC, respectively). Despite the finding of seasonal variation in SO<sub>4</sub>, Pollman et al. (2017) found that SO<sub>4</sub> is one of the primary variables that control pore water sulfide concentrations. Seasonal variation in SO<sub>4</sub> concentrations undoubtedly contributes to noise in the statistical relationship documented by Pollman et al. (2017). Myrbo et al. (2017) found that the microbial production of pore water sulfide in a wild rice sediment is proportional to the long-term average SO<sub>4</sub> surface water concentration.

### 3.8. Cultivated Wild Rice Paddies

Commercial wild rice paddies have been reported with healthy stands of wild rice growing under surface water  $\text{SO}_4$  levels as high as  $170 \text{ mg L}^{-1}$  (Aiken et al., 1988). Our limited measurements in seven different paddies ranged from  $0.3 \text{ mg L}^{-1}$  to  $279 \text{ mg L}^{-1} \text{ SO}_4$ , with a median of  $8.3 \text{ mg L}^{-1}$  ( $n = 7$ , Table S3). Natural wild rice waters in our study had a median  $\text{SO}_4$  concentration of  $4.1 \text{ mg L}^{-1}$ . Surprisingly, median pore water sulfide in paddies was  $182 \mu\text{g L}^{-1}$ , greater than the medians of natural wild rice waters ( $88 \mu\text{g L}^{-1}$ ;  $n = 67$ ) and waters without wild rice ( $126 \mu\text{g L}^{-1}$ ;  $n = 41$ ). The median sediment extractable Fe concentration of paddies ( $4.5 \text{ mg g}^{-1}$ ) is similar to that of natural wild rice waters ( $4.8 \text{ mg g}^{-1}$ ). In contrast, median sediment TOC is much higher in paddies (25.2%) than natural wild rice waters (9.1%). It thus appears that the greater TOC driving enhanced  $\text{SO}_4$  reduction (Pollman et al., 2017) and driving median pore water Fe lower than in natural wild rice sediments ( $1.6$  and  $5.7 \text{ mg L}^{-1}$ , respectively), despite the similar reservoirs of Fe in the sediment. Elevated production of sulfide coupled with the consumption of the available Fe may put some paddies on the brink of sulfide toxicity to wild rice.

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

### 3.9. Conclusions and Implications

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

Aside from low pore water sulfide, favorable wild rice habitat has long, cold winters and transparent surface water in the ice-free season. *Zizania palustris* seeds germinate at low rates if the winter is too warm or too short. The probability that wild rice seedlings successfully grow to maturity is reduced if photosynthesis is inhibited by low water clarity. Thus, pore water sulfide (itself a function of surface water  $\text{SO}_4$ , sediment Fe, and sediment organic matter (Pollman et al., 2017)), water temperature, and water transparency together largely determine wild rice presence and absence. These three factors are independent of one another but may act synergistically on other related processes.

In addition to generating sulfide,  $\text{SO}_4$  reduction supports organic matter mineralization that releases nutrients and alkalinity to the surface water, which has the potential to change plant community structure even if Fe is high enough to keep pore water sulfide from reaching phytotoxic levels (Myrbo et al., 2017). Natural and anthropogenic  $\text{SO}_4$  loading to freshwaters may thus strongly affect ecosystem composition and function, despite the low direct toxicity of  $\text{SO}_4$  under oxic conditions.

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