



RESEARCH ARTICLE

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Sulfur Geochemistry Destabilizes Population Oscillations of Wild Rice (*Zizania palustris*)Sophia LaFond-Hudson^{1,2} , Nathan W. Johnson¹ , John Pastor³, and Brad Dewey³¹Department of Civil Engineering, University of MN Duluth, Duluth, MN, USA, ²Now at Oak Ridge National Laboratory, Oak Ridge, TN, USA, ³Department of Biology, University of Minnesota Duluth, Duluth, MN, USA

Key Points:

- Populations exposed to elevated sulfate went extinct in 6 years, overriding interannual biomass oscillations
- Iron addition and litter removal slightly alleviated sulfide toxicity but did not prevent population extinction
- Stability of oscillating populations can be evaluated with only a few years' data using an eigenvalue

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Abstract Elevated inputs of sulfate to freshwater systems can increase sulfide concentrations in anoxic soils and subsequently destabilize aquatic plant populations, but the interactions between sulfate, other geochemical cycles, and interannual plant population cycles are poorly understood. Increased sulfate loading increases mineralization of nitrogen from litter, but the sulfide produced during this process can limit nitrogen uptake by plants. In some cases, iron may mitigate sulfide's impacts on plants by precipitating iron sulfide. We examined the interannual effects of sulfate loading on mesocosm populations of wild rice, an emergent aquatic plant that undergoes population oscillations and is sensitive to sulfide. Using experimental mesocosms with self-perpetuating populations, we investigated how population dynamics respond to manipulations of surface water sulfate (10 mg L⁻¹ or 300 mg L⁻¹), sediment iron (4.3 mg g⁻¹ or 10.9 mg g⁻¹ dry weight), and shoot litter (present or removed). Populations exposed to constant 10 mg L⁻¹ sulfate concentrations had stable biomass oscillations of approximately 3-year periods, consistent with previous studies that demonstrated litter-driven oscillations in nitrogen availability. Populations exposed to 300 mg L⁻¹ sulfate concentrations produced fewer and smaller seeds and declined to extinction in 6 years or less. We did not find a strong effect of iron loading or litter removal on wild rice biomass or seed production. Our observations show the potential of elevated surface water sulfate to rapidly destabilize wild rice populations under varying iron and organic carbon concentrations.

Plain Language Summary Plants that naturally grow in freshwater do not survive well if the water contains elevated concentrations of sulfate. Sulfate reduction produces sulfide that subsequently inhibits the uptake of nitrogen, an essential plant nutrient. Some annual plants go through boom-bust cycles with years alternating between high and low biomass because nitrogen takes more than a year to be released from dead plant matter. We investigated the combined effect of sulfate and natural biomass cycles on the stability of wild rice populations by growing plants in large tanks and exposing them to high-sulfate and low-sulfate concentrations, high and low iron concentrations, and with plant matter from the previous growing season either returned or removed. Nearly all plant populations exposed to high sulfate had died by 6 years into the experiment, regardless of iron concentration or litter removal. We show a method to analyze population stability with just a few years of data.

1. Introduction

Northern wild rice (*Zizania palustris*) is one of four species in the genus *Zizania*, which are the only native aquatic grains in North America. The range of northern wild rice (hereafter wild rice) is centered across the Great Lakes region and is most abundant in the rivers and lakes of the watersheds of Lakes Superior and Michigan in northern Minnesota, Wisconsin, and Ontario. Wild rice beds are usually very large (tens or hundreds of hectares) and monotypic. Because of its widespread distribution and tendency to form large monotypic stands, wild rice has great potential to control the quality of waters draining into Lakes Superior and Michigan and influence the food supply for waterfowl, muskrats, and other members of the food web. In addition, harvesting and eating wild rice are essential traditional practices that provide food sovereignty and well-being for the native Ojibway people of the watersheds of Lakes Superior and Michigan (Fond du Lac Band of Lake Superior Chippewa, 2018). Therefore, the productivity, perpetuation, and restoration of wild rice are of great ecological and cultural significance.

Production of wild rice biomass is limited by the supply of nitrogen from decomposing plant litter, sediment organic matter, and hydrologic inputs (Pastor & Walker, 2006; Sims et al., 2012; Walker et al., 2006, 2010). Because it is an annual plant, wild rice's nitrogen requirements must be fully supported by uptake during each year. Over 60% of nitrogen uptake happens during a 2-week window in early summer (Grava & Raisanen, 1978;

Sims et al., 2012). Nitrogen, however, is not released from the previous year's litter until later in summer or even the following year (Hildebrandt et al., 2012; Sain, 1984; Walker et al., 2010). In fact, there is considerable microbial immobilization of nitrogen into fresh litter during the period when the demands of wild rice growth for nitrogen are greatest (Hildebrandt et al., 2012; Walker et al., 2010). The coincidence of microbial nitrogen immobilization with the period of rapid nitrogen uptake causes wild rice biomass and litter production to cycle with a period of approximately 4 years (Pastor & Walker, 2006; Walker et al., 2010).

Inputs of sulfate from bedrock weathering, mine drainage, and agriculture enhance sulfide production in natural wild rice ecosystems (Bailey et al., 2017; Lamers et al., 2013; Myrbo et al., 2017a). Wild rice production appears to be adversely impacted by sulfide in the vicinity of its rooting zone. The survival of juvenile seedlings and weights of seeds decrease with increased hydrogen sulfide concentrations in wild rice's rooting zone in aquatic sediments (Pastor et al., 2017). The production of sulfide may be coupled to increased litter deposited in sediment during productive years of the wild rice population cycle. These large litter cohorts could reduce sediment redox potential (Eh) by providing additional labile carbon to support additional bacterial growth and hence oxygen demand the following year, thereby enhancing the potential for reduction of sulfate to sulfide (Azam et al., 1991; Gao et al., 2003, 2004).

Other biogeochemical reactions in the sediments may impede the bioavailability of sulfide to wild rice roots. The most important reaction is precipitation of sulfide with reduced iron (Morse et al., 1987). In both mesocosm and lake studies (Bailey et al., 2017; Myrbo et al., 2017a), iron in sediments appear to exert a strong control on the accumulation of dissolved sulfide in sediments. Bulk sediment iron content is strongly associated with lower porewater sulfide in field conditions and mitigates sulfide toxicity to macrophytes in other aquatic ecosystems (Lamers et al., 2002; Ruiz-Halpern et al., 2008; Van der Welle et al., 2007).

However, iron sulfide can precipitate on roots of mature plants and is associated with impaired nitrogen uptake and inhibited seed production (LaFond-Hudson et al., 2018, 2020a). Plant-mediated gas transport of oxygen from the atmosphere into the rhizosphere allows formation of iron oxides on root surfaces, and oxygen fluxes are typically highest when plants are photosynthetically active (Blossfeld et al., 2011; Han et al., 2018; Marzocchi et al., 2019). As observed on many emergent macrophytes, iron oxide forms on wild rice roots as the plant grows (Jorgenson et al., 2012; Mendelsohn et al., 1995; Sundby et al., 1998). At maturity and the start of seed production, however, root plaques transition from iron oxide to iron sulfide if porewater sulfate is abundant (LaFond-Hudson et al., 2018). We have imaged iron sulfide plaques and quantified plaque iron and sulfide concentrations from plants grown in mesocosms with 300 mg L⁻¹ sulfate (Pastor et al., 2017) and have visually observed black root plaques in the field at lower sulfate concentrations (unpublished data). Plants that accumulate greater concentrations of iron sulfide plaques have lower seed nitrogen mass (LaFond-Hudson et al., 2018, 2020a).

There are, therefore, complex and as yet poorly understood couplings among biomass and litter cycles, nitrogen availability, sulfide inhibition of seed production, control of sulfide concentrations in sediments by iron and litter, and precipitation of iron sulfide on roots during seed production. Here, we investigate how litter-driven population oscillations interact with sulfate geochemistry in wild rice using controlled mesocosm experiments that allow us to scale rhizosphere geochemistry-plant physiology interactions from individual plants to an entire population for several generations. In our mesocosms, we elevated geochemical inputs of sulfate and iron and manipulated carbon through the presence or absence of litter. We investigated: (a) the patterns of biomass oscillations in high-sulfate and low-sulfate conditions, and (b) whether litter and iron enhance or alleviate sulfate's effects on biomass oscillations through the production and precipitation of sulfide.

2. Methods

2.1. Experimental Design

The interactions of sulfate, iron, and litter in wild rice sediment and their effect on wild rice population dynamics were studied using 40 mesocosms. Polyethylene stock tanks (High Country Plastics 400 L, 132 × 78 × 61 cm) were used to assemble the mesocosms (Figure 1). Sediment in the tanks was taken from Rice Portage Lake (MN Lake ID 09003700, 46.7038, -92.6829) on the Fond du Lac Band of Lake Superior Ojibway Reservation in Carlton County, Minnesota (Table 1). This lake is a productive wild rice lake with little surrounding development and its sediment has been used successfully to grow wild rice in previous experiments (Pastor et al., 2017). Sediment was homogenized before it was added to the tanks. Clean sand (10 cm) was added to the bottom of the

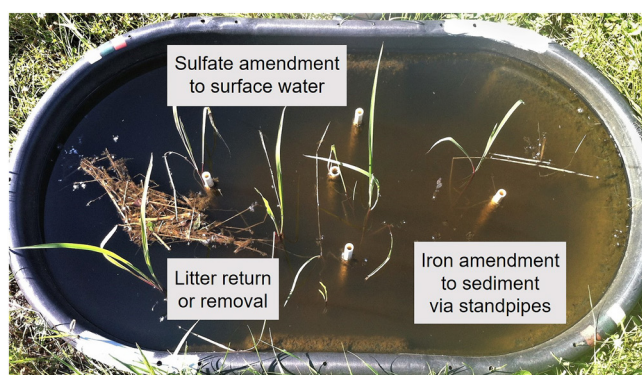


Figure 1. Picture of a mesocosm showing the application of sulfate, iron, and litter treatments. The center standpipe was used to moderate the water level after rain events. The other four standpipes connect to a ring of perforated PVC pipes in the sediment to allow release of iron(II) chloride. Sulfate was applied as sodium sulfate several times over each growing season to maintain surface water concentrations near 300 mg L^{-1} sulfate. Litter was weighed at the end of each growing season, and either returned to the mesocosms, or permanently removed.

tanks before 50 L of lake sediment were placed in each tank, resulting in a sediment depth of about 10 cm on top of 10 cm of sand. Water levels were maintained at 22 cm with a drain standpipe during precipitation and well water additions to account for evaporation. These depths of sand, sediment, and water represent typical wild rice rooting depth and water column heights in the field and have been used in several wild rice mesocosm experiments previously (Hildebrandt et al., 2012; Pastor et al., 2017; Walker et al., 2010).

To test the effects and interactions of sulfate, iron, and litter, we used a factorial design with five replicates for each of eight combinations of elevated or background sulfate, elevated or background iron, and the presence or absence of litter (Figure 1). These combinations were randomly assigned to the tanks in the first year of the experiment. For high-sulfate treatments, enough sodium sulfate was added to the surface water to bring the sulfate concentration to a target level of 300 mg L^{-1} . Surface water sulfate concentrations were tested weekly and sodium sulfate was added as required to maintain concentrations at the target level of 300 mg L^{-1} throughout the growing season for the duration of the experiment. The low-sulfate tanks were filled with water from an on-site well with concentrations around 10 mg L^{-1} and received no additional sulfate additions except for precipitation, which averaged $2.3 \pm 1.5 \text{ mg L}^{-1}$ sulfate. The sulfate concentrations in the low-sulfate tanks averaged around 7 mg L^{-1} over several years (Pastor et al., 2017). The

low-sulfate conditions for our experiment are still higher than the median sulfate concentration of Minnesota wild rice waters, 1.8 mg L^{-1} (Myrbo et al., 2017a), but is just below Minnesota's protective sulfate standard for wild rice waters. At 300 mg L^{-1} , our high-sulfate treatment is close to the EPA's secondary standard for sulfate in drinking water (250 mg L^{-1}) and represents surface water concentrations of a few lakes and rivers in Minnesota that contain wild rice (Myrbo et al., 2017a). Prior to both sulfate and iron amendment, sediment iron was extracted from homogenized sediment samples using 1 M HCl and quantified on a Varian fast sequential flame atomic absorption spectrometer with an acetylene torch (Federation & Association, 2005). The sediment initially contained $77 \mu\text{mol Fe g}^{-1}$ dry weight (Table 1), 85% of which was Fe(II) (Phenanthroline method, see Section 2.2). Each iron-amended tank received 96 g Fe^{2+} , bringing total iron concentrations up to approximately $196 \mu\text{mol Fe g}^{-1}$, or 10.9 mg g^{-1} dry weight. This amendment level aimed to noticeably increase iron concentrations without causing iron toxicity (Kinsman-Costello et al., 2015). Iron was applied gradually in four separate aliquots during the first growing season. For each addition, $75 \text{ g FeCl} \cdot 4\text{H}_2\text{O}$ was dissolved in 400 mL well water and added directly into the sediment through PVC standpipes connected to a buried perforated PVC ring. The

standpipes and ring were flushed with 100 mL of tank water immediately after the iron(II) chloride was injected. Samples of pore water iron were taken several times over the course of the first growing season in 10 points distributed across the tank to ensure that the iron loading was distributed evenly. Noniron tanks did not receive additional iron except for occasional well water that contained 0.17 mg L^{-1} Fe. Noniron tanks did not have a buried PVC ring, but all mesocosms had a center standpipe draining into an external bucket to restore the water level after rain events.

To test the effect of shoot litter cohorts on sulfide production, shoot litter produced by the wild rice population was retained in half the tanks and removed from the remaining tanks. Litter removal was chosen as the experimental treatment rather than litter addition because in typical freshwater, organic-rich wild rice habitats, sulfide production is generally limited by sulfate rather than organic carbon, so increasing litter may have had little effect. Only aboveground litter was removed for two reasons: (a) to minimize sediment disturbance, and (b) to focus on litter effects on sulfide production. Litter-driven biomass oscillations are driven primarily by recalcitrant root litter (Walker et al., 2010), so aboveground litter manipulation was not

Table 1

Initial Bulk Sediment Physical and Chemical Characteristics

Sediment property	Value
Porosity	0.87
Bulk density	0.29 g cm^{-3}
Percent solids	30
Solid-phase acid volatile sulfide (AVS)	$0.346 \pm 0.054 \mu\text{mol g}^{-1}$
Solid-phase extractable iron	$76.7 \pm 5.1 \mu\text{mol g}^{-1}$
Solid-phase ferrous iron	$65.2 \pm 4.8 \mu\text{mol g}^{-1}$
Porewater sulfide	$0.659 \pm 0.239 \mu\text{mol L}^{-1}$
Porewater ferrous iron	$435 \pm 200 \mu\text{mol L}^{-1}$
Initial solid phase S:Fe ratio	0.00450
Initial porewater $\Sigma\text{S}^{2-}:\text{Fe}^{2+}$ ratio	0.0015

Note. Sulfide was measured from initial sediment and porewater iron is an average from fall measurements in tanks unamended with iron.

expected to disrupt interannual cycles. Initial organic carbon content of the sediment was $14.8 \pm 1.7\%$ by dry weight (Pastor et al., 2017).

2.2. Geochemical Sampling and Analysis

In 2019 (year 5 of study), passive diffusion samplers (peepers) were installed in the tanks during vegetative growth (July) and seed production (September) to obtain porewater measurements from discrete depths in the top six cm of sediment. The peepers were placed in deionized water that was bubbled with nitrogen for 1 week prior to installation (Johnson et al., 2019). The peepers were transported to mesocosms in degassed water and installed in three tanks for each treatment. Each peeper contained four wells, the top of which was in the flocculant litter layer at the sediment surface and the bottom of which was approximately six cm below the sediment surface. Two weeks after peepers were installed, each peeper was removed and quickly placed in a large, resealable plastic bag purged with nitrogen gas to keep porewater anoxic during porewater extraction. Approximately 6 mL of porewater from each well was extracted with a syringe and allocated for immediate sulfide and iron measurements in vials preloaded with reagents. A separate aliquot was used to measure pH within 30 s.

Iron(II) and sulfide were quantified colorimetrically using the phenanthroline and methylene blue methods, respectively, on a HACH DR5000 UV-Vis spectrophotometer (Federation & Association, 2005). The pH of the pore water was measured by placing a calibrated ThermoScientific Orion pH electrode in porewater immediately after it was extracted from peepers.

2.3. Biological Sampling and Analysis

Seedlings usually began to germinate around mid-May. When seedlings grew to the water surface, populations in each mesocosm were thinned to approximately 30 plants per tank, which is the optimal density to limit competition and minimize overlapping rhizospheres (Lee, 2002). In August, as plants began to flower, six plants from each tank were randomly selected and tagged. Seed data were collected from these six plants and extrapolated to the total number of plants in the tank. Seeds from the remaining plants were left in the tank to reseed the sediment for the next year. Seeds were sorted as filled or unfilled by visual inspection, counted, and dried at 65°C and weighed to determine seed mass. After all plants had completely senesced in October, all aboveground biomass was removed and weighed with a small subsample dried at 65°C and weighed for moisture correction. The litter that was not dried was returned to the mesocosms assigned to retain litter within a few days.

2.4. Data Analysis

The data collected in this experiment are available at the Digital Repository for University of Minnesota (LaFond-Hudson et al., 2020b). Porewater data collected from peepers were examined to understand how porewater sulfide to iron ratios and saturation with respect to FeS were changed 5 years after geochemical manipulation. A three-way ANOVA was used to test the effect of each geochemical manipulation (sulfate, iron, litter). Repeated measures ANOVAs were used to determine the effect of time, sulfate addition, iron addition, and litter removal on biological traits and porewater measurements. Data were checked for normality and heteroscedasticity using R's standard diagnostic plots and for sphericity using the ez R package (Lawrence, 2016), which provides sphericity corrections in the case of violated assumptions. For porewater data, nondetects (12% of samples) and lost samples (11%) were removed. Zeros were not removed in biological data, as these were true absences of plant or seed tissue. Including zeros in biological data resulted in large variance under high-sulfate conditions, making it harder to detect differences between treatments using ANOVA tests. However, averages calculated including zeros (extinction of the population) represent the effects of high sulfate more completely than removing zeros.

To test the propensity of the population to oscillate in high-sulfate and low-sulfate conditions, we regressed the change in vegetative biomass ($B(t) - B(t - 1)$) against the vegetative biomass from the previous year ($B(t - 1)$). A negative slope of this regression indicates that high productivity 1 year leads to lower productivity the following year and, conversely, that years with low productivity are followed by years with higher productivity (Walker et al., 2010). The slope of this line is $\partial(\text{dB}/\text{dt})/\partial B$, which is effectively an eigenvalue of system. A critical value of -1 of this eigenvalue defines a Hopf bifurcation giving birth to stable limit cycles (Pastor & Walker, 2006; Strogatz, 1994; Walker et al., 2010). A slope of -1 or more negative indicates propensity for stable oscillations,

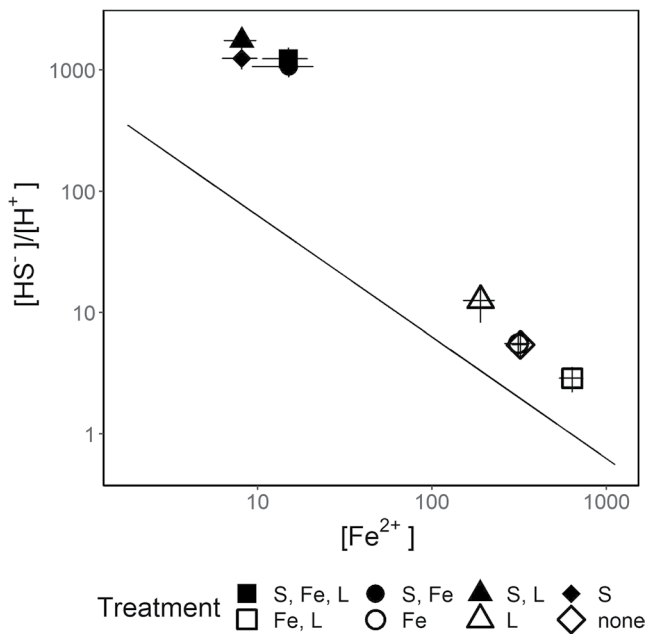


Figure 2. Comparison of ferrous iron ($\mu\text{mol L}^{-1}$) and sulfide concentrations (both sulfide and H^+ in mol L^{-1}) in sediment porewater after 5 years of sulfate amendment (S), iron amendment (Fe), and litter retention (L). Data points represent average porewater measurements for each treatment ($n = 12\text{--}30$; most $n = 24$). Error bars represent the standard error around the mean. Porewater was collected 2, 4, and 6 cm below the sediment surface using peepers. Open symbols are for mesocosms with background sulfate concentrations and closed symbols are for mesocosms with nominal sulfate concentrations of 300 mg L^{-1} in overlying water. The line depicts the ion activity product at saturation for iron and bisulfide at pH 7.0 ($\log K_{\text{sp}} = -3.2$). Porewater pH in elevated sulfate mesocosms ranged from 6.85 to 8.56 with a mean of 7.33 while porewater pH in low-sulfate mesocosms averaged ranged from 6.18 to 8.83 with a mean of 6.67.

while a slope between -1 and 0 indicates dampened oscillations (Walker et al., 2010). Dampened oscillations will eventually converge on a stable value of biomass, which may include zero (extinction). When no biomass is produced the following year, the population is at the boundary line $y = -x$ and the population is extinct. Vegetative biomass, rather than total biomass (vegetative + seeds), was used in this analysis because asynchrony in nitrogen mineralization and nitrogen uptake is expected to affect plants most during vegetative growth (Grava & Raisanen, 1978; Walker et al., 2010).

3. Results

3.1. Geochemical Context

Both high-sulfate and low-sulfate conditions contained sulfide and iron concentrations that favored precipitation of FeS (ion activity product $> K_{\text{sp}}$, Figure 2) in the fifth year of the study. Sulfate amendments to the surface water increased porewater sulfide and pH, and decreased porewater ferrous iron concentrations ($p < 0.001$ for all, Table 2). Sulfate addition raised porewater sulfide from an average of $4 \mu\text{mol L}^{-1}$ in unamended tanks to $110 \mu\text{mol L}^{-1}$ and lowered porewater ferrous iron concentrations by a similar order of magnitude, from 309 to $12 \mu\text{mol L}^{-1}$. The pH in high-sulfate mesocosms was 0.65 units higher than in low-sulfate conditions ($7.32, 6.67$). Iron amendment increased porewater ferrous iron concentrations by approximately $70\text{--}80\%$ ($p < 0.001$, Table 2) but did not notably change sulfide or pH. Litter removal did not significantly change sulfur or iron geochemistry.

3.2. Geochemical Effects on Biomass and Reproduction

All measured traits of wild rice growth and reproduction changed with time (Table 3). Sulfate addition strongly and consistently decreased all measured traits of wild rice growth (Table 3). The main effects of iron addition and litter removal were much weaker and inconsistent relative to sulfate addition, therefore we examined how iron and litter affected wild rice growth and reproduction using separate repeated measures ANOVAs for the high and low sulfur populations, respectively.

Total aboveground biomass density (hereafter referred to as biomass) was similar between high-sulfate and low-sulfate populations during the first year of the experiment (100 g m^{-2}), but biomass in high-sulfate populations declined to less than 5 g m^{-2} during the subsequent 5 years (Figure 3). In 2017, 4 years into the experiment, 8 out of the 20 populations receiving high sulfate loads produced no biomass regardless of iron addition and litter removal. Seven populations recovered partially in 2018, possibly from the germination of seeds from previous years buried in the sediment, but by 2019, 16 out of these 20 populations produced no biomass. In low-sulfate

Table 2

The Effect of Five Years of Sulfate, Iron, and Litter Additions on Porewater Concentrations of Sulfide and Iron, Porewater pH, and the Saturation Index With Respect to FeS Calculated From the Former Three Measurements in 2019 (Three-Way ANOVA)

Variable	Sulfate	Iron	Litter	Significant interactions
Porewater sulfide	$p < 0.001$	$p = 0.53$	$p = 0.64$	$S \times \text{Fe}$, $\text{Litter} \times \text{Fe}$, $S \times \text{Litter} \times \text{Fe}$
Porewater iron	$p < 0.001$	$p < 0.001$	$p = 0.16$	
Porewater pH	$p < 0.001$	$p = 0.64$	$p = 0.57$	$S \times \text{Litter} \times \text{Fe}$
Saturation index of FeS	$p < 0.001$	$p = 0.19$	$p = 0.96$	

Note. Significant interactions (in bold) refer to combinations of sulfate, iron, and/or litter that are significant at $p < 0.05$.

Table 3

Repeated Measures ANOVA Testing the Influence of Sulfate Amendment, Iron Amendment, and Litter Removal on Wild Rice Growth and Reproduction for 2014–2020

Trait	Year	Sulfate	Iron	Litter	Significant interactions
Vegetative biomass	$p < 0.001$	$p < 0.001$	$p = 0.81$	$p = 0.44$	S × L × Fe × Year, S × L × Year, S × Year, Fe × Year
Population seed mass	$p < 0.001$	$p < 0.001$	$p = 0.59$	$p = 0.09$	S × L × Year, S × Year
Filled seed ratio	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p = 0.25$	S × Year
Mass per filled seed	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.40$	S × Year, Fe × Year
Total biomass	$p < 0.001$	$p < 0.001$	$p = 0.71$	$p = 0.23$	S × Fe × L × Year, S × L × Year, S × Year
High-sulfate conditions					
Vegetative biomass	$p < 0.001$	–	$p = 0.82$	$p = 0.83$	L × Year, Fe × L × Year Fe × Year
Population seed mass	$p < 0.001$	–	$p = 0.28$	$p = 0.67$	
Filled seed ratio	$p < 0.001$	–	$p = 0.01$	$p = 0.42$	
Mass per filled seed	$p < 0.001$	–	$p = 0.04$	$p = 0.20$	
Total biomass	$p < 0.001$	–	$p = 0.74$	$p = 0.97$	L × Year, Fe × L × Year
Low-sulfate conditions					
Vegetative biomass	$p < 0.001$	–	$p = 0.68$	$p = 0.30$	Fe × Year
Population seed mass	$p < 0.001$	–	$p = 0.82$	$p = 0.08$	
Filled seed ratio	$p < 0.001$	–	$p = 0.01$	$p = 0.41$	Fe × Year
Mass per filled seed	$p < 0.001$	–	$p = 0.02$	$p = 0.86$	Fe × Year
Total biomass	$p < 0.001$	--	$p = 0.82$	$p = 0.18$	

Note. Bold values highlight p -values < 0.05 . Separate tests for high-sulfate and low-sulfate conditions are also included. Interactions are listed if significance is $p < 0.05$.

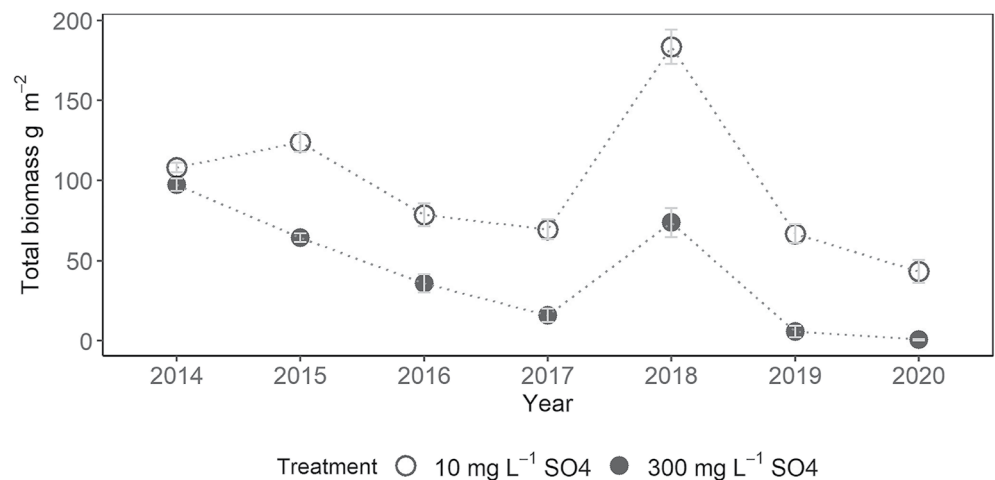


Figure 3. Annual average aboveground biomass (total density (g m^{-2})) in populations grown in high sulfate (300 mg L^{-1} , filled circles) and low sulfate (10 mg L^{-1} , empty circles) in the overlying water. Error bars depict the standard error around the mean ($n = 20$).

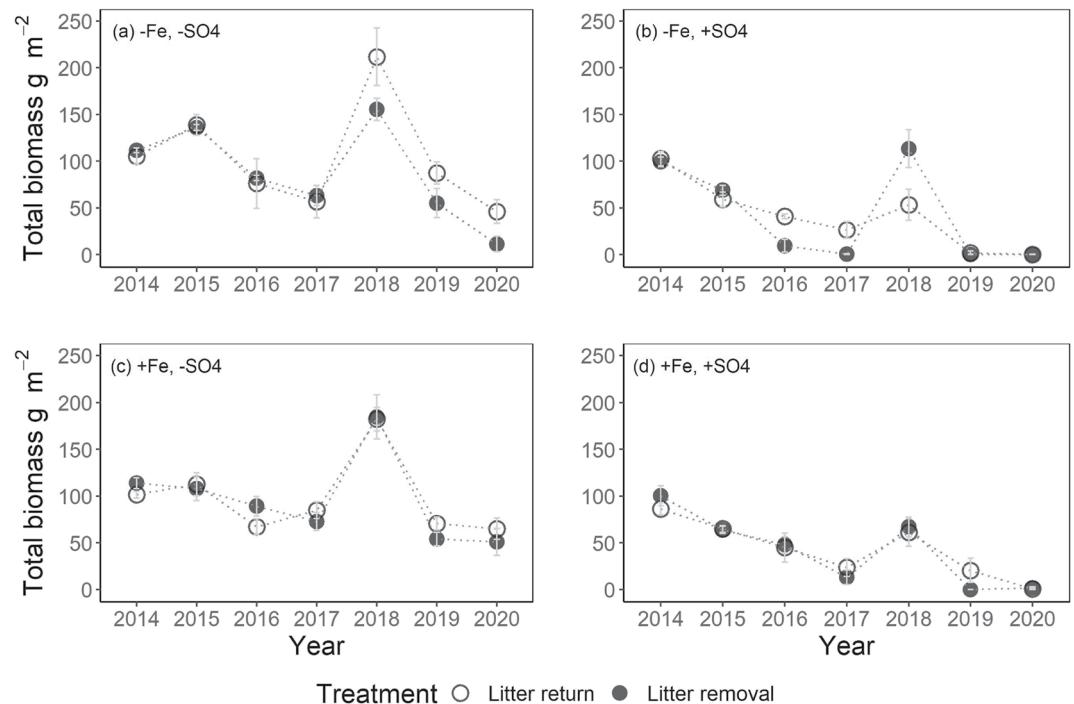


Figure 4. Annual average aboveground biomass (total) density (g m^{-2}) in populations grown with the previous year's aboveground litter returned (empty circles) or removed (filled circles). Populations were treated with combinations of low sulfate ($-\text{SO}_4$, 10 mg L^{-1} ; a, c) or high sulfate ($+\text{SO}_4$, 300 mg L^{-1} ; b, d) and low iron ($-\text{Fe}$, 4.3 mg g^{-1} ; a, b) or high iron ($+\text{Fe}$, 10.9 mg g^{-1} ; c, d). Error bars depict the standard error around the mean ($n = 5$).

conditions, populations showed a stable 3-year cycle of biomass (Figure 3) that oscillated between about 50 and 175 g m^{-2} on average, with peaks in the second and fifth growing season (2015 and 2018).

When biomass was examined separately in high-sulfate and low-sulfate treatments, populations grown in low sulfate were not affected by iron, litter, or their interaction (Figures 4a and 4c). Populations grown in high sulfate were not consistently influenced by iron and litter but were affected by interactions between iron, litter, and year (Table 3). In high-sulfate conditions, litter removal amplified oscillations in the treatments that did not receive iron addition, with lower biomass in years 3–4 of the experiment and higher biomass in year 5 compared to the populations with litter return (Figure 4b). In high-sulfate treatments that received additional iron, no differences in biomass were observed between litter removal and litter return (Figure 4d).

The proportion of filled seeds, individual seed mass, and population seed mass approached zero after six generations in high-sulfate conditions, while the same seed traits remained constant in low-sulfate conditions (Figure 5, seed mass not shown). Sulfate-amended populations that received iron amendment had a 20% higher filled seed ratio for 2015–2017 ($p = 0.01$, Table 3 and Figure 5b), but the effect of iron diminished in 2018 and 2019. In high-sulfate conditions, iron increased the average individual seed mass by approximately 40% in 2016 and by 20–30% in 2017–2018 ($p = 0.04$, Table 3 and Figure 5d). By 2020, only two sulfate-amended populations produced seeds; both also received iron amendment. In low-sulfate conditions, the proportion of filled seeds and average mass per filled seed were slightly increased by iron amendment (Figures 5a, 5c and Table 3). Litter removal did not affect any seed traits (Table 3).

3.3. Stability of Population Cycles

Changes in vegetative biomass from 1 year to the next were regressed against the biomass of the previous year to examine population oscillations for stability (Walker et al., 2010). All treatments with low sulfate were aggregated to compare to all treatments exposed to elevated sulfate since iron and litter manipulations had little effect in comparison to sulfate. In low-sulfate conditions, biomass oscillations are stable, as indicated by the slope of -1.3 for years 2015–2018 (Figure 6a) and a consistent annual biomass production between 50 and 125 g m^{-2} .

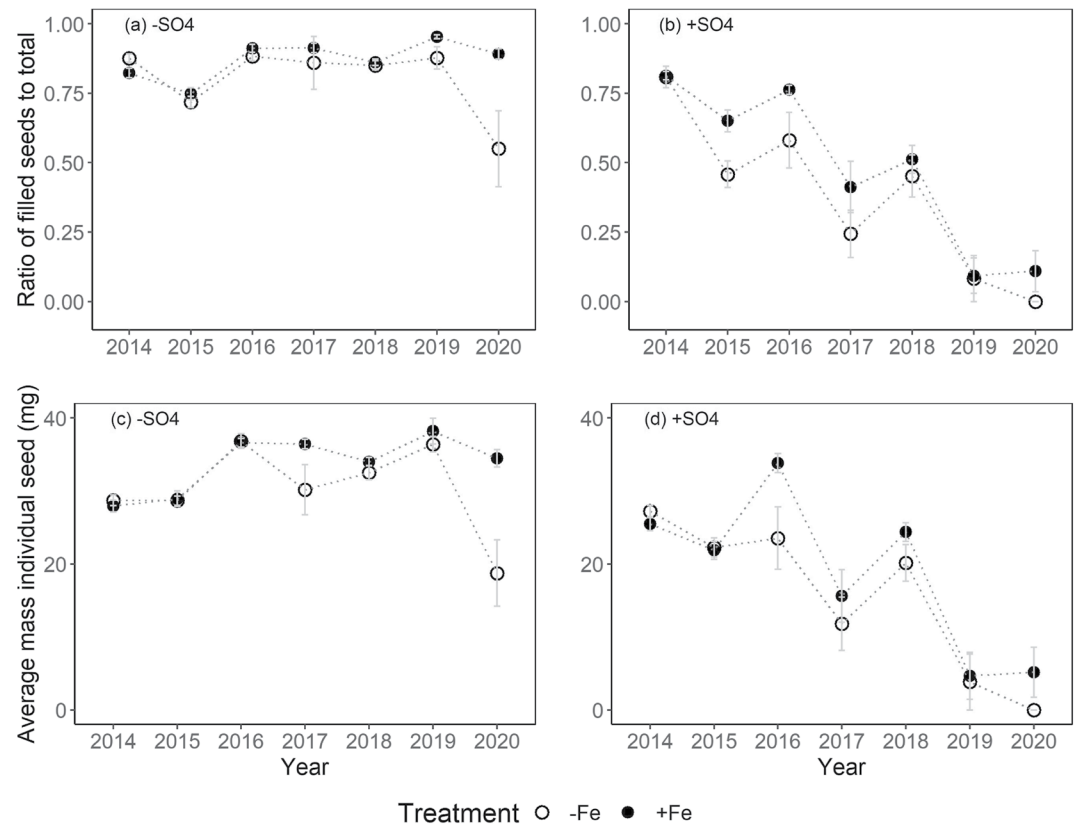


Figure 5. Effects of iron addition (+Fe, 10.9 mg g⁻¹; -Fe, 4.3 mg g⁻¹) on the ratio of filled seeds to total seeds (includes empty husks; a, b) and on average individual seed mass (c), (d) in 10 and 300 mg L⁻¹ sulfate. Error bars depict the standard error around the mean ($n = 10$).

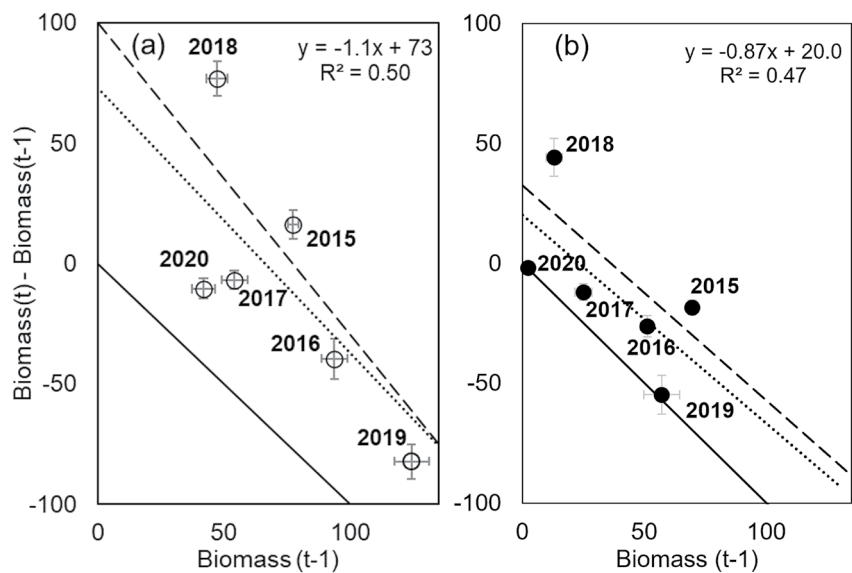


Figure 6. The relationship between average year-on-year change in vegetative biomass (g m⁻²) to the previous year's vegetative biomass in (a) 10 mg L⁻¹ sulfate and (b) 300 mg L⁻¹ sulfate. Error bars show the standard error. The slope of the dashed line represents $\partial(dB/dt)/\partial B$ and is calculated from populations in 2015–2018, representing approximately one population cycle. The dotted line also represents $\partial(dB/dt)/\partial B$ and is calculated from 2015 to 2020, to show the stability after 1.5 population cycles. The solid line represents extinction, when all biomass from the previous year is lost.

This slope is nearly identical to the slopes found a previous experiment (-1.04 to -1.20) that showed wild rice undergoes litter-driven productivity cycles (Walker et al., 2010). When data from 2019 to 2020 are included in this analysis, the slope is -1.1 , still representing stable oscillations. The present experiments and those of Walker et al. (2010) were done during different time periods and in different wild rice populations. Therefore, the two experiments provide independent corroboration of one another.

In high-sulfate conditions, a slope of -0.9 for year 2015–2018 indicates dampened oscillations (Figure 6b). Biomass decreased every year except for 2018, when the populations partially recovered. From 2018 to 2019, almost all points from sulfate-amended mesocosms fell on the boundary line $y = -x$, indicating that populations lost the entirety of the biomass produced during 2018 and produced no new biomass in 2019 (Figures 6b and 3). The data point for 2020 is near zero, indicating two consecutive years of extinct populations. The trajectory of mean biomass over time decreases in the presence of sulfate under our experimental conditions (Figure 3) and oscillations were dampened. In low-sulfate concentrations, biomass peaked in 2015 and 2018, demonstrating a 3-year cycle. Elevated sulfide produced in tanks with high-sulfate concentrations extinguished the population cycle which persisted in tanks with low-sulfate concentrations.

4. Discussion

Elevating surface water sulfate to 300 mg L^{-1} led mesocosm populations of wild rice to extinction in six growing seasons. This study adds to the growing body of literature describing the impacts of sulfate loading and subsequent sulfide exposure on wild rice, including lower rates of seedling survival, delayed phenology, impaired seed production, and declining biomass (Johnson et al., 2019; LaFond-Hudson et al., 2020a; Pastor et al., 2017). In conjunction, field observations both recently as well as decades ago show that wild rice presence becomes increasingly unlikely with elevated surface water sulfate and porewater sulfide (Moyle, 1945; Myrbo et al., 2017a). Statistical modeling of environmental parameters associated with wild rice presence has suggested that iron and organic carbon play the strongest role in controlling sulfide's effects on wild rice populations (Pollman et al., 2017), and similar conclusions have been reached about other sensitive aquatic plant species growing in freshwater wetlands with increasing sulfate loads (Lamers et al., 2002; Van der Welle et al., 2007). In our study, iron addition and organic carbon removal did not limit sulfide accumulation in sediment enough to stabilize wild rice populations.

In anoxic freshwater systems, sulfide production is generally limited by the supply of sulfate, whereas in marine systems or other systems with high sulfate, sulfide production is generally limited by the supply of organic matter (Ruiz-Halpern et al., 2008). Wild rice naturally grows in low-sulfate, organic-rich habitats (Myrbo et al., 2017a), meaning sulfate is the likely limiting factor for sulfide production. In mesocosms with elevated sulfate, litter removal alone did not appear to decrease sulfide concentrations and instead led to slightly faster population decline. It is possible that litter removal decreased the availability of macronutrients or micronutrients, such as nitrogen, potassium, or iron that might be replenished in natural ecosystems with more hydrologic connectivity. Notably, for populations with sulfate added and litter removed, population biomass declined faster in populations with ambient iron compared to populations receiving iron addition (Figures 4b and 4d). Another possibility is that in the litter return treatments, sulfate addition increased rates of litter decomposition and nutrient availability (Myrbo et al., 2017b). Under the conditions in this study and previous studies (Pastor et al., 2017), sulfide's inhibition of nitrogen uptake influenced population biomass more strongly than sulfate-enhanced nutrient mineralization, but the interactions of these two processes warrant further study.

In the presence of excess uncomplexed Fe(II), sulfide reacts quickly with iron, forming relatively stable iron sulfide solid phases. The ratio of sulfur to iron or similar metrics related to the degree of pyritization can be used to determine the capacity of sediment to precipitate iron sulfide and keep porewater sulfide concentrations low (Johnson et al., 2019; Julian et al., 2017). We added iron only during the first growing season of the experiment, making this treatment a pulse (one time) rather than a press (ongoing). In contrast, our sulfate and litter manipulations were maintained throughout the experiment. This decision was made to avoid Fe^{2+} toxicity to the plants (Kinsman-Costello et al., 2015; Sahrawat, 2005) and likely contributed to our findings of little alleviation of sulfide toxicity by iron compared to other studies with freshwater vegetation in environments with natural differences in groundwater upwelling of iron (Lamers et al., 2002) or seagrasses in experiments with monthly iron addition (Ruiz-Halpern et al., 2008). We observed some mitigation of sulfide's effects on seed production by

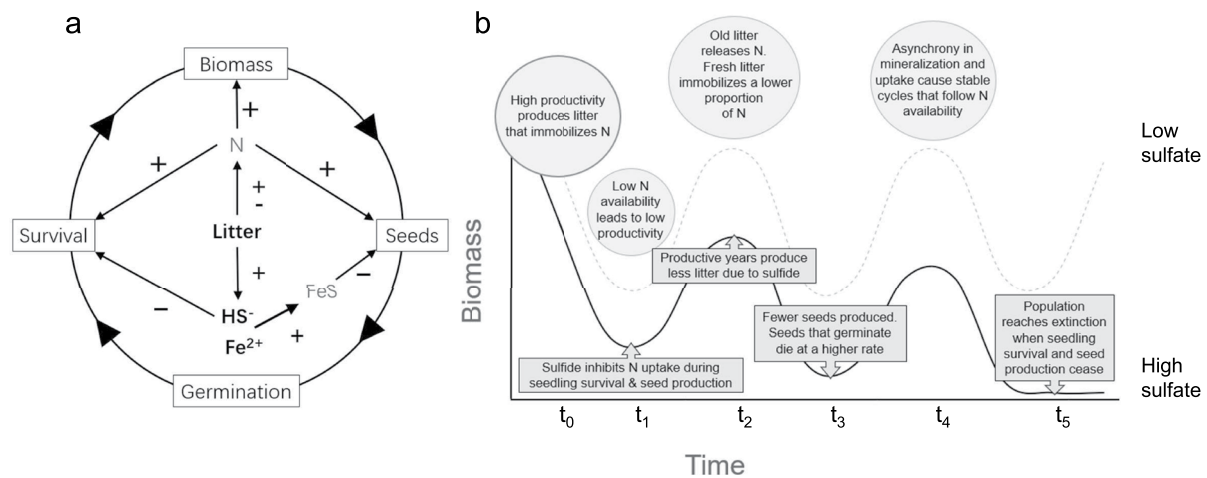


Figure 7. A conceptual model synthesizing the interactions of sulfur, iron, and litter during the life cycle of wild rice (a), and the effect of sulfur on interannual biomass cycles of wild rice (b) based on this study, Walker et al. (2010), and LaFond-Hudson et al. (2018). In (a), the (+) and (−) symbols in (a) represent positive or negative relationships between geochemical constituents and plant traits. In (b), the top, dashed line represents low-sulfate conditions, and the lower, solid line represents conditions with elevated sulfate loading. Time intervals ($t_0 - t_5$) do not represent consecutive years, because time between peaks is typically 3–5 years for wild rice.

iron in the first 4 years of the experiment, but iron's effect diminished by 2018 and 2019 (Figure 5) and porewater measurements in 2019 confirmed that iron concentrations were low in all elevated sulfate treatments, regardless of whether the mesocosms received additional iron (Figure 2).

The effect of sulfur on wild rice population dynamics is further complicated by oscillations due to a lag in nitrogen mineralization from litter relative to the timing of nitrogen uptake during the life cycle (Walker et al., 2010). We present a conceptual model built on the synthesis of previous work elucidating the connection between nitrogen and biomass cycles (Pastor & Walker, 2006; Walker et al., 2010); connections between sulfate, biomass, and seed production (Johnson et al., 2019; LaFond-Hudson et al., 2018; Pastor et al., 2017); and the present study which explores the role of sulfate and biomass cycles (Figure 7). Within each life cycle, nitrogen controls plant growth, and seed production (Grava & Raisanen, 1978). Litter immobilizes then slowly mineralizes nitrogen, leading to alternating negative and positive effects on nitrogen availability (Walker et al., 2010, Figure 7a). When sulfate is added to the system, production of sulfide directly decreases seedling survival and seed production (Figure 7a and Pastor et al., 2017). Iron precipitates sulfide into less-reactive iron sulfide, potentially alleviating some of the effects of sulfide on seedling survival and seed production, although iron sulfide also accumulates on root surfaces concomitant with decreased seed nitrogen (LaFond-Hudson et al., 2018). As the life cycle repeats each year (Figure 7a), these geochemistry-plant interactions lead to different interannual trajectories depending on the level of sulfate present in the system (Figure 7b). In low-sulfate conditions, high biomass production 1 year leads to an above average amount of litter that immobilizes nitrogen and decreases nitrogen availability in the following year(s) (Figure 7b, t_0, t_1). The lower nitrogen availability creates competition among seedlings for scarce nitrogen, leading to diminished biomass and seed production. As litter slowly decays, it releases nitrogen during subsequent years (Figure 7b t_2), and thereby increases seedling survival, biomass, and seed production. Alternating nitrogen availability leads to stable biomass cycles in low-sulfate environments (Figures 3 and 7b, t_4 etc.). At the levels of experimental sulfate addition we used, sulfur impacts overwhelmed the effects of litter-controlled N availability, causing the populations to quickly decrease (Figures 3 and 7b, t_3). When constant sulfate loading sustains declines in seedling survival and seed production, both germination and population biomass decline, eventually to extinction (Figure 7b, t_5).

Although we examined complicated interactions among sulfur, iron, organic carbon, nitrogen, and plant cycles, the mesocosms we used in this study are a relatively simple system containing only one species, homogenized sediment across mesocosms, and little hydrological mixing or external nutrient inputs. Projects that manage or restore plant populations in aquatic ecosystems occur in systems affected by interactions between geochemistry, surface and groundwater hydrology, and competing ecological communities. Many such projects monitor biomass annually, but traditional time series analyses require lengthy monitoring periods. When the monitoring period is

too short to detect population oscillations with a time series analysis, it may be insightful to use annual biomass data from one or two population cycles to calculate and analyze eigenvalues that describe the population's stability. In our experiment, we calculated and analyzed eigenvalues from less than two population cycles and our eigenvalue for populations in low-sulfate conditions corresponded closely to the results of Walker et al. (2010). Even though these were different experiments done during nonoverlapping time periods, the two experiments corroborate one another and strongly suggest that wild rice populations unimpeded by sulfate loadings oscillate stably with an approximate period of 4 years. Additionally, these findings are consistent with observations in some regional lakes and rivers containing wild rice stands (Vogt, 2021). This method is general enough to be applied analogously to data from other field studies and may be useful for identifying at-risk populations or determining whether management and restoration of an oscillating population is effective.

Only some aquatic plants experience population oscillations; however, many are limited by nitrogen and experience sustained or occasional exposure to sulfide, for example, the salt marsh species *Spartina alterniflora* (Mendelssohn & Morris, 2000). Estuarine wetlands receive sulfate from tidal inputs and interannual changes in precipitation can lead to wide variations in interannual nitrogen loading and sulfate intrusion (Sinha & Michalak, 2016). Understanding how plants respond to sulfate and sulfide under fluctuating nitrogen availability may be critical for understanding wetland vegetation dynamics. Perhaps species with different patterns of nitrogen uptake compared to wild rice can be more resilient to sulfide exposure, or plants with greater sulfide tolerance benefit more from increased sulfur-mediated nutrient availability.

5. Conclusion

This study demonstrates the importance of both litter-driven biomass oscillations and sulfate concentrations to population trajectories over several generations and corroborates studies that investigated these processes separately (Johnson et al., 2019; Pastor et al., 2017; Walker et al., 2010). Our observations show predictable and stable biomass oscillations in systems with less than 10 mg L⁻¹ sulfate in surface water and rapid population declines in systems with surface water sulfate elevated to 300 mg L⁻¹. Although population biomass did oscillate in elevated sulfate conditions, oscillations were unstable and less predictable than in low-sulfate conditions. This work aggregates well-understood rhizosphere and geochemical processes to interpret the effects realized at a population scale. We did not find consistent or sustained contributions of iron or litter to stability of wild rice populations. Instead, interactions between sulfate, litter, and iron in this study point to complex couplings among plant life cycles, nutrient availability, and iron and sulfur cycling that become manifest over several generations through at least one population cycle. We conclude that both geochemical context and plant life cycle patterns play a considerable role in determining the stability of oscillating plant populations.

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

Data are publicly available at the Digital Repository for University of Minnesota at <https://doi.org/10.13020/cq0g-r486> and are cited within the data analysis section of the methods.

References

- Azam, F., Lodhi, A., & Ashraf, M. (1991). Availability of soil and fertilizer nitrogen to wetland rice following wheat straw amendment. *Biology and Fertility of Soils*, 11, 97–100. <https://doi.org/10.1007/BF00336371>
- Bailey, L. T., Mitchell, C. P. J., Engstrom, D. R., Berndt, M. E., Coleman Wasik, J. K., & Johnson, N. W. (2017). Influence of porewater sulfide on methylmercury production and partitioning in sulfate-impacted lake sediments. *Science of The Total Environment*, 580, 1197–1204. <https://doi.org/10.1016/j.scitotenv.2016.12.078>
- Blossfeld, S., Gansert, D., Thiele, B., Kuhn, A. J., & Löscher, R. (2011). The dynamics of oxygen concentration, pH value, and organic acids in the rhizosphere of *Juncus* spp. *Soil Biology and Biochemistry*, 43, 1186–1197. <https://doi.org/10.1016/j.soilbio.2011.02.007>
- Federation, W. E., & Association, A. P. H. (2005). *Standard methods for the examination of water and wastewater*. American Public Health Association, American Water Works Association, Water Environment Federation.
- Fond du Lac Band of Lake Superior Chippewa. (2018). Expanding the narrative of tribal health: The effects of wild rice water quality rule changes on tribal health (Vol. 68). Health Impact Assessment.

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- Gao, S., Tanji, K., & Scardaci, S. (2003). Incorporating straw may induce sulfide toxicity in paddy rice. *California Agriculture*, 57, 55–59. <https://doi.org/10.3733/ca.v057n02p55>
- Gao, S., Tanji, K. K., & Scardaci, S. C. (2004). Impact of rice straw incorporation on soil redox status and sulfide toxicity. *Agronomy Journal*, 96, 70–76. <https://doi.org/10.2134/agronj2004.7000>
- Grava, J., & Raisanen, K. A. (1978). Growth and nutrient accumulation and distribution in wild rice 1. *Agronomy Journal*, 70, 1077–1081. <https://doi.org/10.2134/agronj1978.00021962007000060044x>
- Han, C., Ren, J., Wang, Z., Yang, S., Ke, F., Xu, D., & Xie, X. (2018). Characterization of phosphorus availability in response to radial oxygen losses in the rhizosphere of *Vallisneria spiralis*. *Chemosphere*, 208, 740–748. <https://doi.org/10.1016/j.chemosphere.2018.05.180>
- Hildebrandt, L. R., Pastor, J., & Dewey, B. (2012). Effects of external and internal nutrient supplies on decomposition of wild rice, *Zizania palustris*. *Aquatic Botany*, 97, 35–43. <https://doi.org/10.1016/j.aquabot.2011.11.002>
- Johnson, N. W., Pastor, J., & Swain, E. B. (2019). Cumulative sulfate loads shift porewater to sulfidic conditions in freshwater wetland sediment. *Environmental Toxicology and Chemistry*, 38, 1231–1244. <https://doi.org/10.1002/etc.4410>
- Jorgenson, K. D., Lee, P. F., & Kanavillil, N. (2012). Ecological relationships of wild rice, *Zizania* spp. 11. Electron microscopy study of iron plaques on the roots of northern wild rice (*Zizania palustris*). *Botany*, 91, 189–201. <https://doi.org/10.1139/cjb-2012-0198>
- Julian, P., Chambers, R., & Russell, T. (2017). Iron and pyritization in wetland soils of the Florida Coastal Everglades. *Estuaries and Coasts*, 40, 822–831. <https://doi.org/10.1007/s12237-016-0180-3>
- Kinsman-Costello, L. E., O'Brien, J. M., & Hamilton, S. K. (2015). Natural stressors in uncontaminated sediments of shallow freshwaters: The prevalence of sulfide, ammonia, and reduced iron. *Environmental Toxicology and Chemistry*, 34, 467–479. <https://doi.org/10.1002/etc.2801>
- LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2018). Iron sulfide formation on root surfaces controlled by the life cycle of wild rice (*Zizania palustris*). *Biogeochemistry*, 141, 95–106. <https://doi.org/10.1007/s10533-018-0491-5>
- LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2020a). Interactions between sulfide and reproductive phenology of an annual aquatic plant, wild rice (*Zizania palustris*). *Aquatic Botany*, 164, 103230. <https://doi.org/10.1016/j.aquabot.2020.103230>
- LaFond-Hudson, S., Johnson, N. W., Pastor, J., & Dewey, B. (2020b). Supporting data for “Sulfur geochemistry impacts population oscillations of wild rice (*Zizania palustris*)” (Data Repository). University of Minnesota. <https://doi.org/10.13020/cq0g-r486>
- Lamers, L. P. M., Falla, S.-J., Samborska, E. M., van Dulken, I. A. R., van Hengstum, G., & Roelofs, J. G. M. (2002). Factors controlling the extent of eutrophication and toxicity in sulfate-polluted freshwater wetlands. *Limnology & Oceanography*, 47, 585–593. <https://doi.org/10.4319/lo.2002.47.2.0585>
- Lamers, L. P. M., Govers, L. L., Janssen, I. C., Geurts, J. J., Van der Welle, M. E., Van Katwijk, M. M., et al. (2013). Sulfide as a soil phytotoxin—A review. *Frontiers of Plant Science*, 4, 268. <https://doi.org/10.3389/fpls.2013.00268>
- Lawrence, M. (2016). ez [WWW Document]. Retrieved from <https://cran.r-project.org/web/packages/ez/ez.pdf>
- Lee, P. F. (2002). Ecological relationships of wild rice, *Zizania* spp. 10. Effects of sediment and among-population variations on plant density in *Zizania palustris*. *Canadian Journal of Botany*, 80, 1283–1294. <https://doi.org/10.1139/b02-118>
- Marzocchi, U., Benelli, S., Larsen, M., Bartoli, M., & Glud, R. N. (2019). Spatial heterogeneity and short-term oxygen dynamics in the rhizosphere of *Vallisneria spiralis*: Implications for nutrient cycling. *Freshwater Biology*, 64, 532–543. <https://doi.org/10.1111/fwb.13240>
- Mendelsohn, I. A., Kleiss, B. A., & Wakeley, J. S. (1995). Factors controlling the formation of oxidized root channels: A review. *Wetlands*, 15, 37–46. <https://doi.org/10.1007/BF03160678>
- Mendelsohn, I. A., & Morris, J. T. (2000). Eco-physiological controls on the productivity of *Spartina alterniflora* Loisel. In M. P. Weinstein, & D. A. Kreeger (Eds.), *Concepts and controversies in tidal marsh ecology* (pp. 59–80). Springer. https://doi.org/10.1007/0-306-47534-0_5
- Morse, J. W., Millero, F. J., Cornwell, J. C., & Rickard, D. (1987). The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Science Reviews*, 24, 1–42. [https://doi.org/10.1016/0012-8252\(87\)90046-8](https://doi.org/10.1016/0012-8252(87)90046-8)
- Moyle, J. B. (1945). Some chemical factors influencing the distribution of aquatic plants in Minnesota. *The American Midland Naturalist*, 34, 402–420. <https://doi.org/10.2307/2421128>
- Myrbo, A., Swain, E. B., Engstrom, D. R., Wasik, J. C., Brenner, J., Shore, M. D., et al. (2017a). Sulfide generated by sulfate reduction is a primary controller of the occurrence of wild rice (*Zizania palustris*) in shallow aquatic ecosystems. *Journal of Geophysical Research: Biogeosciences*, 122, 2736–2753. <https://doi.org/10.1002/2017JG003787>
- Myrbo, A., Swain, E. B., Johnson, N. W., Engstrom, D. R., Pastor, J., Dewey, B., et al. (2017b). Increase in nutrients, mercury, and methylmercury as a consequence of elevated sulfate reduction to sulfide in experimental wetland mesocosms: SO₄ reduction mobilizes N, P, C, and mercury. *Journal of Geophysical Research: Biogeosciences*, 122, 2769–2785. <https://doi.org/10.1002/2017JG003788>
- Pastor, J., Dewey, B., Johnson, N. W., Swain, E. B., Monson, P., Peters, E. B., & Myrbo, A. (2017). Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments. *Ecological Applications*, 27, 321–336. <https://doi.org/10.1002/eap.1452>
- Pastor, J., & Walker, R. D. (2006). Delays in nutrient cycling and plant population oscillations. *Oikos*, 112, 698–705. <https://doi.org/10.1111/j.0030-1299.2006.14478.x>
- Pollman, C. D., Swain, E. B., Bael, D., Myrbo, A., Monson, P., & Shore, M. D. (2017). The evolution of sulfide in shallow aquatic ecosystem sediments: An analysis of the roles of sulfate, organic carbon, and iron and feedback constraints using structural equation modeling. *Journal of Geophysical Research: Biogeosciences*, 122, 2719–2735. <https://doi.org/10.1002/2017JG003785>
- Ruiz-Halpern, S., Macko, S. A., & Fourqurean, J. W. (2008). The effects of manipulation of sedimentary iron and organic matter on sediment biogeochemistry and seagrasses in a subtropical carbonate environment. *Biogeochemistry*, 87, 113–126. <https://doi.org/10.1007/s10533-007-9162-7>
- Sahrawat, K. L. (2005). Iron toxicity in wetland rice and the role of other nutrients. *Journal of Plant Nutrition*, 27, 1471–1504. <https://doi.org/10.1081/PLN-200025869>
- Sain, P. (1984). Decomposition of wild rice (*Zizania aquatica*) straw in two natural lakes of northwestern Ontario. *Canadian Journal of Botany*, 62, 1352–1356. <https://doi.org/10.1139/b84-183>
- Sims, L., Pastor, J., Lee, T., & Dewey, B. (2012). Nitrogen, phosphorus and light effects on growth and allocation of biomass and nutrients in wild rice. *Oecologia*, 170, 65–76. <https://doi.org/10.1007/s00442-012-2296-x>
- Sinha, E., & Michalak, A. M. (2016). Precipitation dominates interannual variability of riverine nitrogen loading across the continental United States. *Environmental Science & Technology*, 50, 12874–12884. <https://doi.org/10.1021/acs.est.6b04455>
- Strogatz, S. H. (1994). *Nonlinear dynamics and chaos: With applications to physics, biology, chemistry, and engineering*. Westview Press.
- Sundby, B., Vale, C., Caçador, Z., Catarino, F., Madureira, M.-J., & Caetano, M. (1998). Metal-rich concretions on the roots of salt marsh plants: Mechanism and rate of formation. *Limnology & Oceanography*, 43, 245–252. <https://doi.org/10.4319/lo.1998.43.2.0245>
- Van der Welle, M. E. W., Niggebrugge, K., Lamers, L. P. M., & Roelofs, J. G. M. (2007). Differential responses of the freshwater wetland species *Juncus effusus* L. and *Caltha palustris* L. to iron supply in sulfidic environments. *Environmental Pollution*, 147, 222–230. <https://doi.org/10.1016/j.envpol.2006.08.024>

- Vogt, D. (2021). *Wild rice monitoring and abundance in the 1854 Ceded Territory (199–2020)* (Tech. Rep. 1854). Treaty Authority.
- Walker, R., Pastor, J., & Dewey, B. W. (2006). Effects of wild rice (*Zizania palustris*) straw on biomass and seed production in northern Minnesota. *Canadian Journal of Botany*, *84*, 1019–1024. <https://doi.org/10.1139/b06-058>
- Walker, R. E. D., Pastor, J., & Dewey, B. W. (2010). Litter quantity and nitrogen immobilization cause oscillations in productivity of wild rice (*Zizania palustris* L.) in northern Minnesota. *Ecosystems*, *13*, 485–498. <https://doi.org/10.1007/s10021-010-9333-6>