

A. The biogeochemical habitat of wild rice

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C. Key words:

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D. Background

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 but 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 in the lakes and rivers where they are found. 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, the native Ojibway people of the watersheds of Lakes Superior and Michigan teach that they were led to this region to find “the food that grows upon the water”, which is wild rice. The Ojibway identify their origins with wild rice and consider themselves “people of the rice” (Vennum 1988). Therefore, the productivity, perpetuation, and restoration of wild rice are of great ecological and cultural significance.

Native stands of wild rice grow in waters that are circum-neutral pH, of low conductivity and hardness, and generally low in nutrient concentrations. Wild rice is most abundant in waters with sulfate concentrations of less than $10 \text{ mg} \cdot \text{L}^{-1}$ and its abundance falls off rapidly at higher concentrations (Moyle 1944, 1945). Based on Moyle’s observational studies, the Minnesota Pollution Control Agency (MPCA) regulates allowable sulfate concentrations in wild rice waters to $< 10 \text{ mg} \cdot \text{L}^{-1}$.

However, recent proposals to open copper-nickel mines in the Lake Superior watershed of northern Minnesota have given rise to concerns that sulfate discharge from both existing iron mines and proposed copper mines may greatly exceed this sulfate standard. There are discussions proceeding amongst policy makers, the public, and industry representatives about whether this

standard should be relaxed. These discussions and policy decisions are impeded by a paucity of knowledge regarding the fate of sulfate once it enters wild rice waters, its potential toxicity, or that of sulfides derived from it, to wild rice. Our group has begun researching this problem with funds from the MPCA and NSF and our results form the basis of this proposal. **Our objectives here are to determine (1) the fates of sulfate and sulfide in wild rice beds in relation to litter accumulation and availabilities of nitrogen and other nutrients, and (2) the effects of sulfate and sulfide on wild rice production.** Results of this research will be made available to the public and policy makers through our outreach program.

Sediments of wild rice lakes are almost always anoxic and consequently have a high potential for reducing sulfate to sulfide. Because sulfate reduction is more thermodynamically favorable than methanogenesis (Capone and Kiene 1988), increasing concentrations of sulfate are likely to increase the rate of sulfide accumulation in sediment. High sulfide levels can inhibit root growth of wetland plants (Koch and Mendelsohn 1989, Koch et al. 1990, Lamers et al. 2002, van der Welle et al. 2007, Guerts et al. 2009), including white rice (*Oryza sativa*; Gao et al. 2003; Armstrong and Armstrong 2005). If root biomass is reduced by sulfide, then the plant's ability to take up limiting nutrients, especially nitrogen, will be impaired (Gao et al. 2003, Armstrong and Armstrong 2005). Sulfide can also directly inhibit the ability of roots to take up nitrogen (DeLaune et al. 1983).

Production of wild rice biomass is limited by the supply of nitrogen from decomposing plant litter, sediment organic matter, and hydrologic inputs (Pastor and Walker 2006, Walker et al. 2006, 2010, Sims et al. 2012a). Because it is an annual plant, wild rice's nitrogen needs must be supported by uptake during the current year. Over 60% of nitrogen uptake happens during a two-week window in early summer (Grava and Raisinen 1978, Sims et al. 2012a). Nitrogen, however, is not released from the previous year's litter until later in summer or even the following year (Sain 1984, Walker et al. 2010, Hildebrandt et al. 2012); 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 (Walker et al. 2010, Hildebrandt et al. 2012). This asynchrony among the timing of nitrogen uptake and release and the coincidence of microbial immobilization with the period of rapid uptake causes wild rice biomass and litter production to cycle with a period of approximately 4 years (Pastor and Walker 2006, Walker et al. 2010). The increased litter deposited on the sediment from a productive year could reduce sediment redox potential (Eh) by providing additional labile carbon to support additional bacterial growth and oxygen demand the following year, thereby enhancing the potential for reduction of sulfate to sulfide (Azam et al. 1991, Verma et al. 1992, Adachi et al. 1997, Gao et al. 2002, 2003). Nitrogen loading is increasing both regionally and globally and shows no signs of abating (Vitousek et al. 1997, USEPA 2000). Nitrogen loading from atmospheric deposition and runoff could greatly alter productivity and nitrogen cycling in wild rice ecosystems as in other aquatic ecosystems (Valiela et al. 1997).

There are, however, a number of other biogeochemical reactions in the sediments which 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). Precipitation of sulfide with reduced iron liberates phosphate (Caraco et al. 1989, Lamers et al. 2002, Suplee and Cotner 2002), which may be secondarily limiting to wild rice growth (Walker et al. 2010, but see Sims et al. 2012a).

There are, therefore, complex interactions between sulfate inputs to wild rice lakes and its reduction to sulfide, the availability of iron to precipitate the sulfide, plant litter accumulation, and the availability of limiting nitrogen to the plants. Increased inputs of nitrogen to wild rice waters could increase production by satisfying microbial demand during litter decomposition. But increased litter production could in turn increase the rate of sulfate conversion to sulfides via

sulfate reduction. The potential for sulfide toxicity to wild rice, therefore, may vary interannually and be driven by the four-year cycle of productivity. Hence, the effects of sulfate and sulfide on wild rice productivity are likely to be intimately tied to the feedbacks between the asynchrony in the release of nitrogen from litter produced the previous year and the limiting effect of nitrogen on current year's production. An integrated systems view of the biogeochemical habitat of wild rice is needed to provide an adequate understanding of the impacts of sulfur and nitrogen loading on wild rice ecosystems.

E. Hypotheses

We hypothesize that increased nitrogen loading in northern Minnesota watersheds has increased the sediment oxygen demand in wild rice beds, leading to increased sulfide concentrations which inhibit plant growth. As an essential nutrient, increased nitrogen loading should increase rice production and organic matter accumulation. But with greater organic matter accumulation and increased sediment oxygen demand there could be increased denitrification rates if nitrate is present. Consequently, wild rice stands in northern Minnesota face a three part sequence of biogeochemical events that could decrease productivity (Fig. 1). First, although increased nitrogen loading at first increases overall biomass productivity, as organic matter accumulates it increases sediment oxygen demand (SOD) and therefore decreases redox potentials. Second, the decreased redox potential combined with higher sulfate loadings increase sulfide concentrations and fluxes into the sediments. Third, higher concentrations of sulfide in the sediment will decrease wild rice growth. The interactions of all these processes, some inhibiting wild rice growth and some enhancing it, could result in complex dynamics of wild rice populations and production that cycle over many years.

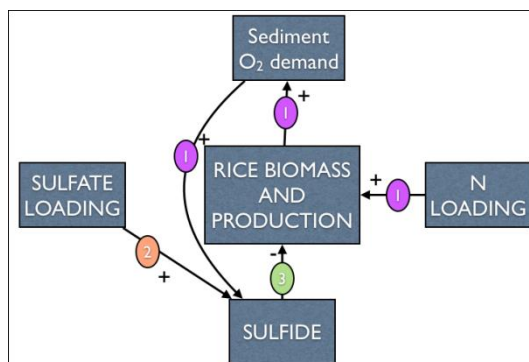


Figure 1. Hypothesized interactions among wild rice production, sediment oxygen demand, and the cycling of nitrogen and sulfur species. Numbers next to arrows correspond to the three part sequence of biogeochemical interactions in wild rice ecosystems.

F. Methods

General approach

Our approach will be to examine redox, S, N and P dynamics, productivity, and litter decay in wild rice populations in experimental mesocosms and populations in natural lakes and rivers. This approach will connect experimental tests of our above hypotheses in long-term experiments in mesocosms with observational studies of wild rice beds in a pristine lake (Lake Itasca) and a major river of the Lake Superior watershed which contains sulfate in excess of other local watersheds (Berndt and Bavin 2012).

With previous funding from NSF and the Fond du Lac and Grand Portage Reservations Pastor has cultivated wild rice in experimental mesocosms for the last 5-8 years under different loadings of nitrogen, phosphorus, and sulfate and with litter removed or left intact. These experiments have already demonstrated short-term effects of sulfate loadings on wild rice growth, but also four-five year cycles of wild rice productivity driven by bacterial nitrogen immobilization in fresh litter (results below). Each of these experiments provides direct tests of our hypothesized chain of

biogeochemical events of the interactions of nitrogen and sulfate loading and litter production on sulfide production and wild rice growth (Fig. 1).

These experiments will provide the mechanistic data to interpret observational data to be collected from three field plots of wild rice on Lake Itasca and three plots containing wild rice in the St. Louis River for two growing seasons. Although the mesocosms are important for understanding the mechanistic interactions that we have hypothesized, the physical environment of wild rice is impossible to simulate perfectly. Therefore, we propose to include several field plots from two ecosystems that support significant wild rice. Field plots chosen for the study will have significant background information on aquatic plant population health. Plots in the St. Louis watershed have been established by Johnson in conjunction with ongoing MPCA efforts. Lake Itasca is adjacent to the University of Minnesota Itasca Biological Station and Laboratories and has been studied by Cotner at various times in the past 5 years.

Direct measurements of sulfur in overlying water, pore waters and solid-phase sediments in these lakes, rivers, and mesocosms will provide data on sulfur exposure in natural wild rice populations and also in populations experimentally subjected to sulfate, nitrogen, and phosphorus additions and litter removal over the course of the growing season. In addition, we will use a flow-through core approach to examine rates of nitrification, denitrification and sulfate reduction in sediments (Gardner et al. 2001). We will define our monitoring based on four ecologically relevant periods: germination or ice-out (April-May), submerged and floating leaf stage (June), emergent and seed deposition stage (July-September), and senescence or ice-over (October-March). In so doing, we will be able to determine whether there are periods when the plant is more sensitive to environmental and physical conditions.

Mesocosm experiments

Proposed mesocosm treatments: We propose to apply the following treatments to the mesocosms: 1. Litter removal/no removal fully crossed with thinning/unthinned treatments. This is a continuation of an eight-year experiment on nitrogen availability and wild rice productivity in relation to litter production and decomposition (Walker et al. 2010). This experiment will allow us to directly test the effect of litter production on redox potential and background sulfide production in wild rice sediments. Our hypothesis predicts greater sulfide production in unthinned populations and in mesocosms with intact litter. 2. Loading wild rice populations with $15 \text{ g} \cdot \text{m}^{-2}$ per year of nitrogen or with $5.5 \text{ g} \cdot \text{m}^{-2}$ per year of phosphorus or maintained as unfertilized controls. This experiment, already in place for five years, will allow us to directly test the effects of nitrogen as well as phosphorus loading on litter production as well as background sulfide production. We expect enhanced sulfide production in mesocosms with higher nitrogen loadings. Phosphorus should enhance wild rice litter decay (Hildebrandt et al. 2012) and sulfide production may also increase in phosphorus amended mesocosms. 3. Loading wild rice populations with water column sulfate concentrations of 0, 50, 100, 150, and $300 \text{ mg SO}_4^{-2} \cdot \text{L}^{-1}$. This experiment has been in operation for the past three year and will allow us to test the effects of sulfate loadings on sulfide production in relation to the amount of litter produced during the previous year. Collectively, the continuation of these experiments through the 2014 and 2015 growing seasons will allow us to test several hypothesized mechanisms of how nitrogen and sulfate loadings and litter accumulation may or may not affect wild rice growth. More importantly, it will allow us to test whether sulfide production oscillates with litter production in the previous year as nitrogen availability does (Pastor and Walker 2006, Walker et al. 2010) as well as examine long-term cumulative effects of sulfate loading on sulfide accumulation in sediment.

Significant findings from these experiments pertinent to this proposal include:

1. In the first year of the sulfate loading experiment when litter was not present, increased sulfate concentrations did not decrease biomass production and mean seed mass of wild rice (Fig. 2).

However, in the second year when litter was present, sulfate loading significantly decreased total stem biomass and mean seed weight. In addition, we found that roots of plants grown with greater than 50 mg L⁻¹ sulfate concentrations in overlying waters were black, which is characteristic of sulfide toxicity of white rice (*Oryza sativa*) roots (Gao et al. 2003). The impaired root function may have resulted in nitrogen stress to the plants; external signs of nitrogen stress in these plants include chlorotic leaves, stunted growth, and fewer and smaller seeds (Pastor and Dewey, personal observation).

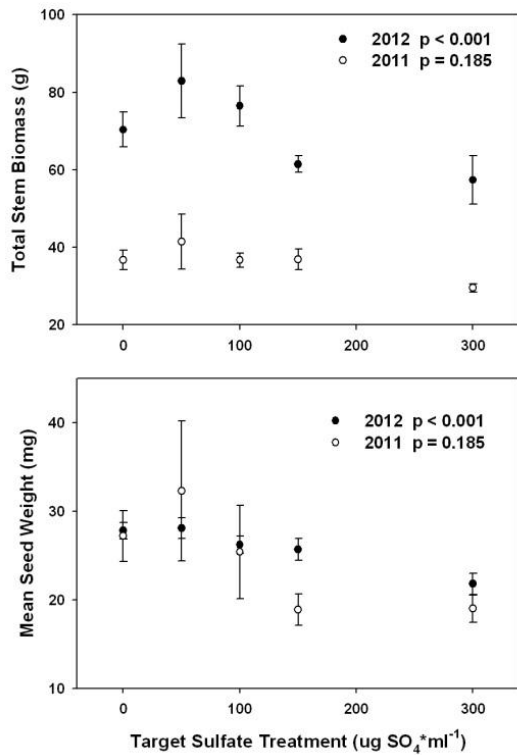


Figure 3. Sulfate loading reduces stem biomass and mean seed weight in year 2 after litter accumulation but not in year 1 when plant populations were initially established in sediment without litter.

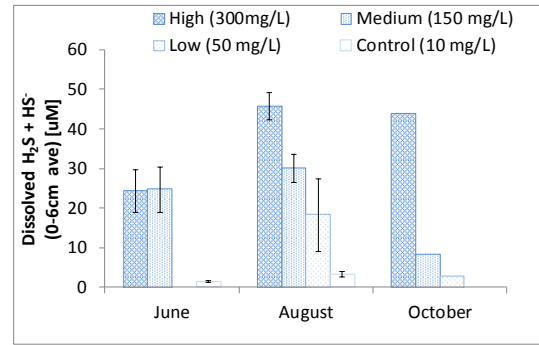


Figure 2. Dissolved reduced sulfide (H₂S + HS⁻) in surficial sediment pore waters of wild rice mesocosms with overlying water sulfate amendments.

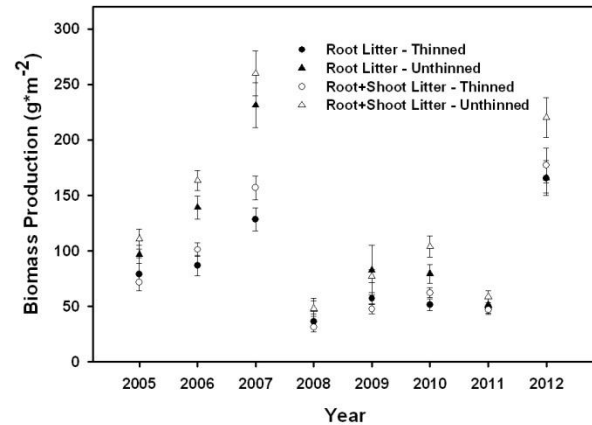


Figure 4. Four-five year concurrent cycles of biomass production and nitrogen availability (data from Walker et al. 2010).

2. In the second year of the sulfate loading experiment, after additions of litter from the first year's growth, total dissolved sulfide in the sediment pore waters increased with increased sulfate loading (Fig. 3). However, during the few measurements made during the first year, when litter was not present, sulfide levels in 300 mg/L amended tanks were below 10 μM (Johnson 2012).
3. A four to five year cycle of biomass/litter production (Fig. 4) which coincides with a four-five year cycle of nitrogen availability (Walker et al. 2010). The amplitude of this cycle is attenuated both by removing shoot litter (leaving only root litter) and by thinning (Fig. 4).

The work proposed herein will allow us to follow the production and decay of rice biomass, litter, and seed production in these experimental populations for several years concurrently with measurements of sediment oxygen demand and sulfur species in sediments, thereby allowing us to determine whether litter production exacerbates sulfide production and toxicity to wild rice.

Mesocosm methods: The mesocosms at the University of Minnesota Duluth Research and Field Studies Station in Duluth, MN are polyethylene stock tanks (Rubbermaid #4242, 378 L capacity, 132 cm long x 79 cm wide x 63 cm deep). There are 54 mesocosms devoted to the productivity oscillation experiments, 18 devoted to the N and P addition experiments, and 30 devoted to the sulfate addition experiments.

The mesocosms are buried to ground level and connected by a drain pipe to 20 L polyethylene overflow buckets (Fig. 5). Water tables are set by the drain pipe at 23 cm above the sediment surface. The tanks were partly filled with 10 cm of clean washed sand covered with 10 cm of surface sediment collected from natural wild rice beds from a lake on the Fond du Lac Band of Lake Superior Ojibway Reservation in Carlton County, Minnesota. Ten to twenty cm of sediment over sand approximates the rooting depths we have observed in natural wild rice lakes. During the growing season, water levels are maintained by weekly additions of water to drain pipe heights or by allowing water to drain through the drain pipe into the overflow buckets. Water used to fill and supplement tank levels is obtained from a nearby well. Rainfall N concentrations as $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ ranged from $0.2 - 1.99 \mu\text{g} \cdot \text{mL}^{-1}$ while the $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in the well water are always $< 0.2 \mu\text{g} \cdot \text{mL}^{-1}$ (Walker et al. 2010). The sulfate concentrations in the well water are between 8 and $10 \mu\text{g} \cdot \text{mL}^{-1}$. The sediments comprise an inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter remain submerged year round with water levels set at approximately 20 cm in late fall.



Figure 5. Mesocosms with wild rice populations subject to different sulfate concentrations. A peeper used to sample sulfate, sulfide, and iron concentrations in the sediment profile is in the middle of the mesocosm.

End-of-season plant density in Minnesota wild rice lakes averages 30-40 plants per square meter (Vogt 2010). Plant density is thinned to this level in spring for the sulfate addition experiments half the mesocosms in the nitrogen and phosphorus addition experiments, and half the mesocosms in the litter removal/thinning experiments. We also count the number of seedlings we remove to obtain an estimate of germination success.

Sulfate is added to the appropriate mesocosms as solutions of sodium sulfate in the appropriate concentrations as reported above (Fisher Chemical S421). Nitrogen and phosphorus are added as NH_4Cl and K_2PO_4 to the sediments in the appropriate mesocosms through perforated PVC pipes inserted into the sediment and in aliquots designed to mimic the seasonal pattern of nitrogen and phosphorus uptake (Sims et al. 2012a).

During the growing season we will sample the water from each tank every week and the overflow buckets and rain gauge as needed and determine ammonium, nitrate, phosphate, and sulfate concentrations with a Lachat Autoanalyzer. Sediment samples from each tank will be collected and analyzed for total carbon, nitrogen, and sulfur on a Thermo Electron Flash EA1112 elemental analyzer at the beginning and end of each growing season. Sediment samples will be collected at the beginning and end of the growing season and analyzed for solid phase sulfide concentrations. In addition tank water pH and temperature will be measured in situ every 2 weeks using a portable meter. Ammonium, nitrate, and phosphate fluxes in the sediment will be analyzed by means of 3 mixed-bed cation-anion resin bags buried into the sediment per mesocosm (Walker et al. 2010). Five plants in each tank will be randomly chosen in early summer for detailed measurements throughout the growing season. In late August to September, ripe seeds will be

collected from these plants biweekly or more often during the period of seed dispersal. The plants will be harvested at the end of the growing season for detailed growth analysis (root:shoot, seed number, seed weight, etc) and elemental analysis for N and S by CHNS analyzer, P by H_2SO_4 and H_2O_2 digestion (Harmon and Lajtha, 1999) followed by spectrophotometric analysis using a molybdate-antimony-ascorbic acid method on a Lachat Autoanalyzer (Lachat Instruments method 13-115-01-1-B), and a suite of metals by ICP analyses. In addition, all aboveground plant material will be collected from each tank and weighed along with a subsample taken to determine wet:dry ratios for moisture correction. All samples will be dried at 60°C before weighing. All stems will be counted in these samples to determine end of growing season plant density. All aboveground plant material except for the five sample plants and the plant material from the litter removal mesocosms will be returned to each mesocosm. Temperature, wind speed and direction, and precipitation will be monitored daily on site with an automated weather station equipped with a recording thermometer, radiosonde anemometer, and rain gauge. Temperature data loggers (HOBO UA-002-08) will be placed in the sediment in each of the treatments, these will collect hourly temperatures throughout the growing season.

We will also make detailed seasonal measurements of pore water chemistry. We will measure oxidized and reduced forms of nitrogen, iron, and sulfur in sediment pore water using Rhizon samplers (“sippers”; Fig. 6). At selected time points, measurements of vertical stratigraphy in pore water redox will be made using microelectrodes and/or peepers (diffusion-equilibrium samplers). In-situ measurements will be made for oxidation-reduction potential and pH while small-volume pore water samples will be collected and analyzed for dissolved anions (sulfate, nitrate, phosphorus, chloride) by ion chromatography, dissolved sulfide and dissolved ferrous iron by spectrophotometry, pH, and other nutrients (phosphorus, copper, zinc). In addition, we will measure dissolved gas concentrations in overlying water and sediment pore water (H_2S , N_2 , O_2 and CO_2) using a membrane inlet mass spectrometer. Changes in the ratio of N_2/Ar will be used to infer rates of denitrification and CO_2/O_2 ratios can be used to determine the proportion of microbial metabolism that is anaerobic (Cotner et al. 2004).

The effect of sulfate levels, N and P additions, litter removal, and thinning on wild rice seed, shoot, and root growth, sediment profiles of sulfate, sulfide, and iron, and N and P availabilities will be determined using both randomized complete block analysis of variance and linear and nonlinear regression. Potential toxicity of enhanced sulfate or sulfide as well as increased nitrogen limitations because of decreased root growth will be analyzed using the graphical analysis technique of Timmer and Stone (1975); we have recently used this technique to analyze response of wild rice to enhanced nitrogen, phosphorus, and light levels (Sims et al. 2012a).

Field measurements in wild rice beds at Lake Itasca and in the St. Louis River

Field site selection: Our field sites and measurements will allow us to examine whether our hypothesized mechanisms can be generalized to native stands of wild rice in both pristine waters and in waters already receiving significant sulfate loadings. To do this, we propose to monitor three plots in healthy native stands of wild rice on Lake Itasca for two growing seasons. Itasca is an area with significant background information on aquatic plant population health, and is



Figure 6. Microelectrodes (right) and pore water sippers (left) for investigating speciation of sulfur and iron concentrations in sediment profiles.

adjacent to the University of Minnesota Itasca Biological Station and Laboratories. Three additional sites containing wild rice stands with relatively high sulfate ($20\text{-}70 \text{ mg} \cdot \text{L}^{-1}$, N. Johnson, *unpubl. data*) will also be chosen from the St. Louis River watershed. These sites will also have significant background data on both aquatic vegetation and sediment geochemistry from the ongoing MPCA wild rice study.

Field sampling and analytical methods: Plots will be monitored continuously for temperature, dissolved oxygen, pH, and light using Hydrolab sondes. These data will provide information on changes in the baseline conditions of our study areas. Furthermore, we use these sondes to measure benthic plant and water column productivity using changes in dissolved oxygen levels and pH. Cotner's lab group currently has 12 of these internally logging sondes that could be used in this project.

Consistent with mesocosm measurements, we will make direct measurements of sulfur in lake water, pore waters and solid-phase sediments at time points corresponding to four ecologically relevant periods; germination or ice-out (April-May), submerged and floating leaf stage (June), emergent and seed deposition stage (July-September), and senescence or ice-over (October-March). By doing so we will be able to determine whether there are periods when the plant is more sensitive to environmental and physical conditions. These measurements will provide characteristic data on sulfur exposure in a healthy wild rice area over the course of the growing season and how they relate to the current standard of 10 mg L^{-1} in surface waters. Potential feedbacks with P availability, production of autochthonous organic C, and sulfate production also exist and will be examined in these plots. Our group has considerable experience investigating P-limitations of microbial communities and primary producers. The remaining biological analyses listed in the table below will aid in characterizing the biogeochemical habitat of wild rice at our field plots for comparison with the mesocosm experiments as well as in other regions (Koch et al. 1990, Gao et al. 2002, Choi et al. 2006).

Chemical measurements in the water column and pore waters will be made several times throughout the summers (at time points defined by rice growth stages) since these are the phases expected to see the most transient seasonal differences. Biological measurements will also be made seasonally at the field plots, while chemical measurements in the solid phase and physical measurements will be made at the beginning and the end of the growing season. Within each field plot, locations having variation in plant density and/or plant monoculture will be chosen.

Samples for chemical measurements will be collected and analyzed using methods consistent with those used in mesocosms. Procedures for collecting sediment porewater and solid phases for analysis will be modified from those outlined by Myrbo (2012). Additionally, we will take depth profiles of porewater concentrations of sulfate, sulfide, iron, and pH consistent with measurements made in mesocosms. To the extent possible, measurements of wild rice growth at field sites will be consistent with those used at mesocosm sites. Plant density will be measured in 1 m^2 (Myrbo 2012) subplots at each site (the number of replicate subplots to be determined by preliminary sampling to obtain an estimate of variance). Within each subplot, six plants will be sampled at the end of the growing season; their growth and allocation to roots, shoots, and seeds and elemental analyses of these tissues will be determined by the same procedures as the sample plants in the mesocosms (see above).

In the field, we will also set up a series of enrichment experiments that parallel mesocosm experiments. At each site, we will fertilize with N and P and S through perforated PVC pipes that are inserted into the sediments. Also, similar to the mesocosm experiments, litter removal/no removal plots will be set up to understand the role of surface sediment organic matter on plant

and nutrient dynamics. Loadings of N, P and S will be at 15, 5.5 and 25 $\text{g}\cdot\text{m}^{-2}\cdot\text{yr}^{-1}$, respectively. These correspond with the nitrogen and phosphorus loadings and the average sulfate loading in the mesocosm experiments. Sulfate will be added as sodium sulfate, N as ammonium chloride and P as dipotassium phosphate.

We will make measurements of water column conditions (temperature, pH, conductivity, PAR [photosynthetically active radiation] and dissolved oxygen) using water quality sondes (Hydrolab H2O) deployed throughout the growing season and measurement in 15 min intervals. In addition, we will measure total dissolved sulfide and sulfate, nitrate plus nitrite, total dissolved N, ammonium, soluble reactive P, total P, dissolved inorganic carbon (DIC), chlorophyll concentration and major cations seasonally. Porewater measurements will include DIC, pH, dissolved oxygen, total dissolved sulfides, sulfate, nitrate, ammonium, total P and dissolved organic C. We will also make measurements of solid phase components including total organic C and N, total S and acid volatile S, and metals. Lastly, we will conduct incubations at in situ temperatures to quantify nitrification, denitrification and respiration rates (Kana et al. 1994). We will quantify plant and root density, water depth and sediment bulk density.

DOC and TDN will be measured on filtered water samples with a high temperature combustion method on a Shimadzu TOC-5000 VCH. Water samples are collected, filtered through a combusted GF/F filter into a no-headspace serum bottle and preserved with HCl prior to analyses.

Particulate organic carbon, nitrogen and phosphorus will be determined using CHN and wet chemistry techniques. Seston will be filtered onto pre-combusted, glass-fiber filters (Whatmann GF/F) for C, N and P measurements. C and N contents will be determined using a CHN analyzer (model 2400, Perkin-Elmer, MA, USA) and P contents will be measured by acid-persulfate digestion and subsequent soluble reactive phosphorus analysis (Apha 1992) using a Lachat flow injection system.

We will measure oxygen (O_2) and N_2 using a membrane inlet mass spectrometer (Kana et al. 1994) that provides high precision measurements of dissolved gases in water. Dissolved oxygen and N_2 are measured as the ratio of these gases to Ar in the sample relative to air-equilibrated Nanopure water at the same temperature (25 °C) and pressure (atmospheric) (Tortelli 2005). Recently we have used this instrument to measure pCO_2 concentrations in lakes. Others have used a very similar instrument to measure H_2S concentrations and we are confident that we will be able to do the same.

G. Expected Results

If our hypotheses are correct, then we expect to see: 1. Increased sulfide production with increased sulfate loadings (Fig. 3); 2. Reduction in wild rice biomass, root, and seed production in relation to sulfate loading and sulfide production (Fig. 2); 3. four-year oscillations in sulfide production driven by a four-year cycle in wild rice litter production (Fig. 4); 4. Inhibition of uptake of other nutrients, especially nitrogen as sulfide inhibits root production and function. The collective set of measurements, including both controlled conditions in mesocosms and natural conditions in field settings, will allow for detailed observations of a system having detailed manipulations of the biogeochemical habitat of wild rice as well as validation of the observations in natural ecosystem settings.

H. Usefulness

The work proposed here will provide the State of Minnesota and other states grappling with similar issues a more fundamental basis for understanding the interactions between wild rice and

water quality. There is a great deal of interest in the State of Minnesota in developing a stronger scientific basis for the current standard or in determining whether other standards need to be developed to protect wild rice waters. Specifically, we need to determine mechanistically how sulfate/sulfide and nutrients interact with the growth and persistence of wild rice considering the complex geochemical cycles that occur in wild rice sediment. Past work by Moyle (1944, 1945) suggested that wild rice does not grow effectively above a limit of about 10 mg L^{-1} sulfate, but it is unclear if sulfate per se has any effect on wild rice growth or if the inhibitory agents are the products of sulfate reduction in the sediments, namely reduced sulfides. The mechanistic work proposed here will help determine where the threshold is and more importantly what causes it if there is one. It will also indicate whether future work should focus more on other water quality parameters as a standard. Furthermore, by examining interactions between sulfate and other nutrients such as N and P, we will be much more capable of putting the sulfate standard work in a larger ecosystem context and in the context of a wide range of conditions throughout the state. This latter point is particularly important given the increased potential for nutrient enrichment throughout the state from increased agricultural use of fertilizers and increased nitrogen and phosphorus loadings associated with population growth.

I. Economic Benefits

Wild rice is harvested in Minnesota from both by machine from cultivated wild rice beds and by hand from canoes from natural stands. The cultivated beds are exempt from the State $10 \text{ mg SO}_4 \cdot \text{L}^{-1}$ standard, which applies only to natural stands. Approximately 4000-5000 individuals hand-harvest natural wild rice from canoes each year, almost entirely from the St. Louis and Upper Mississippi Watersheds in northern Minnesota. At least 3000 of these harvesters are members of the Ojibway tribe. Hand harvesting of rice from natural beds is an important source of nutritious food and funding to the Ojibway people. This harvest not only generates an important staple food for tribal members, but also at least \$500,000 for tribal budgets (statistics from Minnesota Dept. of Natural Resources 2008). Protection of wild rice beds is of high economic and cultural importance to the various reservations of the Lake Superior Chippewa (Ojibway) and the 1854 Treaty Organization, a tribal organization authorized to inventory and protect natural resources covered under the 1984 Treaty between the State of Minnesota and the Ojibway tribe. The effects of sulfate draining from existing iron and proposed copper-nickel mines as well as other sources into wild rice beds, especially those in the St. Louis River, is of great cultural and economic concern to tribal biologists (see letter of support from Nancy Schuldt, Water Quality Specialist with the Fond du Lac Reservation). The St. Louis River flows through the Fond du Lac Reservation just before entering its estuary into Lake Superior, and most of the sulfate in these waters originates almost certainly from mines upstream. Therefore, how sulfate affects wild rice growth and distribution poses very large economic challenges to tribal members and the mining industry in Minnesota.

J. Outreach

This work will involve the training of undergraduates, and one or more graduate students. Undergraduates will be supported directly through this grant as well as University of Minnesota internal funds. Our research group has always had numerous undergraduates working in our laboratories and published papers and presented research based on their work (Gardner et al. 1995, Cotner et al. 1995, Gardner et al. 1996, Cotner et al. 2001, Biddanda et al. 2001, Amado et al. 2007, Johnson and Beck 2011, Dahlberg and Pastor in preparation). This past year, there were as collectively over a dozen undergraduates working in our laboratories at any given time.

Cotner will be teaching a new course on Biogeochemical Processes this coming spring at the Itasca Biological Station and Laboratories with the intent that it will continue to be offered for the

next 5-10 years. As discussed above, Lake Itasca has an extremely well-developed wild rice population; as part of the class, 5-10 students will conduct biogeochemical studies of these populations. Students will collect samples for C, N and P as well as dissolved gas (N_2 , O_2 , H_2S , CO_2) measurements. In addition to training these students in the interpretation of biogeochemical data, we will use data from the class and our proposed study to develop a long-term dataset on wild rice dynamics. These data will help put the work that we are doing in Pastor's mesocosms in the context of natural population dynamics.

A graduate student will work with Cotner both on the research- focused aspects of this project as well as a transformational program that was developed at the Mayo Clinic in Rochester, MN called InSciedOut (<http://insciedout.org>). This innovative program trains students at the K-8 grades via project-based science developed with research scientists. The goals of the program are to a) build a science literate public, b) improve science education curriculum, c) to facilitate the return of science into education and d) develop 'hybrid' science faculty capable of fluency in science and education. The principal and co-principal investigators will work with the InSciedOut educators and personnel at both Lincoln Elementary in Rochester and the Richfield Schools (Minneapolis) to develop a freshwater science-focused curriculum.

In the past year Cotner has been involved with the Minnesota International Center and their "Great Decisions Speaker Series". In this series, member clubs (of which there are several dozen) throughout the Twin Cities invite speakers to come and speak about some of the important issues of our time. Cotner has given one of these talks so far and have 8 more scheduled for the fall on the topic of "The state of the oceans". In addition, through this group, Cotner has been invited to write an op-ed piece for the Star Tribune newspaper on the same topic. If funded, we would like to continue to develop this relationship with a focus on freshwater resources in the future.

In the past year Pastor and Johnson have been working closely with the Minnesota Pollution Control Agency and their Sulfate Standards for Wild Rice Waters Advisory Committee. Much of the research presented in this proposal (especially Figs. 1 and 2) has resulted from funding provided by this Agency. We have had weekly conference calls with Agency scientists and have made presentations about our findings during two interim meetings of the Committee. We will continue to maintain these relationships and provide findings to the Minnesota Pollution Control Agency as they develop (see letter of support from Ed Swain, Research Scientist, MPCA).

Finally, Pastor has had a long-standing collaboration with tribal biologists from the 1854 Treaty Organization and the Fond du Lac and Grand Portage Reservations of the Lake Superior Ojibway (see letter of support from Nancy Schuldt, Water Quality Specialist, Fond du Lac). This collaboration began with his long-term mesocosm experiments under NSF funding (Fig. 3) and continued during the first two years of the mesocosm experiment (Fig. 2), which was supported with tribal funds. Several tours of the mesocosm experiment have been given to tribal students and several talks have been given at the Fond du Lac Tribal and Community College. We will make every effort to continue and strengthen these collaborations.

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Data Management Plan

All data will be entered for statistical analysis in Excel spreadsheets. Mr. Brad Dewey, senior technician for J. Pastor, will oversee data entry and proof checking for the data from the mesocosm experiments, Cotner's technician will oversee data entry and proof checking for data from the Lake Itasca measurements and Johnson will supervise data entry and proof checking for the St. Louis River measurements. All data files will be made available to appropriate agency scientists and managers who request them.

After proof checking, appropriate data files will also be posted on a publically accessible project website. This website will also contain metadata about experimental design, sampling protocols, and analytical procedures, as well as photos of the experiments and sites. Users who wish to access these data will be requested to send us a brief description of what questions they are seeking the data to answer and how they will use the data. This request is to educate ourselves about how our data is being used so that we can document its value to other researchers. We will also request that they acknowledge the source of the data should it lead to a publication.

Obviously, we must determine the level of data which we will post on this website as it is impractical to post all data, not matter how raw. We place a high priority on posting data which is required to verify our published conclusions (eg. data to which statistical analyses such as ANOVA or regression are applied) rather than raw data about sample weights or aliquots and standard curves for the chemical or physical analyses. The website will also contain contact information for those researchers who wish to obtain additional data not posted or who wish to ask us questions about methodological procedures. We will update the website as needed based on the types of requests which are made, assuming that if one person asks for more information others may also wish for that same information.