EXPERT OPINIONS & ATTACHMENTS

Submitted with WaterLegacy Comments on Proposed Wild Rice Rule
November 22, 2017

John Pastor, Ph.D., Technical Review Comments on MPCA's Proposed Flexible Standard for Sulfate in Wild Rice Beds, November 2017, with Attachments A through G. (143 pages)

Technical Review Comments on MPCA's Proposed Flexible Standard for Sulfate in Wild Rice Beds

Proposed Minnesota Pollution Control Agency Rulemaking

John Pastor, PhD (November 2017)

Background and Research

I am a Professor of Biology at the University of Minnesota Duluth, past Co-Chair of the Natural History Section of the Ecological Society of America, and an Honorary Member of the Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden.

I received my B.S. in Geology from the University of Pennsylvania in 1974, and my Ph.D. in Forestry and Soil Science in 1980 from the University of Wisconsin-Madison. I've also done post-doctoral research in the Environmental Sciences Division at Oak Ridge National Laboratory. I've authored two books on ecology, over 100 peer-reviewed papers, and over 20 book chapters. My papers have been cited over 17,000 times by other scientists. My *curriculum vitae* is provided (attachment A) with these comments.

For the past ten years, my research has focused on the ecology of wild rice, including the effects of sulfate pollution and iron on wild rice. This work has been funded by the National Science Foundation, Minnesota Pollution Control Agency, Fond du Lac and Grand Portage Bands of Lake Superior Chippewa, and Minnesota Sea Grant. I was the lead researcher for the hydroponic experiments and tank mesocosm studies of sulfate and wild rice coordinated by the Minnesota Pollution Control Agency (MPCA) in the Wild Rice Sulfate Standard Study funded by the Minnesota Legislature. However, our mesocosm studies of wild rice and sulfates began several years before the MPCA study and have continued through 2017.

Results of the first several years of my research regarding effects of sulfate and sulfide on the life cycle of wild rice in hydroponic and mesocosm experiments were published in a peer-reviewed journal article (Pastor *et al*. 2017) provided (attachment B) with these comments.

For the past several years, I have continued mesocosm research designed to test the MPCA's hypothesis that sediment iron would protect wild rice from the effects of high surface water concentrations of sulfate. The results of this research are reflected in a Minnesota Sea Grant Progress 2016 report (attachment C) and a Minnesota Sea Grant Progress 2017 report (attachment D) provided with these comments. One of my graduate students, Sophia LaFond-Hudson, studied iron and sulfur cycling in the root zones of wild rice in an experimental growing wild rice in buckets. Her 2016 Master's thesis on this research (LaFond-Hudson, 2016) is also provided with my comments (attachment E). The 2016 Sea Grant Progress Report and Ms. LaFond-Hudson's thesis were provided to the MPCA in the summer of 2016. I also presented a slide presentation on the experimental effects of iron and sulfate on wild rice to the MPCA and Wild Rice Sulfate Standard Study Advisory Committee in August 2016. That slide presentation is also provided with my comments (attachment F).

I was contacted by WaterLegacy to review the MPCA's proposal to replace Minnesota's existing fixed standard of 10 milligrams per liter (mg/L) sulfate applicable to water used for the production of wild rice (Minn. R. 7050.0224, subp. 2) with a flexible standard derived through the use of an equation. Throughout the past six years, I have read numerous MPCA draft proposals, internal memos, peer review materials, submitted and published articles and comments of various entities and experts. In preparing these comments, I also reviewed the MPCA's draft rule, Statement of Need and Reasonableness and Exhibit 1 Technical Support Document.

Summary

1) Our recent research at the University of Minnesota Duluth demonstrates that sulfide, not sulfate, is toxic to seedlings of wild rice. The MPCA proposes that iron can protect wild rice by precipitating with the sulfide. However, the addition of iron to mesocosms with high sulfate concentrations did not entirely mitigate the toxic effects of sulfide to seedlings. Our research also demonstrates that precipitation of iron sulfide on wild rice roots can inhibit nutrient uptake needed to ripen seeds, so iron sulfide can have negative effects on wild rice sustainability. Setting sulfate limits based on the level of sediment iron is premature and is not reasonable.

2) In addition, the MPCA's model assumes that concentrations of sulfide, sulfate, reactive iron and organic matter are in a steady state. This is not a reasonable assumption, especially once sulfate loading increases from various sources of pollution.

3) Both historic field data and the recent field surveys performed by the University of Minnesota as part of the Wild Rice Sulfate Standards Study demonstrate that concentrations of sulfate in surface water above 10 mg/L proposed in the MPCA's flexible standard may not adequately protect wild rice.

Statement of the problem

The State of Minnesota now has a fixed standard of "10 mg/L sulfate applicable to water used for production of wild rice during periods when the rice may be susceptible to damage by high sulfate levels" (Minn. R. 7050.0224, subp. 2). This standard, developed during the 1970s, is based on research by DNR botanist John Moyle, who found that "No large stands of rice occur in water having sulfate content greater than 10 ppm [parts per million, or mg/L], and rice generally is absent from water with more than 50 ppm" (Moyle 1944).

Application of Minnesota's sulfate standard has been rare and controversial. To put this in perspective, EPA drinking water standards for sulfate are 250 mg/L, while EPA standards for sulfide in surface waters to protect aquatic life are very low; 2 parts per billion (2ug/L). Although ecologists, including John Moyle, have long believed that wild rice toxicity resulted from conversion of sulfate to sulfide in sediments with low concentrations of oxygen, little experimental data confirmed that hypothesis. Research was designed to evaluate what factors resulted in wild rice toxicity and whether limiting sulfate was necessary to prevent sulfide-induced toxicity.

Sulfate, Sulfide and Iron Research

Sulfate is released to surface waters by several industrial processes, but *sulfate* is transformed into *sulfide* in waterlogged sediments with low concentrations of oxygen. Our initial investigations of the effects of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris* L.) in hydroponic solutions and in outdoor mesocosm tanks demonstrated that sulfide, not sulfate, is toxic to seedlings of wild rice. In hydroponic solutions, sulfate had no effect on seed germination or juvenile seedling growth and development, but sulfide greatly reduced juvenile seedling growth and development at concentrations greater than 320 μg/L.

When we added sulfate to experimental mesocosm tanks where wild rice was grown in sediments from a wild rice lake under low oxygen conditions similar to those in a natural environment, sulfate additions to overlying water increased sulfide production in sediments. Seedling emergence, seedling survival, vegetative growth and seed production all declined in proportion to the amount of sulfate added and the amount of sulfide produced.

In each spring after the initial planting in 2011, the number of seedlings that emerged from the sediment declined significantly with increased sulfate concentrations ($p < 0.001$). The rate of seedling survival also declined significantly with increased sulfate concentrations ($p < 0.001$) and became worse in each subsequent year ($p < 0.001$). The rate of decline in seedling survival with amended sulfate was twice as high in 2014 and 2015 as it was in 2012 and 2013 (Pastor *et al*. 2017).

Elevated sulfate and presumably sulfide concentrations decreased vegetative growth, measured as plant biomass ($p < 0.001$), and the rate of decline increased significantly during the course of the experiment. Although the overall number of seeds produced per plant did not change across sulfate concentrations, the proportion of seeds produced that were filled and thus able to propagate declined significantly with increasing sulfate concentrations ($p < 0.001$). The proportion of filled seeds declined more steeply with each successive year ($p < 0.001$) (Pastor *et al.* 2017).

These declines in seed production and seedling survival lead to the extinction of wild rice populations after 5 years at sulfate concentrations comparable to drinking water standards (Pastor *et al*. 2017). Populations of wild rice exposed to sulfate concentrations of 150 mg/L have continued to decline over the course of the mesocosm experiments, nearing the point of extinction (Progress Report 2017). In addition, we have noticed a parallel decline in other species in the tanks with enhanced sulfate concentrations. These species include the larvae of dragonflies and caddisflies, which are important foods for fish such as walleye that typically inhabit wild rice lakes. Therefore, the decline in population densities with enhanced sulfate concentrations may not be limited to wild rice but in fact may happen to other important species of the food web.

The MPCA also coordinated a parallel field study of over 100 wild rice lakes. The MPCA's preliminary findings seemed to support retaining the existing 10 mg/L sulfate limit to protect wild rice from sulfide-induced toxicity. However, the MPCA is currently proposing to replace its 10 mg/L fixed sulfate standard with a flexible standard based on a model which attempts to predict sulfide concentrations in sediment of each individual lake from the concentration of sulfate in surface waters and the concentrations of reactive iron and organic matter in sediments from these lakes.

Geochemistry supports the MPCA's basic premise that iron may reduce sulfide concentrations in sediments. Sulfate is converted to sulfide by microorganisms that also obtain energy by decomposing organic matter. Iron is present in many forms in wild rice beds but the more important form for the purpose of this model is ferrous iron, a form that can reduce the reactivity of sulfide in sediment.

However, MPCA's proposed model relies on a critical assumption that is tenuous and has not been experimentally verified. The MPCA assumes that any precipitation of sulfide by iron helps to protect wild rice. Our experimental mesocosm research has substantially undermined this assumption. During the course of our initial mesocosm (tank) experiments, we noticed that wild rice roots in tanks with more than 50 mg/L sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle. Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques, whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides. (Pastor *et al*. 2017; Sea Grant Report 2017).

Figure 1. Orange healthy roots (left) of wild rice grown under low sulfate concentrations near the current standard and black iron sulfide coatings on roots of plants grown with high sulfate concentrations.

We learned that iron sulfide precipitates rapidly on wild rice roots in midsummer at the time when the plants are beginning to flower and take up additional nutrients for the ripening seeds. The iron sulfide precipitates gave the roots a black appearance, compared to amber or rust colored roots on healthy plants exposed to sulfate concentrations near the current fixed standard of 10 mg/L. Seed nitrogen, seed count and seed weight were all markedly reduced in plants with back root surfaces exposed to high sulfate surface water concentrations (300 mg/L) because these black iron sulfide precipitates inhibit the uptake of nutrients necessary for the filling and ripening of seeds necessary for propagation of wild rice. This happened even though the amount of iron remaining in the sediment was sufficient to remove sulfide from sediment porewater. These experiments are detailed in Progress Report (2017) and LaFond-Hudson (2016). Plants grown at lower concentrations of sulfate had black iron sulfide coatings in proportionally lower amounts, as well as proportionally reduced seed production (Pastor et al. 2017).

Our experimental mesocosms contained sediment iron near the median of that observed in field conditions. Our more recent experiments, in which we tripled the amount of sediment iron in the first growing season and removed litter to reduce carbon supply for microbes under sulfate conditions of 300 mg/L, began in 2015. During the three years of this experiment, sulfate amendments had the greatest effect on outcomes, reducing seedling survival, plant growth, and seed production. Litter removal had no effect on seedlings, vegetative growth, or seed production. Adding iron without sulfate had no effect on seedling survival, plant growth, or seed production. Iron amendments in the presence of sulfate increased seedling survival compared with seedlings grown under sulfate amendments alone, but seedling survival in the tanks with both iron and sulfate additions was still less than in control tanks. (Progress Report 2017). Our experiments found that precipitation of iron sulfide in the sediment may temporarily ameliorate the effects of sulfate on seedling survival, but by the spring of year three, iron amendment no longer had an effect on seedling survival, possibly because almost all the added iron had been precipitated. (Progress Report 2017).

Our experiments demonstrate that precipitation of sulfide in the presence of high levels of iron has both ameliorative and negative effects on wild rice growth. Iron additions may partly ameliorate sulfide toxicity to seedlings in spring. However, precipitation of iron sulfide plaques on roots during the flowering and seed production period of wild rice's life cycle appears to block uptake of nitrogen, leading to fewer and smaller seeds with reduced nitrogen content. The net effect of sulfate additions to wild rice populations is to drive the populations to extinction within 4 or 5 years at high concentrations of sulfate (300 mg/l), even when iron was added to the sediments. Sulfate loading greatly reduce population viability at lower concentrations.

How and whether iron mitigates sulfide toxicity to wild rice is not fully understood and appears not to be related to the amount of reactive iron in sediments in the simple way assumed by MPCA's model. Therefore, setting sulfate standards based on the amount of reactive iron in sediments is premature at best. Based on current scientific evidence, an equation determining "protective" sulfate levels based on iron in sediments and available carbon is not a defensible strategy to protect wild rice.

Finally, MPCA claims, on p. 82 in their Statement of Need and Reasonableness, that concentrations of sulfate above the allowable standard in one year out of ten would not have a significant impact on wild rice populations in the long run. They cite our experiments in support of this conclusion. While I agree that it is important to determine the allowable frequency and degree of excursions to avoid impacts on wild rice, I must also point out that our experiments were not designed to determine what these might be. At present, a one-in-ten year allowable excursion is premature and requires further experiments designed specifically to determine what level of excursions does not harm the long term sustainability of wild rice populations.

Steady State Concentrations

In addition to assuming a simple relationship between iron in sediments and survival of wild rice, MPCA's model assumes that the concentrations of sulfide, sulfate, reactive iron, and organic matter in the sites from which the equation was developed are in steady state, which means that their concentrations do not change over long periods of time.

MPCA claims that the assumption of steady state is verified by data that concentrations of these elements of the model did not change during one growing season. But one growing season is insufficient to test the assumption of steady state. The steady state assumption must be tested against data across years, particularly in systems subject to transient changes to sulfate from industrial discharges. Until longer-term information is obtained, we do not know if these ecosystems are in a steady state from one year to the next. If the ecosystems are not in steady state, then the calculation that a certain sulfate concentration in surface water creates lower-than-toxic levels of sulfide during one year may not apply to subsequent years. A sulfate concentration deemed "protective" in year one could become toxic in subsequent years.

Once sulfate inputs to a wild rice bed increase as a result from discharge of wastewater, ecosystems will no longer be in steady state. Microbes in the sediments will convert some of this sulfate to additional sulfide and the sulfide will precipitate with some of the reactive iron and convert it to iron sulfide precipitates. But the iron in these precipitates will no longer be available to precipitate any additional sulfide. The reactive iron removed by precipitation with sulfide must be replenished by inputs of additional iron for the initial calculation to remain valid. In an ecosystem, it cannot be assumed that natural inputs of reactive iron from streams and groundwater or from weathering of sediments will keep pace with sulfate pollution.

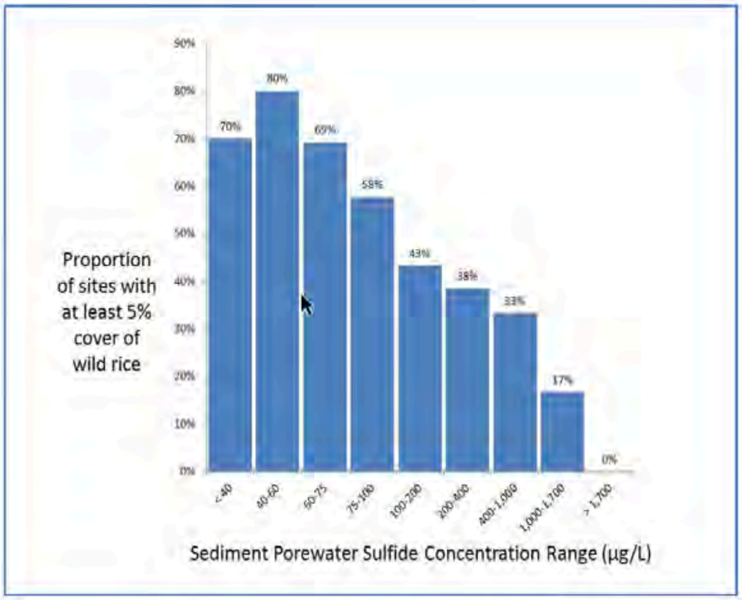
The amount of reactive iron in a localized area will decline with increased sulfate loading, just as a checkbook balance declines when withdrawals increase without a matching increase in deposits. MPCA's model does not demonstrate that natural inputs of iron would replenish the reactive iron in the sediment commensurate with sulfate discharge. The model assumes, without evidence, that iron input will remain at a rate sufficient to ameliorate sulfide toxicity from the additional sulfate without creating additional adverse consequences for wild rice survival.

As also pointed out by Prof. David Schimpf (Schimpf, 2015), a decision to allow sulfate concentrations in surface waters above their current levels in certain sites could look reasonable for a while, but become inadvisable and fail to protect wild rice over time.

Concentrations of Sulfate Greater than 10 mg/L May Not Adequately Protect Wild Rice

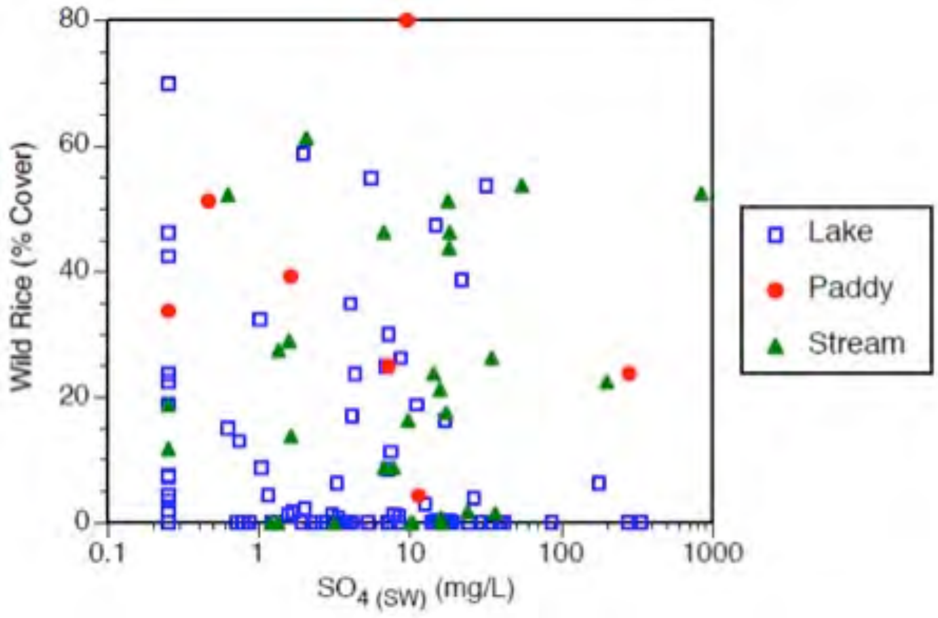
Professor Shimpf has also raised the concern that the MPCA's proposal, by focusing on the presence of wild rice may redefine "protect wild rice" in a weaker sense than that of the existing standard, which was based on John Moyle's field research finding no large stands of wild rice in Minnesota where sulfate exceeded 10 mg/L and that wild rice was "generally absent" where sulfate exceeded 50 mg/L. (Schimpf, 2015)

Data from MPCA's survey lakes demonstrate a decline in wild rice abundance at sulfide concentrations above 75 µg/L, which is below MPCA's proposed EC10 of 120 µg/L. (MPCA, 2014). In addition, a standard that is based on 5% wild rice cover may not protect wild rice sustainability.



MPCA's flexible standard, based on calculating a "protective sulfate concentration" to attain a sulfide level of 120 ug/L, would allow sulfate concentrations more than an order of magnitude above the current sulfate limit of 10 mg/L in many cases and could sometimes result in allowing sulfate concentrations two orders of magnitude higher than the current standard. For example, the MPCA has calculated that a "protective sulfate concentration" for the St. Louis Estuary would range from 99.5 mg/L to 241.1 mg/L, while a "protective" concentration of sulfate for the Embarrass River would be 1248.9 mg/L. (See MPCA spreadsheet, attachment G).

Current data collected by MPCA demonstrate that allowing sulfate concentrations much greater than 10 mg/L (the current standard) may not protect wild rice. This chart prepared by an MPCA staff scientist from the 119 field study sites [1] shows that over 70% of wild rice ecosystems are found in sulfate concentrations of 10 mg/L or less and 94 % are found in lakes or streams with sulfate concentrations below 50 mg/L. Even though the MPCA field survey was designed to study sites with wild rice present despite high sulfate levels (MPCA, 2014), field survey findings strongly corroborate Moyle's (1944) conclusions.



This figure illustrates the infrequency of wild rice presence and density in waters with sulfate concentrations above the current standard of 10 mg/L. Based on its model and equation, MPCA's proposed flexible standard would allow for much higher concentrations of sulfate to be defined as "protective" if high levels of iron were present. Sulfate limits set for individual water bodies above the current standard of 10 mg/L incur increased risk to the sustainability of wild rice populations.

Sandy Lake provides an example of the decline of wild rice populations in the presence of sulfate exceeding the existing 10 mg/L standard despite high sediment iron concentrations. Sandy Lake (MN DNR ID 69-0730-00, in St. Louis County) had extensive and productive wild rice populations in the past. Sandy Lake has received discharge from a nearby tailings pond of an iron mine since the

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[1] Edward Swain, MPCA, "The world's 4 species of wild rice," slide presentation to Minnesota Native Plant Society, Feb. 4, 2016.

mid-1960s. The MPCA sampled water and sediment and counted wild rice stem density in Sandy Lake 10 times from June through September in 2013 (Appendix G). The sulfate concentration in Sandy Lake during 2013 averaged 95 mg/L, which is not significantly different from the calculated average allowable sulfate concentration using MPCA's flexible standard model of 79 mg/L, although it is significantly higher than the existing wild rice sulfate limit of 10 mg/L. The sediment of Sandy Lake has high iron content, 23,540 ug/g, which is nearly three times the statewide average (8800 µg/mg) for all non-paddy wild rice water bodies sampled by MPCA. Despite this high iron content, wild rice was largely absent at all times and sampling locations in Sandy Lake, except for two locations with very low population densities (0.6 stems per m^2 at one location on Sept. 17 and 3.8 stems per m^2 at another location on Sept. 21). These low densities are highly unlikely to be viable in the long run.

If MPCA's model is correct, then wild rice should be present and abundant in Sandy Lake because of the high sediment iron content and the similarity of the concentration of sulfate in the water compared to the allowable sulfate concentrations. And yet, despite the high iron content of the sediment, MPCA could barely find any wild rice in Sandy Lake. Although wild rice is present in Sandy Lake and thus appears in MPCA's modeling as a lake with wild rice despite high sulfate concentrations the populations of wild rice in Sandy Lake are clearly not healthy, especially compared to what is known to have been present in the past.

Conclusion

The Wild Rice Sulfate Standard Study wild rice research funded by the Minnesota Legislature and coordinated by the MPCA has made important contributions to our understanding of the process of sulfide-induced toxicity resulting from sulfate concentrations in surface waters in the presence of iron and other factors. However, based on my training and experience, it is my opinion that the weight of the scientific evidence supports retaining Minnesota's existing sulfate standard of 10 mg/L to protect wild rice. As sulfate concentrations rise above the current standard, the risk to sustainable wild rice populations increases because of increased sulfide production.

Although the MPCA's conceptual framework pertaining to sulfate reduction to sulfide and iron sulfide precipitation has substantial merit, making the leap from this conceptual understanding to the MPCA's proposed flexible standard equation makes important assumptions about the ameliorative effects of iron and the continuation of a steady state over time despite sulfate addition to the ecosystems. These assumptions cannot be defended based on scientific evidence. Both experimental research and field data suggest that sulfate concentrations above 10 mg/L may not protect wild rice and that sulfate concentrations an order of magnitude or more above 10 mg/L, as would be allowed in some water bodies by MPCA's proposed flexible standard, are likely to result in decline and extinction of wild rice over time.

Attachments

A. John Pastor *curriculum vitae*.

B. John Pastor *et al*., Effects of sulfate and sulfide on the life cycle of Zizania palustris in hydroponic and mesocosm experiments, Ecological Applications, 27(1), 2017, pp. 321-336.

C. John Pastor, Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*), August 18, 2016 slide presentation.

D. John Pastor, The biogeochemical Habitat of Wild Rice, Minnesota Sea Grant Report May 5, 2016.

E. John Pastor, Progress Report on Experiments on Effects of Sulfate and Sulfide on Wild Rice, June 28, 2017.

F. Sophia LaFondn Hudson, Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*) May 2016, Masters dissertation.

G. MPCA, Field Data with CPSC (All MN Data), Aug. 17, 2016.

Additional References

John Moyle, Wild Rice in Minnesota, Journal of Wildlife Management, Vol. 8, No. 3 (1944)

MPCA, Analysis of the Wild Rice Sulfate Standard Study: Draft for Scientific Peer Review, June 9, 2014.

David Schimpf, Comments on the Minnesota Pollution Control Agency's draft proposed approach for Minnesota's sulfate standard to protect wild rice (March 24, 2015), Dec. 14, 2015.

Ed Swain, MPCA, Plant-of-the-month: The world's 4 species of wild rice (Zizania Linnaeus) slide presentation at Minnesota Native Plan Society, Feb. 4, 2016.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment A

(27 pages)

JOHN PASTOR

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Education

Ph.D., Forestry and Soil Science, University of Wisconsin, Madison, June 1980
M.S., Soil Science, University of Wisconsin, Madison, December 1977
B.S., Geology, University of Pennsylvania, May 1974

Present Positions

Professor, Dept. of Biology, University of Minnesota Duluth (July 1996 – present)

Director, Natural History Minor, University of Minnesota Duluth (March 2009 – present)

Previous Positions

Associate Director of Graduate Studies, Ecological, Organismal, and Population Biology Track, Integrated Biosciences Graduate Program, University of Minnesota Duluth (March 2006 – May 2009)

Director of Graduate Studies, Biology Graduate Program, University of Minnesota Duluth (July 2000 – August 2009)

Visiting Scientist, Dept. of Animal Ecology, Swedish University of Agricultural Sciences, Umeå, Sweden (June – July 1998, and annually thereafter)

Visiting Scientist, Macaulay Land Use Research Institute, Aberdeen, Scotland (May 1997)

Distinguished Visiting Professor, College of Forestry, University of Washington, Seattle, Washington (March 1991)

Visiting Scientist, Institute of Applied Ecology, Shenyang, People's Republic of China (July – August 1988)

Senior Research Associate, Natural Resources Research Institute, University of Minnesota Duluth (July 1985 – 2006)

Postdoctoral Fellow, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831 (August 1983 – June 1985)

Postdoctoral Research Associate, Department of Forestry, University of Wisconsin, Madison, WI 53706 (June 1980 – July 1983)

Graduate Student, Departments of Soil Science and Forestry, University of Wisconsin, Madison, WI 53706 (September 1975 – May 1980)

Staff Geologist, Ralph Stone Engineers, Los Angeles, CA 97821 (September 1974 – August 1975)

Research Interests

Species effects on nutrient cycling, plant-herbivore interactions, northern ecosystems, mathematical ecology

Awards and Honors

Honorary Life Member, Finnish Society of Forest Science, elected May 1999

First Recipient, Chancellor's Distinguished Research Award, University of Minnesota Duluth, November 1999

Institute of Scientific Information, Highly Cited List, Ecology and Environment, 2002 – 2012

Sabra and Dennis Anderson Scholar/Teacher Award, College of Science and Engineering, University of Minnesota Duluth, May 2007

University of Minnesota Council of Graduate Students Outstanding Faculty Award, April 2010

Doctores honoris causa, Faculty of Forest Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden, October 2010

Distinguished Ecologist Lecture, Colorado State University, April 2012

Teaching

Courses

Dept. of Biology, University of Minnesota Duluth: Biology 5776, "Ecosystem Ecology" (Spring 1990, Fall 1993, Fall 1998 and alternate years to present)

Dept. of Fisheries and Wildlife, University of Minnesota, St. Paul: Fisheries and Wildlife 8579, "Ecosystem Analysis and Simulations" (Winter 1993)

Province of Ontario and Lakehead University: "Ontario Advanced Forestry Program", Lecturer, 1992 and 1993

Dept. of Biology, University of Minnesota Duluth: Biology 5774, "Forest Ecology" (Summer 1994), with George Host

Dept. of Biology, University of Minnesota Duluth: Biology 5155, "Evolutionary Biology" (Fall 1994), with Carl Richards

Dept. of Biology, University of Minnesota Duluth: Biology 8871, "Graduate Seminar: Soil Genesis" (Winter 1994)

Dept. of Biology, University of Minnesota Duluth: Biology 8871, "Graduate Seminar: Measurement of Ecological Diversity" (Winter 1995 and Winter 1998)

Dept. of Biology, University of Minnesota Duluth: Biology 3871, "Issues in Global Change" (Winter 1996)

Dept. of Biology, University of Minnesota Duluth: Biology 5821, "Mathematical Ecology" (Fall 1997 and alternate years to present)

Dept. of Biology, University of Minnesota Duluth: Biology, "Graduate Seminar: Species Diversity in Time and Space" (Winter 1997)

Dept. of Biology, University of Minnesota Duluth: Biology 1102, "Biology & Society" (Spring 1998)

Dept. of Biology, University of Minnesota Duluth: Biology, "Graduate Seminar: Ecological Stoichiometry" (Spring 2005)

Dept. of Biology, University of Minnesota Duluth: Biology 5583, "Animal Behavior" (Spring 1999 – present)

Dept. of Biology, University of Minnesota Duluth: Biology 1097, "Biological Illustration" (Fall 1999 – present)

Dept. of Biology, University of Minnesota Duluth: Biology 8099, "The Biological Practitioner" (Fall 1997 – 2005)

Dept. of Biology, University of Minnesota Duluth: Integrated BioSciences 8011, "Integrated Biological Systems" (Fall 2006 – present)

Dept. of Biology, University of Minnesota Duluth: Integrated BioSciences 8201, "Ecological Processes" (Spring 2007 – present)

Graduate Students and Postdoctoral Fellows

Pamela McInnes, M.S. Wildlife Conservation, 1989 (co-advised with Y. Cohen)
Thesis title: Moose browsing and boreal forest dynamics, Isle Royale, Michigan, USA

Carmen Chapin, M.S. Biology, 1994
Thesis title: Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*.

Ron Moen, Ph.D. Wildlife Conservation, 1995 (co-advised with Y. Cohen)
Thesis title: Evaluating foraging strategies with linked spatially explicit models of moose energetics, plant growth, and moose population dynamics

Cindy Hale, M.S. Biology, 1996
Thesis title: Comparison of structural and compositional characteristics and coarse woody debris dynamics in old-growth versus mature hardwood forests of Minnesota, USA

John Terwilliger, M.S. Biology, 1997
Thesis title: Small mammals, ectomycorrhizae, and conifer succession in beaver meadows

Jean Fujikawa, M.S. Wildlife Conservation, 1997 (co-advised with Y. Cohen)
Thesis title: Interfacing songbird habitats with simulation processes

Scott McGovern, M.S. Biology, 1999
Thesis title: The effects of nitrogen, bacteria, and tachinid parasitoids on the nutrition of the spruce budworm (*Choristoneura fumiferana* Clem.)

Bingbing Li, M.S. Applied and Computational Mathematics, 2001
Thesis title: Mapping and modelling change in a boreal forest landscape

David VanderMeulen, M.S. Water Resources Science, 2001
Thesis title: Decay and nutrient dynamics of litter from peatland plant species

Nathan DeJager, M.S. Biology, 2004
Thesis title: Interactions between moose and the fractal geometries of birch (*Betula pubescens* and *B. pendula*) and Scots Pine (*Pinus sylvestris*)

Wendy Graves, M.S. Applied and Computational Mathematics, 2004 (co-advised with B. Peckham)
Thesis title: A Bifurcation Analysis of a Differential Equations Model for Mutualism

Laura Zimmerman, M. S., Applied and Computational Mathematics, 2006 (co-advised with B. Peckham)
Thesis title: A producer-consumer model with stoichiometry

Rachel Durkee Walker, Ph.D. Water Resources Science, 2008
Thesis title: Wild rice: the dynamics of its population cycles and the debate over its control at the Minnesota Legislature

Laurence Lin, M.S. Applied and Computational Mathematics, 2008 (co-advised with B. Peckham and H. Stech)
Thesis title: A stoichiometric model of two producers and one consumer

Nathan DeJager, Ph.D. Ecology, Evolution, and Behavior, 2008
Thesis title: Multiple scale spatial dynamics of the moose-forest-soil ecosystem of Isle Royale National Park, MI, USA

Rachel MaKarrall, M.S. Biology, 2009 (co-advised with T. Craig)
Thesis title: Creating useful tools for learning insect anatomy

Diana Ostrowski, M.S. Integrated BioSciences, 2009
Thesis title: White-tailed deer browsing and the conservation of forest songbirds and understory vegetation: A natural experiment within the Apostle Islands National Lakeshore

Angela Hodgson, Ph.D. Ecology, Evolution, and Behavior, 2010
Thesis title: Temporal changes in spatial patterns in a boreal ecosystem, causes and consequences

Lauren Hildebrandt, M.S., Integrated BioSciences, 2011
Thesis title: Decay and nutrient dynamics of wild rice litter in response to N and P availability and litter quality

Lee Sims, M.S. Integrated BioSciences, 2011
Thesis title: Light, nitrogen, and phosphorus effects on growth, allocation of biomass and nutrients, reproduction, and fitness in wild rice (*Zizania palustris* L.)

Angelique Edgerton, M.S. Integrated BioSciences, 2013
Thesis title: Structure of relict arctic plant communities along the north shore of Lake Superior

David Wedin, Postdoctoral Fellow, 1990 – 1992

Scott Bridgham, Postdoctoral Fellow, 1993 – 1995 (co-advised with C. Johnston)

Ron Moen, Postdoctoral Fellow, 1995 – 1998 (co-advised with Y. Cohen)

Terry Brown, Postdoctoral Fellow, 1997 – 2000 (co-advised with C. Johnston)

Thesis Opponent for the Following Ph.D. students

Otso Suominen, Ph.D. Biology, Turku University, Turku, Finland, 1999
Thesis title: Mammalian herbivores, vegetation, and invertebrate assemblages in boreal forests: feeding selectivity, ecosystem engineering and trophic effects

Johan Olofsson, Ph.D. Ecology and Environmental Science, Umeå University, Umeå, Sweden, 2001
Thesis title: Long term effects of herbivory on tundra ecosystems

Sari Stark, Ph.D. Biology, University of Oulu, Oulu, Finland, 2002
Thesis title: Reindeer grazing and soil nutrient cycling in boreal and tundra ecosystems

Caroline Lundmark, Ph.D. Wildlife, Fish, and Conservation, Swedish University of Agricultural Sciences, 2008
Thesis title: Morphological and behavioural adaptations of moose to climate, snow, and forage

Professional Service

National Science Foundation

Ad Hoc Reviewer for Ecosystems, Ecology, Long-Term Research in Environmental Biology, Computational Biology, Mathematics, Geography, Hydrology, and Polar Programs

Review Team, Louisiana State University's application to National Science Foundation's EPSCOR Program (January 1986)

Ecosystems Studies Panel (March 1989 – October 1991; reappointed October 2004 – October 2008)

Review Team, Central Plains Long-Term Ecological Research Site (June 1990)

Review Team, Jornada Long-Term Ecological Research Site (May 1991)

Terrestrial Ecology and Global Change (TECO) Research Panel (June 1995)

Research Training Centers Panel (April 1996)

Board, National Center for Ecological Analysis and Synthesis (September 1998 – September 1999)

Long Term Ecological Research Panel (April 2000; reappointed April 2010)

Biocomplexity Panel (June 2000)

Frontiers in Integrated Biological Research Panel (December 2002; reappointed November 2004)

Long-Term Research in Environmental Biology (LTREB) Workshop (September 2003)

Review Team, Coweeta Long-Term Ecological Research Site (June 2005)

Review Team, Bonanza Creek and Toolik Lake Long-Term Ecological Research Sites (June 2007)

Review Team, Virginia Coast Reserve Long-Term Ecological Research Site (September 2009)

National Academy of Sciences / National Research Council

Committee on Scholarly Communications with the People's Republic of China (March 1991 – December 1991)

Committee to Review the Environmental Protection Agency's Environmental Monitoring and Assessment Program (July 1991 – March 1995)

Committee to Review the U.S. Navy’s Extremely Low Frequency Submarine Communication Ecological Monitoring Program (March 1995 – June 1997)

Committee to Evaluate Indicators for Monitoring Aquatic and Terrestrial Environments (January 1997 – July 2000)

Review Coordinator for Progress Towards Adaptive Monitoring and Assessment for the Comprehensive Everglades Restoration Plan (September 2002 – February 2003)

Department of Interior

Review Team, Value of Downed Logs in Second Growth Douglas-Fir, Bureau of Land Management (August 1986)

Technical Advisor, U.S. Fish and Wildlife Service and Bell Museum, Endangered Species Exhibition (October 1993 – October 1994)

Department of Agriculture

Committee to Review U.S. Dept. of Agriculture's Research Initiative Program on Water Quality and Ecosystems (August 1993)

U.S. Dept. of Agriculture, National Research Initiative Program, Ecosystems Panel (March 1994)

Environmental Protection Agency

Review Team, Environmental Protection Agency's Research Initiative on Forest Ecosystems (March 1988)

Chair, Review Team, Corvallis Laboratory (August 2001)

NASA

Panel Member, Earth Observing System satellite (September 1988)

U.S. Congress

Testimony on Voyageurs National Park and Boundary Waters Wilderness, U.S. House of Representatives, Subcommittee on National Parks, Forests, and Lands (October 28, 1995 and July 16, 1996)

Testimony on Voyageurs National Park and Boundary Waters Wilderness, U.S. Senate, Committee on Energy and Natural Resources (July 18, 1996)

The White House

National Environmental Monitoring and Research Workshop, National Science and Technology Council (September 1996)

National Sciences and Engineering Research Council, Canada

Grant Selection Committee for Evolution and Ecology (August 1996 – June 1998)

State of Minnesota

Expert Witness on the Effects of Global Climate Change on Minnesota's Ecosystems, Attorney General's Office (1994)

Testimony on the Effects of Global Climate Change on Minnesota's Ecosystems, House Environmental Policy Committee (April 1998)

Local Governments

Co-Founder, City of Duluth Tree Commission (October 1994); Board Member (October 1994 – October 1999); Chair (October 1998 – October 1999)

City of Duluth Secondary Education Mathematics Curriculum Committee (October 1995 – October 1996)

City of Duluth Cities for Climate Protection Program, Steering Committee (November 2001 – October 2002)

University of Minnesota

Chair, Search Committee, Director of the Center for Water and the Environment, Natural Resources Research Institute (1990)

University of Minnesota Duluth Campus Planning Committee (1994)

College of Science and Engineering Executive Committee (May 1998-June 1999; reappointed September 2004 – June 2005)

Chair, Search Committee, Vertebrate Physiologist, Dept. of Biology (September 1998 – June 1999)

Research Ethics Advocates Committee (November 2000 – November 2001)

College of Science and Engineering Academic Standards Committee (September 2001 – 2002)

College of Science and Engineering Integrated Biosciences Program Executive Committee (June 2000 – May 2009)

College of Science and Engineering Single Semester Leave Committee (October 2003)

Chair, University of Minnesota Duluth Graduate Council (September 2004 – May 2005)

College of Science and Engineering Curriculum Committee (September 2007 – June 2009)

Office of Vice-President for Research, Research and Scholarship Advisory Panel (September 2010 – present).

Office of Vice-President for Research, Minnesota Futures Proposal Review Committee (June 2012).

Professional Journals and Societies

Member, Society of American Naturalists, American Mathematical Society, Ecological Society of America

Ad Hoc Reviewer for Science, Nature, Ecology, Forest Science, Canadian Journal of Forest Research, Canadian Journal of Botany, Biogeochemistry, Climatic Change, and other journals

Chair, Committee on Ecosystems and Macroscale Phenomenon, Society of Conservation Biology (April 1988).

Secretary, Association of Ecosystem Research Centers (November 1993 – November 1994)

Associate Editor, The American Naturalist (September 1990 – June 1994)

Associate Editor, Silva Fennica (December 1993 – December 1998)

Ad Hoc Associate Editor, Ecology (May 1994 – August 1996)

Associate Editor, Vegetatio (now Plant Ecology) (March 1995 – March 1998)

Associate Editor, Conservation Ecology (October 1995 – June 2004)

Associate Editor, Ecosystems (January 2001 – present)

R.H. MacArthur Award Committee, Ecological Society of America (2012)

Private Organizations

Joint Coordinating Committee, Climate Systems Modeling Initiative, University Corporation for Atmospheric Research (January 1989 – January 1990)

Technical Advisor, North Central Caribou Corporation (January 1992 – October 1995)

Board of Directors, Voyageurs Region National Park Association (January 1993 – January 2003)

Board of Directors, Sigurd Olson Environmental Institute, Northland College (May 1995 – September 1998)

Board of Directors, Biodiversity Fund, Duluth-Superior Area Community Foundation (October 2010-present)

Board of Trustees, Minnesota, South Dakota, and North Dakota Chapter of The Nature Conservancy (July 2013-present)

Symposia and Workshops, Co-Organizer

"Geomorphology and Ecosystem Processes," Ecological Society of America Annual Meeting, Syracuse, New York, August 1986 (co-organizer with D. Schimel)

"Sustainability of Boreal Regions: Sources and Consequences of Variability," MacArthur Foundation and the Beijer Institute, Itasca State Park, Minnesota, October 1997 (co-organizer with C.S. Holling and S. Light). The papers from this symposium were published in a special issue of *Conservation Ecology*.

"The Role of Large Herbivores in Ecosystem Processes", World Wildlife Fund, Hällnäs, Sweden, May 2002 (co-organizer with K. Danell). The papers from this symposium were published in Danell, K., R. Bergström, P. Duncan, and J. Pastor, (editors). 2006. *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*. Cambridge University Press, Cambridge, Great Britain.

"Mathematical Problems of Global Climate Change", Mathematical Biosciences Institute, Columbus, Ohio, June 2006. (co-organizer with D. Schimel and J. Harte).

"Modeling Nutrient Constraints: Stoichiometry of Cells, Populations, and Ecosystems", Society of Industrial and Applied Mathematics Conference on Applications of Dynamical Systems, Snowbird, Utah, May 2007 (co-organizer with B. Peckham).

Symposia and Workshops, Invited Speaker

"Predicting the Consequences of Intensive Forest Harvesting on Long-Term Productivity," Swedish University of Agricultural Sciences, Jaadrås, Sweden, May 1986

"Positive Feedbacks and the Global Carbon Cycle," Oak Ridge National Laboratory, Tennessee, May 1987

"Influence of Large Mammals on Ecosystem Processes," Symposium at the Ecological Society of America Annual Meeting, Columbus, Ohio, August 1987

"Ecology and Forest Policy for the Lake States," Society of American Foresters Annual Meeting, Minneapolis, Minnesota, October 1987

"Problems in Conservation Biology," Society of Conservation Biology, Hawk's Kay, Florida, June 1988

"Modeling Forest Response to Climatic Change," Scientific Committee on Problems of the Environment, Oxford, England, September 1988

"Ecology for a Changing Earth," National Science Foundation, Santa Fe, New Mexico, December 1988

"Climate Systems Modeling Initiative - First Workshop," University Corporation for Atmospheric Research, Boulder, Colorado, January 1989

"Production-decomposition linkages in northern forests and grasslands and response to climate change," Scientific Committee on Problems of the Environment, Woods Hole, Massachusetts, April 1989

"Explaining Records of Past Global Changes," Global Change Institute, Aspen, Colorado, July 1989

"New Perspectives for Watershed Management: Balancing Long-Term Sustainability with Cumulative Environmental Change," University of Washington and Oregon State University, Seattle, Washington, November 1990

"Hydrological-Geochemical-Biological Interactions in Forested Catchments," Gordon Conference, Holderness School, New Hampshire, July 1991

"Workshop on Northern Herbivory," National Science Foundation, LTER Program, Ecosystems Center, Woods Hole, Massachusetts, November 1992

"Biodiversity of Arctic and Alpine Tundra," Scientific Committee on Problems of the Environment, Kongsvold Biological Station, Oppdal, Norway, August 1993

“Functional Roles of Biodiversity: A Global Perspective,” Scientific Committee on Problems of the Environment, Asilomar, California, March 1994

“Ungulates in Temperate Forest Ecosystems,” Netherlands Institute for Forestry and Nature Research, Wageningen, The Netherlands, April 1995

“Control and Chaos,” National Science Foundation, Hawaii, June 1995

“Managing Ungulates as Components of Ecosystems,” The Wildlife Society Annual Conference, Portland, Oregon, September 1995

“Synthesis, Science, and Ecosystem Management,” National Center for Ecological Analysis and Synthesis, Santa Barbara, California, November 1996

“Hydrobiogeochemistry of Forested Catchments,” Gordon Conference, Colby-Sawyer College, New London, New Hampshire, August 1997

“Herbivore-Plant Interactions,” Third European Congress of Mammalogy, Jyväskylä, Finland, June 1999

“How Nutrient Cycles Constrain Carbon Balances in Boreal Forests and Arctic Tundra,” GCTE-IGBP, Abisko, Sweden, June 1999

“Understanding Ecosystems: The Role of Quantitative Models in Observation, Synthesis, and Prediction,” Cary Conference IX, Institute of Ecosystem Studies, Millbrook, New York, May 2001

“Third North American Forest Ecology Conference,” Duluth, Minnesota, June 2001

“Biogeochemistry of Wetlands,” Duke University Wetland Center, Durham, North Carolina, June 2001

“Twenty-fifth National Indian Timber Symposium” Intertribal Timber Council, Fond du Lac Reservation, Minnesota, June 2001

“Fifth International Moose Symposium”, Lillehammer, Norway, August 2002

“The Importance of Spatial Heterogeneity on Ecosystem Ecology”, Cary Conference X, Institute of Ecosystem Studies, Millbrook, New York, May 2003

“Third ManOMin Watershed Conference: Rainy River Basin”, International Falls, Minnesota, November 2003

“New Directions in Research in Grazing Ecology”, The Macaulay Institute, Aberdeen, Scotland, December 2003

“Novel Approaches to Climate Change”, Aspen Institute of Physics, Aspen, Colorado, June 2005

“Wild Rice Roundtable”, Ecological Society of America Annual Meeting, Milwaukee, Wisconsin, Aug. 4, 2008

"Understanding the Vegetation and Hydrology of Upper Midwest Wetlands", Fond du Lac Band of Lake Superior Ojibway, Carlton, MN, Sept. 22, 2010.

Research Grant Support

Dept. of Energy, "Changes in forest carbon storage with intensive management and climatic change," $93,567 (1985 – 1987). To Pastor

Environmental Protection Agency, "Factors controlling the recovery of aquatic systems from disturbance," $221,032 (1986 – 1987). To Niemi, Naiman, and Pastor

National Science Foundation, "The effects of large mammal browsing on the dynamics of northern ecosystems," $258,645 (1987-1989) to Pastor and Naiman; $419,170 (1989 – 1992) to Pastor and Mladenoff

National Science Foundation, "Reconstructing forest stand histories and soil development from paleoecological evidence," $405,000 (1987 – 1989). To Davis and Pastor

National Science Foundation, "A cooperative facility for research on the ecology of spatial heterogeneity," $403,066 (1988 – 1990). To Johnston and Pastor

Dept. of Energy, "Response of northern ecosystems to global change," $45,150 (1989). To Pastor, Gorham, and Shaver

National Science Foundation, "Animal influences on the aquatic landscape: vegetative patterns, successional transitions, and nutrient dynamics," $430,974 (1989 – 1992). To Naiman, Johnston, and Pastor and $660,000 (1992-1995) to Johnston and Pastor

NASA, "Regional modeling of trace gas production in grassland and boreal ecosystems," $240,000 (1989 – 1992). To Johnston and Pastor

Legislative Commission on Minnesota's Resources, "The relationship between heavy metal biogeochemistry and airborne spectral radiometry as an exploration method," $250,000 (1989 – 1991). To Hauck and Pastor

U.S. Forest Service and The Nature Conservancy, "A landscape approach to biological diversity management using geographic information systems and a forest succession model," $32,000 (1989 – 1991). To Mladenoff and Pastor

U.S. Forest Service and The North Central Caribou Corporation, "Woodland caribou assessment of northern Minnesota," $40,000 (1990 – 1991). To Pastor and Mladenoff

National Science Foundation, "The use of fractal and chaos theory to verify, simplify, and extend forest ecosystem models," $220,975 (1991 – 1993). To Cohen and Pastor

National Science Foundation, "Spatial modelling of forest ecosystem landscapes and bird species diversity," $200,000 (1994 – 1996). To Cohen, Pastor, and Niemi

U.S. Forest Service, "Investigating ecological and economic interactions between soil and forest conditions and harvesting regimes on the Chippewa National Forest," $25,000 (1992 – 1993). To Pastor and Mladenoff

National Science Foundation, "Moose foraging strategy, energetics, and ecosystem processes in boreal landscapes," $90,000 (1993 – 1994). To Pastor, Mladenoff, and Cohen

National Science Foundation, "Long-term dynamics of moose populations, community structure, and ecosystem properties on Isle Royale," $250,000 (1993 – 1998). To Pastor, Mladenoff and Cohen

National Science Foundation, "Direct and indirect effects of climate change on boreal peatlands," $800,000 (1993 – 1997). To Bridgham, Pastor, Malterer, and Janssens

National Science Foundation, "Landscape control of trophic structure in arctic Alaskan lakes," $200,000 (1995 – 1997). To Hershey, McDonald, Pastor, and Richards

Legislative Commission on Minnesota's Resources, "Forest management to maintain structural and species diversity," $160,000 (1995 – 1997). To Pastor and Rusterholz

National Science Foundation, "Moose foraging strategy, energetics, and ecosystem processes in boreal landscapes," $765,000 (1995 – 2000). To Pastor and Cohen

National Science Foundation, "Grizzly bear digging in subalpine meadows: Influences on plant distributions and nitrogen availability," $111,549 (1995 – 1998). To Stanford and Pastor

National Science Foundation, "Control of productivity and plant species segregation by nitrogen fluxes to wetland beaver meadows," $600,000 (1997 – 2000). To Johnston, Pastor, and Mooers

National Science Foundation, “Carbon and energy flow and plant community response to climate change in peatlands,” $1,200,000 (1997-2001). To Bridgham, Pastor, and Chen

National Science Foundation, “Moose population cycles, ecosystem properties, and landscape patterns on Isle Royale,” $300,000 (1998 – 2003). To Pastor, Cohen, Moen, and Dewey

NASA, “Mapping and modeling forest change in a boreal landscape,” $350,000 (2000 – 2003). To Pastor and Wolter

National Science Foundation, “Wild rice population dynamics and nutrient cycles.” $543,046 (2002 – 2006). To Pastor

National Science Foundation, “LTREB: Spatial dynamics of the moose-forest-soil ecosystem on Isle Royale.” $300,000 (2004 – 2009). To Pastor and Cohen

National Science Foundation, “OPUS: A synthesis of long-term research on moose-boreal forest interactions.” $143,911 (2007 – 2009). To Pastor and Cohen

National Science Foundation, “GK-12: Graduate Fellows in Science and Mathematics Education.” $2,931,828 (2007 – 2011). To Latterell, Hale, Munson, Morton, and Pastor

National Science Foundation, “Wild rice population oscillations, allocation patterns, and nutrient cycling.” $547,000 (2007 – 2012). To Pastor and Lee

Biodiversity Fund, Duluth-Superior Area Community Foundation. “Tundra conservation and monitoring along the North Shore of Lake Superior”, $8,396 (2011-2012). To Pastor

Minnesota Pollution Control Agency, “Wild rice sulfate standards study”, $88,000 (2012-2014). To Pastor

Minnesota Sea Grant, “The biogeochemical habitat of wild rice”. $200,000 (2014-2016). To Pastor, Johnson, and Cotner

Books

Danell, K., R. Bergström, P. Duncan, and J. Pastor, (editors). 2006. *Large Mammalian Herbivores, Ecosystem Dynamics, and Conservation*. Cambridge University Press, Cambridge, Great Britain.

Pastor, J. 2008. *Mathematical Ecology of Populations and Ecosystems*. Blackwell, Oxford, Great Britain.

Peer-reviewed Journal Articles

Pastor, J., and J.G. Bockheim. 1980. Soil development on moraines of the Taylor Glacier, Lower Taylor Valley, Antarctica. Soil Science Society of America Journal 44: 341-348.

Pastor, J., and J.G. Bockheim. 1981. Biomass and production of an aspen-mixed hardwood-spodosol ecosystem in northern Wisconsin. Canadian Journal of Forest Research 11: 132-138.

Aber, J.D., J. Pastor, and J.M. Melillo. 1982. Changes in forest canopy structure along a site quality gradient in southern Wisconsin. American Midland Naturalist 108: 256-265.

Pastor, J., J.D. Aber, C.A. McClaugherty, and J. Melillo. 1982. Geology, soils, and vegetation of Blackhawk Island, Wisconsin. American Midland Naturalist 198: 266-277.

Pastor, J., J.D. Aber, and J.M. Melillo. 1984. Biomass prediction using generalized allometric regressions for some northeast tree species. Forest Ecology and Management 7: 256-274.

Pastor, J., J.D. Aber, C.A. McClaugherty, and J.M. Melillo. 1984. Aboveground production and N and P cycling along a nitrogen mineralization gradient on Blackhawk Island, Wisconsin. Ecology 65: 256-268.

Pastor, J., and J.G. Bockheim. 1984. Distribution and cycling of nutrients in an aspen-mixed hardwood-spodosol ecosystem in northern Wisconsin. Ecology 65: 339-353.

Pastor, J., and W.M. Post. 1984. Calculating Thornthwaite's and Mather's actual evapotranspiration using an approximating function. Canadian Journal of Forest Research 13: 466-477.

McClaugherty, C.A., J. Pastor, J.D. Aber, and J.M. Melillo. 1985. Forest litter decomposition in relationship to soil nitrogen dynamics and litter quality. Ecology 66: 266-275.

Post, W.M., J. Pastor, P. Zinke, and A. Stangenberger. 1985. Global patterns of soil nitrogen storage. Nature 317: 613-616.

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, C.A. McClaugherty, and J. Pastor. 1985. Fine root turnover in forest ecosystems in relation to quantity and forms of nitrogen availability: a comparison of two methods. Oecologia 66: 317-321.

Pastor, J., and W.M. Post. 1986. Influence of climate, soil moisture, and succession on forest carbon and nitrogen cycles. Biogeochemistry 2: 3-27.

Binkley, D., J.D. Aber, J. Pastor, and K.J. Nadelhoffer. 1986. Nitrogen availability in some Wisconsin forests: comparisons of resin bags and on-site incubations. Biology and Fertility of Soils 2: 77-82.

Norby, R.J., J. Pastor, and J.M. Melillo. 1986. Carbon-nutrient interactions in response to CO_2 enrichment: physiological and long-term perspectives. Tree Physiology 2: 233-242.

Pastor, J., M.A. Stillwell, and D. Tilman. 1987. Nitrogen mineralization and nitrification in four Minnesota old fields. Oecologia 71: 481-485.

Pastor, J., M. A. Stillwell, and D. Tilman. 1987. Little bluestem litter dynamics in Minnesota old fields. Oecologia 72: 327-330.

Pastor, J., R.H. Gardner, V.H. Dale, and W.M. Post. 1987. Successional changes in soil nitrogen availability as a potential factor contributing to spruce dieback in boreal North America. Canadian Journal of Forest Research 17: 1394-1400.

Pastor, J., R.J. Naiman, and B. Dewey. 1987. A hypothesis of the effects of moose and beaver foraging on soil nitrogen and carbon dynamics, Isle Royale. Alces 23: 107-124.

Pastor, J. and W.M. Post. 1988. Response of northern forests to CO_2-induced climatic change. Nature 334: 55-58.

[*]Pastor, J., R.J. Naiman, B. Dewey, and P. McInnes. 1988. Moose, microbes, and the boreal forest. BioScience 38: 770-777.

[†]Naiman, R.J., H. Décamps, J. Pastor, and C.A. Johnston. 1988. The potential importance of boundaries to fluvial ecosystems. Journal of the North American Benthological Society 7: 289-306.

O'Neill, R.V., D.L. DeAngelis, J. Pastor, B.J. Handley, and W.M. Post. 1989. Multiple nutrient limitations in ecological processes. Ecological Modeling 46: 147-163.

Pastor, J. and M. Broschart. 1990. The spatial pattern of a northern conifer-hardwood landscape. Landscape Ecology 4: 55-68.

Cohen, Y. and J. Pastor. 1991. The responses of a forest ecosystem model to serial correlations of global warming. Ecology 72: 1161-1165.

Ågren, G.I., R.E. McMurtrie, W.J. Parton, J. Pastor, and H.H. Shugart. 1991. State-of-the-art of models of production-decomposition linkages in conifer and grassland ecosystems. Ecological Applications 1: 118-138.

Bryant, J.P., F.D. Provenza, J. Pastor, P.B. Reichardt, T.P. Clausen, and J.T. du Toit. 1991. Interactions between woody plants and browsing mammals mediated by secondary metabolites. Annual Review of Ecology and Systematics 22: 431-446.

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[*] Included in the anthology *Readings in Ecology*, S. I. Dodson et al. (editors). Oxford University Press, 1999.

[†] Included in the anthology *Foundation Papers in Landscape Ecology*, J. Wiens et al. (editors). Columbia University Press, 2006.

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, J. Pastor, and R. Boone. 1991. Factors controlling nitrogen cycling and nitrogen saturation in northern temperate forest ecosystems. Ecological Applications 1: 303-315.

Moen, R., J. Pastor, and Y. Cohen. 1991. Effects of moose and beaver on the vegetation of Isle Royale National Park. Alces 26: 51-63.

Pastor, J. and R.J. Naiman. 1992. Selective foraging and ecosystem processes in boreal forests. The American Naturalist 139: 690-705.

Post, W.M., J. Pastor, A.W. King, and W.R. Emanuel. 1992. Aspects of the interaction between vegetation and soil under global change. Water, Air, and Soil Pollution 64:345-363.

McInnes, P.F., R.J. Naiman, J. Pastor, and Y. Cohen. 1992. Effects of moose browsing on vegetation and litterfall of the boreal forest, Isle Royale, Michigan, USA. Ecology 73: 2059-2075.

Pastor, J., B. Dewey, R.J. Naiman, P.F. McInnes, and Y. Cohen. 1993. Moose browsing and soil fertility in the boreal forests of Isle Royale National Park. Ecology 74:467-480.

Pastor, J. and W.M. Post. 1993. Linear regressions do not predict the transient responses of eastern North American forests to CO_2 induced climate change. Climatic Change 23:111-119.

Geng Xiaoyuan, J. Pastor, and B. Dewey. 1993. Studies on leaf decomposition of some tree species on Changbai Mountain. Acta Phytoecologica et Geobotanica Sinica 17:90-96 [in Chinese].

Mladenoff, D.J., M.A. White, J. Pastor and T.R. Crow. 1993. Comparing spatial pattern in unaltered old-growth and disturbed forest landscapes for biodiversity design and management. Ecological Applications 3:294-306.

Hershey, A.E., J. Pastor, B.J. Peterson, and G.W. Kling. 1993. Stable isotopes resolve the drift paradox for *Baetis* mayflies in an arctic river. Ecology 74:2315-2326.

Alban, D.H. and J. Pastor. 1993. Decomposition of aspen, spruce, and pine boles on two sites in Minnesota. Canadian Journal of Forest Research 23: 1744-1749.

Geng Xiaoyuan, J. Pastor, and B. Dewey. 1993. Decay and nitrogen dynamics of litter from disjunct, congeneric tree species in Wisconsin and northeastern China. Canadian Journal of Botany 71: 693-699.

Wedin, D.A. and J. Pastor. 1993. Nitrogen mineralization dynamics in grass monocultures. Oecologia 96: 186-192.

Frelich, L.E., R.R. Calcote, M.B. Davis, and J. Pastor. 1993. Patch formation and maintenance in an old-growth hemlock-hardwood forest. Ecology 74: 513-527.

Mladenoff, D.J., M.A. White, T.R. Crow, and J. Pastor. 1994. Applying principles of landscape design and management to integrate old-growth forest enhancement and commodity use. Conservation Biology 8: 752-762.

Updegraff, K., J. Pastor, S.D. Bridgham, and C.A. Johnston. 1995. Environmental and substrate quality controls over carbon and nitrogen mineralization in a beaver meadow and a bog. Ecological Applications 5: 151-163.

Bridgham, S.D., J. Pastor, C.A. McClaugherty and C.J. Richardson. 1995. Nutrient-use efficiency: a litterfall index, a model, and a test along a nutrient availability gradient in North Carolina peatlands. The American Naturalist 145: 1-21.

Wedin, D.A., L.L. Tieszen, B. Dewey, and J. Pastor. 1995. Carbon isotope dynamics during grass decomposition and soil organic matter formation. Ecology 76: 1383-1392.

Bridgham, S.D. C.A. Johnston, J. Pastor, and K. Updegraff. 1995. Potential feedbacks of northern wetlands on climate change. Bioscience 45: 262-274.

Chapin, C.T. and J. Pastor. 1995. Nutrient limitations in the northern pitcher plant *Sarracenia purpurea*. Canadian Journal of Botany 73: 728-734.

Pastor, J., B. Dewey, and D. Christian. 1996. Carbon and nutrient mineralization and fungal spore composition of vole fecal pellets in Minnesota. Ecography 19: 52-61.

Post, W.M. and J. Pastor. 1996. Linkages - an individual-based forest ecosystem model. Climatic Change 34: 253-261.

Bridgham, S.D., J. Pastor, J.A. Janssens, C. Chapin, and T. J. Malterer. 1996. Multiple nutrient limitations in peatlands: a call for a new paradigm. Wetlands 16: 45-65.

Moen, R., J. Pastor, Y. Cohen, and C.C. Schwartz. 1996. Effect of moose movement and habitat use on GPS collar performance. Journal of Wildlife Management 60: 659-668.

Cohen, Y., and J. Pastor. 1996. Interactions among nitrogen, carbon, plant shape, and photosynthesis. The American Naturalist 147: 847-865.

Sarkar, S., Y. Cohen, and J. Pastor. 1996. Mathematical formulation and parallel implementation of a spatially explicit ecosystem control model. In: Conference Proceedings, Grand Challenges in Computer Simulations, Society for Computer Simulation, New Orleans.

Pastor, J., A. Downing, and H. E. Erickson. 1996. Species-area curves and diversity-productivity relationships in beaver meadows of Voyageurs National Park, U.S.A. Oikos 77: 399-406.

Keenan, R.J., C.E. Prescott, J.P. Kimmins, J. Pastor, and B. Dewey. 1996. Litter decomposition in western red cedar and western hemlock forests on northern Vancouver Island, British Columbia. Canadian Journal of Botany 74: 1626-1634.

Moen, R., J. Pastor, and Y. Cohen. 1997. A spatially-explicit model of moose foraging and energetics. Ecology 78: 505-521.

Moen, R., J. Pastor, and Y. Cohen. 1997. Accuracy of GPS telemetry collar location with differential correction in theory and practice. Journal of Wildlife Management 61: 530-539.

Moen, R., J. Pastor, and Y. Cohen. 1997. Interpreting behavior from activity counters in GPS collars on moose. Alces 32: 101-108.

Pastor, J. and Y. Cohen. 1997. Herbivores, the functional diversity of plants species, and the cycling of nutrients in ecosystems. Theoretical Population Biology 51: 165 -179.

Pastor, J., R. Moen, and Y. Cohen. 1997. Spatial heterogeneities, carrying capacity, and feedbacks in animal-landscape interactions. Journal of Mammalogy 78: 1040-1052.

Moen, R., Y. Cohen, and J. Pastor. 1998. Evaluating foraging strategies with a moose energetics model. Ecosystems 1: 52-63.

Moen, R. and J. Pastor. 1998. Simulating antler growth and energy, nitrogen, calcium, and phosphorus metabolism in caribou. Rangifer Special Issue No. 10: 85-97.

Bridgham, S. D., K. Updegraff, and J. Pastor. 1998. Carbon, nitrogen, and phosphorus mineralization in northern wetlands. Ecology 79: 1545-1561.

Jordan, P.A., J.L. Nelson, and J. Pastor. 1998. Progress towards the experimental reintroduction of woodland caribou to Minnesota and adjacent Ontario. Rangifer Special Issue No. 10: 169-181.

Pastor, J. and D. Binkley. 1998. Nitrogen fixation and the mass balances of carbon and nitrogen in ecosystems. Biogeochemistry 43: 63-78.

Pastor, J., B. Dewey, R. Moen, M. White, D. Mladenoff, and Y. Cohen. 1998. Spatial patterns in the moose-forest-soil ecosystem on Isle Royale, Michigan, USA. Ecological Applications 8: 411-424.

Updegraff, K., S.D. Bridgham, J. Pastor, and P. Weishampel. 1998. Hysteresis in the temperature response of carbon dioxide and methane production in peat soils. Biogeochemistry 43: 253-272.

Hale, C.M. and J. Pastor. 1998. Nitrogen content, decay rates, and decompositional dynamics of hollow versus solid hardwood logs in old-growth and mature hardwood forests of Minnesota, U.S.A. Canadian Journal of Forest Research 28: 1276-1285.

Pastor, J., S. Light, and L. Sovell (editors). 1998. Sustainability and Resilience in Boreal Regions: Sources and Consequences of Variability. Conservation Ecology 2 (Special Issue).

Moen, R., J. Pastor, and Y. Cohen. 1999. Antler growth and extinction of the Irish elk. Evolutionary Ecology Research 1: 235-249.

Cohen, Y., J. Pastor, and R. Moen. 1999. Bite, chew, and swallow. Ecological Modelling 116: 1-14.

Pastor, J. and S.D. Bridgham. 1999. Nutrient efficiency along nutrient availability gradients. Oecologia 118: 50-58.

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John Pastor Technical Review Comments - Wild Rice Rule
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Attachment B

(16 pages)

Ecological Applications, 27(1), 2017, pp. 321–336

Effects of sulfate and sulfide on the life cycle of *Zizania palustris* in hydroponic and mesocosm experiments

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Abstract. Under oxygenated conditions, sulfate is relatively non-toxic to aquatic plants. However, in water-saturated soils, which are usually anoxic, sulfate can be reduced to toxic sulfide. Although the direct effects of sulfate and sulfide on the physiology of a few plant species have been studied in some detail, their cumulative effects on a plant's life cycle through inhibition of seed germination, seedling survival, growth, and seed production have been less well studied. We investigated the effect of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris* L.) in hydroponic solutions and in outdoor mesocosms with sediment from a wild rice lake. In hydroponic solutions, sulfate had no effect on seed germination or juvenile seedling growth and development, but sulfide greatly reduced juvenile seedling growth and development at concentrations greater than 320 μg/L. In outdoor mesocosms, sulfate additions to overlying water increased sulfide production in sediments. Wild rice seedling emergence, seedling survival, biomass growth, viable seed production, and seed mass all declined with sulfate additions and hence sulfide concentrations in sediment. These declines grew steeper during the course of the 5 yr of the mesocosm experiment and wild rice populations became extinct in most tanks with concentrations of 250 mg SO_4/L or greater in the overlying water. Iron sulfide precipitated on the roots of wild rice plants, especially at high sulfate application rates. These precipitates, or the encroachment of reducing conditions that they indicate, may impede nutrient uptake and be partly responsible for the reduced seed production and viability.

Key words: hydroponics; life cycles; sulfate; sulfide; toxicity; wetlands; wild rice; Zizania palustris.

Introduction

Under oxygenated conditions, sulfate, the most abundant form of dissolved sulfur in aquatic systems, is relatively non-reactive, and is therefore relatively non-toxic. However, where oxygen is absent and organic matter is present, sulfate can serve as an electron acceptor for heterotrophic microbial metabolism, producing reactive reduced sulfur species. When sulfate concentrations limit the activity of sulfur-reducing microbes, an increase in sulfate can enhance the decomposition of organic matter and initiate a cascade of interrelated biogeochemical reactions (Garrels and Christ 1965) that alter the bioavailability of phosphorus and other nutrients (Lamers et al. 2002), and generate alkalinity (Giblin et al. 1990). One of the most reactive products of sulfate reduction is hydrogen sulfide, which we here term "sulfide." If dissolved sulfide persists in the rooting zone of aquatic plants, it can inhibit root growth and metabolism (Mendelssohn and McKee 1988, Koch and Mendelssohn 1989, Koch et al. 1990, Lamers et al. 2002, 2013, Gao et al. 2003, Armstrong and Armstrong 2005, Geurts et al. 2009, Martin and Maricle 2015) and photosynthesis (Pezeshki 2001). If root biomass and metabolism are reduced by elevated sulfide concentrations, then the plant's ability to take up limiting nutrients may be impaired (DeLaune et al. 1983, Koch et al. 1990, Gao et al. 2002, 2003, Armstrong and Armstrong 2005, Lamers et al. 2013).

Although the direct effects of sulfide on the physiology of individual plants of a few species have been studied in some detail, the cumulative effects of sulfide on a plant's life cycle through possible inhibition of seed germination, seedling survival, and seed production have been less well studied. Sulfide could affect any or all of these stages of a plant's life cycle, either directly by toxicity to seeds and seedlings or indirectly by decreasing nutrient uptake through roots during seed formation. If so, then populations may become sparser and less viable over several life cycles. Population effects could be realized rapidly in non-clonal annual aquatic emergent plant species that

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rely exclusively on seed production, germination, and seedling survival to produce the next generation of emergent shoots. A seed bank in the sediment would facilitate recovery of a population after one or two catastrophic growing seasons, but would become depleted if chronic sulfide toxicity does not allow occasional successful growth and reproduction to restock the seed bank.

Northern wild rice (*Zizania palustris* L., hereafter wild rice) is an annual graminoid (Family Poaceae, Tribe Oryzeae), which is most abundant in the rivers and lakes in the Lake Superior region. Because of its widespread distribution and tendency to form large monotypic stands, wild rice is an important component of the food supply for the aquatic and avian herbivores and seed consumers, such as muskrats and waterfowl. Reduction of these wild rice populations could, therefore, have cascading effects on diverse aquatic food webs. In addition, the native Ojibwe people of the Lake Superior and Lake Michigan region teach that they were led to this region to find "the food that grows upon the water," which is wild rice. The Ojibwe identify their origins with wild rice and consider themselves "people of the rice" (Vennum 1998). The resource is also important to Menominee and Dakota peoples of the region. Efforts to enhance the productivity, perpetuation, and restoration of natural wild rice populations are of great importance to state and tribal natural resource agencies for both ecological and cultural reasons.

The wild rice life cycle begins when seeds from the previous year or years germinate in mid to late May. Juvenile seedlings grow through the water column in early to mid-June. Upon reaching the surface, the seedling generates a floating leaf that fixes carbon into carbohydrates for root production and nutrient uptake. By the end of June, nitrogen and other nutrients are translocated out of the floating leaf into an aerial shoot emerging from the leaf axil, and the floating leaf dies. The early stages of the vegetative growth of the aerial shoot happen during the next two weeks and vegetative growth continues until the emergence of flowering heads in late July. Seed production and ripening begins in early to mid-August with seed production completed by early- to mid-September. The productivity of wild rice is primarily limited by nitrogen and secondarily by phosphorus; increased nitrogen supply accelerates development of the life cycle and reduces allocation to roots (Sims et al. 2012*a*) and increases the number of inflorescences, seeds per inflorescence, and mean seed mass, resulting in more seedlings produced the following year, and hence greater fitness (Sims et al. 2012*b*).

Historic observations suggested that wild rice usually occurs in waters where sulfate concentrations were near or below 10 mg/L and populations are uncommon where sulfate concentrations exceeded 50 mg/L (Moyle 1944, 1945). Based on Moyle's (1944, 1945) research, the State of Minnesota sulfate standard for waterbodies supporting wild rice is 10 mg/L; Wisconsin, Michigan, and Ontario currently do not have sulfate standards for wild rice waters. For comparison, the EPA non-enforceable, aesthetic (taste) secondary water quality sulfate standard for human consumption is 250 mg/L (*available online*).[7]

This research is part of a larger study coordinated by the Minnesota Pollution Control Agency on the effect of sulfate on wild rice, which included an extensive survey of potential wild rice waters across Minnesota containing surface water sulfate ranging from <2 mg/L to >600 mg/L. This study was carried out because of recent interest in the nature of the relationship between sulfate and wild rice, especially with respect to potential anthropogenic sulfate enhancements to wild rice ecosystems such as sewage treatment plants, agricultural runoff, and mining of ores containing metallic sulfides. The mechanisms responsible for the decreased wild rice density with increased sulfate concentrations observed by Moyle (1944, 1945) have not been investigated until this study.

Although we have a fairly extensive understanding of the general aspects of the life cycle of wild rice in natural stands in relation to nutrient availability and sediment chemistry (Keenan and Lee 1988, Day and Lee 1990, Meeker 1996, Lee 2002, Pastor and Walker 2006, Walker et al. 2010, Hildebrandt et al. 2012, Sims et al. 2012*a, b*), the way in which sulfate in surface water can affect the life cycle of wild rice, and hence its population dynamics, is much less well understood. The objectives of our research are to (1) determine the relative effects of sulfate and sulfide on seed germination, seedling viability, vegetative growth, and seed production; (2) determine the response of wild rice populations and population viability to sulfate in the overlying water and the production of sulfide in sediment porewaters.

METHODS

The effects of sulfate and sulfide on wild rice were tested in two different ways: (1) a laboratory hydroponic culture system and (2) an outdoor mesocosm system that better mimicked natural wild rice waters, but does not control the chemical exposures as precisely as the hydroponic experiments did. Short-term (10 or 11 days) hydroponic exposures of seeds and seedlings to sulfate and sulfide were conducted to examine effects on seed germination, seedling growth, and survival. Full life cycle tests were conducted in mesocosms where wild rice grew in sediment taken from a natural wild rice lake. These multi-year outdoor tests examined the effects of elevated surface water sulfate and the associated increased sedimentary sulfide concentrations on germination, survival, growth, and reproduction.

Hydroponic experiments

Li et al. (2009) published one of the few dose-response studies of aquatic macrophytes (*Typha* and *Cladium*) to sulfide, which requires the maintenance of anaerobic

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[7] http://water.epa.gov/drink/contaminants/secondarystandards.cfm

conditions. Malvick and Percich (1993) developed a simple hydroponic system to investigate effects of nutrients on germination and early growth of wild rice, but their system could only be implemented under aerobic conditions. We used these two studies as starting points for the development of our methods.

Wild rice seeds used for all hydroponic experiments were collected on 30 August 2012 from Little Round Lake (Minnesota Lake ID 03-0302, 46.97° N, 95.74° W; average surface water sulfate <0.5 mg/L and porewater sulfide = 77 μg/L, $n = 5$). The seeds were stored at 4°C in polyethylene bottles in a darkened room until needed for experiments. Immediately before each experiment, a subsample of these seeds was selected that were intact, filled, not green (unripe), and not moldy. To obtain seedlings for juvenile seedling response to sulfate or sulfide, the selected seeds were allowed to germinate in aerobic deionized water until a 1–2 cm long mesocotyl shoot appeared, which usually occurred 5–7 days after germination. The mesocotyl is the embryonic stem that will develop into the mature stem.

Once the seeds or seedlings were selected, they were picked up with forceps and transferred to the appropriate test in appropriate containers. The hydroponic solution was one-fifth strength Hoagland's solution in 5 mmol/L PIPES buffer to maintain a pH of 6.8 ± 0.03 (mean ± SD) in the solution, similar to that observed in the porewater of mesocosm experiments. Nitrogen was supplied only as ammonium (0.16 mmol/L NH_4Cl) to mimic natural concentrations of inorganic nitrogen in wild rice waters (Walker et al. 2010). The Hoagland's solution contained sulfate only in trace amounts as $ZnSO_4$ (0.5 μmol/L) and $CuSO_4$ (0.15 μmol/L). This nutrient solution was then augmented with appropriate amounts of anhydrous Na_2SO_4 or $Na_2S·9H_2O$ to achieve desired sulfate or sulfide treatment concentrations. The one-fifth Hoagland's solution and PIPES buffer were chosen based on previous trials to determine proper strengths and buffers that would support seedling growth without adverse effects (see Appendix S1 for composition of our modification of Hoagland's Solution).

Germination of wild rice seeds under aerobic conditions subject to various concentrations of sulfate.—The selected seeds were placed into each of six numbered plastic cups to total 50 seeds each, then randomly assigned and transferred to each of six 1-pint Mason jars (1 pint = 473 mL) containing six sulfate treatment concentrations of 0 (trace), 10, 50, 100, 400, or 1600 mg SO_4/L. These sulfate treatments (trace to 1600 mg/L) bracket the large range encountered across Minnesota's geologically diverse landscape (10th and 90th percentiles of 0.2 and 285 mg/L, respectively; MPCA 2016), plus some mine pits over 1000 mg/L that may overflow into wild rice waters. This seed counting and random transfer was repeated twice more to result in six treatment levels with three replicate jars per treatment. The jars were covered with plastic covers fitted with rubber stoppers to facilitate solution exchanges. Two holes in the plastic lids were left open to facilitate air exchange and to prevent the solutions from becoming anaerobic. The experiment proceeded in a growth chamber at 20°C in the dark to simulate conditions measured in sediments during the growing season, which we have measured in our mesocosms (see *Results*). The solutions were exchanged with fresh solution of the appropriate treatment concentration every three days. Dissolved oxygen in the solutions across all treatments was initially 8.280 ± 0.218 mg/L (mean ± SD) and dropped to 2.85 ± 0.60 mg/L by the end of three days, still well above anoxic levels required for production of sulfide. Solution pH and sulfate were measured on each initial batch of sulfate treatment and on the exchanged solution from each jar. The germinated seedlings were harvested after 11 days. The number of successfully germinated seeds, determined as those that produced a mesocotyl at least 1 cm in length, were counted. The length of the mesocotyl was measured for each seed. The germinated seeds were then dried at 65°C for 3 d. The mesocotyl was then carefully separated from the seed hull and weighed.

Germination of wild rice seeds under anoxic conditions subject to various concentrations of sulfide.—The techniques used here were the same as for the germination trials under various sulfate concentrations, except that extra care was necessary to ensure anaerobic conditions. Fifty seeds were chosen as above and then placed in 700 mL borosilicate glass bottles capped using phenolic screw caps with chlorobutyl septa 5 mm thick. The one-fifth Hoagland's nutrient solution was deoxygenated with oxygen-scrubbed nitrogen before being added to the bottles. PIPES buffer was added to the test solution to maintain consistent pH levels of 6.8 ± 0.03 throughout an experiment. Bottles were filled completely with the deoxygenated nutrient solution and without introducing any air bubbles and then capped with the septa. Stock sulfide solutions (20–30 mmol/L) were prepared as needed by adding $Na_2S·9\ H_2O$ (sodium sulfide nonahydrate) to deionized and deoxygenated water. The concentration of the stock sulfide solution was checked periodically against a stock solution that had been standardized using an iodimetric titration. An appropriate amount of the stock solutions was added to each bottle with a Hamilton gas-tight glass syringe through the septa while simultaneously withdrawing an equivalent volume of the Hoagland's solution by means of a second syringe through the septum. All of the syringes used in this and other experiments were purged three times with oxygen-scrubbed ultra-pure nitrogen from a tilled PVDF gas sampling bag (Saint-Gobain No. D1075016-10), which had also been purged three times before filling. Added stock sulfide solution volumes range between 0.2 and 3.0 mL depending on target exposure concentrations and the nominal concentration of stock sulfide solution. The target sulfide concentrations were 0 (trace), 96, 320, 960, and 2880 μg/L. These sulfide treatments (trace to 2880 μg/L) bracket the range encountered across shallow aquatic systems in Minnesota that potentially could host wild rice (5th and 95th percentiles of 26 and 1631 μg/L, n = 108; A. Myrbo, *unpublished data*).

The bottles were placed in a growth chamber in continuous darkness at 20° ± 1°C. Solutions were exchanged every two days if during the week or three days if over a weekend. The solution in each jar was sampled for sulfide analysis at the beginning and end of each two- or three-day cycle. The pH of the solution in each jar was measured at the end of each two- or three-day cycle. To obtain the initial pH of the solution, one additional replicate jar for each treatment but without seeds was filled with one-fifth Hoagland's solution, then the sulfide treatment was added using syringes as above and the jar was opened and pH was measured immediately. Total dissolved sulfide (H_2S + HS^-) was measured on a Hach DR5000 spectrophotometer using a colorimetric methylene blue method (4500 S2-D; Eaton et al. 2005) as implemented with Hach method 8131. The method was adapted for a lower detection limit (~15 μg/L) using a photo cell with a 5 cm path length. All measurements of dissolved sulfide in both hydroponics and mesocosm experiments refer to the sum of all dissolved inorganic reduced sulfur (H_2S + HS^-). The samples of hydroponic water were added directly from the gas tight syringe to the sulfuric acid reagent, followed immediately by the potassium dichromate reagent. After 11 days, the germinated seeds were harvested and measured as described for the experiments on effects of sulfate on germination.

Growth of juvenile wild rice seedlings under aerobic conditions subject to various concentrations of sulfate.— We examined growth of juvenile seedlings at concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L. Twenty replicated 70-mL unsealed glass Kimax tubes (Cole-Parmer, Vernon Hills, IL, USA) were used for each test concentration. One seedling germinated and selected as described was placed with forceps into each Kimax tube, which was then filled with one-fifth Hoagland's solution and an appropriate amount of sulfate. The filled tubes (solution and seed) were placed into every other opening in Nalgene Resmer (ThermoFisher Scientific, Waltham, MA, USA) test tube holding racks so that light could penetrate to all sides of each tube. A total of six 40-tube racks, each containing 20 tubes, were used to hold the test tubes. Screw caps were placed loosely on the tubes to allow for oxygen exchange across the solution surface and thereby prevent the development of anaerobic conditions. The tubes were placed in a Percival environmental growth chamber where we measured 288 ± 22 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of photosynthetically active radiation immediately above the plants using a Decagon PAR – 80 Ceptometer (Decagon Devices, Pullman, WA, USA). Tests were performed under a 16 h:8 h light:dark schedule. All racks were placed in the growth chamber so that the spaces between the racks were the same as the spaces within the racks and the tops of the tubes are within 30 cm of the bottom of the lights. The location of each rack in the growth chamber remained the same for the test duration. Test solutions in the tubes were renewed every two days. Temperature was maintained at 21°C during lighted periods and 19°C during dark periods and the humidity was maintained at 85%. Plants were harvested after 10 days and the seed hull was carefully removed. Stem and leaf length was measured to the nearest millimeter by placing the stem with leaf stretched out on a flat surface next to ruler with the zero mark aligned with the point of stem-root transition. Total root lengths were measured in duplicate scans of the entire root system using the program WinRhizo (Regent Instruments, Quebec, Canada). Seedlings were weighed after drying at 100°C for 48 h. Control juvenile seedlings did not have any visible phytotoxic or developmental symptoms at any time and the controls had additional stem growth of at least 5.0 cm during the 10-d test.

Growth of juvenile wild rice seedlings under anaerobic conditions subject to various concentrations of sulfide.— Germinated seedlings were chosen using the same techniques described for aerobic conditions. Seven seedlings 1–2 cm in length that fit the criteria as described, were placed with a forceps in 125-mL borosilicate glass jars capped using phenolic screw caps with 5 mm thick chlorobutyl septa. Each sulfide concentration was replicated in this way in three separate jars. Deoxygenated Hoagland's nutrient solution was added as described above. Seedlings were grown in the same environmental growth chamber under the same temperature and light conditions as for the sulfate experiments but with solution sulfide concentrations of 0, 96, 320, 960, and 2880 μg/L. Solutions were exchanged every two days if during the week or three days if over a weekend. Sulfide concentrations were measured at the beginning and end of each two–three day solution exchange period. Because the plants were photosynthesizing and producing oxygen, the sulfide concentration declined during these two–three day periods. This was especially so for the lowest sulfide concentrations (less than ~300 μg/L) in which less than 10% remained after two days, but 70–90% of sulfide remained after two days for sulfide concentrations greater than 650 μg/L. We therefore used the time-weighted average sulfide concentration over the 10 days period to characterize the sulfide concentrations the plants were exposed to. Seedlings were harvested after 10 days, the seed hull was carefully removed, and the stem and leaf lengths and total plant mass were determined. Because many of the plants, especially at high sulfide concentrations, did not grow at all (see Results below) the roots and shoots were very fragile and no attempt was made to dissect the plants into subcomponents as with the experiment on the effects of sulfate on seedling growth.

Statistical analyses of hydroponic experiments.—The general procedure for each set of sulfate and sulfide exposure experiments was first to examine seed germination or seedling growth response across a wide range of concentrations spanning three orders of magnitude of either sulfate or sulfide as noted. The main effect of sulfate or sulfide concentrations on the variable of interest was then tested with an analysis of variance using SigmaPlot (SYSTAT Software, San Jose, CA), USA. When the residuals were not normally distributed or the data did not have equal variance between treatments, then the data were transformed by taking the natural logarithms, which then passed normality and equal variance tests. If there were no effects across this wide range of concentrations in this experiment, then it was repeated to test whether the results were a false negative. If there were significant main effects, then Tukey's pairwise comparisons were performed to determine in which part of the range of concentrations significant effects occurred. Further experiments were then conducted twice using this narrower range of concentrations centered on the region of significant change to more precisely refine the range of response of seedling germination or growth to sulfate or sulfide concentrations.

If there was a significant effect of sulfide on seedling growth, then the biomass growth of seedlings (mg) over the 10-d period was regressed against the time-weighted total dissolved sulfide concentrations (µg/L) with a four-parameter sigmoidal function using SigmaPlot nonlinear regression

$$\text{Plant growth} = y_{\min} + \frac{y_{\max}}{1+\exp\{-[(S^{2-}-x0)/b]\}} \quad (1)$$

where $y_{\min}$ is the right-side (minimum) horizontal asymptote (minimum growth response) $y_{\max}$ is the height of the left-side horizontal asymptote (maximum growth response) above $y_{\min}$, S^{2-} is total dissolved inorganic sulfide ($H_2S + HS^-$), $x0$ is the sulfide concentration at the inflection point of the curve, and b is a parameter that scales µg/L of sulfide concentration to mg of biomass growth. The 50% effects concentration (EC50, the concentration of sulfide that caused a 50% reduction in change in plant mass compared to controls) was calculated from this regression.

The sulfate experiment had to be conducted under aerobic conditions while the sulfide experiment had to be conducted under anaerobic conditions. Therefore, redox statuses of the solutions were necessarily confounded with sulfur speciation. To test the effect of redox status on seedling growth, we compared the growth of plants from both the lowest concentrations of the sulfate (aerobic) and sulfide (anaerobic) experiment using a single-factor analysis of variance.

Mesocosm experiments

Experimental design.—We constructed mesocosms using the same procedures and designs previously reported by Walker et al. (2010) for a 5-yr experiment on the interaction of the nitrogen cycle and wild rice population dynamics.

In late spring of 2011, polyethylene stock tanks (400 L, 132 × 78 × 61 cm; High Country Plastics, Caldwell, ID, USA) were fitted with overflow drain pipes and buried to ground level. The drain pipes are connected to 20-L polyethylene overflow buckets buried adjacent to each tank. Water tables were set by the inflow to the drain pipe at 23 cm above the sediment surface. The tanks were leveled and then partly filled with 10 cm of clean sand washed with the same well water later added to the tanks (see next paragraph). The sand layer was then covered with 12 cm of surface sediment collected from a natural wild rice bed in Rice Portage Lake (Minnesota Lake ID 09-0037, 46.70° N, 92.70° W) on the Fond du Lac Band of Lake Superior Chippewa Reservation, Minnesota. Rice Portage Lake is approximately 337 ha, of which approximately 50 ha are wild rice beds (Minnesota Department of Natural Resources 2008). Ten to 20 cm of sediment over sand is sufficient to support the rooting depths we have observed in natural wild rice lakes. The sediments were kept saturated and then thoroughly homogenized in a large stock tank prior to distribution into the tanks. Analyses of five volumetric samples of the mixed sediment indicate a homogenous material (C = 14.8% ± 1.7%, N = 1.12% ± 0.13%, S [acid volatile sulfur] = 0.005% ± 0.003%). Sediment bulk density was 0.27 ± 0.01 g/cm^3 (Walker et al. 2010). These nutrient and bulk density values are similar to those of other wild rice beds (Keenan and Lee 1988, Day and Lee 1990). No new sediment has been added to the stock tanks since the mesocosms were established in 2011.

The tanks were immediately filled with water obtained from a nearby well after sediment additions to prevent the sediment from drying. Water was added cautiously from a garden hose to prevent redistribution and suspension of sediment. During the growing season, water levels were maintained at 23 cm above the sediment surface by weekly additions of water to the drain pipe heights or by allowing water to drain through the pipe into the overflow buckets. Rainfall N concentrations as NO_3-N and NH_4-N ranged from 0.2 to 1.99 mg/L while the NO_3-N and NH_4-N concentrations in the well water are always <0.2 mg/L (Walker et al. 2010). Sulfate concentrations in well water averaged 10.73 ± 0.75 mg/L (n = 36) and in rainwater averaged 2.13 ± 1.02 mg/L (n = 16). The sediments comprise a natural inoculation source for microbes and a background supply of nutrients for plant growth source. The sediments and plant litter remain submerged in the mesocosms year round with water levels set at approximately 20 cm in late fall.

Wild rice was planted once in late spring 2011 from seeds obtained from Swamp Lake (Minnesota Lake ID 16-0256, 47.85° N, 90.58° W), a 37-ha lake on the Grand Portage Band of Lake Superior Chippewa Reservation, Minnesota. Seeds from each year's crop were allowed to fall unimpeded into the tanks to provide the seed source for the next year's population; no further seeding from external seed sources occurred.

End-of-season plant density in Minnesota wild rice lakes monitored by the 1854 Treaty Authority averages 40 plants/m^2 (Vogt 2010). Accordingly, the seedlings were thinned to this density (30 plants per tank) in late spring or early summer each year before the floating leaf stage was achieved. The seedlings removed from each tank during thinning in 2012–2015 were counted to estimate seed germination and early seedling success.

Immediately after installation and seeding, beginning in late June 2011, the tanks were treated with different amounts of sulfate to achieve several target sulfate concentrations in the overlying water. There were five overlying water sulfate concentrations and six replicate tanks per sulfate concentration, for a total of 30 tanks. Nominal water column sulfate concentrations of 50, 100, 150, and 300 mg SO_4/L were maintained in sulfate-amended tanks. Aside from incidental sulfate in the make-up water from a well and rainwater, control tanks did not receive any sulfate amendments and overlying water concentrations ranged from 2 to 10 mg/L (average of 7 mg/L) depending on rainfall, evapotranspiration, and loss via sulfate reduction in the sediment. The overlying water sulfate concentrations in the mesocosm experiments bracket both the existing 10 mg/L Minnesota statutory standard for wild rice waters and the EPA drinking water standard of 250 mg/L. Samples of the water column were taken weekly and analyzed for sulfate concentration using a Lachat QuikChem 8000 Autoanalyzer (Method 10-116-10-1-A, Hach Co., Loveland, CO, USA). When necessary (approximately every two weeks), the sulfate concentration was adjusted to near the desired nominal concentrations with appropriate amounts of 10 g/L sodium sulfate (Na_2SO_4; Fisher Chemical S421, Thermo Fisher Scientific, Waltham, MA, USA) stock solution and well water. The sodium sulfate stock solution was first mixed in 1–2 L of water from the tank, then added back to the tank's overlying water with mild mixing.

Plant, sediment, and water sampling and analyses.—In each year from 2011 to 2015, five plants in each tank were randomly chosen in early summer for detailed measurements throughout the growing season and to be destructively sampled at the end of the growing season. In late August to September, ripe seeds from these plants were collected every two or three days by gently removing them, leaving unripe seeds behind for the next collection date. The seeds from each individual plant were placed in a paper envelope and marked with the tank identification number. The plants were then harvested for determination of biomass, root:shoot mass ratios and total seed production by counting seed peduncles along the flowering stem.

Seeds from each of the five sampled plants were separated into filled (viable) seeds and empty (nonviable) seeds, counted, and weighed. A subsample of seeds collected in all years except 2013 were dried at 60°C for determination of moisture content to convert wet mass to dry mass. The five sample plants were separated into root and shoot (stem + leaves), and then weighed. Root:shoot ratios and seed masses and numbers from the five sampled plants were applied to total aboveground population masses and total plant numbers to determine total root and seed biomass and number and total biomass in each tank.

While harvesting the plants for growth and biomass measurements, we noticed that plants in the tanks amended with sulfate had blackened roots while plants grown in the control tanks had white or light tan or orange roots. To investigate this further, a sample of roots from a plant from one control tank and a plant from one 300 mg/L amended tank were collected and placed immediately in water in which dissolved oxygen had been purged by bubbling with oxygen-free N_2. These samples were analyzed for Fe and S concentrations by energy-dispersive X-ray spectroscopy (EDS) using a Hitachi TM-1000 scanning electron microscope (Hitachi High Technologies, Schaumburg, IL, USA) fitted with a Quantax EDS unit (Bruker Corporation, Billerica, MA, USA). The nominal spot size was 0.2 μm and the analysis volume was ~5 μm^3. The sample of blackened roots was analyzed at seven points and the sample of tan/orange control roots was analyzed at five points.

All aboveground plant material was collected from each tank at the end of the growing season and weighed to determine total aboveground biomass. A subsample was taken to determine wet:dry ratios for moisture correction after drying at 60°C. All aboveground plant material except for the five sample plants were returned to each tank. All stems in each stank were counted at the time of harvesting the aboveground plant biomass to determine end of growing season plant density.

In 2013, significant seedling mortality occurred in all tanks after thinning but before the floating leaf stage. We believe this early season mortality was due to a record cold and late spring in northern Minnesota in April and May of 2013; ice stayed on lakes an average of 3 weeks later than the median ice-out date (data *available online*).[8] The reduced overall emergence of plants in the spring of 2013 precluded the destructive sampling of five sample plants in each tank at the end of the 2013 growing season because this harvesting would have greatly decreased the number of viable seeds returned to the sediment for the following growing season. Instead, during 2013 all seeds were harvested from each and every plant in the tanks, sorted as described above on each collection day, and returned to the tanks within 24 h of collection without drying in order to maintain their viability for future populations. To determine wet-dry conversion ratios for these seeds, additional seeds were collected at the same collection times from an adjacent experiment on wild rice (Walker et al. 2010) for moisture determination after drying them at 60°C.

Polycarbonate porewater equilibrators (peepers) with sampling ports spaced 1.5 cm intervals were used to make in situ measurements of geochemical profiles of sulfur and iron species at discrete depths in the sediment porewater of a subset of tanks in August of 2013. Care was taken that the installation and extraction of the peepers did not disturb any plants. The method for collecting samples for sulfate, sulfide, and ferrous iron with peepers was modified from Koretsky et al. (2007). Sulfide and iron were quantified in samples immediately with minimal oxygen exposure using a colorimetric methylene blue method (4500 S2-D; Eaton et al. 2005) as implemented with Hach method 8131 for sulfide and a colorimetric phenanthroline method for iron (3500-Fe-B; Eaton et al. 2005). Sulfate was quantified with ion chromatography on a Dionex ICS 1100 system (Thermo Fisher Scientific, Waltham, MA, USA) after acidifying samples to pH < 3

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[8] http://climate.umn.edu/doc/journal/ice_out_recap_2013.htm

using hydrochloric acid and purging gently with oxygen-free nitrogen gas.

In August 2013 and 2015, we also used 10-cm long Rhizon samplers (Rhizosphere Research Products B.V., Wageningen, The Netherlands) to obtain porewater for sulfide analysis. The sampler was inserted vertically into the sediment and connected to an evacuated 125-mL serum bottle. Sulfide samples were prepared without removing the butyl rubber stopper for inline distillation by automated flow injection colorimetric analysis (4500 S2-E; Eaton et al. 2005).

On 6 October 2015, a 10-cm long sediment core was taken from each mesocosm and homogenized. Extractable iron was quantified following a 30-min exposure to 0.5 mol/L HCl, following Balogh et al. (2009), at the Minnesota Department of Health Environmental Laboratory. Total organic carbon was determined using the method of oxidative combustion-infrared analysis (U.S. EPA 2004), after pre-treatment with acid to remove inorganic carbon, at Pace Analytical Services in Virginia, Minnesota, USA.

Statistical analyses of mesocosm experiments.—The effects of sulfate concentrations on plant attributes were tested by repeated measures analysis of variance followed by pairwise comparisons between attributes of plants in the control tanks and each higher sulfate concentration. We also regressed each plant attribute against average annual sulfate concentration for each year. Correlations were assessed using Pearson's correlation test. This combination of both analysis of variance and regression was used as recommended by Cottingham et al. (2005). We used target sulfate concentrations as categorical variables in analyses of variance and growing season actual sulfate concentrations in regression analyses.

RESULTS

Hydroponic experiments

Effect of sulfate on seed germination.—Between 71% and 76% of the seeds pre-selected as filled and mold-free germinated at each sulfate concentration. Sulfate exposure concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L did not affect germination success, mesocotyl lengths, or the masses of the stem plus leaf (if any) and roots ($P > 0.10$ for each test). The experiment was repeated with the same results.

Effect of sulfide on seed germination.—Sulfide concentrations of 0, 96, 320, 960, and 2880 µg/L did not affect germination success of seeds, mesocotyl masses, or mesocotyl lengths ($P > 0.10$ for each test). The experiment was repeated with the same results.

Effect of aerobic and anaerobic conditions on seed germination.—There were no differences in germination rates under anaerobic compared with aerobic conditions when concentrations of sulfur were at trace (<1 µmol/L) amounts of $CuSO_4$ and $ZnSO_4$ in the Hoagland's solution. Mean mesocotyl lengths in the anaerobic solutions (7.8 cm) were significantly reduced ($P < 0.05$) by 38% compared with mean mesocotyl lengths in the aerobic solutions (12.5 cm).

Effect of sulfate on seedling growth.—Sulfate concentrations of 0, 10, 50, 100, 400, and 1600 mg SO_4/L did not affect the growth of juvenile seedling stem length, juvenile stem mass, juvenile root mass, or total juvenile seedling mass ($P > 0.10$ for each test). Sulfate decreased juvenile root length slightly ($P < 0.02$) but only at 1600 mg SO_4/L compared with 50 mg SO_4/L. The experiment was repeated with the same results.

Effect of sulfide on seedling growth.—To examine the effects of sulfide on early seedling growth, we began by growing juvenile seedlings under a wide range of nominal sulfide exposure concentrations of 0, 96, 320, 960, and 2880 µg/L in anoxic solutions in a first trial. Both roots and stems of control plants (no added sulfide) increased significantly ($P < 0.05$) over the exposure, approximately doubling in size compared with initial lengths and masses. In seedlings exposed to sulfide concentrations 320 µg/L or more, stem and leaf masses ($P < 0.01$) and total plant masses ($P < 0.001$) were significantly depressed by an average of 60% and 75%, respectively, relative to controls. Root lengths were only weakly depressed with increasing sulfide concentration ($P < 0.10$).

To narrow the range of toxicity, we then conducted two additional trials focusing on the effects of sulfide on juvenile seedling growth at concentrations less than 1600 µg/L sulfide. The second trial examined growth at exposure concentrations of 0, 200, 400, 800, 1600 µg/L sulfide and the third trial examined growth at exposure concentrations of 0, 160, 320, 640, and 1280 µg/L sulfide. Consistent with the first trial, the biomass of all control plants increased significantly ($P < 0.05$) during the 10 d of

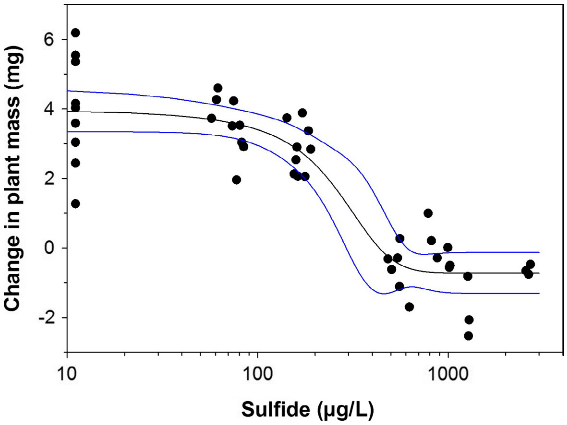

FIG. 1. Growth of wild rice seedlings declines with increasing sulfide concentrations in hydroponic solutions. Individual data points are from three separate experimental runs (see *Methods* and *Results* sections). Fitted sigmoidal response curve (Eq. 1) is shown in black, 95% confidence intervals in blue; $r^2 = 0.80$, $y_{min} = -0.7172$, $y_{max} = 5.1353$, $x0 = 245.9051$, $b = -103.8853$.µ (Color figure can be viewed at wileyonlinelibrary.com.)

exposure, approximately doubling in size compared with initial lengths and masses, and exposure to sulfide across these narrower ranges of concentration again significantly depressed stem plus leaf lengths and total masses of juvenile seedlings.

Because all three trials produced similar effects, we performed a pooled analysis of variance using data from all three. Exposures of seedlings to sulfide concentrations of 320 μg/L or greater significantly reduced growth rates ($P < 0.01$) of wild rice seedlings compared to the control by 88% or greater; Fig. 1). Seedlings exposed to sulfide concentrations at 320 μg/L or greater hardly grew at all and in some cases their mass decreased during the 10-d course of the exposure (Fig. 1). But exposures at sulfide concentrations less than 320 μg/L did not significantly reduce growth rates ($P > 0.10$) compared with the controls (Fig. 1). There was a sigmoidal response of seedling growth to elevated sulfide concentrations, with an inflection point at approximately 245 μg/L (Fig. 1; see figure caption for parameter values and r^2 for Eq. 1). The EC50 calculated from this regression was 227 μg sulfide/L.

Effect of aerobic and anaerobic conditions on seedling growth.—Under micromolar concentrations of sulfur from trace amounts of $CuSO_4$ and $ZnSO_4$ in the Hoaglands solution, stem lengths were 10% longer ($P < 0.02$), root lengths were 73% shorter ($P < 0.001$), and total plant masses were 16% less ($P < 0.01$) under anaerobic conditions compared to aerobic conditions.

Mesocosm experiment

Sulfate concentrations in overlying water.—The average monthly measured sulfate concentrations in amended tanks were consistently within 80–100% of nominal target concentrations of 50, 100, 150, and 300 mg/L (Table 1). The sulfate concentrations sometimes decreased after large rainfall events.

Porewater sulfide concentrations with sulfate additions.—Profiles of sulfate, sulfide, and iron in the mesocosm porewaters showed patterns consistent with sulfate diffusion from the overlying water into the surficial 5 cm of sediment with subsequent reduction to sulfide (Fig. 2). Concentrations of sulfide were typically highest in upper 3–5 cm, which is the rooting zone of seedlings. Sediment in tanks contained on average 8.3 ± 0.8 mg/g extractable iron; extractable iron did not vary with average surface

TABLE 1. Target and measured sulfate concentrations in overlying water in the mesocosm experiment.

Target sulfate concentration	Measured growing season mean sulfate concentrations (mg/L)					
	12 Jul–30 Aug 2011	6 Jun–28 Aug 2012	5 Jun–27 Aug 2013	27 May–26 Aug 2014	5 May–4 Sep 2015	Average over all years
0	8.05 (0.34)	8.0 (0.31)	7.05 (0.18)	5.8 (0.16)	6.16 (0.25)	7.01 (0.45)
50	50.0 (1.58)	34.0 (1.26)	37.2 (1.02)	43.3 (0.8)	41.7 (1.26)	41.2 (2.73)
100	97.7 (4.33)	77.1 (1.76)	79.7 (1.41)	87.2 (1.29)	85.3 (2.03)	85.4 (3.58)
150	135.0 (3.73)	126.0 (2.08)	127.0 (1.55)	131.0 (1.68)	132.0 (2.56)	130.0 (1.57)
300	254.0 (7.35)	263.0 (3.32)	268.0 (2.37)	273.0 (2.52)	272.0 (4.08)	266.0 (3.50)

Note: Values in parentheses are SE.

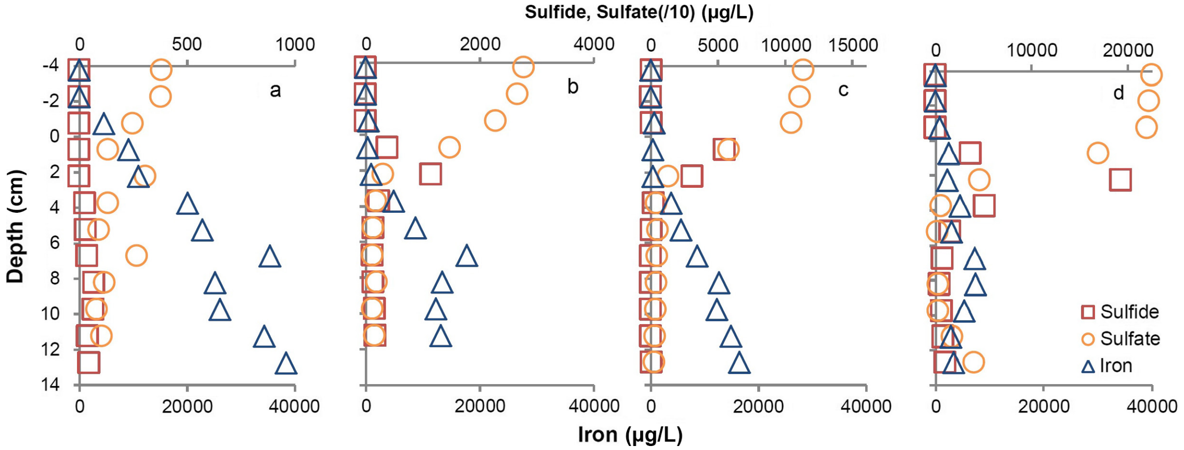

FIG. 2. Vertical profiles of sulfate, sulfide, and iron in mesocosms with different measured sulfate concentrations in the overlying water measured during August 2013. Average annual overlying water sulfate concentrations were (a) 7.05 mg/L, (b) 37.2 mg/L, (c) 127 mg/L, and (d) 268 mg/L. Note different scales for sulfate and sulfide in panels b, c, and d. (Color figure can be viewed at wileyonlinelibrary.com.)

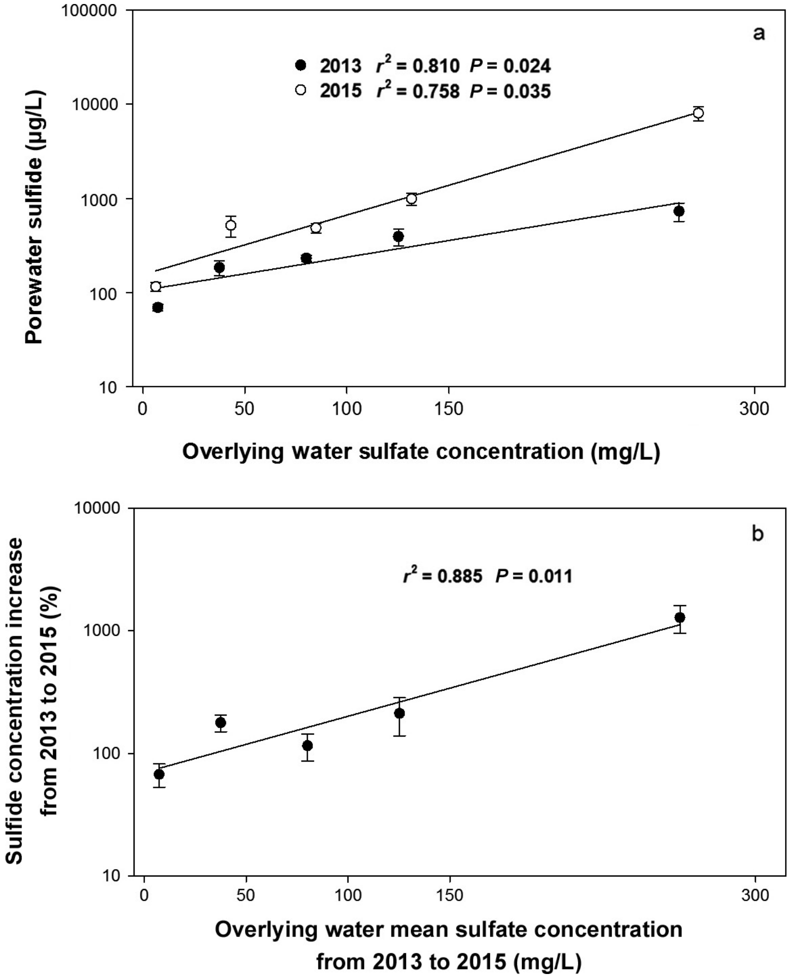

FIG. 3. (a) Porewater sulfide concentrations are strongly correlated with measured concentrations of sulfate in overlying water in the mesocosms and (b) the sulfide concentrations increased from 2013 to 2015 in proportion to sulfate concentrations. Symbols are means and standard errors.

water sulfate concentration (linear regression $r^2 = 0.02$). Sediment in control tanks contained less than 0.15 mg/g acid volatile sulfides (1 mol/L hydrochloric acid, Allen et al. 1991) while sediment in 300 mg/L sulfate tanks contained over 1.75 mg/g in 2013.

Porewater sulfide concentrations obtained from the upper 10 cm of sediment with Rhizon samplers were highly correlated with sulfate concentrations in the overlying water in both 2013 and 2015 (Fig. 3a). Concentrations were higher in 2015, and disproportionately higher in the higher sulfate treatments (Fig. 3b), which could be a consequence of progressively less precipitation with iron, which was a limited quantity.

Effects of sulfate and sulfide on seedling emergence rate and seedling survival.—In each spring after the initial planting in 2011, the number of seedlings that emerged from the sediment (Fig. 4a) declined significantly with increased sulfate concentrations ($P < 0.001$). Emergence rates differed from year to year ($P < 0.001$) but the rate of decline in seedling emergence with amended sulfate concentrations (slopes of regressions in Fig. 4a) did not change significantly from year to year (sulfate × year interaction $P = 0.598$).

The subsequent survival of those seedlings remaining after thinning (Fig. 4b) also declined significantly with increased sulfate concentrations ($P < 0.001$) and year ($P < 0.001$). The rate of decline in seedling survival with amended sulfate was twice as high in 2014 and 2015 than in 2012 and 2013. The number of surviving seedlings was not correlated with the number of seedlings that had been removed by thinning in any given year ($P > 0.10$), so the magnitude of thinning itself had no effect on seedling survival in the same year. The number of surviving seedlings was also not correlated ($P > 0.10$) with the production of straw litter from the previous year, so the decline in seedling survival was not an artifact of inhibition by thatch accumulation or nitrogen immobilization into fresh litter (Walker et al. 2010).

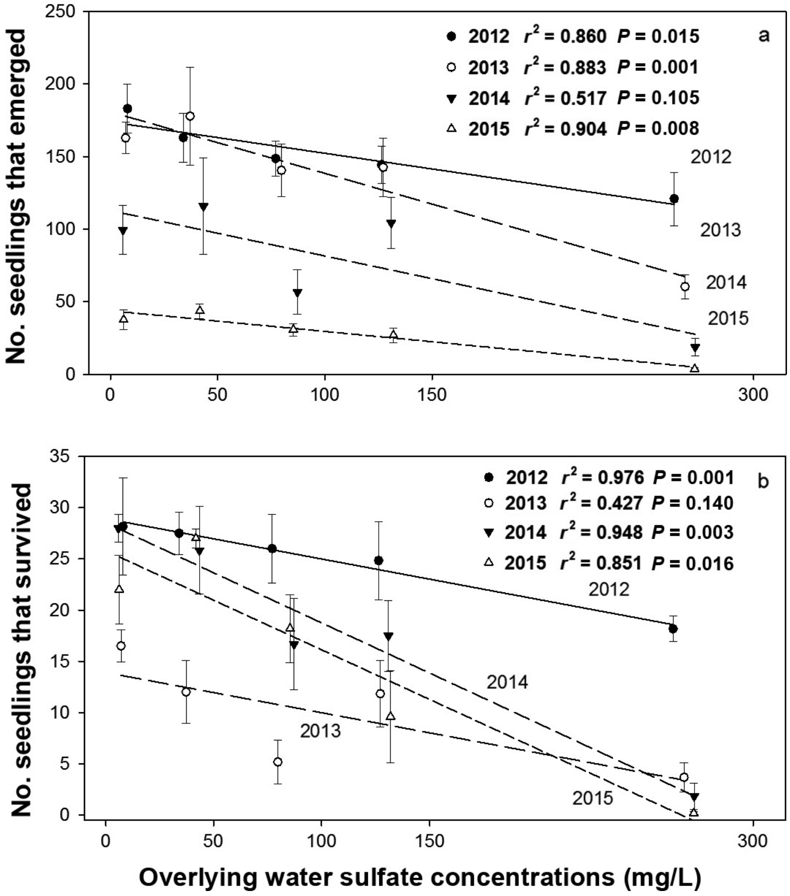

FIG. 4. Emergence (a) and survival (b) of seedlings in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

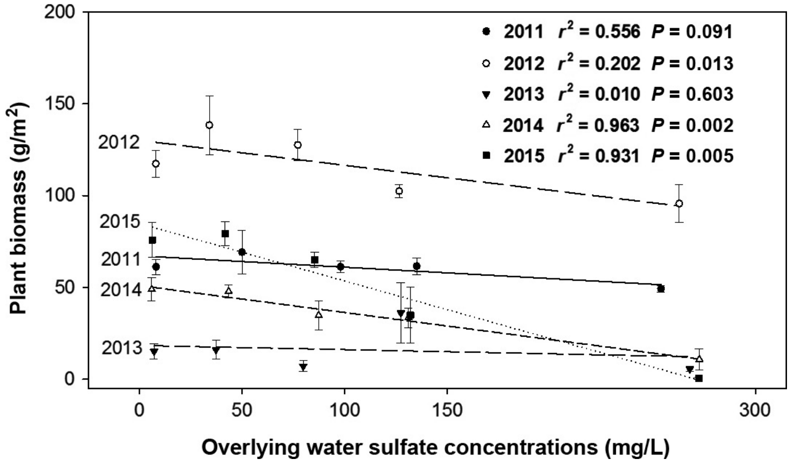

FIG. 5. Vegetative biomass in mesocosms declines with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

In each year, there were no differences between control tanks and tanks amended to 50 mg/L SO_4, but seedling emergence and survival were significantly lower ($P < 0.05$) in tanks amended to 100 mg/L SO_4 or greater compared to control tanks.

Effects of sulfate and sulfide on vegetative growth.—Elevated sulfate and presumably sulfide concentrations decreased plant biomass ($P < 0.001$) and the rate of decline increased significantly during the course of the experiment, but most especially in 2015 (sulfate × year

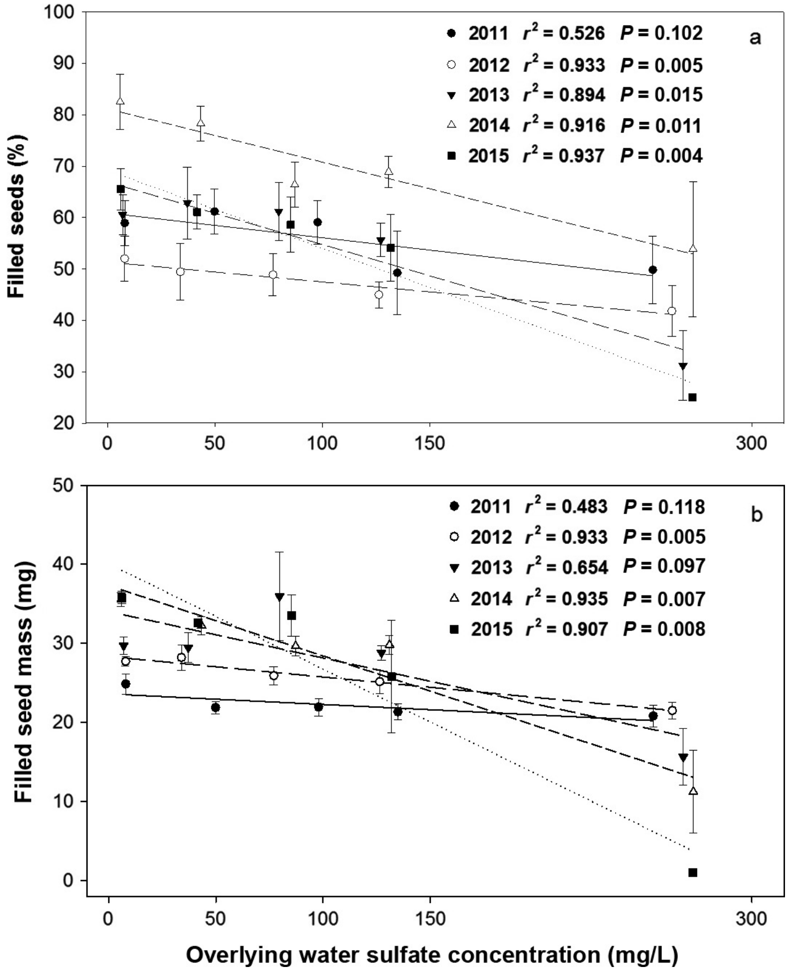

FIG. 6. (a) The proportion of seeds that were filled and (b) the mean seed mass in mesocosms both declined with increasing measured sulfate concentrations in the overlying water. Symbols are means and standard errors.

interaction statistically significant at P < 0.001; see Fig. 5 and the figure legend for r^2 and P levels). By 2015, wild rice was extinct in all but one replicate in the 300 mg/L treatment, which supported only two plants. Root and shoot masses of individual plants were highly correlated (r = 0.998, P < 0.001) and root:shoot ratios were nearly constant between 0.210 and 0.224. Therefore, while the amounts of root and shoot productions were significantly affected by elevated sulfate concentrations, the proportional allocation of production between roots and shoots was not.

Effects of sulfate and sulfide on seed production.—The number of seeds produced per plant (both filled and empty, as determined from peduncle counts) did not change significantly across all sulfate concentrations (not displayed), but the proportion of seeds produced that were filled declined significantly with increasing sulfate concentrations (Fig. 6a, P < 0.001). Although 55–80% of seeds from control plants were filled during all four years, the slopes of the regressions of the proportions of filled seeds against sulfate concentration declined more steeply with each successive year (sulfate × year interaction significant at P < 0.001). By 2015, the proportions of filled seeds were as low as 25% in the tanks with the highest sulfate concentrations.

Individual seed masses declined with increased sulfate concentrations (Fig. 6b, $P < 0.001$). The seed masses declined more steeply with increasing sulfate concentrations with each successive year (sulfate × year interaction significant at $P < 0.001$).

In each year, seed production did not differ between control tanks and tanks amended to 50 mg/L SO_4, but seed mass and the proportion of viable seeds were significantly lower ($P < 0.05$) in tanks amended to 100 mg/L SO_4 or greater compared to control tanks.

Blackened roots associated with elevated sulfate.—Beginning in 2012 and continuing for each subsequent year, plants in the tanks amended with sulfate had blackened roots while plants grown in the control tanks had white or light tan or orange roots when we

Table 2. Summary of the effects of sulfate and sulfide on the stages in the life cycle of wild rice.

	Effects of increased sulfate and/or sulfide	
Wild rice life cycle stage	Hydroponic experiments	Mesocosm experiments
Germination rate	no effect of sulfate or sulfide	not assessed
Juvenile seedling growth	significant negative effect of sulfide, no effect of sulfate	not assessed
Seedling emergence from sediment	not assessed	significant negative effect of sulfate addition, probably a result of reduced seed viability rather than direct effects of sulfide
Seedling survival	not assessed	significant negative effect of sulfate addition, most likely through sulfide production
Mature plant growth	not assessed	significant negative effect of sulfate addition, most likely through sulfide production
Seed production (number of seeds per plant)	not assessed	no effect of sulfate or sulfide
Seed viability, both individual seed mass and proportion of filled seeds	not assessed	significant negative effect of sulfate addition, most likely through sulfide production

harvested them at senescence. Visual estimates of the proportion of blackened roots increased progressively from approximately 50% in the tanks with sulfate concentrations approximately 50 mg/L to 100% in tanks with sulfate concentrations approximately 300 mg/L. These roots were pliable and white in cross sections cut with a knife, so they appeared to be still alive. In these cross sections, the blackening appeared to be crusted plaques on the root surfaces. The blackened roots from the 300 mg/L amended tank averaged 28.3% ± 9.8% Fe and 13.4% ± 4.6% S by mass, both much greater than tan/orange roots from the control tanks, which averaged 5.0% ± 3.9% Fe and 0.34% ± 0.29% S. We are investigating the chemistry of these plaques further, but our analyses thus far suggest that the blackening was caused by precipitation of some form of iron sulfide.

Discussion

Table 2 summarizes the major effects of sulfate and sulfide in these experiments. In the mesocosms, the correlation between sulfate concentrations in overlying water and sulfide concentrations in porewater (Fig. 3a) is so strong within a given year that we can reasonably use sulfate concentrations in overlying water as a surrogate for increased sulfide concentrations in sediment porewater. Porewater sulfide increased substantially between 2013 and 2015 (Fig. 3a, b). The sulfide production in these sulfate-amended mesocosms will eventually overwhelm the available iron and accumulate free sulfide in the porewater, which may be responsible for the disproportionately higher sulfide in the highest treatment in 2015 (Fig. 3b). The mesocosms did not mimic the steady state that occurs in the natural environment because sulfate in overlying water was resupplied but iron was not. Mechanistic models that include the interaction between sulfide and iron (e.g., Wang and Van Cappellen 1996, Eldridge and Morse 2000) include the continuous addition of iron from the overlying to the sediment, successfully modeling the steady-state relationship between sulfate, sulfide, and iron observed in the environment. The sedimentation of new iron to the sediment occurs in the natural environment, but was not included in this mesocosm experiment. Nevertheless, the experiment successfully exposed wild rice to progressively higher concentrations of porewater sulfide and documented the biological effects.

The porewater sulfide concentrations observed in natural waterbodies will vary depending on each site's surface water sulfate and sedimentary concentrations of organic matter and iron (Eldridge and Morse 2000). The sediment organic matter and extractable iron in this experiment (8.1% and 8.3 mg/g) are within the range of 67 Minnesota wild rice waterbodies; organic matter is lower than the median of 9.1%, and the iron is higher than the median of 4.8 mg/g (5th to 95th percentiles of 0.9–31.0% and 1.6–15.3 mg/g, respectively; A. Myrbo, *unpublished data*).

Upwelling groundwater through sediment would cause a waterbody to deviate from the conceptual model presented here; upward groundwater flow would not only counter downward diffusion of sulfate, but could also supply water with chemistry completely different than the overlying water. In a survey of 46 Wisconsin lakes, Nichols and Shaw (2002) found that the occurrence of wild rice is associated with areas of inflowing groundwater. In some cases, upwelling groundwater may supply sulfate to the reduction zone in littoral sediments (Krabbenhoft et al. 1998), so the effect of groundwater is unpredictable. Wild rice waters most likely to exhibit elevated porewater sulfide are those with relatively high organic matter, which allows enhanced microbial activity, and relatively low iron, which minimizes removal of porewater sulfide as a FeS precipitate (Heijs et al. 1999, Eldridge and Morse 2000).

Elevated sulfate concentrations were not directly toxic to wild rice seedlings in hydroponic solutions, in agreement with results reported by Fort et al. (2014). But adding sulfate to overlying waters in the mesocosms with wild rice sediment increased porewater sulfide concentrations most strongly in the upper 5 cm of sediment in 2013, after three field seasons of sulfate amendments (Fig. 2).

Sulfide was clearly toxic to early seedling growth in hydroponic experiments at concentrations above 320 μg/L, as indicated by slower growth or even zero or negative growth in a few cases (Fig. 1). Sulfide concentrations in excess of 320 μg/L were observed in the upper 5 cm of sediment when sulfate concentrations in the overlying water exceeded 20–50 mg/L (depending on season, Fig. 2).

The upper 2–5 cm of sediment is where seed germination and very early seedling growth most likely takes place. Wild rice seeds are shaped like torpedoes and penetrate the sediment aided by their long awns, which act as rudders and keep the seed vertical as it falls through the water column (Ferren and Good 1977). It is likely that the seeds are buried in the upper 2–5 cm of this sediment where oxygen is low and sulfide concentrations are greatest (Fig. 2). To survive, the seedling must germinate in and grow through this zone of high sulfide concentrations. In nature, the mesocotyl may elongate up to 6 cm (Aiken 1986), allowing a buried seed to emerge through up to "3 inches of flooded soil" (Oelke et al. 1982). After emergence into the overlying oxygenated water, the mesocotyl differentiates into the mature stem. Wild rice is unusual among grasses in that the stem develops before the root, probably because the seedling may have to grow between 50 and 100 cm before reaching the water surface, at which time floating leaves supply energy for root development (Aiken 1986). This is consistent with the enhanced stem plus leaf growth of seedlings we observed under anaerobic conditions without elevated sulfide concentrations. Root growth, in contrast, was reduced by anaerobic conditions in our hydroponic experiments, as it has been previously observed for wild rice (Campiranon and Koukkari 1977) and white rice (Kordan 1972, 1974*a, b*).

Elevated sulfide concentrations greatly reduced shoot and leaf elongation in our hydroponic experiments, particularly at concentrations greater than 320 μg/L. The toxic effect of sulfide on shoot and leaf elongation and seedling growth (Fig. 1) overrides the enhanced growth that normally happens under anaerobic conditions. Seedlings in the mesocosms with elevated sulfate (and hence sulfide) concentrations likely were inhibited from emerging successfully from the sediment and reaching aerobic conditions higher in the water column, resulting in reduced survival in the mesocosms.

It is possible that high ionic strength or salinity in the mesocosms with the higher concentrations of elevated sulfate could be the cause of reduced seedling emergence and survival. However, the hydroponic experiments demonstrated that seeds and seedlings could withstand sulfate concentrations of up to 1600 mg SO_4/L without adverse effects. This sulfate concentration is half the salinity of seawater (Schlesinger 1991). Electrical conductivity in the mesocosms was correlated with sulfate concentrations but, in 2012, we saw only small effects of sulfate on seedling emergence and survival even though electrical conductivity was high then as it was in 2015. High ionic strength alone is therefore probably not the cause of the progressively greater declines in seedling emergence and survival in the mesocosms.

It is likely that the observed negative effects on wild rice seedling growth and survival can be directly attributed to the toxic effects of sulfide because of the coherence between the mesocosm experiments and the hydroponic experiments, which isolated the toxic effect of sulfide on seedling growth from any direct effect of sulfate. The progressive decline in seedling emergence and survival during the 5-yr course of the experiment could have resulted from increasingly greater sulfide concentrations (Fig. 3) and progressive titration of reactive forms of ferrous iron out of the system as insoluble iron sulfide. The cumulative effects of this progressive loss of reactive ferrous iron could have allowed more sulfide to remain in solution (Fig. 3) and thereby have increasingly toxic effects on seedling emergence and survival. The possible loss of reactive ferrous iron during the 5-yr course of the experiment may have been partly responsible for the declines in population densities, even to extinction at the highest sulfate concentrations.

Elevated sulfate concentrations in the mesocosm water progressively reduced vegetative production over the five years, but to much less extent than seed production was reduced. The proportion of seeds that were filled, as well as their mean masses, decreased by over 30% and as much as 50% in the 300 mg/L mesocosm treatment by year five of the experiment. Reduced seed production and seed masses followed by reduced seedling emergence and survival the following year depressed population growth in successive years eventually driving wild rice populations to extinction at high sulfate concentrations. It is likely that this extinction was driven by reduced seed production, seedling emergence, and seedling survival that depleted the seed bank over the fine years of the experiment, and cumulative impacts on sediment chemistry from repeated sulfate additions could have exacerbated the decline.

The strong decline in measures of seed viability with increased sulfate concentrations at the end of the growing season (Fig. 6) compared with the weaker decline in vegetative growth in early to mid-growing season (Fig. 5) could not have been due to decreased N or P availability late in the growing season. Litter from the previous year has begun mineralizing N and P at this point in the growing season (Walker et al. 2010, Hildebrandt et al. 2012). The production of sulfide is correlated with many other chemical changes associated with the sulfate-enhanced anaerobic decay of organic matter (Lamers et al. 2002), including increased phosphate solubility. Phosphorus availability could not be controlled independent of sulfide in sediment, and sediment porewater and overlying water phosphate concentrations were elevated in sulfate amended tanks (A. Myrbo, *unpublished data*) most likely because precipitation of sulfide with reduced iron liberates phosphate (Caraco et al. 1989, Lamers et al. 2002). Since N and P availability were likely not limiting late in the growing season, it is unlikely that reduced N or P availability were responsible for the decline in seed production with increased sulfate concentrations. Therefore, by deduction, it must have been uptake that was limiting.

Sixty percent of annual N uptake in wild rice plants occurs early in the growing season but there is a second burst of nitrogen and phosphorus uptake in August during seed filling and ripening (Grava and Raisanen 1978, Sims et al. 2012*a*). Even though N and P were most bioavailable in August when wild rice seeds were being developed and filled, there was coincident peak accumulation of sulfide in the sediment porewater (Fig. 2). When exposed to high sulfide concentrations, roots of white rice (*Oryza sativa*) often become suberized (Armstrong and Armstrong 2005) with subsequent possible reduction in nutrient uptake across the thicker root membranes (DeLaune et al. 1983, Koch et al. 1990, Armstrong and Armstrong 2005, Lamers et al. 2013). Suberization of roots in response to high sulfide concentrations at this stage in wild rice's life cycle might inhibit nutrient uptake, resulting in fewer and smaller filled seeds.

Another possible mechanism for impaired nutrient uptake might be the precipitation of black iron sulfide plaques on the roots of plants that grew in mesocosms with elevated sulfate and sulfide concentrations. Our EDS analyses suggest that the tan or orange coatings on roots of plants grown under low sulfate concentrations may be iron hydroxide plaques, which are often found on healthy wild rice roots (Jorgenson et al. 2012). The existence of tan or orange coatings, consistent with iron hydroxide plaques, strongly suggests that the immediate vicinity of the roots is oxidized when sulfate concentrations are low, most likely due to radial oxygen loss through the aerenchyma tissues within the roots (Stover 1928, Colmer 2003, Yang et al. 2014). Blackened roots, however, are often observed in white rice (*Oryza sativa*) populations subjected to elevated sulfate concentrations or organic carbon (Jacq et al. 1991, Gao et al. 2003, Sun et al. 2015) and our EDS observations suggest that the blackened plaques on our roots are some form of iron sulfide. Sun et al. (2015) also found that these black plaques contain substantial amounts of iron sulfides. Precipitation of iron sulfide plaques on roots, whether a direct inhibitor of nutrient uptake or a harbinger of the encroachment of reducing conditions to nearer the root tissue, may be partly responsible for the reduced proportion of filled seeds as sulfate concentrations increased (Fig. 6). Further experiments using labeled ^{15}N would be useful to determine whether reduced nutrient uptake during seed filling is the cause of reduced seed production.

Suberization of roots and precipitation of iron sulfide plaques may not be independent. Enhanced suberization when the root tissue is exposed to sulfide (Armstrong and Armstrong 2005) might cause decreased radial oxygen loss from roots of wetland plants (Joshi et al. 1975, Gao et al. 2002, Armstrong and Armstrong 2005). If radial oxygen loss from roots is essential to maintaining low concentrations of hydrogen sulfide in the immediate vicinity of roots (Eldridge and Morse 2000), then sulfide concentrations in the rhizosphere could encroach nearer to the root surface when radial oxygen loss from roots is impaired. Iron (hydr)oxide present on or near the roots under these conditions could be reduced to iron sulfide and precipitated on the roots. Nutrient uptake during the stage of seed filling therefore might be impaired directly by suberization of roots followed by precipitation of iron sulfides on the roots if suberization reduces radial oxygen loss.

Conclusions

In our hydroponic experiments, elevated sulfide concentrations are directly toxic to seedlings. In our mesocosm experiments, sulfate amendments increased sulfide concentrations in the rooting zone, which then apparently decreased seedling emergence and survival. The reductions in seedling emergence and survival in the mesocosms are consistent with the toxic effects of sulfide on seedling growth in the hydroponic experiments.

The vegetative growth phase of wild rice's life cycle did not appear to be as strongly affected by sulfide as the production of viable seeds. The mechanisms behind reduced seed production and viability with increased sulfate and hence sulfide production in sediments are more difficult to discern, but may involve reduction of nutrient uptake during seed set by iron sulfide plaques on roots of mature plants (Jacq et al. 1991) or by increased suberization with elevated sulfide concentrations later in the summer (Armstrong and Armstrong 2005).

In natural wild rice ecosystems, the extent to which sulfate is reduced to sulfide, and to which sulfide persists in porewaters, are controlled by factors such as the sedimentary concentrations of iron and organic matter, and groundwater flow, among others, all of which may differ from the conditions in our mesocosms. But our experiments strongly suggest that the reduction of sulfate to sulfide in sediments, to the extent that it occurs in natural systems, may cause populations to decline by adversely affecting the reproductive phases of wild rice's life cycle.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1452/full

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment C

(25 pages)

Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*)

John Pastor
Dept. of Biology
University of Minnesota Duluth

Does Iron Control Sulfide Toxicity to Wild Rice?

- **Long term Mesocosm Experiment**

- **Bucket Experiment**

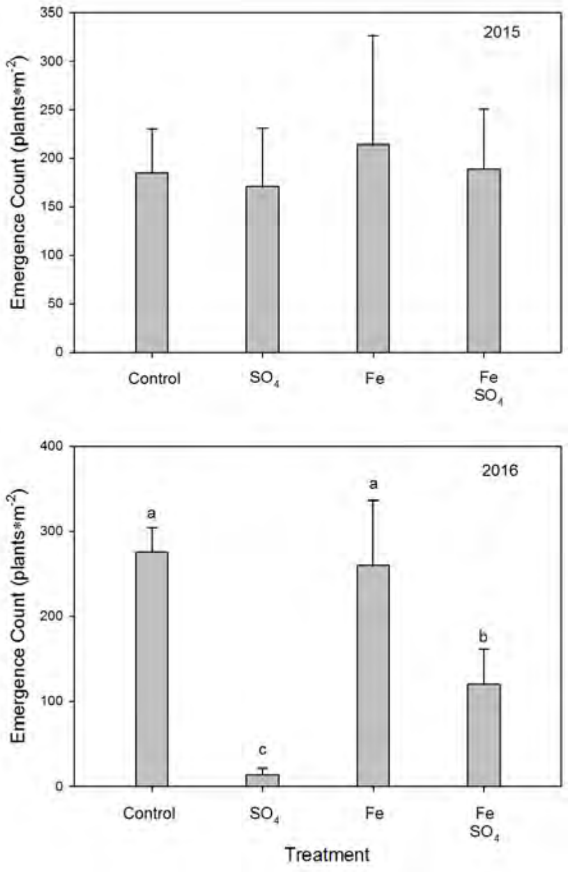
Mesocosm Experimental Design:

- 40 stock tanks
- Sulfate – control (c. 7 mg/L) & 300 mg/L
  added as Na_2SO_4 to water column
- Fe – control & tripled extractable Fe in sediment (220 g/m^2 added as $FeCl_2$ in four aliquots into sediment in July and August 2014)
- Litter – present or removed (no significant effect)
- Thinned to 30 plants per tank
- Sediment from Rice Portage Lake

- 6 plants marked and harvested for seeds, plant growth, and allocation to roots and shoots
- Rest of tank harvested and weighed but returned to tank (or not if no litter)
- 2014 & 2015

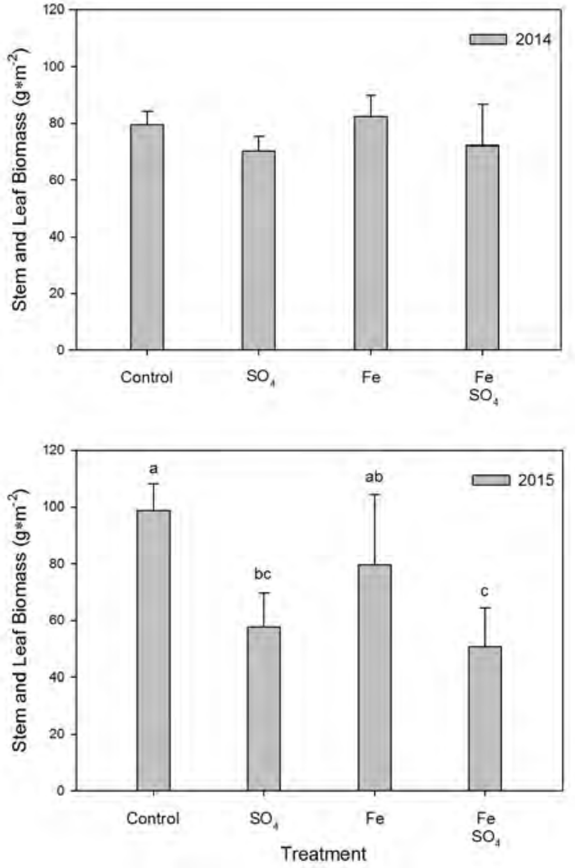
Seedling emergence depressed in the presence of sulfate by 2015

Fe partly compensated for the effect of sulfate/sulfide



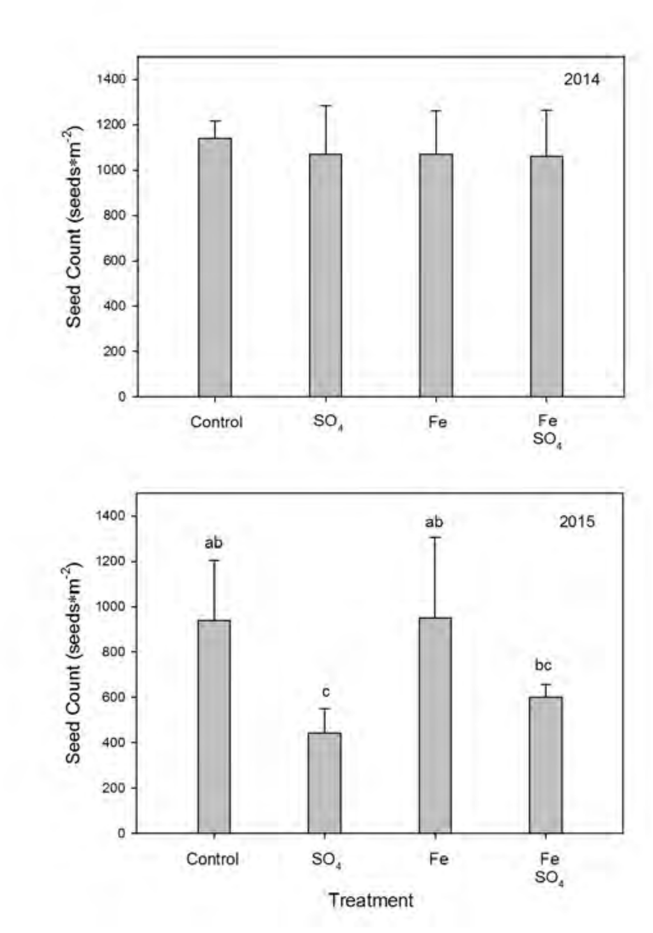
Vegetative growth depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



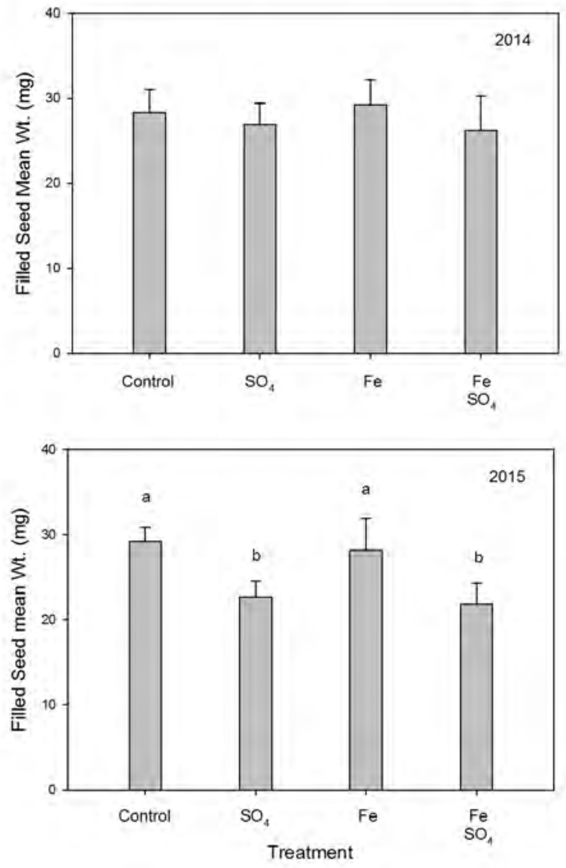
Seed count depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



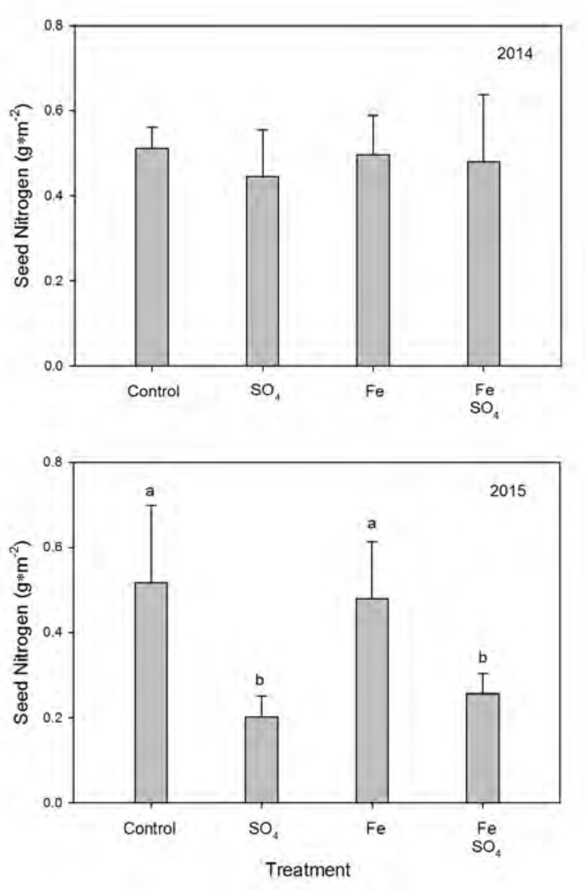
Seed weight depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



Seed nitrogen depressed in the presence of sulfate by 2015

Fe had no effect by itself and no compensating effect in the presence of sulfate



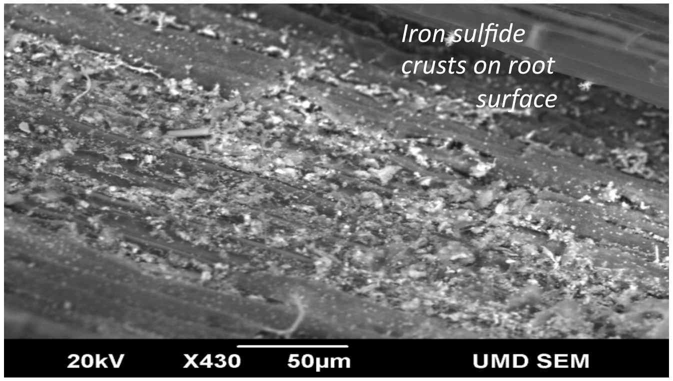
Preliminary Conclusions – Mesocosm Experiment

- Fe additions partly compensated for toxic effect of sulfide on seedling emergence, possibly by precipitating FeS

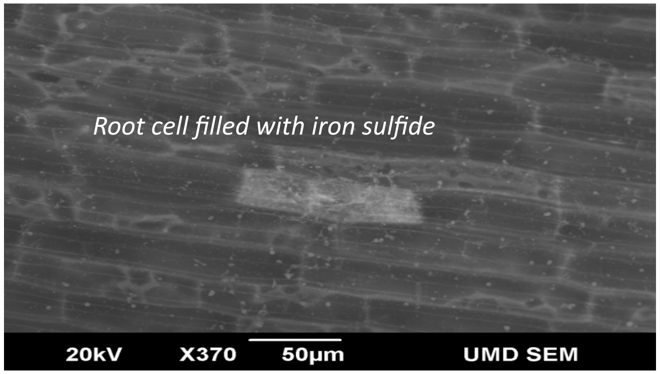
- Fe additions did not compensate for depression of vegetative growth or seed production and nitrogen content

Iron plaques

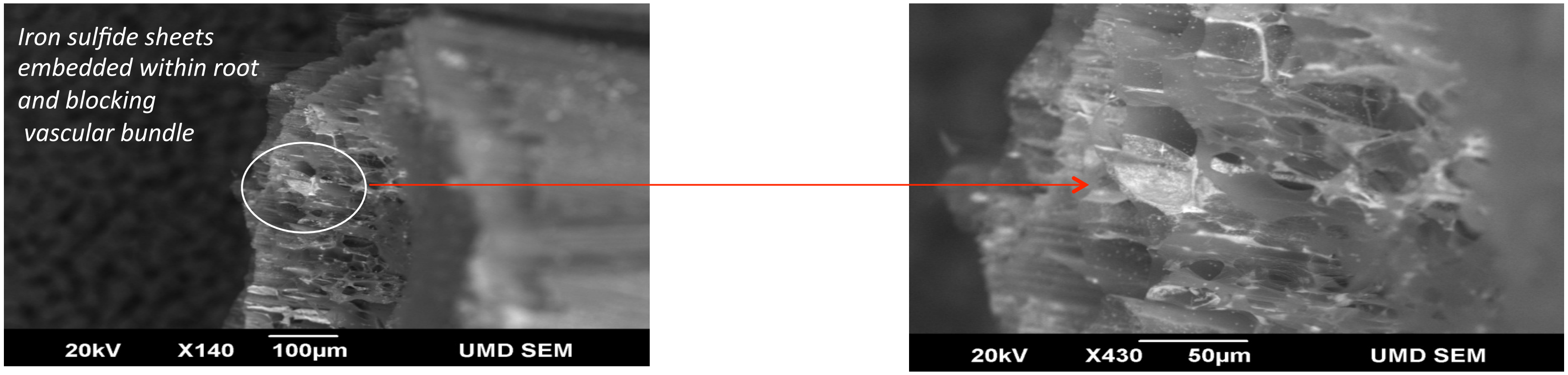
SEM Scans of Iron Sulfide Precipitates on Roots



Iron sulfide crusts on root surface



Root cell filled with iron sulfide



Iron sulfide sheets embedded within root and blocking vascular bundle

Scans courtesy of Dr. Bryan Bandli, UMD

What geochemical conditions are associated with iron sulfide plaque formation?

How do iron sulfide plaques change seasonally?

Do iron sulfide plaques inhibit nitrogen uptake?

Bucket Experimental Design:

- 40 buckets: 300 mg/L SO_4
- 40 buckets: control
- 1 wild rice plant per bucket
- Sediment from Rice Portage Lake

- 8 plants harvested per sample date
  - every 2 weeks during flowering
  - weekly during seed production
- Pore water sampled one day prior to harvest
- Sediment sampled start and end of growing season

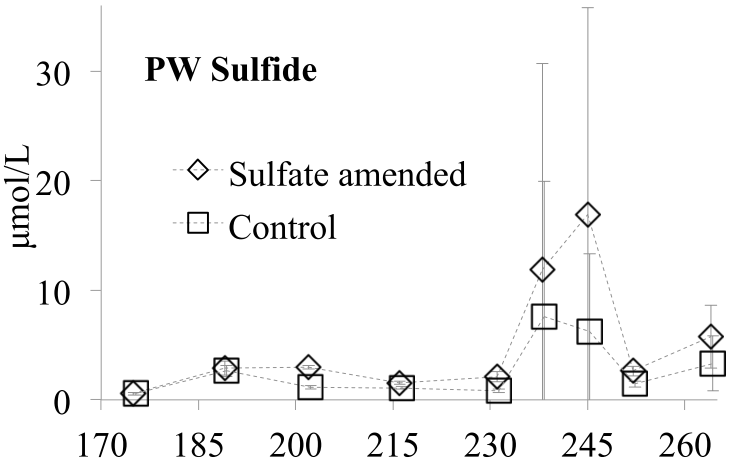
Methods: Pore water collection & analysis

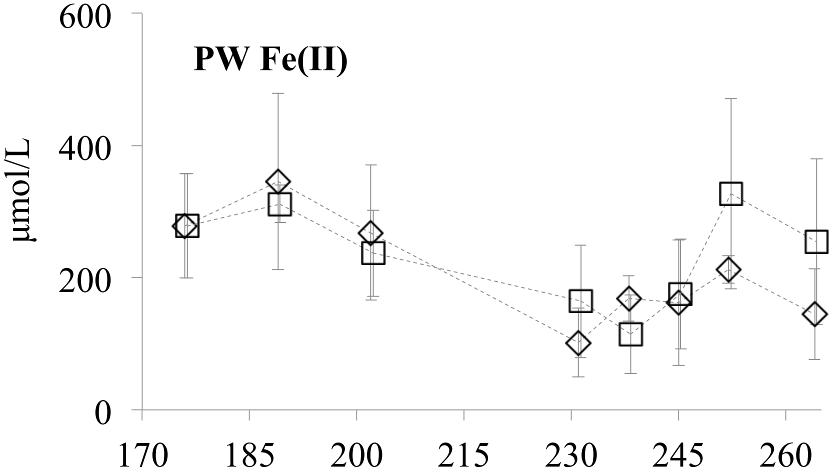
- Sampling procedure: rhizons attached to preloaded, vacuumed bottles

Analyte	Analysis
Sulfide	spectrophotometry (methylene blue)
Sulfate	ion chromatrography
Fe2+	spectrophotometry (phenanthroline)
pH	electrode

Methods: Root AVS & Fe

- Root collection
  - Placed in jar underwater in degassed DI water
- AVS quantification
  - Extracted for 4 hours with 1M HCl
  - Quantified with a sulfide ion-selective electrode
- Fe quantification
  - Aliquot of acid analyzed on AA
  - Ferrous iron quantified on spec

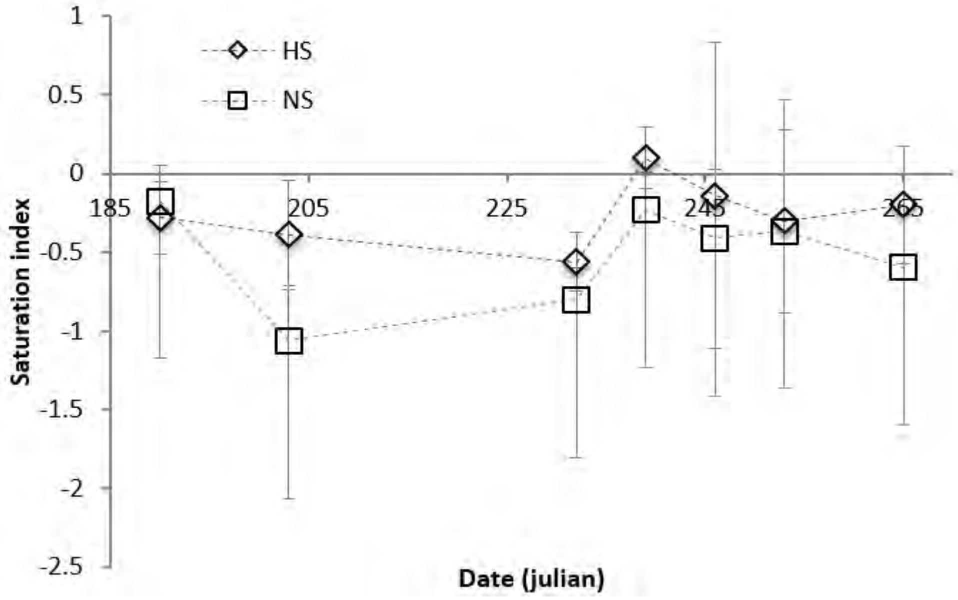

PW Sulfide
μmol/L
◇ Sulfate amended
□ Control
0
10
20
30
170
185
200
215
230
245
260

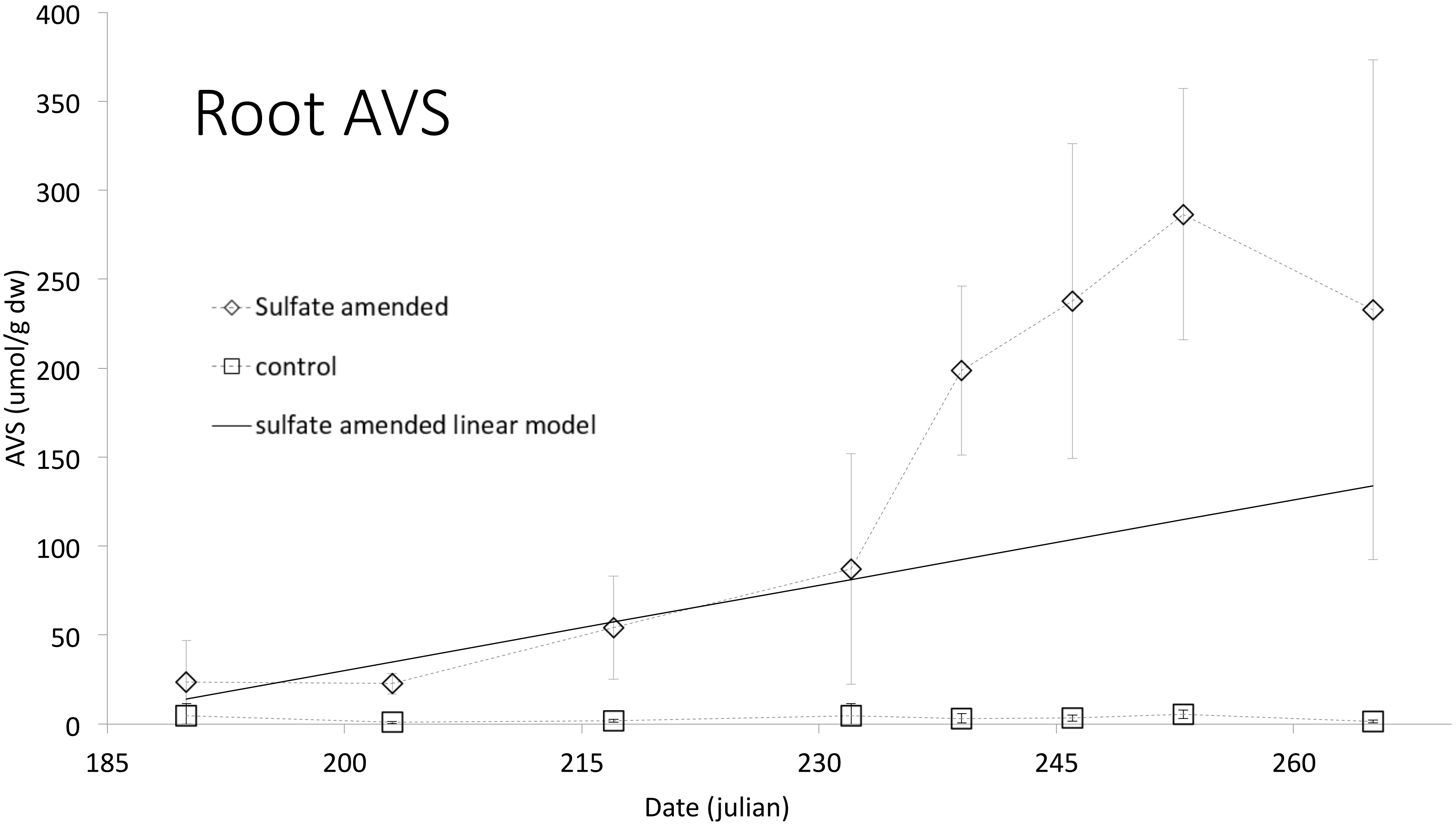

PW Fe(II)
μmol/L
0
200
400
600
170
185
200
215
230
245
260

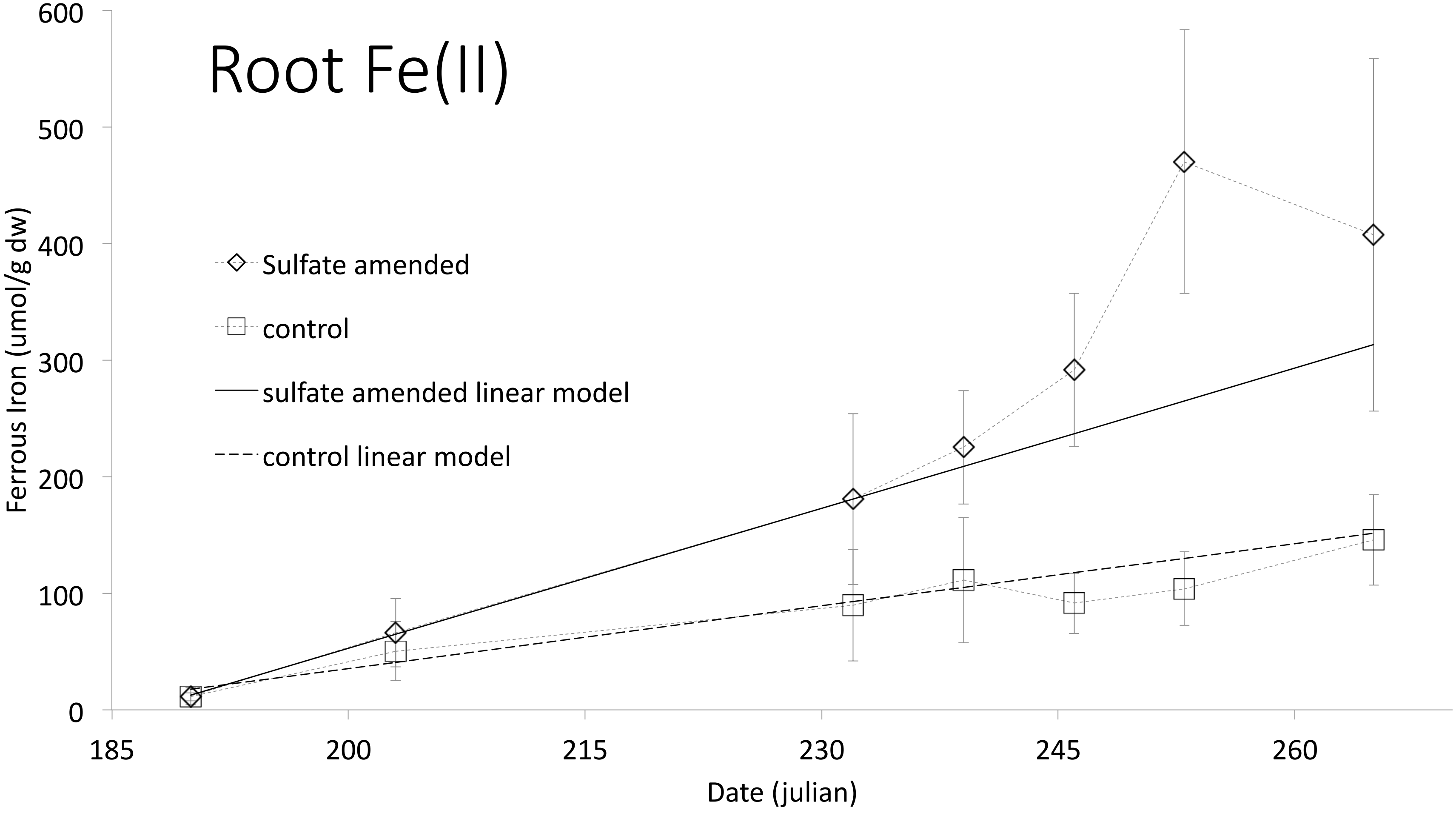
Saturation Index in Bulk Sediment

Pore water 2 cm from roots is *undersaturated* with respect to FeS

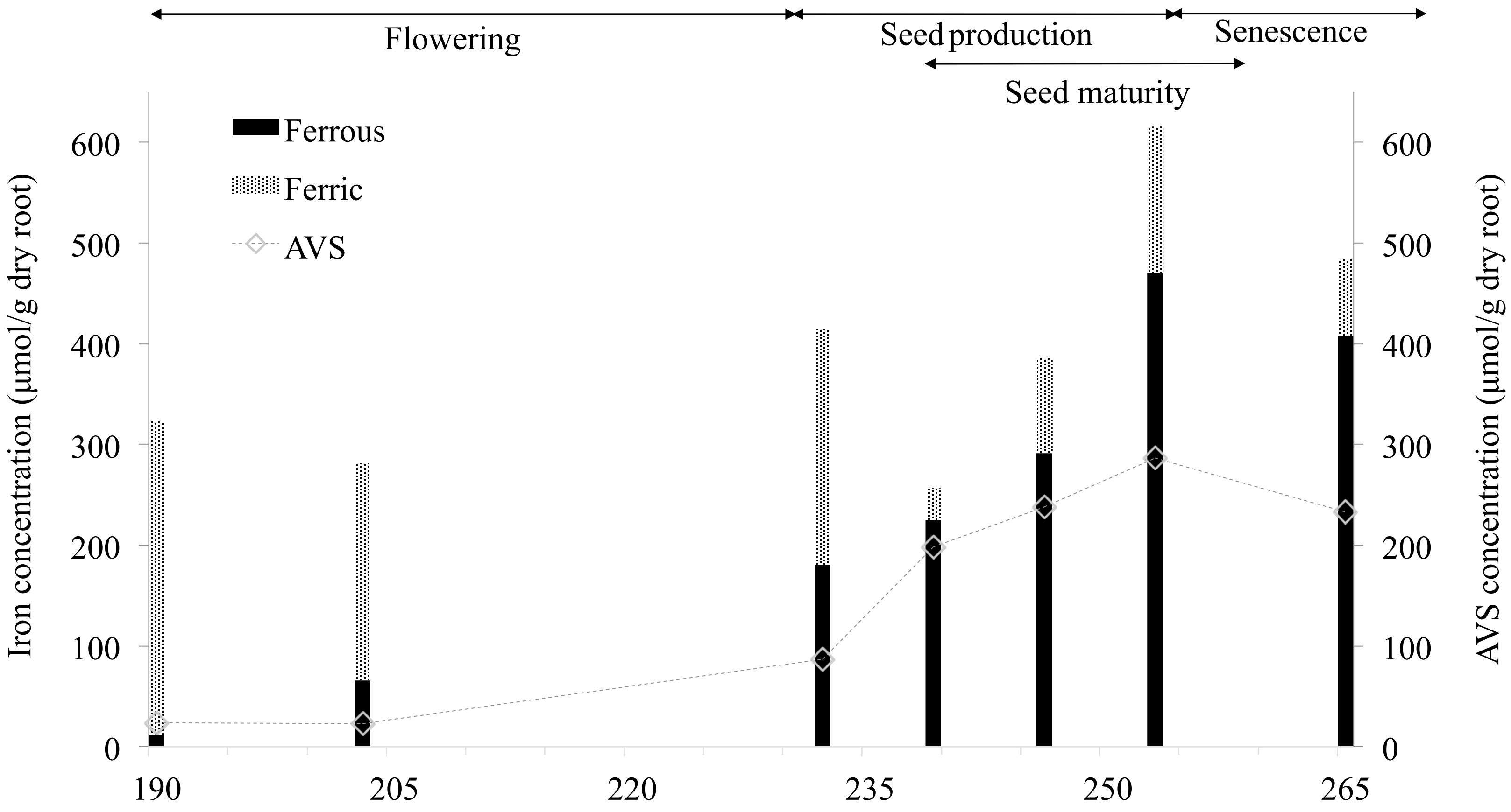
$SI=\log[IAP]/K_{sp}$, where $IAP=[Fe^{2+}][HS^{-}]/[H^{+}]$ and $K_{sp} = 10^{-2.95}$

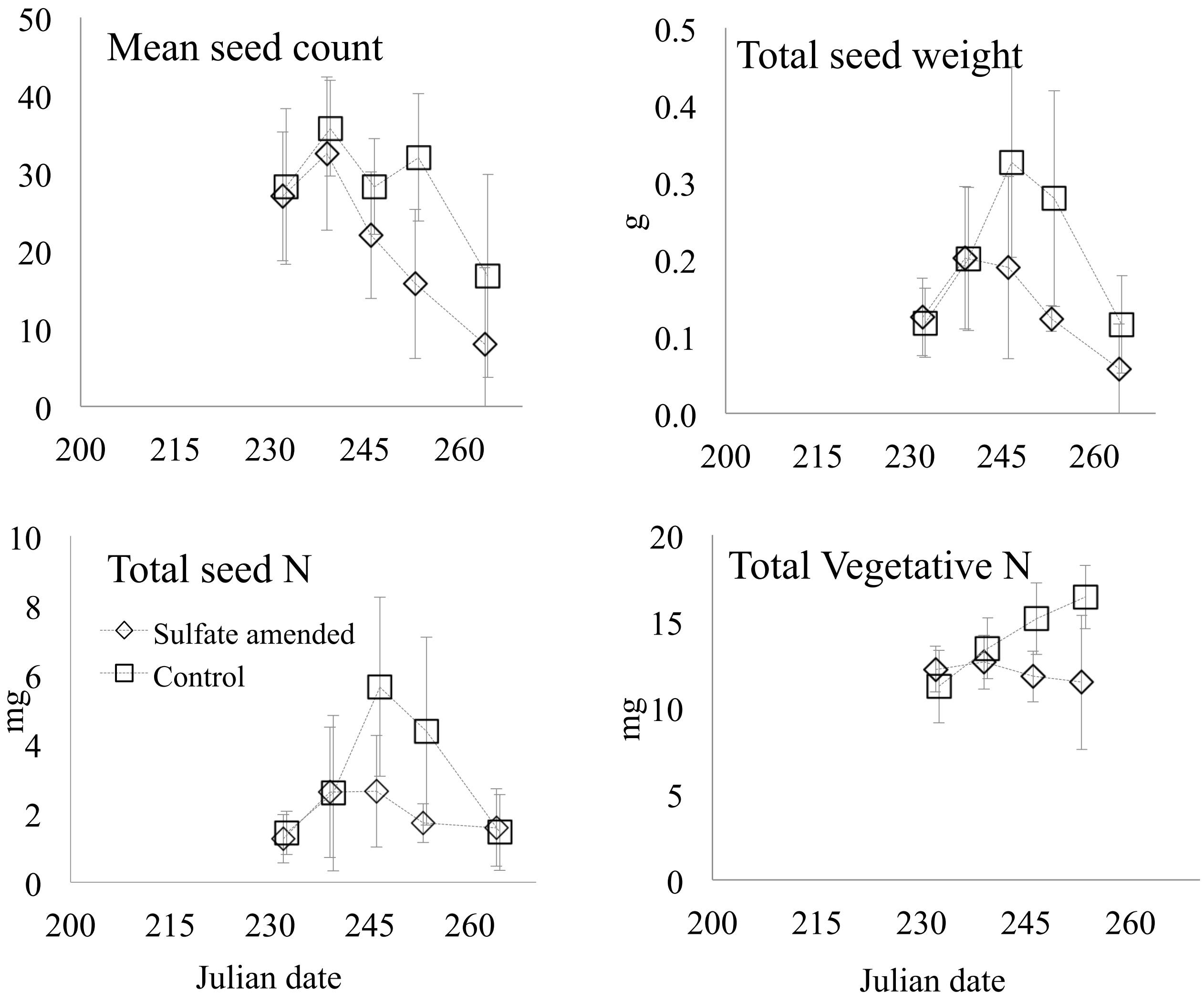



Root AVS
AVS (umol/g dw)
400
350
300
250
200
150
100
50
0
185
200
215
230
245
260
Date (julian)
◇ Sulfate amended
□ control
sulfate amended linear model


Root Fe(II)
Ferrous Iron (umol/g dw)
Date (julian)
0
100
200
300
400
500
600
185
200
215
230
245
260
Sulfate amended
control
sulfate amended linear model
control linear model

Root iron speciation on amended roots




Mean seed count
50
40
30
20
10
0
200
215
230
245
260
Total seed weight
g
0.5
0.4
0.3
0.2
0.1
0.0
200
215
230
245
260
Total seed N
mg
10
8
6
4
2
0
◇ Sulfate amended
□ Control
200
215
230
245
260
Julian date
Total Vegetative N
mg
20
15
10
5
0
200
215
230
245
260
Julian date

Repeated measures ANOVA (F values)	Sulfate	d.f.	Time	Sulfate x Time	d.f.
Pore water geochemistry					
Iron	5.16	1, 5	5.51***	1.14	6, 35
pH	3.25	1, 6	12.5***	1.45	6, 36
Saturation index	2.68	1, 4	2.19*	0.50	6, 34
Sulfide	239***	1, 3	8.17***	1.09	5, 27
Root geochemistry					
AVS (during flowering)	66.1***	1, 5	1.10	0.40	3, 17
AVS (during seed production)	148***	1, 6	5.46**	1.76	4, 24
Weak acid extractable iron	0.53	1, 6	2.65**	2.42**	7, 42
Ferrous Iron	127***	1, 6	57.2***	3.34**	6, 36
% Ferrous Iron	235***	1, 6	41.5***	4.91***	6, 36
Biological variables (during seed maturity)					
Plant weight	5.00*	1, 6	0.40	0.31	3, 18
Seed N (total mass)	5.84*	1, 6	1.10	1.22	2, 12
Seed weight	4.88*	1, 6	0.59	0.94	2, 12
Seed count	5.00*	1, 6	1.89	0.70	2, 12
Vegetative N (plant+seed mass)	5.43*	1, 6	0.32	1.71	2, 12

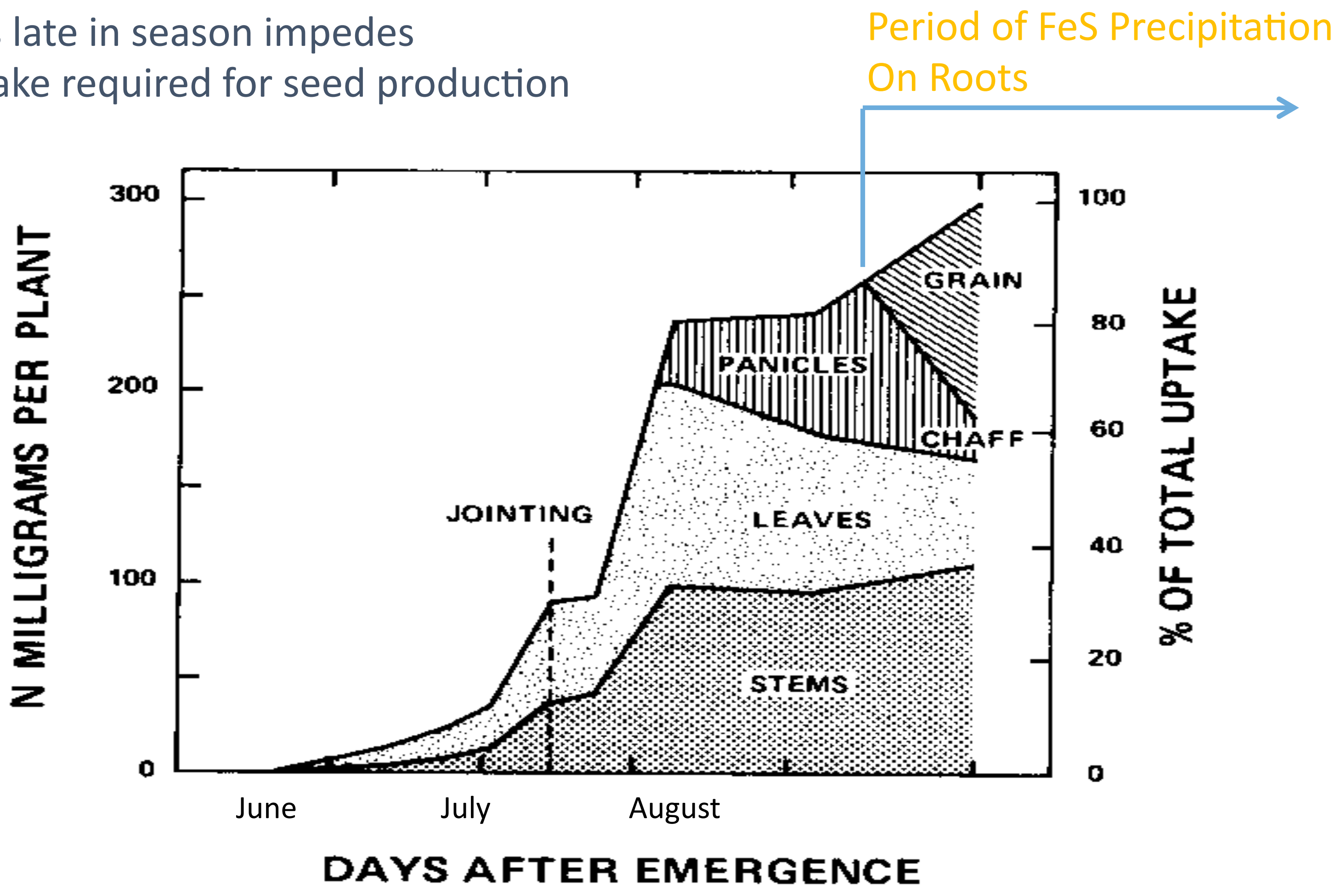
Significance levels

* $0.05 < p < 0.10$

** $0.001 < p < 0.05$

*** $p < 0.001$

FeS$_x$ on roots late in season impedes nitrogen uptake required for seed production



Grava and Raisanen 1978

Preliminary Conclusions – Bucket Experiment

- Iron oxides act as oxidized buffer during early-mid season
- Iron oxide buffer is overwhelmed by sulfide around the start of seed production
- Seed stage may be disproportionately harmed by sulfide because it coincides with iron sulfide precipitation on roots

Acknowledgements

NAH-GAH-CHI-WA-NONG
FOND DU LAC
RESERVATION

Minnesota Pollution Control Agency

Sea Grant
Minnesota

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment D

(4 pages)

[redacted]

MINNESOTA
SEA GRANT COLLEGE PROGRAM
RESEARCH ANNUAL REPORT

PI NAME: John Pastor

PROJECT NUMBER: R/CE-04-14
Chart String: 1000 10340 20857 00041968

PROJECT END DATE: June 30, 2016

REPORT DATE: May 5, 2016

PROJECT TITLE: The Biogeochemical Habitat of Wild Rice

PROGRESS TOWARD OBJECTIVES: (summarize your progress over the last 12 months)

With Sea Grant funding, we continued one long-term experiment and initiated two others. The long-term experiment consisted of adding sulfate to tanks containing wild rice grown in wild rice sediment to achieve surface water concentrations of ambient (7), 50, 100, 150, and 300 ppm SO4. After five years (two under SeaGrant funding, the wild rice populations in the 300 ppm tanks have gone extinct and the populations in the 150 ppm tanks are nearing extinction (Pastor et al. submitted). Extinction was caused by toxic levels of sulfide (from sulfate reduction) to seedlings and from reduced seed production. Proportional decreases in population productivity have happened in the other amended tanks.

During the course of these experiments, wild rice roots in tanks with more than 50 mg/L sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle (Fig. 1). Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides.

To sort out these two potential effects of FeS precipitation in roots and on sediments, we initiated two additional experiments. One is a large scale tank experiment in which additions of sulfate to 300 ppm, a tripling of sediment iron, and removal of litter (to reduced labile carbon for microbes) were applied in a crossed factorial design. After two years, sulfate amendments had the greatest effect, reducing production as in the first experiment regardless of iron amendment and litter removal. Iron amendment had no statistically significant effect, but plants grown under both sulfate and iron amendments had the lowest vegetative and seed production of all. Litter removal had no effect. While we cannot yet conclude from this experiment that iron has a strong depressive effect on wild rice growth via FeS plaques on roots, we can conclude that iron has no beneficial effect by reducing the toxicity of sulfide.

We also initiated a third experiments aimed at quantifying the development of these FeS root plaques. In this experiment, wild rice was grown individually in buckets with and without sulfate amendments (to 300 ppm). We sampled plants every two weeks to determine the phenology of the development of FeS plaques on the roots. We made two surprising observations. First, accumulation of FeS plaques on roots of plants grown under high sulfate concentrations increased very rapidly and suddenly in midsummer even while porewater sulfide in the bulk sediment remained unchanged. And second, by the end of the growing season, FeS concentrations were two orders of magnitude higher on black root surfaces than in the surrounding sediment; after a single annual growing season, the black roots contained approximately 5% (by mass) of the total amount of sulfur in the experimental sediments. FeS in the bulk sediment also increased during the growing season but much more slowly and without an obvious breakpoint in accumulation rate. These observations suggest an overwhelmingly dominant, plant-induced change towards conditions more conducive to FeS precipitation in the immediate vicinity of the roots that begins in the middle of the growing season and controls the rates and location of sulfur transformations.

Plants with the black FeS plaques on their roots produced fewer and less viable seeds, perhaps because the plaques potentially impair the uptake of phosphorus and nitrogen (Pastor et al. submitted). The rapid accumulation of FeS plaques occurs at the time that wild rice plants are beginning to flower and take up additional nutrients for the ripening seeds. This suggests that even if the precipitation of FeS in the bulk sediment reduces aqueous sulfide, precipitation on the root surfaces somehow impedes seed formation, perhaps by blocking nutrient uptake.

Last summer, we also added ^{15}N periodically throughout the growing season to plants amended with 300 mg/L sulfate and plants without sulfate addition. These experiments are providing a more detailed look at the plant-side nutrient fluxes in the context of the changing rates of sulfur accumulation on root surfaces. Preliminary results suggest that nitrogen uptake by wild rice may be inhibited by plaque formations, especially during the period of seed filling and ripening. If nitrogen uptake is inhibited by FeS plaques, then this may explain why wild rice plants with FeS plaques on roots had smaller seeds and a greater proportion of the seeds were not filled (Pastor et al. submitted).

DIFFICULTIES ENCOUNTERED AND ACTIONS TAKEN TO OVERCOME THEM:

Before we began the ^{15}N experiment last year, we had to spend the previous summer in pilot trials determining how much ^{15}N to add to create a measureable signal in the plants while overcoming the strength of the microbial sink in the sediment. This took up one entire summer The following summer was spent determining the approximate joint phenology of FeS plaque formation and ^{15}N uptake. Now that we know the proper amount of ^{15}N to add and the approximate joint phenology of its uptake in relation to FeS plaque formation, we have devised a sampling schedule wherein we will sample at high frequencies during the time of FeS plaque formation to determine how it coincides with nitrogen uptake. This will allow us to determine whether FeS plaques form at a constant increment controlled entirely by inorganic geochemistry of the sediments, or whether FeS plaques grow exponentially as they progressively cut off radial oxygen losses from the roots. We are, under separate documentation, requesting a no-cost extension of unspent graduate student funds to support Ms. Sophie LaFond-Hudson to continue these experiments which will be part of her Ph.D. thesis in Water Resources Sciences at the University of Minnesota.

RESULTS TO DATE: (please provide a brief summary of your results)

See above. Paper submitted acknowledging SeaGrant support:

Pastor, J., B. Dewey, N. W. Johnson, E.B. Swain, P. Monson, E.B. Peters, and A. Myrbo. Effects of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris*) in hydroponic and mesocosm experiments. Ecological Applications: submitted.

ASSESS PROGRESS RELATIVE TO ORIGINAL SCHEDULE AND FINAL DEADLINE:

We have accomplished all of our original goals involving the tank experiments. The ^{15}N experiments were begun in response to a recommendation of the proposal review panel that we include some isotopic amendments to determine the effect of sulfate amendments on nutrient cycling. However, in order to do that with any precision, we needed to spend two years in pilot experiments to determine the amount of ^{15}N to add and its phenology relative to the growth of FeS plaques at high sulfate concentrations. With one more year's fieldwork we will be able to accomplish this objective.

OUTREACH OR PRODUCTS: Please list any products (Web or print), presentations, articles, media interviews, teacher training, K-12 education, etc. that you or your student(s) have from this research thus far. Is there anything our Communications or Extension staff can do to help you connect your research with stakeholders?

PERFORMANCE MEASURES: We are required to provide performance measures to National Sea Grant each year. You may not have anything at all in some of these categories, and that is expected. All we need at this point is your best guess and an explanation of how you arrived at your answer.

Measure 1: Economic and societal benefits derived from the discovery and application of new sustainable coastal, ocean, and Great Lakes products from the sea.

We are reporting these results to the Minnesota Pollution Control Agency and to the various tribal units of Lake Superior Chippewa who are in discussion about setting sulfate standards for waters entering wild rice beds. Many of these waters also enter Lake Superior and the estuaries of some major rivers such as the St. Louis and Fish Rivers once supported extensive wild rice beds which the states of Minnesota and Wisconsin are trying to restore. These results will help inform these restoration efforts by helping the state agencies determine how many and which acres could be restored to wild rice populations.

Measure 2: Cumulative number of coastal, marine, and Great Lakes issue-based forecast capabilities developed and used for management. *(typically interpreted to include most computer models)*

Not applicable

Measure 3: Percentage/number of tools, technologies, and information services that are used by managers (NOAA and/or its partners and customers) to improve ecosystem-based management.

See answer to Measure 1.

Measure 4: Acres of ecosystems protected or restored as a result of Sea Grant's involvement.

Not directly applicable, but see answer to Measure 1.

Measure 5: Number of environmentally-responsible fisheries and/or aquaculture production or harvesting techniques implemented.

Not applicable.

Measure 6: Number of communities who adopt/implement sustainable, economic and environmental development practices and policies, or hazard resiliency practices.

See answer to Measure 1.

Measure 7: Number of environmental curricula adopted by formal and informal educators.

John Pastor uses these results in his class in Integrated Biological Systems and Nathan Johnson uses these results in his class in Environmental Modelling. In addition, classes from Fond du Lac Community College routinely tour these experiments as part of their curriculum in wild rice management.

OTHER METRICS OF INTEREST TO NOAA: Please answer any that apply to your project (none may, and that is fine).

1. Did or will your project help develop or update sustainable development ordinances, policies, or plans? If so, in what community?

   See answer to Measure 1 above. The communities are the States of Minnesota and Wisconsin and the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa.

2. Did your project help a community implement a sustainable development plan? If so, what community?

   Potentially it will help the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa.

3. Did your project help develop or update a port or waterfront redevelopment ordinance, policy, or plan? If so, what port or community?

Not applicable

4. Did you help a port or waterfront implement a redevelopment plan? If so, what port or community?

Not applicable

5. Did your project help develop or update polluted runoff management ordinances, policies, or plans? If so, for what community?

Potentially the results of this research will help inform the State of Minnesota as it reviews its sulfate criteria for wild rice beds, especially in regard to runoff from iron and copper-nickel mines in northern Minnesota.

6. Did your project help implement a polluted runoff management ordinance, policy, or plan? If so, for what community?

Not applicable (yet).

PLANS FOR THE NEXT 6 MONTHS:

Continue to monitor the changes in wild rice populations in the tank experiments and initiate another ^{15}N addition experiment to distinguish between different models of FeS plaque formation and their effect on nitrogen uptake.

NAMES OF STUDENTS BEING SUPPORTED BY THIS GRANT AND THEIR LEVEL (e.g, grad (MS, PhD), undergrad, etc). For grad students, please indicate whether their thesis research is related to this project.

Ms. Sophie LaFond-Hudson, completed MS - WRS research on this project and is initiating Ph.D. –WRS research on it as well. Advisors: Profs. Nathan Johnson and John Pastor

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment E

(3 pages)

June 28, 2017

Progress Report on Experiments on Effects of Sulfate and Sulfide on Wild Rice

John Pastor, Dept. of Biology, University of Minnesota Duluth

This memo is a brief report on our ongoing experiments on the effects of sulfate and sulfide on wild rice, funded by EPA through the Fond du Lac and Grand Portage Bands of Lake Superior Chippewa Water Quality Programs, the State of Minnesota, and Minnesota Sea Grant.

Our hypothesis is that sulfate amendments are detrimental to wild rice populations when it is reduced to the more toxic sulfide. We have initiated several long-term experiments to test this hypothesis and elucidate the underlying mechanisms. The longest experiment consisted of adding sulfate to 100 gallon stock tanks containing wild rice grown in wild rice sediment to achieve surface water concentrations of ambient (7), 50, 100, 150, and 300 mg/l SO_4. Sulfide concentrations in sediments increased in proportion to sulfate concentrations (Pastor et al. 2017). After five years (2011-2015), the wild rice populations in the 300 mg/l tanks have gone extinct and the populations in the 150 mg/l tanks are nearing extinction (Pastor et al. 2016; Fig. 1). Extinction was caused by toxic levels of sulfide (from sulfate reduction) to seedlings (Fig. 1) and from reduced seed production (Fig. 2). Proportional decreases in population productivity have happened in the other amended tanks. Raw data from this experiment has been archived at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1452/full

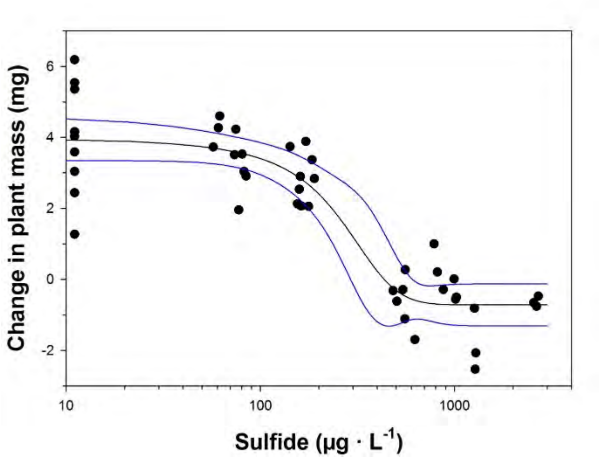


Figure 1. Reduction in seedling growth with increased sulfide concentrations in a hydroponucs experiment (Pastor et al. 2017).

During the course of these experiments, wild rice roots in tanks with more than 50 mg/l sulfate had become blackened. In contrast, plants grown in the low sulfate treatments had orange stains on the roots throughout the annual life cycle. Using SEM elemental scans, we identified the black plaques as iron sulfide (FeS) plaques whereas the orange stains had iron but no sulfide and are most likely iron (hydr)oxides. Precipitation of iron sulfide on roots may inhibit nutrient uptake, thus leading to reduced seed production. On the other hand, precipitation of iron sulfide in sediments could neutralize the toxicity of sulfide to seedlings.

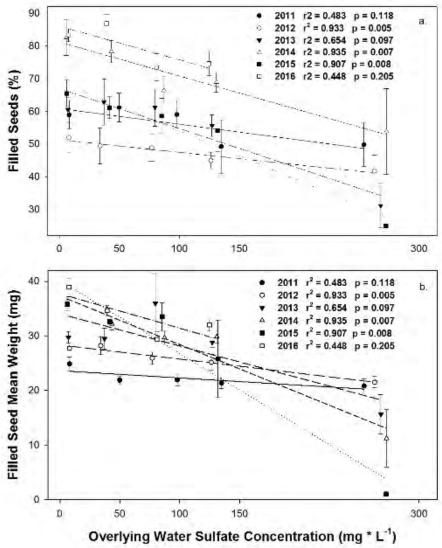


Figure 2. Reduction in seed production with increased sulfate concentrations in stock tank experiments (2011-2015 data from Pastor et al. 2017, with 2016 data added).

To sort out these two potential effects of FeS precipitation in roots and on sediments, we initiated two additional experiments. One is a long-term tank experiment in which additions of sulfate to 300 mg/l, a tripling of sediment iron in the first growing season, and removal of litter (to reduced labile carbon for microbes) were applied in a crossed factorial design. This experiment began at the beginning of the 2015 growing season. During the first three years of this experiment, sulfate amendments had the greatest effect, reducing seedling survival, plant growth, and seed production regardless of iron amendment and litter removal. Litter removal had no effect on seedlings, vegetative growth, or seed production. In the first two growing seasons, adding iron without sulfate had no effect on seedling survival, plant growth, or seed production. Iron amendments in the presence of sulfate increased seedling survival compared with seedlings grown under sulfate amendments alone, but seedling survival in the iron + sulfate tanks was still less than in control tanks. We believe the partially ameliorative effects of iron on seedling survival was due to precipitation of iron sulfide in the sediment, thus partly neutralizing sulfide toxicity to seedlings. However, by the spring of year 3 (2017), the amendment of iron no longer appears to have any effect on seedling survival, possibly because all the iron we added has been titrated out of the tanks by precipitation with sulfide either in the sediment or on the plant roots.

Figure 3. Orange iron (hydr(oxide) stains on healthy wild rice roots in low sulfate environments (left) and black iron sulfide plaques on roots in high sulfate environments (right).

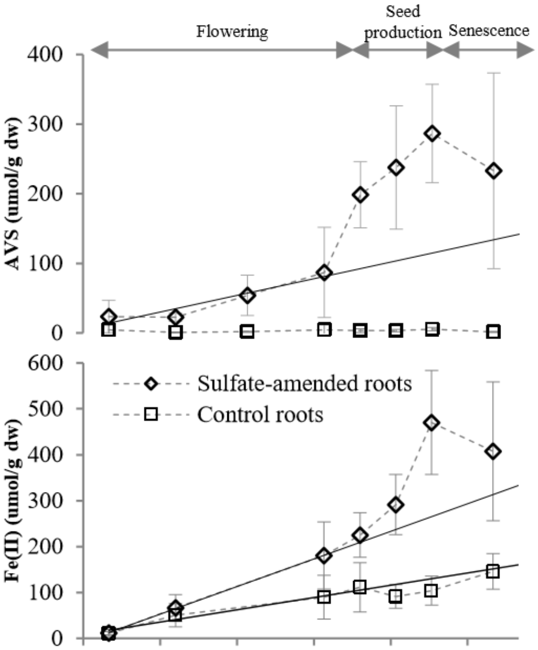


Figure 4. Time course of (top) sulfide and (middle) ferrous iron accumulation on plant roots in sulfate amended and control conditions (LaFond-Hudson et al. submitted).

We also initiated a third experiment aimed at quantifying the development of FeS root plaques (Fig. 3). In this experiment, wild rice was grown individually in buckets with and without sulfate amendments (to 300 mg/l). We sampled plants every two weeks to determine the phenology of the development of FeS plaques on the roots. We made two surprising observations. First, accumulation of FeS plaques on roots of plants grown under high sulfate concentrations increased very rapidly and suddenly in midsummer at the time that wild rice plants are beginning to flower and take up additional nutrients for the ripening seeds (Fig. 4). And second, by the end of the growing season, FeS concentrations were two orders of magnitude higher on black root surfaces than in the surrounding sediment; after a single annual growing season, the black roots contained approximately 5% (by mass) of the total amount of sulfur in the experimental sediments. FeS in the bulk sediment also increased during the growing season but much more slowly and without an obvious breakpoint in accumulation rate. These observations suggest an overwhelmingly dominant, plant-induced change towards conditions more conducive to FeS

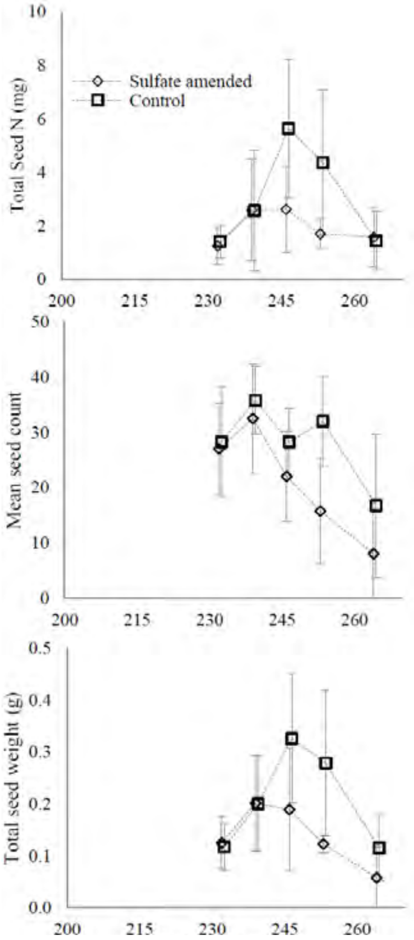


Figure 5. Seed nitrogen, seed count, and seed weight are higher in control plants with orange roots compared with plants with black roots grown under 300 mg/L sulfate (Lafond-Hudson

precipitation in the immediate vicinity of the roots that begins in the middle of the growing season and controls the rates and location of sulfur transformations.

Plants with the black FeS plaques on their roots produced fewer and smaller seeds containing less nitrogen (Fig. 5), perhaps because the plaques potentially impair the uptake of nitrogen. This suggests that even if the precipitation of FeS in the bulk sediment reduces aqueous sulfide and partly ameliorates sulfide toxicity to seedlings, precipitation on the root surfaces somehow impedes seed formation, perhaps by blocking nutrient uptake.

In summary, our long-term experiments on the biogeochemistry of sulfate in wild rice habitat demonstrates that sulfate is not toxic in and of itself to wild rice, but when reduced to sulfide is directly toxic to seedlings. Iron additions may partly ameliorate sulfide toxicity to seedlings in spring, but precipitation of iron sulfide plaques on roots during the flowering and seed production period of wild rice's life cycle appears to block uptake of nitrogen, leading to fewer and smaller seeds with reduced nitrogen content. The net effect of sulfate additions to wild rice populations is to drive the populations to extinction within 4 or 5 years at high concentrations of sulfate (300 mg/l) and to greatly reduce population viability at lower concentrations.

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Pastor, J., B. Dewey, N. W. Johnson, E.B. Swain, P. Monson, E.B. Peters, and A. Myrbo. 2017. Effects of sulfate and sulfide on the life cycle of wild rice (*Zizania palustris*) in hydroponic and mesocosm experiments. Ecological Applications 27: 321-336.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment F

(39 pages)

Iron and Sulfur Cycling in the Rhizosphere of Wild Rice (*Zizania palustris*)

A thesis
SUBMITTED TO FACULTY OF THE
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BY

Sophia LaFond-Hudson

IN PARTIAL FULFILLMENT OF THE REQUIERMENTS FOR THE DEGREE OF
MASTER OF SCIENCE

Nathan Johnson, John Pastor

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Abstract

Iron (hydr)oxides typically form on roots of many wetland plants, including wild rice (*Zizania palustris*), an annual macrophyte with significant cultural, economic, and ecological value. Iron (hydr)oxides are thought to protect macrophytes from toxic reduced species, such as sulfide, by providing an oxidized barrier around the roots. However, wild rice grown under high sulfate loading develops a black iron sulfide precipitate on the root surface, and produces fewer and lighter seeds, leading to a decreased population in the long term. In order to investigate the role of iron sulfide root precipitates in impaired seed production, wild rice plants grown in buckets were exposed to sulfate loading of 300 mg/L, and harvested biweekly for extraction of root acid volatile sulfide (AVS) and weak acid extractable iron and analysis of plant and seed N. In sulfate-amended plants, AVS on roots accumulated over the course of the growing season, and accumulated rapidly just prior to seed production. Simultaneously, iron speciation of the root precipitate shifted from Fe(III) to Fe(II), consistent with a transition from iron (hydr)oxide to iron sulfide. A mechanism is herein proposed by which sulfide-induced suberization of roots decreases radial oxygen loss that keeps the rhizosphere oxidized, leading to reduction of iron (hydr)oxides and subsequent iron sulfide accumulation. Plants amended with sulfate produced fewer, lighter seeds with less nitrogen. We suggest that sulfide inhibits N uptake, and seeds are disproportionately harmed because rapid AVS accumulation occurs during the reproductive life stage.

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Introduction

Iron (hydr)oxide plaques have been observed on the roots of wild rice (*Zizania palustris*), a culturally significant macrophyte that forms large monotypic stands in the lakes and rivers of Minnesota, Wisconsin, northern Michigan, and Ontario (Lee and McNaughton 2004, Jorgenson et al. 2013). Iron (hydr)oxide plaques commonly form on the roots of wetland plants growing in anoxic, reduced sediments as a result of a redox gradients found in the rooting zone (Mendelssohn and Postek 1982, Jacq et al. 1991, Snowden and Wheeler 1995, Christensen and Sand-Jensen 1998). Redox gradients in the rhizosphere are caused by radial oxygen loss, a process in which wetland plants release oxygen into the rhizosphere through their roots via arenchyma tissue (Armstrong and Armstrong 2005, Schmidt et al. 2011). When Fe(II) is transported from anoxic sediment into the oxygenated rhizosphere, it is oxidized to Fe(III), which combines with oxygen from the roots to form insoluble iron oxides or hydroxides. Iron plaque formation can occur abiotically, but it is also associated with iron-oxidizing bacteria in many cases (St. Cyr 1993, Neubauer et al. 2007). Iron plaques have been proposed as a mechanism to protect plants from reduced toxic substances such as hydrogen sulfide, because they form an oxidized barrier around the roots (Koch and Mendelssohn 1989, Mendelssohn et al. 1995). However, during previous sulfur addition experiments, black iron sulfide root coatings, characteristic of iron sulfide minerals, have been observed on wild rice roots (Pastor et al., in review). Black root coatings have also been observed in white rice grown in surface water with high sulfate concentrations (Jacq et al. 1991, Gao et al. 2003, Sun et al. 2015).

The iron and sulfur chemistry of aquatic plant rooting zones involves a set of interrelated biogeochemical processes. Sulfate and iron (III) oxides are both redox active species that play a role in degradation of organic matter in aquatic sediments. During aerobic respiration, electrons are transferred from organic compounds to oxygen, but in anaerobic respiration alternative electron acceptors are used, including nitrate, ferric iron, sulfate, and carbon dioxide. Organisms use the more thermodynamically favorable electron acceptors first; nitrate is used before ferric iron, and carbon dioxide is used only when more favorable electron acceptors have been consumed. This thermodynamic ordering manifests itself as stratified microbial communities with distance away from an oxic-anoxic boundary (Boudreau 1996, Van Cappellen and Wang 1996). Anaerobic respiration produces reactive reduced species as byproducts, including ammonia, ferrous iron, sulfide, and methane. Iron-reducing and sulfate-reducing bacteria facilitate production of ferrous iron and sulfide respectively, after which ferrous iron and sulfide can combine to produce iron monosulfide (FeS) or pyrite (FeS_2). Alternatively, ferrous iron and sulfide can undergo oxidization back to ferric iron and sulfate abiotically via bioturbation or water level fluctuations (Thamdrup et al. 1994, Eimers et al. 2003) or biotically via iron or sulfide oxidizing bacteria (lithoautotrophy). Despite the predictability of the sequence of electron acceptors used in anaerobic respiration, coincident iron reduction and sulfate reduction in close proximity has been documented, during which the subsequently produced sulfide reacts abiotically with nearby iron (hydr)oxides to produce reduced iron and elemental sulfur (Hansel et al. 2014, Kwon et al. 2013).

Macrophytes can accelerate iron and sulfur cycling by enhancing redox gradients when radial oxygen loss creates an oxic layer around the root surface. Oxidation of Fe(II) to Fe(III) oxides immobilizes iron on or very near the root surface. Conversely, oxidation of sediment FeS by radial oxygen loss mobilizes previously bound sulfur as soluble sulfate (Choi et al. 2006). Cycling is dynamic near the rhizosphere because oxidation potential (Eh) changes abruptly over just a few millimeters. Just outside the oxic layer, the sediment can be strongly reducing. Heterotrophic iron and sulfate reduction can be stimulated by root exudates released by the plant (Kimura et al., 1981), and, in the case of an annual plant like wild rice, senesced plant material at the end of the growing season each year (Jacq et al. 1991). Several studies have compared sediment with and without vegetation and found higher sulfide or FeS concentrations in sites with plants (Holmer & Nielsen, 1997, Jacq et al. 1991, Lee & Dunton 2000). The increase in reduced species is attributed to larger pools of organic matter to drive reduction.

In Minnesota, surface water sulfate concentrations are regulated in wild rice waters because high surface water sulfate concentrations are associated with decreased wild rice abundance (Moyle, 1945, MPCA Analysis of the Wild Rice Sulfate Standard Study, 2014). It has recently been shown that sulfide, the reduced form of sulfate, is toxic to wild rice seedlings (Pastor et al., in review). In other wetland plants, sulfide is thought to interrupt metabolism by inhibiting metallo-enzymes in the electron transport chain during respiration (Allam and Hollis 1972, Koch and Mendelssohn 1989, Koch et al. 1990, Lamers et al. 2013; Armstrong and Armstrong 2005, Martin and Maricle 2015). Inhibition of ATP production deprives a plant of energy required for nutrient uptake. Sulfide has been shown to reduce nutrient uptake in white rice (*Oryza sativa*), a plant physiologically similar to wild rice (Joshi et al. 1975), so it is plausible that sulfide may also inhibit nutrient uptake in wild rice.

Pastor et al. (in review) found that exposure to sulfide decreased mean seed weight and the proportion of filled seeds more significantly than by having immediate toxic effects on plant growth and physiology. Wild rice takes up nitrogen, its limiting nutrient, in three main bursts: 30% is taken up during early season vegetative growth, 50% is taken up during early flowering, and 20% is taken up during late flowering and seed production (Grava and Raisanen, 1978). The effects of sulfide exposure on wild rice are consistent with nitrogen limitation during seed production, but it is not well understood why the seed production life stage is disproportionately harmed by sulfide. Is iron sulfide plaque accumulation a geochemical mechanism that controls the impact of sulfide on nitrogen uptake?

The objective of this study is to understand how iron and sulfur cycle near root surfaces and how this cycling affects nitrogen uptake by wild rice during its life stages, especially seed production. We investigate the drivers of iron sulfide plaque formation and seek to answer if plant and seed nitrogen uptake are adversely affected by iron sulfide accumulation on root surfaces.

Methods

Experimental Design

Sediment was collected from Rice Portage Lake (MN Lake ID 09003700, 46.703810, -92.682921) on the Fond du Lac Band of Lake Superior Chippewa Reservation in Carlton County, Minnesota in late May, 2015 and placed in a 400L Rubbermaid stock tank where it was homogenized by shovel. Initial carbon in the sediment was 14.8 ± 1.70% and initial nitrogen was 1.12 ± 0.13 %. Eighty 4 L plastic pails were then filled with 3 L of the sediment. Each 4 L pail was placed inside of a 20 L bucket which was filled with 12 L of water to provide a 12-15 cm water column. The overlying water of 40 randomly chosen buckets was then amended with an aliquot of stock solution (5.15g of Na_2SO_4 dissolved in 200ml of deionized water) to result in 300 mg/L (3.125 mM) sodium sulfate. The amendment concentration was chosen as such because when used in previous mesocosm experiments, wild rice populations went extinct within five years (Pastor et al. in review), but it is only slightly higher than the EPA drinking water secondary standard (250mg/L) and is a concentration found in some Minnesota lakes (MPCA Analysis of the Wild Rice Sulfate Standard Study, 2014). The overlying water was sampled twice throughout the trial and adjusted to 300mg/L SO_4 with appropriate amounts of Na_2SO_4 stock solution. The other 40 buckets did not receive any sulfate and on 6/23/15 (day 174, Julian date) had an average surface water sulfate concentration of 14.44 ± 1.01 mg/L, consistent with the local groundwater sulfate concentration. In each bucket, two seeds which were harvested in 2014 from Swamp Lake on the Grand Portage Reservation (MN Lake ID 16000900, 47.951856, -89.856844) were planted on 5/15/15 (Julian day 135). Once shoots reached a height of approximately 20 cm during the aerial stage, plants were thinned to one plant per bucket.

Sampling of pore water, roots, and stems began midsummer (63 days after planting/germination), at the start of flowering and the second burst of nitrogen uptake (Grava and Raisanen, 1978), and continued until plants had thoroughly senesced, for a total of eight sample dates, not including initial sediment and pore water sampling. Sampling occurred every two weeks for the first four sample dates, (flowering, days 189-232) and weekly for the last four sample dates (seed production, days 238-265), for a total of eight sample dates. One week prior to each sampling date, 40 ml of enriched ^{15}N

solution were injected into the sediment of four randomly selected sulfate-amended buckets and four control buckets. For the first two sample dates, the labeling solution was prepared by adding 0.88 mg of 10% ^{15}N-NH_4Cl to 500 ml DI water. For all other sample dates, 2.2mg of 10% ^{15}N-NH_4Cl were added to 500 ml of DI water to account for an increase in plant biomass later in the growing season. The solution was injected into the sediment of the 4L pail in four locations uniformly spaced around the center of the pail, approximately 2 cm from the outer edge and 2 cm from the bottom. Immediately before injection, the overlying water was removed from the outer pail, leaving 2-5 cm above the sediment in the internal pail, to keep the ^{15}N-NH_4Cl contained in the sediment for uptake by the wild rice roots. On each sample date, one week after injection of ^{15}N, the four sulfate-amended and four control buckets were sampled for pore water sulfide, pore water sulfate, pore water iron, and pH. After pore water sampling, the wild rice plant was destructively harvested for analysis of vegetative ^{15}N, vegetative total N, and root AVS and weak acid extractable iron. The bulk sediment was sampled for solid phase S and Fe analysis at the beginning and at the end of the growing season.

Pore water sampling and analysis

Prior to extracting pore water samples, pH was measured *in-situ* with a ThermoScientific Orion pH electrode at a depth of 5 cm below the sediment surface and 2 cm from the stem of the wild rice plant. Pore water was sampled using 5-cm length, 2-mm diameter tension lysimeter filters (Rhizons, Seeberg-Elverfeldt et al., 2005) attached with a hypodermic needle to an evacuated, oxygen-free serum bottle sealed with a 20 mm thick butyl-rubber stopper (Bellco Glass, Inc). The entire filter end of the Rhizon was inserted vertically into the sediment just below the surface. The goal was to draw water from approximately the upper 5 cm of sediment without drawing surface water. The filter was placed with minimal jostling to avoid creating a cavity around the filter that would allow surface water to enter the sediment and contaminate the pore water. The Rhizon was placed approximately 2 cm away from the stem of the wild rice plant and on the opposite side from where pH was measured.

Pore water sulfide samples were drawn into 50-mL serum bottles preloaded with 0.2% 1 M ZnAc and 0.2% 6 M NaOH to preserve sulfide. Sulfide bottles were left to fill overnight, then stored at 4C in the sealed serum bottles used for sample collection for approximately 30 days before sulfide was quantified. Samples for pore water sulfate analysis were withdrawn from sulfide sampling bottles and filtered through a Dionex 1cc metal cartridge and a 0.45 μm polyethersulfone filter approximately three months after they were collected. Pore water iron was collected in 8-mL serum bottles preloaded with 40% deionized water, 40% phenanthroline, 20% acetate buffer, and 1% concentrated hydrochloric acid. Iron bottles were filled until the solution turned light red, approximately ten minutes. If the solution turned red before 8 mL were collected, samples were diluted with deionized water to bring the total solution to 8 mL. Iron samples were quantified within two hours of sampling. Iron and sulfide were quantified colorimetrically using the phenanthroline and methylene blue methods, respectively, on a HACH DR5000 UV-Vis spectrophotometer (Eaton et al., 2005). Sulfate was quantified using a Dionex ICS-1100 Integrated IC system (AS-DV Autosampler) (Eaton et al., 2005).

Solid phase sampling and analysis

Samples for the bulk sediment initial conditions were obtained after homogenization of the sediment prior to placement in the buckets (day 152). Five replicate samples were placed in jars and analyzed for AVS and simultaneously extracted iron. At the end of the season, mini-cores of intact sediment were retrieved immediately before wild rice plants were sampled.

On each sample date throughout the summer, wild rice roots were collected for AVS and weak acid extractable iron. Each plant was removed from the sediment and immediately rinsed in buckets of deoxygenated water continuously bubbled with nitrogen. While submerged in deoxygenated water, the stem was cut just above the root ball so that the shoots and seeds could be saved for ^{15}N analysis. Roots were then placed in jars full of deoxygenated water, which were immediately placed in a plastic bag flushed with nitrogen and transported to an oxygen-free glove box. In the glove box, the roots were cleaned of extra organic matter prior to removing a 1-2 g section of wet root mass for AVS and iron analysis. From both sediment and roots, AVS was extracted using 7.5 ml 1 N HCl for 4 hours using a modified diffusion method (Brouwer and Murphy 1994). During a room temperature acid incubation with gentle mixing, sulfide was trapped in an inner vial containing Sulfide Antioxidant Buffer (SAOB) and subsequently quantified using a ThermoScientific sulfide ion-selective electrode with a detection limit ranging from 0.01-40 mmol/L. Ferrous iron was quantified colorimetrically using the phenanthroline method on a HACH DR5000 UV-Vis spectrophotometer (Eaton et al., 2005), and weak acid extractable iron was quantified using a Varian fast sequential flame atomic absorption spectrometer with an acetylene torch.

A subset of roots was tested for chromium(II)-reducible sulfur (CRS) to determine whether AVS was extracting all total reduced inorganic sulfur on the roots. A diffusion-based CRS method was used, which can fully extract amorphous iron sulfide and pyrite and can partially extract elemental sulfur (Burton et al. 2008). Chromic acid for CRS analysis was prepared according to Burton et al. (2008). Inside an oxygen-free glove box, a section of root from a plant previously analyzed for AVS was placed in the analysis bottle. An inner vial containing SAOB was also placed inside the bottle prior to sealing. Bottles were taken out of the glove box and injected with chromic acid. CRS was extracted for 48 hours and quantified using a ThermoScientific sulfide ion-selective electrode.

Isotope sampling and analysis

For analysis of ^{15}N uptake, the plants were sub-sampled by cutting at the stem to root transition. If seeds were present, they were removed prior to sampling the plant and saved for separate analysis. The plants and seeds were rinsed with deionized water and dried in paper bags for seven days at 65C. The dried plants were weighed, placed in polycarbonate vials with stainless steel balls, and shaken in a SPEX 800M mixer mill until the samples were in a powdered form. Seeds were counted, weighed, and powdered using the same method. The samples were transferred to glass vials and dried again overnight at 65C with caps loosely covering the vials. Samples were quantified for total N and δ^{15}N on a Finnigan Delta Plus XP isotope ratio monitoring mass spectrometer.

Data analysis

Geochemical parameters and measured attributes of plants were analyzed using repeated measures analysis of variance to determine differences between sulfate amendments and controls. A paired *t* test was used to determine differences between AVS and CRS concentrations on roots. A two-factor ANOVA was used to compare preplanting and post-senescence sediment concentrations of iron and AVS between treatments. Analyses were performed using the statistical software SAS. Logarithmic transformations were used when data was non-normal. A reciprocal transformation was used for dry weight of plants, as a logarithmic transformation was not effective. Data for root AVS were split into pre-seed production and post-seed production because the full-season data was not able to be transformed.

The saturation index was calculated to determine if the pore water was saturated enough to precipitate iron sulfide (equation 1). A positive saturation index value indicates precipitation, and a negative value indicates dissolution. The K_{sp} value used was $10^{-2.95}$ (Stumm and Morgan, 1995).

$$SI = log\frac{[IAP]}{K_{sp}} \text{ where } IAP = \frac{[Fe^{2+}][HS^{-}]}{[H^{+}]} \qquad \text{Equation 1}$$

Changes in the accumulation rates of root AVS and ferrous iron were tested by fitting linear regressions to the concentrations of root AVS and Fe^{2+} prior to seed production (days 189-231). The model was extrapolated to late season sample dates (days 232-264) to test if accumulation rates changed between flowering and seed production.

A mixing model was used to determine the proportion of seed nitrogen originating from the pore water and the proportion translocated from the stems (equations 2 and 3). The $\delta^{15}N$ of the seeds was measured, and the $\delta^{15}N$ of the pore water and the stems were approximated. In equation 2, δ_{sample} is the isotopic signature of nitrogen in the seed, $\delta_{source1}$ is the isotopic signature of the pore water ammonium, f_1 is the proportion of nitrogen coming from the pore water, $\delta_{source2}$ is the isotopic signature of nitrogen in the plant stem, and f_2 is the proportion of the nitrogen sourced from the plant stem. Seed nitrogen can be sourced only from the pore water or the stems, so the proportions from both components must sum to one (equation 3).

$$\partial_{sample} = \partial_{source1} \times f_1 + \partial_{source2} \times f_2 \qquad \text{Equation 2}$$

$$f_1 + f_2 = 1 \qquad \text{Equation 3}$$

Results

Pore water

Although sulfate was 40x higher in the overlying water of sulfate-amended plants, pore water sulfide concentrations were only approximately twice as high in the in the rooting zone of sulfate-amended plants compared to the control over the entire growing season. Sulfide concentration and variability increased in the pore water of both amended and control rooting zones one week after the first seeds were produced (day 238, Julian date) and returned to initial concentrations two weeks later (day 245, Fig. 1a). Pore water sulfide data did not fit any parametric model, so a repeated measures ANOVA was not performed.

Pore water iron concentrations were not correlated with sulfate amendment (Table 1). Pore water iron decreased until shortly after seed production began (day 238) in both amendments. The minimum iron concentration occurred at the same time that a peak in pore water sulfide developed (Fig 1b). Shortly before senescence (days 252 and 264), the iron concentrations returned to values similar to concentrations during the first month of data collection.

The pore water pH and saturation index were not correlated with sulfate amendment (Table 1). The pH of the pore water peaked at the start of seed production (days 231-238, Fig.1c). This peak occurred approximately one week before the iron minimum and the sulfide maximum. The saturation index peaked one week after the first seeds were produced, when pH and sulfide were elevated and iron was low (day 238, Appendix Table 1). The average saturation index was above zero only in the sulfate-amended buckets on day 238. The saturation index gradually declined for the rest of the growing season.

Sulfate concentrations ranged from 10-30 times higher in the pore water of plants amended with sulfate (Table 1). Sulfate increased in the amended pore water until seed production began, when it declined precipitously from 2300 μmol/L to 770 μmol/L over 15 days (Fig 1d). In the pore water of control plants, sulfate concentrations followed a similar trend, but at lower concentrations. Control sulfate peaked at 230 μmol/L before decreasing to 34 μmol/L. Sulfate declined just prior to an increase in pore water sulfide.

Table 1. Results of repeated measures ANOVA testing effect of sulfate, time and interaction of sulfate and time on geochemical and biological variables. Tests for pore water and root parameters include data from the entire growing season, whereas tests for biological parameters only include data from mature seed production. *F* values and degrees of freedom (*d.f.*) are given. Tests for time and sulfate x time have the same number of degrees of freedom. Significance levels are shown using asterisks (***indicates $p < 0.001$, **indicates $0.001 < p < 0.05$, *indicates $0.05 < p < 0.10$).

Repeated measures ANOVA (F values)	**Sulfate**	**d.f.**	**Time**	**Sulfate x Time**	**d.f.**
Pore water geochemistry					
Iron	5.16	1, 5	5.51***	1.14	6, 35
pH	3.25	1, 6	12.5***	1.45	6, 36
Saturation index	2.68	1, 4	2.19*	0.50	6, 34
Sulfate	239***	1, 3	8.17***	1.09	5, 27
Root geochemistry					
AVS (during flowering)	66.1***	1, 5	1.10	0.40	3, 17
AVS (during seed production)	148***	1, 6	5.46**	1.76	4, 24
Weak acid extractable iron	0.53	1, 6	2.65	2.42**	7, 42
Ferrous Iron	127***	1, 6	57.2***	3.34**	6, 36
% Ferrous Iron	235***	1, 6	41.5***	4.91***	6, 36
Biological variables (during seed maturity)					
Plant N (total mass)	1.53	1, 6	0.35	0.25	2, 12
Plant weight	5.00*	1, 6	0.40	0.31	3, 18
Seed N (total mass)	5.84*	1, 6	1.10	1.22	2, 12
Seed weight	4.88*	1, 6	0.59	0.94	2, 12
Seed count	5.00*	1, 6	1.89	0.70	2, 12
Seed δ15N	1.47	1, 6	2.45	0.05	2, 12
Seed N%	1.70	1, 6	3.04*	0.40	2, 12
Vegetative N (plant+seed mass)	5.43*	1, 6	0.32	1.71	2, 12

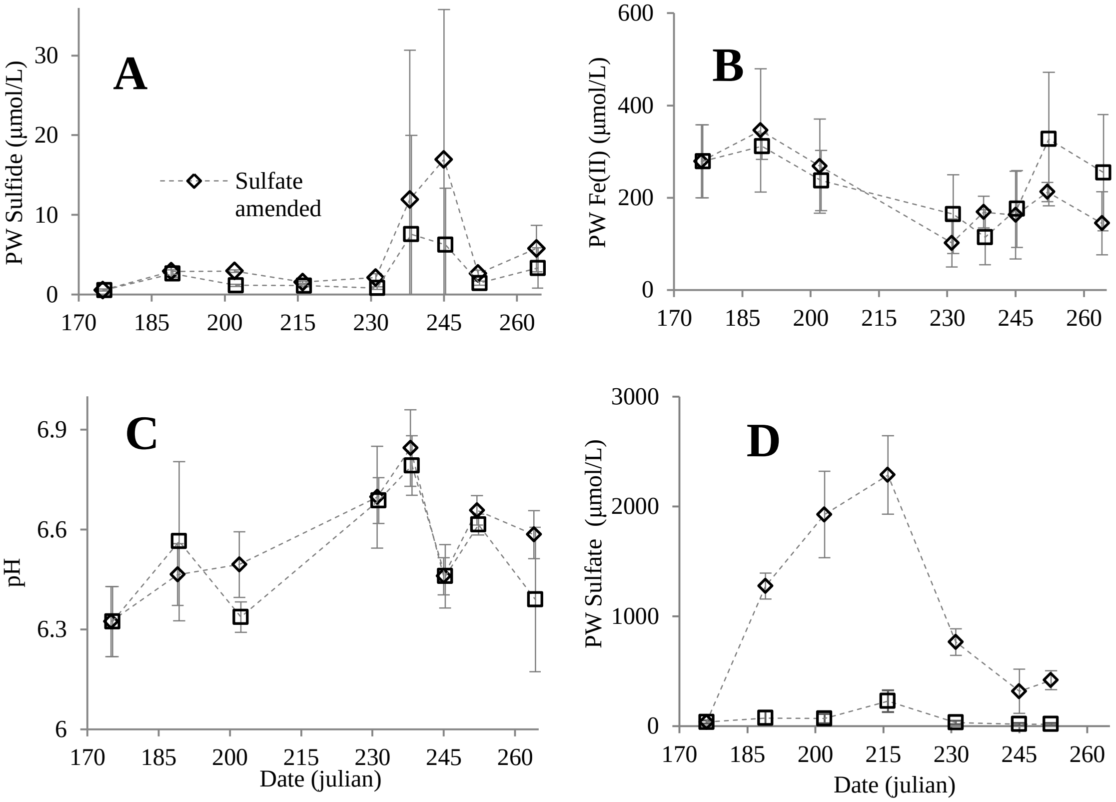

Figure 1. Pore water (PW) data measured in buckets during summer of 2015. Diamonds indicate data from buckets amended with 300 mg/L sulfate. Squares represent data from control buckets. Time is shown in Julian days. Error bars indicate one standard deviation. Control data points are slightly offset to show overlap in error bars.

Roots

Wild rice plants grown in sediment with high overlying water sulfate concentrations developed a black coating on their root surfaces (Appendix Fig. 1). A SEM scan of the roots showed that the root precipitate contained iron and sulfur in approximately a 1:1 ratio (Dan Jones, unpublished data). The oxic/anoxic interface was often recorded on the root; the black coating started on the stem just above the root ball and extended downwards along the entire length of the roots. Adventitious roots that grew at the surface of the sediment remained white, the natural color of wild rice root tissue. Control plants, grown in sediment with low overlying water sulfate, formed very little black color on their roots, instead appearing amber, a color characteristic of iron (hydr)oxides.

Roots grown under elevated sulfate (hereafter “amended roots”) accumulated AVS concentrations up to two orders of magnitude higher than the control roots by late summer. Amended root AVS peaked at 298 ± 74 umol/g dw immediately prior to senescence (Fig 2a). Concentrations of AVS on roots grown under control surface water sulfate (hereafter “control roots”) did not consistently increase, and averaged of 3.2±1.7 umol/g dw. For amended roots, the rate of accumulation of root AVS appeared relatively constant (linear) until the first day seeds were produced (day 232), when the rate of AVS accumulation appeared to increase abruptly. During seed production, AVS concentrations were greater than that predicted by a linear model (constant accumulation rate), suggesting that the net rate of AVS accumulation on amended roots increased rapidly when seed production began. Points after the first day of seed production (day 231) fell outside of a 95% CI of a linear regression on the points during flowering (days 190-231, Appendix Fig. 2). Concentrations of CRS on both amended and control roots did not differ from AVS concentrations on the same roots, indicating that crystalline forms of FeS did not make up a significant proportion of reduced sulfur (paired *t* test, $p=0.27$, $t=0.63$, $n=20$).

Ferrous iron accumulation paralleled AVS accumulation on amended roots (Fig 2b). Root ferrous iron concentrations were elevated and accumulated faster on the amended roots compared to the control (Table 1). Ferrous iron on control roots and amended roots increased linearly, but ferrous iron on amended roots increased at a higher rate until the first seeds were produced (day 232). During seed production, ferrous iron concentrations on amended roots were greater than those predicted by a linear model, while Fe(II) accumulation on control roots appeared to slow.

Weak acid extractable iron (sum of Fe(II) + Fe(III) concentrations on roots, hereafter "total extractable iron") was variable, but did not differ significantly between treatments (Table 1). The average total extractable iron remained relatively constant in both treatments during flowering; however, during the first week of seed production (days 232 and 239) the total extractable iron dropped by about 150-250 umol/g on both the amended and control roots, and then gradually increased over the following three weeks (Fig. 3). Total extractable iron changed seasonally from mostly Fe(III) to mostly Fe(II) on sulfate-amended roots, especially during the first week of seed production (days 232 and 239). This abrupt shift in iron speciation occurred the same week that total extractable iron decreased and at about the same time as the increase in AVS accumulation rate (Fig. 3). Immediately prior to seed production, total extractable iron on the amended roots was 46 ± 11% Fe(II), and after one week of seed production, the composition of iron was 87 ± 10% Fe (II). During this same week, control root Fe(II) increased from 20 ± 11% to 48 ± 16%.

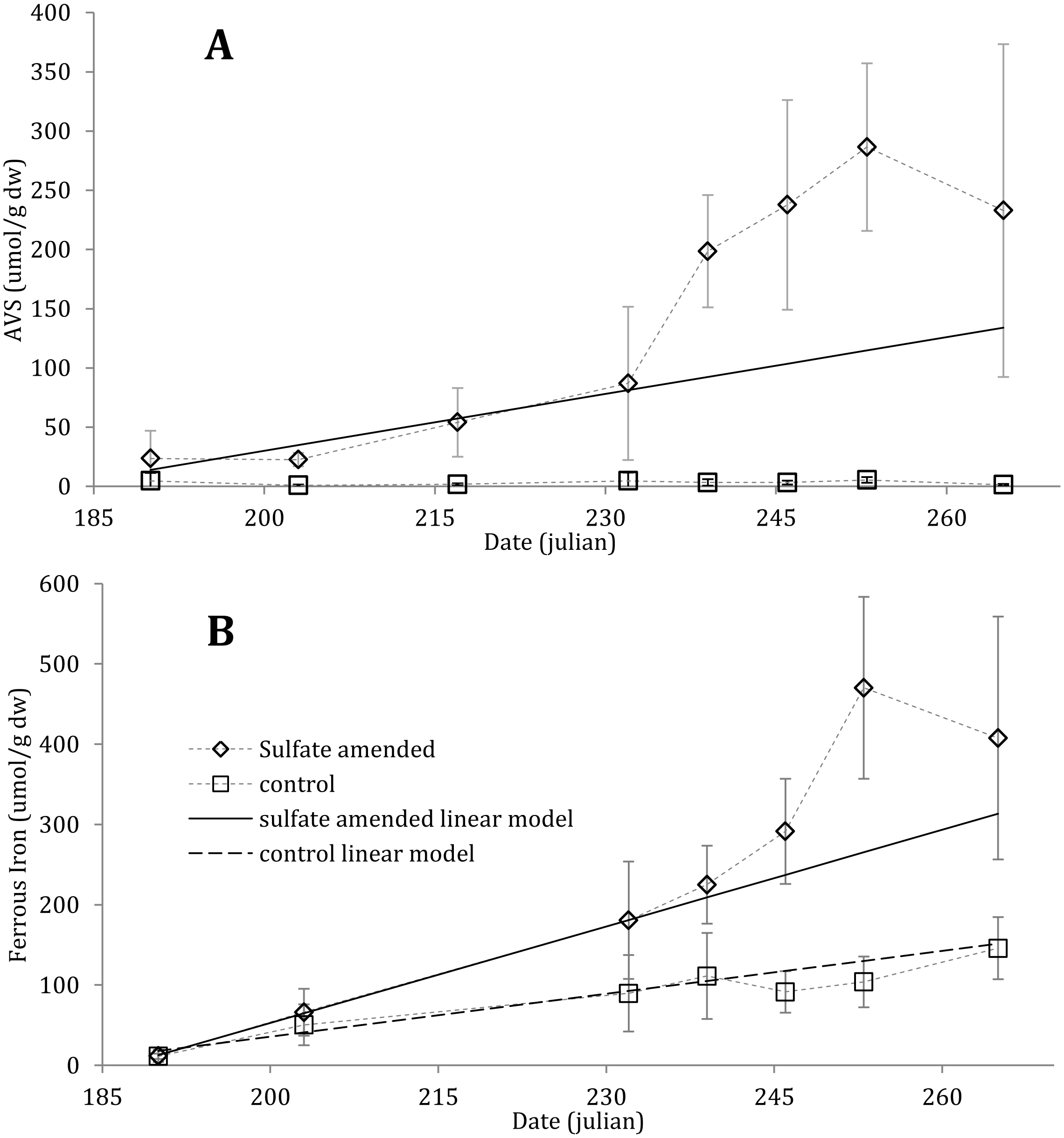

Figure 2. Solid phase acid volatile sulfide (A) and ferrous iron (B) concentrations on roots. Diamonds represent the average concentration on roots of four sulfate-amended plants, and squares represent the average of four control plants. The dashed line shows a linear model fit to the data from day 190 to day 232. Time is expressed in Julian dates. Error bars show one standard deviation.

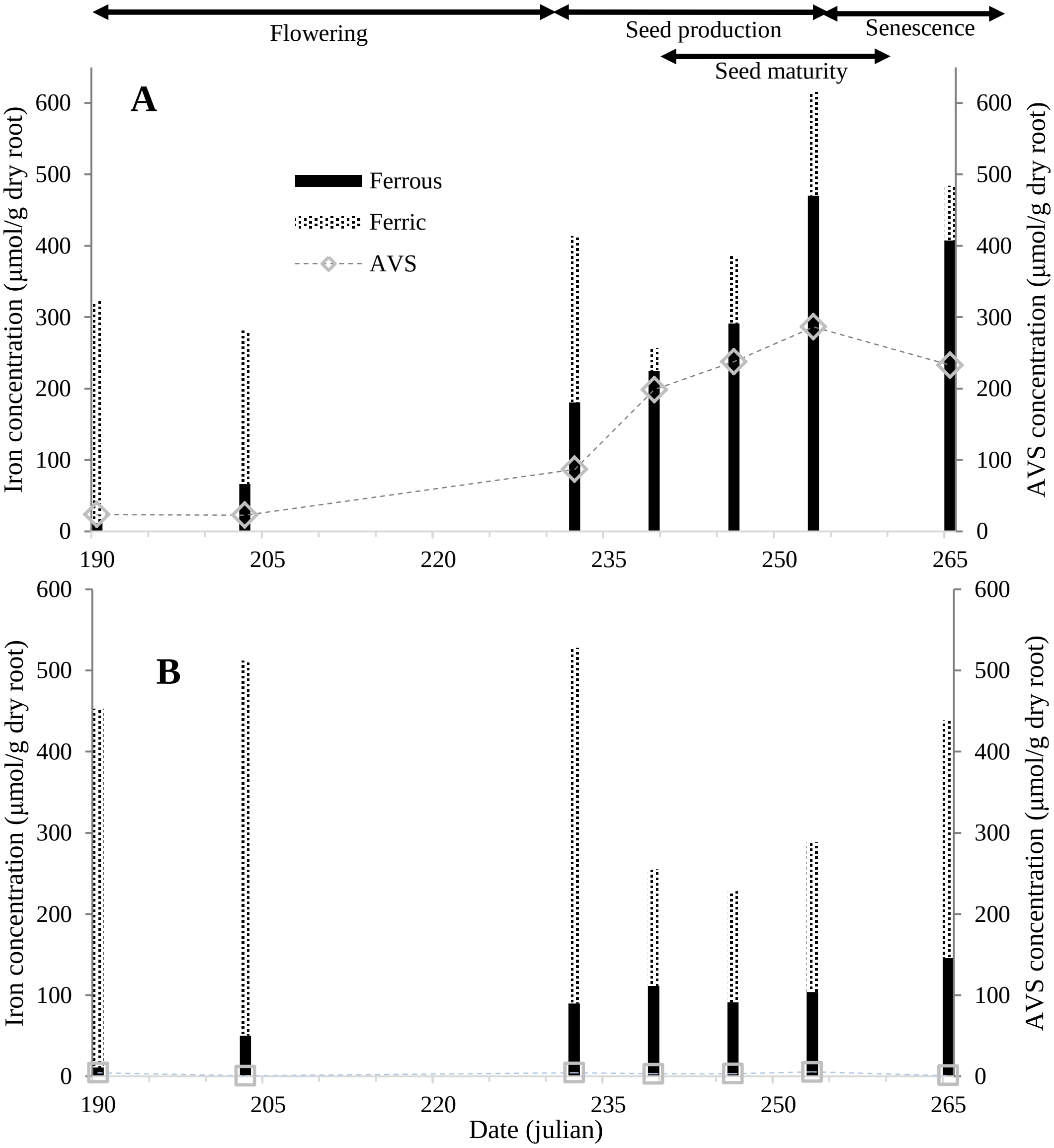

Figure 3. Seasonal iron speciation with root AVS overlain in sulfate-amended bucket. The dotted pattern indicates ferric iron and the solid black represents ferrous iron. A). Sulfate-amended bucket iron. Grey diamonds show root AVS concentrations in sulfate-amended buckets. B). Control bucket iron. Grey squares show root AVS concentrations in control buckets. Error bars are omitted for clarity.

Sediment

Sediment AVS was significantly different between treatments, but total extractable iron was not. In both the sulfate-amended and control sediment, AVS increased during the growing season, but more AVS accumulated in the amended sediment (2-factor ANOVA, time x treatment interaction, f=5.08, df=1,18, p=0.037). Amended sediment AVS increased from 0.39 umol/g in early summer to 4.7 μmol/g at the end of the growing season, whereas the control sediment only increased from 0.39 μmol/g to 0.88 umol/g. There was no difference in total extractable iron between the amended and control sediment at the beginning or end of the growing season (2-factor ANOVA, f=0.65, df=1,18, p=0.429).

Biological effects

Plant sampling began at the start of the flowering stage (days 190-230). The first seeds were collected on 8/20/15 (day 232), but were unripe and not yet filled. In this paper, seed production is referred to as days 230 to day 264, but mature seeds were not produced until one week after the start of seed production (day 239). On the last sample date (day 265) seeds were collected, but were unfilled. Stems and leaves were no longer green, indicating that the plants had senesced. Of the four replicates in the sulfate amendment on this date, two plants did not produce seeds. Thus, “mature seed production” refers to dates 239-253.

Total seed nitrogen, total seed weight, and seed count were all lower in sulfate-amended plants during mature seed production, a time that coincided with elevated FeS on roots (days 239-253, Table 1, Fig 4). Sulfate addition was not correlated with seed δ^{15}N or seed N %. During mature seed production and senescence, the dry weight of the sulfate-amended plants was lower than that of control plants. Total vegetative (plant + seeds) N was unaffected by sulfate until the last two sample dates prior to senescence, when it was lower in sulfate-amended plants (Fig 4d, two-sample t test, p=0.031, p=0.047, n=8 for both dates).

A mixing model was used to determine the fraction of total seed nitrogen coming from the pore water and the fraction translocated from the stem (Appendix Fig. 3). In the days following a spike of enriched nitrogen to sediment pore water, there were two possible sources of nitrogen in the seeds; wild rice can translocate nitrogen from its stem or take nitrogen up from the pore water. The plant $\delta^{15}N$ was estimated to be 4.5‰ from the average of 12 unlabeled plants harvested on the first two sample dates. The pore water $\delta^{15}N$ was approximated to be 180‰ and calculated from the percent by mass of $^{15}NH_4$ added ($\delta^{15}N$ =26,200‰) and the percent by mass of ammonia already present in the pore water ($\delta^{15}N$ assumed to be 0‰). The two-component mixing model showed no difference in fraction of nitrogen uptake from pore water between the amended and control plants (repeated measures ANOVA, p=0.83, f=0.05, df=1,6). In both control and amended plants, the fraction of total seed nitrogen originating from the pore water increased two weeks into seed production (day 246) from 27 ± 18 % to 51 ± 19%, but returned to 29 ± 19 % a week later (day 253). The elevated proportion coming from the pore water coincides with the day seeds contained the most nitrogen (Fig 4c). On this day, total seed nitrogen was significantly lower in the sulfate amended plants than in the control plants (two-sample t test, p=0.047, n=8). Plant N (excluding seeds), however, was not different between amended and control plants on this day (two-sample t test, p=0.41, n=8).

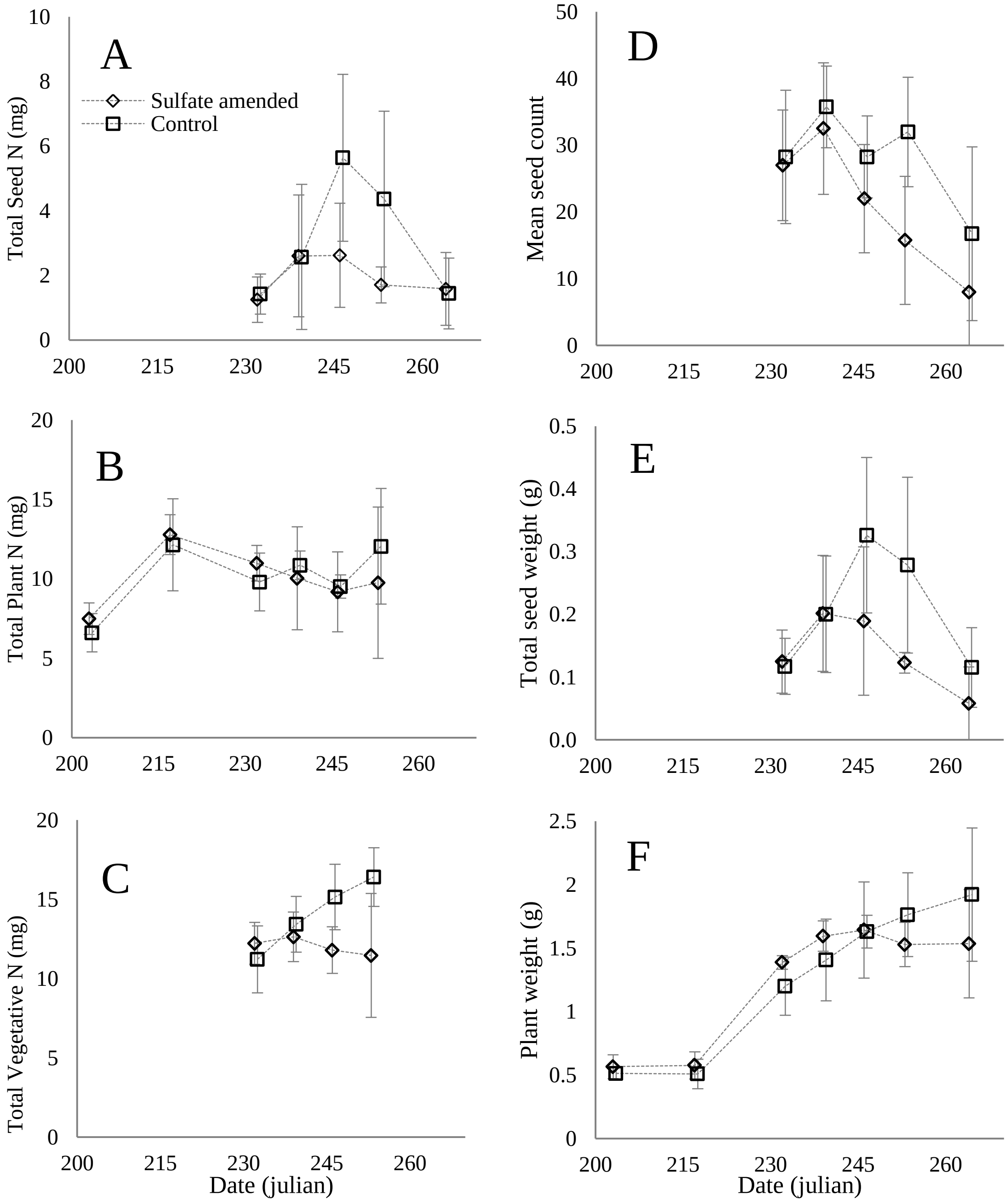

Figure 4. Biological endpoints. Diamonds represent plants grown in surface water with 300 mg/L sulfate added while squares show data from control plants. Each data point represents four replicates. Error bars represent one standard deviation. A) Weekly total mass of nitrogen in seeds of sulfate amended and control plants. B) Total mass of nitrogen in the plant (stems+leaves), excluding seeds, over the course of the growing season. C) Weekly total vegetative nitrogen in amended and control plants. Total vegetative nitrogen was calculated by summing nitrogen from seeds, stems, and leaves. D) Weekly seed count in amended plants and control plants. E) Weekly total seed mass in amended plants and control plants. F) Dry mass of plants over the course of the growing season.

Discussion

Our observations suggest a tight coupling of iron and sulfur cycling in the rooting zone of wild rice. Iron (hydr)oxides form on wild rice roots early in the growing season, but roots that are exposed to high sulfate loading (300 mg/L) develop iron sulfides later in the growing season. An inflection point in iron sulfide accumulation occurs at the start of seed production, shortly after rapid depletion of sulfate in the pore water, and defines an increase in the net rate of FeS accumulation. The rapid increase in net FeS accumulation suggests a change in a process that controls the way iron and sulfur cycle in the rhizosphere, and the timing suggests that this process may be tied to and have important implications for rice physiology. Previous research has suggested that an accumulation of FeS occurs after plant senescence (Jacq 1991), but our observations clearly show accumulation of FeS during the reproductive life stage of wild rice.

The change in FeS accumulation rate is consistent with an inhibition of radial oxygen loss. Sulfate accumulation in the pore water during the flowering stage suggests that the rhizosphere is relatively oxidized. The initially linear FeS accumulation rate on plant roots suggests constant rates of sulfide production and sulfide oxidation, with a higher rate of sulfide production than oxidization (net accumulation). However, sulfide exposure in white rice leads to the formation of suberin in the cell walls of roots which is hypothesized to create a barrier that limits diffusion of toxic solutes into the plant (Armstrong and Armstrong, 2005). The barrier not only excludes toxic solutes like sulfide, but also traps oxygen inside the roots, suppressing radial oxygen loss (Krishnamurthy et al. 2009, Soukup et al. 2006). A relatively rapid transition to anoxia of the rhizosphere appears to have occurred at the onset of seed production, possibly as a result of suberin-induced suppression of radial oxygen loss. Under the anoxic conditions, the net accumulation of reduced species likely increased because fewer reduced species cycled back to their oxidized form.

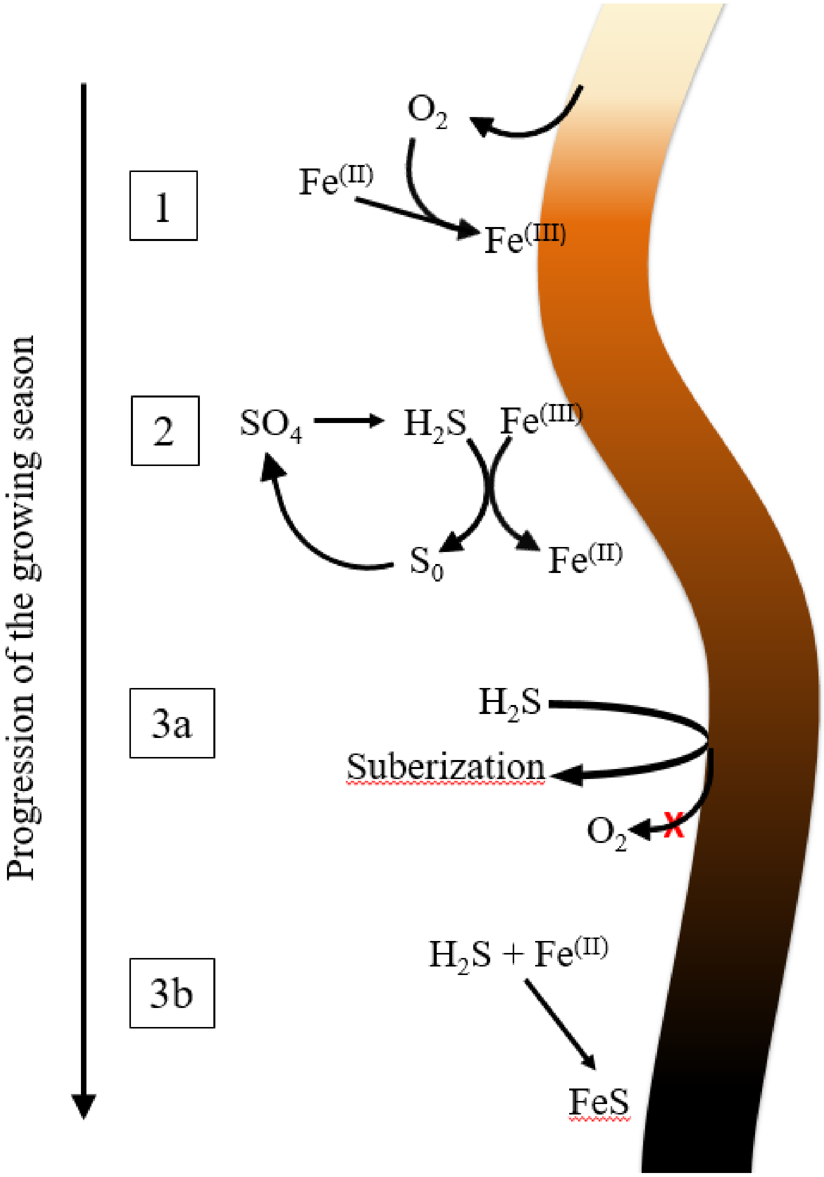

Figure 5. Proposed mechanism of iron sulfide formation on wild rice roots. Roots are protected by iron (hydr)oxides [1], but reduced by sulfide [2]. Exposure of roots to sulfide induces suberization of root cells, which leads to decreased radial oxygen loss [3a]. Rhizosphere anoxia allows iron sulfides to precipitate [3b].

A hypothesized pathway for how the rice roots might transition from iron (hydr)oxide plaques to iron sulfide plaques over the growing season is outlined in Figure 5. Initially, radial oxygen loss creates oxic conditions in the rooting zone, causing ferrous iron within the rhizosphere to precipitate as iron (hydr)oxides and accumulate on root surfaces (Fig. 5, [1] label). At this initial stage, the root is protected from reduced species by both radial oxygen loss and iron (hydr)oxide plaques, an electron accepting sink. Before sulfide can penetrate to the root, the iron (hydr)oxide plaques, effectively acting as an electron accepting buffer, must be reduced (Fig. 5, [2] label). As sulfide erodes the accumulated ferric iron barrier (Hansel et al. 2014, Kwon et al. 2013), sulfide can then reach the root surface and cause suberization (Fig. 5, [3a] label). Once radial oxygen loss is suppressed by suberin formation, the electron accepting buffer capacity of iron (hydr)oxides can no longer be replenished. The remaining quantity of iron (hydr)oxides can be more rapidly reduced due to a net change in the flow of electrons to the rooting zone. Upon depletion of iron (hydr)oxides, sulfide accumulates rapidly, since neither iron (hydr)oxides or a supply of radial oxygen loss are available to oxidize sulfide (Fig 5, [3b] label). As sulfide penetrates closer to the root surface, it precipitates with available iron, and the redox potential of the rhizosphere shifts to more reducing conditions.

The rapid accumulation of sulfur on roots in amended plants seems inconsistent with the relatively small difference in sulfur and iron concentrations in pore water. The saturation index (SI), which is calculated from pore water concentrations two centimeters from the stem, indicates that the pore water is undersaturated with respect to iron sulfide. The thermodynamic understanding of mineral precipitation and dissolution is that minerals precipitate when pore water is saturated and dissolve when pore waters are undersaturated (Stumm & Morgan, 1995). The rapid accumulation of iron sulfide on roots in the setting of undersaturated pore water suggests that the transition of iron (hydr)oxide to iron sulfide on the roots occurs very close to the surface of the root, and thus depends on near-root-surface processes more than on pore water concentrations. Sulfide on root surfaces must be supplied externally, either from reduction of surface water sulfate, or from mobilization of AVS on sediment, but ferrous iron in the FeS plaques could be sourced from the reduction of iron (hydr)oxides already accumulated on the root surface earlier in the season. Indeed, a decrease in solid-phase iron on the roots, a shift in iron speciation, and an accumulation of pore water iron all occur simultaneously, which is consistent with loss of soluble ferrous iron off of the root surface during the redox transition. Thus, if the ferrous iron in FeS plaques is sourced from the iron (hydr)oxides on the root, saturation index calculations based on pore water iron concentrations may not be relevant to understanding FeS formation on roots. Additionally, the decline of pore water sulfate followed by rapid accumulation of AVS on the root surfaces suggests that a large amount of sulfur passes through the pore water pool very quickly. Iron sulfide formation is strongly favorable thermodynamically and kinetically rapid (Rickard, 1995). Using pore water sulfide concentrations to calculate the saturation index may underestimate the amount of sulfur available to precipitate on root surfaces, as pore water sulfide may act as a transient phase between pore water sulfate and root AVS. The transience of sulfide in pore waters near rice rhizospheres was noted by Hara (2013) who observed black iron sulfide zones around white rice seeds grown in sulfate-amended sediment, but was unable to quantify any sulfide, despite measuring redox potentials low enough to support sulfide production.

In this experiment, iron sulfide plaques occurred concomitantly with lower seed nitrogen and fewer seeds. Less nitrogen was present in the total seed mass of the amended plants, and fewer seeds were produced. This is likely a strategy for optimizing reproduction; amended plants produce fewer filled seeds but each filled seed is fully viable (Pastor et al., in reveiw). The two-component isotope mixing model suggests that the amended plants were not able to compensate for inhibition of nitrogen uptake by translocating a greater percentage of seed nitrogen from the stem and leaves. Between the sulfate and control, no difference was observed in the fraction of N uptake from the pore water. The decreased total seed N in sulfate amended plants appears to be an equally proportioned result of decreased uptake from pore water and decreased translocation from the plant.

Biological variables were only affected during seed production. During the biomass growth life stages, little difference in total plant weight and total plant N was observed. Biomass may not have been impacted because sulfide can produce a fertilization effect by sequestering iron bound with phosphate, releasing free phosphate

(Geurts et al. 2009, Caraco et al. 1989, Smolders et al. 2003, Lamers et al. 2002). However, nitrogen, rather than phosphorus, is the limiting nutrient for wild rice (Sims et al. 2012), so the fertilization effect is likely minimal in wild rice. In the long term, Pastor et al. (in review) showed that sulfide takes several years to affect a population of wild rice, because although sulfide showed no effect on germination and very little effect on biomass of wild rice, sulfide greatly decreased the number of juvenile seedlings that survive and the number of filled seeds produced by the plant. The results from our study suggest that during seed production, the buffering capacity of iron (hydr)oxides has been overwhelmed by sulfide and no longer protects the plant from sulfide. Similarly, juvenile seedlings may be vulnerable to sulfide because they have not yet grown out of the water column and are thus unable to transport oxygen from the atmosphere to their roots. The life stages of wild rice affected by sulfide are consistent with times during which an oxic barrier around the roots is absent.

Accumulation of FeS on roots may have implications for wetland cycling of iron and sulfide. After senescence, roots coated with FeS decay and become incorporated into the bulk sediment. Jacq et al. (1991) found significant accumulation of FeS on white rice roots after senescence, likely because the dead root material stimulated continued iron and sulfate reduction. Additionally, Jacq et al. (1991) found that sediment in a planted rice paddy contained higher FeS concentrations than an unplanted rice paddy. Because wild rice is an annual plant, the amount of root FeS that accumulates over a growing season is added to the sediment each year. Choi et al. (2006) likewise found that in a riparian wetland containing *Phragmites australis* and *Zizania latifolia*, AVS concentrations were higher in the top 6 cm of non-vegetated sediment, but vegetated sediment had higher concentrations of AVS 6-14 cm below the sediment-water interface. If AVS on roots is supplied mainly from reduction of surface water sulfate, burial of FeS coated roots may be supplying sulfide to the sediment faster than pore water precipitation of iron sulfide in the bulk sediment. If root AVS is supplied largely by mobilization of sediment AVS, which Choi et al. suggests can be caused by radial oxygen loss, then sediment AVS concentration may be an important parameter in determining iron sulfide accumulation and concomitant inhibition of nitrogen uptake in wild rice. Knowledge of the main sources of sulfur for root AVS will be crucial in managing wild rice in sulfur-impacted systems.

Conclusion & Directions for Future Work

The timing of our observations of rhizosphere AVS accumulation in conjunction with decreased total seed N in sulfate-amended plants suggests that nitrogen uptake by wild rice is affected only after significant sulfide accumulation on root surfaces. In this experiment, elevated sulfide on plant roots coincides with the plant's reproductive stage. We propose that root surface iron (hydr)oxides delay sulfide from entering the plant, effectively acting as a buffer against early and mid-season sulfide exposure. When the oxic barrier on the root surface is overwhelmed, iron sulfide accumulates rapidly, as shown by the doubling of AVS and the shift in iron speciation from about 50% Fe(II) to 90% Fe(II) within just one week. In this experiment, the oxic barrier was overwhelmed just prior to seed production; concurrently, reduced seed count, total seed weight, and total seed nitrogen were observed.

Many questions remain about the cause of the redox shift in the rhizosphere. We propose a mechanism in which sulfide-induced suberization of roots facilitates reduction of the oxic barrier, but a seasonal change in wild rice physiology could also facilitate a rapid transition to anoxia. Control roots, like sulfate-amended roots, lost about half of their total extractable iron at the start of seed production, and accumulated some ferrous iron even in the absence of significant S accumulation. Is there a seasonal shift in redox potential in wild rice rhizospheres, regardless of the presence of sulfur? Seasonal measurements of redox potential and magnitude of radial oxygen loss may provide insight into the comparative influence of plant processes and sulfur loading on shifting redox conditions in the rhizosphere. Is the bacterial community affected more by rhizosphere geochemistry or by life stages of the plant? Seasonal microbial community analysis could also elucidate the relative causes of the rhizosphere anoxia, as a significant seasonal shift in the microbial community of control plants would indicate plant controlled redox conditions. If the redox conditions of the rhizosphere are controlled by iron and sulfur geochemistry as proposed, would a lower initial concentration of iron on roots result in erosion of the iron (hydr)oxide barrier and subsequent inhibition of nitrogen uptake earlier in the growing season? If so, would plant biomass and nitrogen also be decreased? A similar study to this one could be done in which total iron concentrations of the sediment were varied to produce different initial concentrations of iron (hydr)oxides on roots.

Finally, from a management perspective, it would be useful to understand the sources of sulfur on root surfaces and the sediment parameters that control those sources. Is the sulfide on the roots sourced primarily from surface water sulfate or from mobilization of sediment AVS? Could a lake that has previously received high sulfur loads but currently has low surface water sulfate contain wild rice with significant iron sulfide plaques? This question has implications for restoration of wild rice in sulfur-impacted lakes.

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Appendix

Table 1. Average and standard deviation of the saturation index in sulfate amended and control pore waters. The K_{sp} value used was $10^{-2.95}$.

Date (julian)	Sulfate-amended	Control
177	-1.436 ± 0.228	-1.436 ± 0.228
190	-0.282 ± 0.346	-0.175 ± 0.354
203	-0.390 ± 0.189	-1.061 ± 0.204
232	-0.560 ± 0.195	-0.802 ± 0.242
239	0.099 ± 0.969	-0.232 ± 0.435
245	-0.140 ± 0.580	-0.410 ± 0.837
256	-0.302 ± 0.376	-0.365 ± 0.333
263	-0.199 ± 0.198	-0.597 ±0.581

Figure 1. Sulfate-amended root (left) and control root (right). Sulfate-amended root has black color extending from about 0.5 cm above the root ball down to the tips of the roots (not shown). Control root has amber color characteristic of iron (hydr)oxides, especially 2-3 cm below root ball.

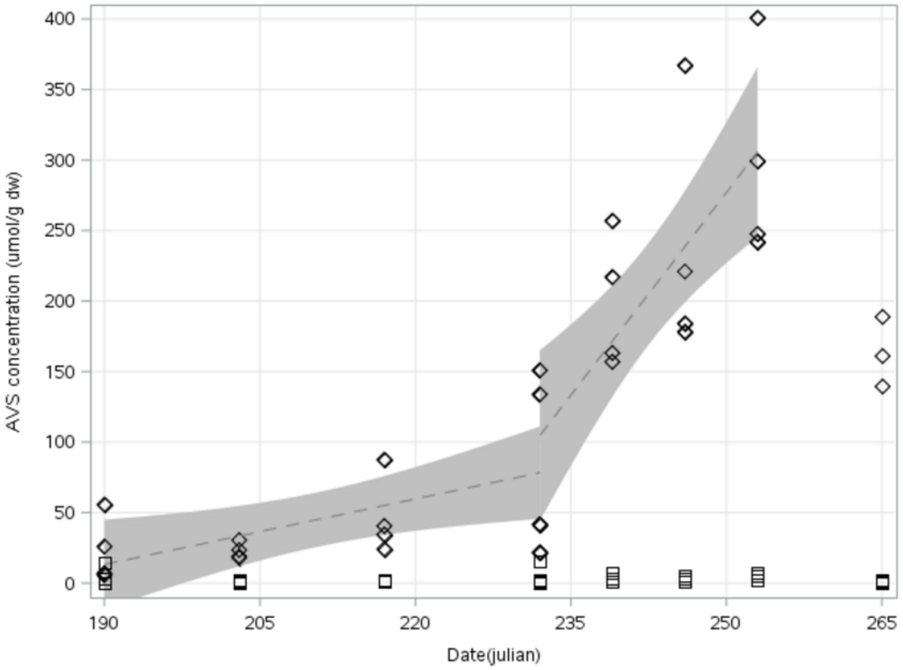

Figure 2. A 95% confidence interval around a regression of time and AVS on sulfate amended roots depicting the change in rate of sulfide accumulation. Diamonds represent sulfate amended plants, and squares represent control plants. The plant is in the flowering stage until day 232, when it starts producing seeds. The last sample date was during senescence, and is therefore not included in the 95% confidence interval.

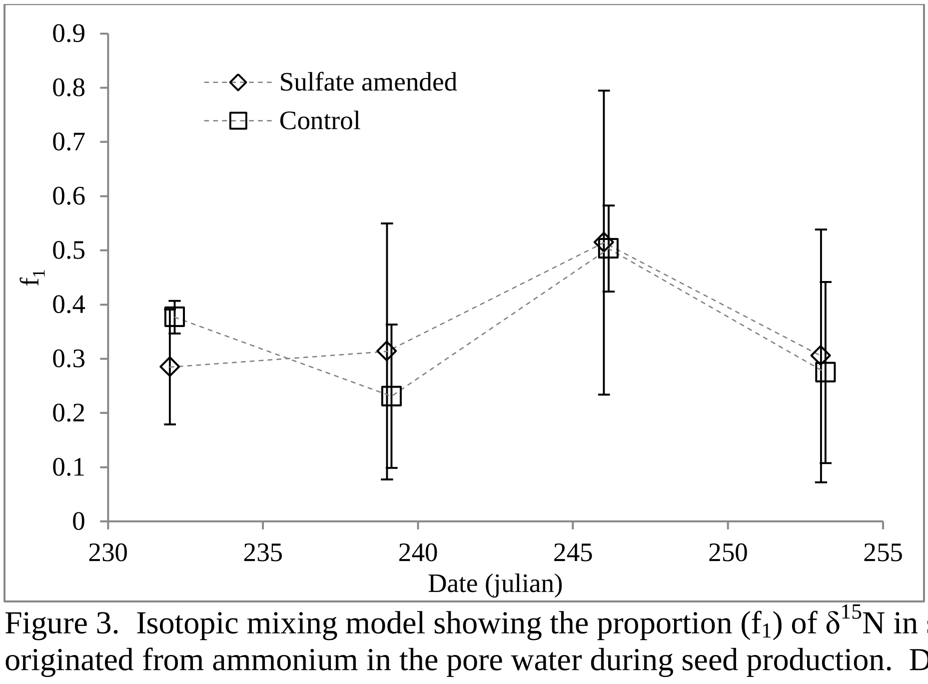

Figure 3. Isotopic mixing model showing the proportion (f_1) of $\delta^{15}N$ in seeds that originated from ammonium in the pore water during seed production. Diamonds represent sulfate amended plants, and squares represent control plants. Each data point is the average of four replicates. Error bars are one standard deviation.

John Pastor Technical Review Comments - Wild Rice Rule
November 2017

Attachment G

(13 pages)

LacCore_field_ID	Site_name	Unique site ID	DNR/State ID	Date	Lat	Long	Calculated Wild rice ave stems/m2	surface water SO4 (mg SO4/L)	pore water Total Sulfide (TS, mg S/L)	Sediment Fe (µg/g)	Sediment TOC (%)	potential SO4 standard CPSC120
P-35	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7377	3.0	2.23	0.493	2170	14.84	1.2
FS-192	Anka	26	21-0353-00-202	8/29/12	46.07689	-95.7292	2.3	8.44	0.53	1498	22.85	0.4
P-34	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7292	25.9	2.23	0.671	1485	23.57	0.3
FS-134	Bass	43	31-0576-00-207	9/18/12	47.2844	-93.6276	64.0	1.01	0.0664	3740	26.12	1.8
FS-85	Bean	8	03-0411-00-201	8/21/12	46.9337	-95.8706	0.0	85	16	1967	11.85	1.4
FS-87	Bee	60	60-0192-00-202	8/23/12	47.6527	-96.0504	39.8	11	0.67	3054	13.62	2.7
FS-193	Big Mud	79	71-0085-00-201	8/30/12	45.4529	-93.7418	14.3	< 0.5	0.0308	12943	18.63	29.5
FS-216	Big Sucker	39	31-0124-00-203	9/12/12	47.3919	-93.2658	3.8	7.78	0.145	3559	21.45	2.1
FS-205	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.7418	56.3	5.47	0.0527	1719	4.81	3.1
FS-204	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.742	133.7	5.49	0.0914	1731	5.94	2.4
FS-89	Birch	67	69-0003-00-205	9/10/12	47.7358	-91.943	33.1	8.61	0.1	16938	31.2	26.7
P-12	Birch	67	69-0003-00-205	8/30/11	47.7357	-91.9428	68.6	3.58	0.104	12431	26.8	17.7
FS-52	Blaamyhre	48	34-0345-00-203	8/1/12	45.364	-95.186	102.2	0.62	0.078	3517	9.33	5.5
FS-214	Bowstring	116	S007-219	9/11/12	47.7024	-94.0608	69.7	1.34	0.256	1974	24.34	0.6
FS-126	Bray	58	56-0472-00-202	8/20/12	46.4518	-95.8783	7.6	1.65	0.072	3937	21.95	2.5
FS-63	Caribou	72	69-0489-00-206	9/3/12	46.8913	-92.3135	0.0	1.21	0.0938	13791	29.44	19.3
P-53	Carlos Avery Pool 9	4	02-0504-00-201	8/19/11	45.3179	-93.0587	43.0	0.35	0.029	37965	16.51	270.0
FS-109	Carlos Avery Pool 9	4	02-0504-00-202	7/3/12	45.3192	-93.0611	52.8	< 0.5	< 0.011	14736	12.51	61.0
FS-339	Christina	28	21-0375-00-315	7/31/13	46.0734	-95.7567	0.6	14.6	1.93	1741	8.96	1.5
FS-373	Clearwater	96	S002-121	9/9/13	47.9372	-95.6909	3.2	34.4	0.0354	5315	3.33	41.8
FS-189	Clearwater	96	S002-121	8/28/12	47.9372	-95.6906	4.5	23.8	0.117	2856	1.27	40.2

FS-327	Clearwater	96	S002-121	7/17/13	47.9371	-95.6906	0.3	23.7	0.117	3521	1.82	39.1
FS-314	Clearwater	96	S002-121	6/24/13	47.9372	-95.6907	0.6	28	0.0664	3946	2.68	30.6
FS-337	Clearwater	98	S004-204	7/29/13	47.5175	-95.3906	69.1	0.95	0.0608	14564	24.58	26.6
FS-88	Clearwater	98	S004-204	8/24/12	47.5174	-95.3904	148.3	2.04	0.0488	9874	22.17	14.2
P-31	Cloquet	52	38-0539-00-201	9/14/11	47.4313	-91.4844	74.4	0.81	0.024	4252	6.58	12.1
FS-128	Cromwell	14	14-0103-00-201	8/22/12	46.9651	-96.3171	0.0	41.2	1.22	2948	2.85	16.2
FS-369	Dark	77	69-0790-00-202	9/5/13	47.6389	-92.7781	11.8	176	0.052	2037	0.82	35.4
FS-352	Dark	77	69-0790-00-202	8/15/13	47.6388	-92.7782	2.9	173	0.136	5120	3.61	35.3
FS-368	Dark	77	69-0790-00-202	9/5/13	47.6387	-92.7782	11.1	175	0.305	3354	1.94	33.0
FS-322	Dark	77	69-0790-00-202	7/10/13	47.6389	-92.7781	3.2	175	0.131	2480	1.48	25.5
FS-64	Dead Fish	12	09-0051-00-202	9/4/12	46.7454	-92.6865	0.0	0.71	0.0608	14387	22.4	29.0
P-44	Dead Fish	12	09-0051-00-202	9/20/11	46.7451	-92.6863	48.7	0.3	0.056	9685	16.6	19.4
FS-378	Duck Lake WMA	22	18-0178-00-202	9/12/13	46.7521	-93.8851	113.0	< 0.5	0.0251	12151	26.57	17.1
FS-86	Eighteen	61	60-0199-00-202	8/22/12	47.6397	-96.0607	40.1	4.29	0.164	1860	3.1	6.1
FS-309	Eighteen	62	60-0199-00-203	6/13/13	47.6369	-96.0599	0.0	4.36	0.127	4478	16.52	4.4
FS-328	Eighteen	62	60-0199-00-203	7/18/13	47.6369	-96.0599	44.2	3.34	0.25	5106	24.65	3.5
FS-359	Eighteen	62	60-0199-00-203	8/20/13	47.6367	-96.06	21.0	2.83	0.118	5500	30.88	3.1
P-6	Elk	15	15-0010-00-203	8/25/11	47.1946	-95.2254	25.9	0.28	0.04	8480	10.24	26.8
FS-137	Elk	15	15-0010-00-204	9/19/12	47.1952	-95.2249	42.7	< 0.5	0.0936	6334	10.07	15.6
FS-333	Embarrass	73	69-0496-00-203	7/26/13	47.5333	-92.2976	0.0	18.2	0.0866	11179	0.47	1821.2
FS-95	Embarrass	73	69-0496-00-203	9/14/12	47.5334	-92.2979	0.0	18.8	0.0298	21847	1.89	1248.9
FS-76	Field	45	34-0151-00-201	7/25/12	45.2964	-94.9058	0.0	< 0.5	0.0687	7586	8.68	26.3
FS-195	Fisher	78	70-0087-00-201	8/31/12	44.7942	-93.4061	20.7	6.85	0.136	11140	5.76	90.1
FS-81	Flowage	1	01-0061-00-204	8/7/12	46.688	-93.337	0.0	0.78	0.134	12470	32.34	14.2
P-51	Flowage	1	01-0061-00-205	9/22/11	46.6896	-93.338	160.2	0.56	0.014	5627	20.1	5.4
P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	0.56	0.018	4641	18.1	4.2
P-52	Flowage	1	01-0061-00-205	9/22/11	46.6895	-93.338	123.1	0.56	0.018	3706	16.52	3.1

P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	0.56	0.018	4302	21.79	2.9
FS-194	Gilchrist	91	86-0064-00-201	8/31/12	45.2309	-93.824	0.0	6.98	0.355	3117	20.81	1.7
FS-51	Glesne Slough	49	34-0353-00-201	7/31/12	45.3514	-95.1887	99.6	< 0.5	0.061	7983	3.01	103.2
P-23	Gourd	10	04-0253-00-201	9/7/11	47.812	-94.9654	38.4	0.69	0.038	2675	27.4	0.9
FS-104	Gourd	10	04-0253-00-201	6/27/12	47.8121	-94.965	0.0	0.27		1776	36.87	0.3
FS-213	Gull	9	04-0120-00-204	9/10/12	47.6558	-94.6945	9.5	1.14	0.0778	3527	16.01	2.9
P-20	Gull	9	04-0120-00-203	9/6/11	47.6559	-94.6944	15.6	0.78	0.103	1608	5.08	2.5
FS-367	Hay	33	31-0037-00-202	9/4/13	47.287	-93.1009	141.0	22.1	0.0447	15436	3.44	312.7
P-45	Hay	33	31-0037-00-201	9/21/11	47.2874	-93.1017	0.0	10.24	0.087	12403	4.36	154.6
P-46	Hay	33	31-0037-00-201	9/21/11	47.2869	-93.1018	0.0	10.24	0.026	16139	7.69	130.0
FS-130	Hay	33	31-0037-00-202	9/6/12	47.2874	-93.102	141.0	31.7	0.0738	13154	5.79	123.3
FS-221	Hay Creek Flowage	59	58-0005-00-202	9/17/12	46.0894	-92.4104	97.7	1.95	0.119	9456	22.05	13.2
FS-375	Height of Land	5	03-0195-00-210	9/10/13	46.913	-95.6111	117.5	< 0.5	< 0.011	1795	0.86	26.2
FS-127	Height of Land	5	03-0195-00-210	8/21/12	46.9133	-95.6095	111.1	< 0.5	< 0.011	2112	1.32	21.5
FS-318	Height of Land	5	03-0195-00-210	6/26/13	46.9135	-95.6124	43.0	1.21	0.0658	1349	1.13	10.9
FS-338	Height of Land	5	03-0195-00-210	7/30/13	46.913	-95.6116	94.2	< 0.5	0.0554	2641	4.58	7.4
P-1	Height of Land	5	03-0195-00-209	8/22/11	46.9129	-95.6095	62.9	0.24	0.053	1298	1.76	6.0
FS-131	Hinken	113	S007-207	9/5/12	47.7271	-93.9923	46.8	< 0.5	0.0876	2960	4.53	9.4
FS-185	Hoffs Slough	85	76-0103-00-201	8/1/12	45.3255	-95.7059	0.0	273	0.0343	3512	0.75	112.3
FS-353	Holman	42	31-0227-00-202	8/12/13	47.3009	-93.3444	0.0	68	0.583	5094	30.6	2.7
FS-218	Holman	42	31-0227-00-202	9/13/12	47.3005	-93.3445	0.0	24.2	1.01	3035	29.74	1.0
FS-182	Hunt	65	66-0047-00-208	7/27/12	44.3275	-93.4443	0.0	17.1	0.0729	2412	1.21	30.8
FS-191	Ina	27	21-0355-00-202	8/29/12	46.0715	-95.7281	30.2	7.08	0.274	2216	9.09	2.3
FS-136	Itasca	16	15-0016-00-208	9/19/12	47.2343	-95.2049	23.6	< 0.5	0.0636	1496	2.23	5.9
P-7	Itasca	16	15-0016-00-207	8/25/11	47.2332	-95.1985	20.1	0.26	0.064	1650	6.01	2.2
P-5	Itasca	16	15-0016-00-208	8/25/11	47.2381	-95.2065	45.8	0.26	0.056	1355	7.4	1.2
FS-207	Kelly Lake	64	66-0015-00-204	8/13/12	44.3542	-93.3743	0.0	1.92	0.0927	4387	27.33	2.3

FS-79	Lady Slipper	53	42-0020-00-203	7/27/12	44.5723	-95.6216	0.0	330	1.63	3314	1.85	34.1
FS-78	Lady Slipper	53	42-0020-00-202	7/27/12	44.5699	-95.6275	0.0	335	1.68	2719	1.66	26.5
P-55	Lady Slipper	53	42-0020-00-204	9/22/11	44.5702	-95.6274	0.0	107.71	14.84	2814	2.09	21.5
P-61	Lily	90	81-0067-00-202	9/28/11	44.194	-93.6469	51.5	0.66	0.041	6180	14.06	10.0
P-62	Lily	90	81-0067-00-202	9/28/11	44.194	-93.6469	0.0	0.64		5069	13.39	7.2
FS-180	Lily	90	81-0067-00-202	7/26/12	44.1947	-93.647	38.2	< 0.5	0.0295	5095	28.07	3.0
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.05	4503	4.46	21.4
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	2236	1.75	17.1
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	3544	5.11	11.5
P-47	Little Birch	87	77-0089-00-101	9/21/11	45.7747	-94.7996	25.9	3.2	0.191	2253	8.37	2.7
FS-54	Little Birch	87	77-0089-00-207	8/3/12	45.7779	-94.7978	70.0	7.4	0.0353	1794	6.02	2.6
P-4	Little Flat	6	03-0217-00-201	8/24/11	46.9981	-95.6641	83.1	0.22	0.011	7479	33.13	5.2
FS-250	Little Rice	75	69-0612-00-201	9/20/12	47.7086	-92.4389	29.3	1.03	0.0293	9488	26.45	10.7
FS-342	Little Round	7	03-0302-00-203	8/5/13	46.9721	-95.7358	58.3	< 0.5	0.0676	4447	25.16	2.6
FS-138	Little Round	7	03-0302-00-203	9/20/12	46.9726	-95.735	78.0	< 0.5	0.128	3069	27.48	1.2
FS-374	Little Round	7	03-0302-00-202	9/10/13	46.9745	-95.738	37.6	0.12	0.0391	2018	14.8	1.1
FS-319	Little Round	7	03-0302-00-203	6/27/13	46.9724	-95.735	17.5	< 0.5	0.117	3579	39.84	1.0
P-3	Little Round	7	03-0302-00-202	8/24/11	46.9759	-95.7404	57.2	0.46	0.032	1689	20.91	0.5
FS-223	Little Sucker	40	31-0126-00-202	9/14/12	47.3765	-93.246	0.0	13.7	0.534	6297	16.56	8.5
FS-203	Long Prairie	110	S007-203	8/9/12	45.9729	-95.1603	58.3	6.66	0.0391	5074	4.35	27.8
FS-202	Long Prairie	110	S007-204	8/9/12	46.0072	-95.2634	13.4	7.71	0.0793	2897	2.85	15.7
FS-200	Louisa	94	86-0282-00-205	8/8/12	45.2998	-94.258	0.0	7.04	0.192	7824	8.76	27.6
FS-226	Louise	25	21-0094-00-202	8/14/12	45.9331	-95.4148	46.5	4.09	0.0746	1833	0.83	28.5
FS-60	Lower Panasa	38	31-0112-00-205	8/29/12	47.3018	-93.2521	0.0	33.6	0.243	8048	14.12	16.5
FS-357	Lower Panasa	38	31-0112-00-204	8/15/13	47.3026	-93.2561	0.0	28.5	1.26	2347	2.42	12.7
P-25	Lower Rice	107	S006-985	9/8/11	47.3793	-95.4834	114.4	1.02	0.097	2337	17.76	1.2
P-26	Lower Rice	109	S007-164	9/8/11	47.3817	-95.4926	120.1	0.55	0.07	2364	6.76	3.8

FS-133	Mahnomen	21	18-0126-02-201	9/17/12	46.4985	-93.9958	0.0	16.9	0.308	18746	7.7	173.2
FS-377	Mahnomen	21	18-0126-02-201	9/11/13	46.4986	-93.9956	0.0	21.1	0.0283	16540	7.47	141.1
FS-175	Maloney	88	79-0001-00-201	7/23/12	44.2251	-91.9321	0.0	3.15	0.0608	15126	4.57	214.0
P-64	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	0.0	1.83		10382	4.05	119.9
P-63	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	148.7	1.83	0.01	10269	4.24	111.2
FS-187	McCormic	81	73-0273-00-203	8/2/12	45.722	-94.9121	8.9	1.54	0.144	1512	1.1	14.0
FS-230	Mill Pond	23	21-0034-00-202	8/16/12	46.0715	-95.2218	80.9	7.36	0.192	3969	3.14	25.6
FS-229	Mill Pond	23	21-0034-00-202	8/16/12	46.0716	-95.2218	102.2	7.16	0.109	5143	7.86	14.0
FS-225	Miltona	24	21-0083-00-205	8/13/12	46.0496	-95.4217	0.0	4.11	0.0694	2624	1.77	22.9
FS-201	Mink	92	86-0229-00-206	8/8/12	45.274	-94.0269	0.0	1.31	0.0373	1740	1.53	12.4
FS-129	Mink	92	86-0229-00-207	8/23/12	45.2767	-94.0299	0.0	1.22	0.182	4247	13.63	5.0
FS-80	Mission	95	S001-646	8/6/12	45.8623	-93.0011	87.5	0.62	0.0485	9231	4.83	77.5
FS-83	Mississippi Crow Win	111	S007-205	8/8/12	46.4386	-94.1251	0.0	3.13	0.127	13451	3.88	207.8
FS-211	Mississippi Pool 4/Ro	89	79-0005-02-201	8/16/12	44.3611	-91.9897	57.6	17.7	0.0714	9265	1.55	304.2
FS-336	Mississippi Pool 4/Ro	89	79-0005-02-201	7/30/13	44.3613	-91.9901	46.5	55.3	0.0602	8193	1.41	269.0
FS-210	Mississippi Pool 4/Ro	89	79-0005-02-202	8/16/12	44.3593	-91.9881	35.3	15.7	0.07	6450	1.16	214.5
FS-371	Mississippi Pool 5 / Sp	123	S007-660	9/10/13	44.2016	-91.8443	39.8	34.4	0.069	3582	0.11	1161.0
FS-335	Mississippi Pool 5 / Sp	123	S007-660	7/30/13	44.1953	-91.841	63.0	47.7	0.0342	4362	0.25	634.7
FS-212	Mississippi Pool 5 / Sp	123	S007-660	8/17/12	44.1993	-91.8461	29.6	17.2	0.0224	3674	0.22	531.7
FS-372	Mississippi Pool 5 / Sp	123	S007-660	9/10/13	44.2016	-91.8443	26.7	34.8	0.0536	3330	0.33	270.9
FS-312	Mississippi Pool 5 / Sp	123	S007-660	6/21/13	44.2018	-91.8444	35.7	28.3	0.0844	3563	0.67	132.2
FS-370	Mississippi Pool 8 at C	118	S007-222	9/9/13	43.5765	-91.2337	17.8	33.3	0.062	6558	1.43	172.4
FS-208	Mississippi Pool 8 at C	118	S007-222	8/14/12	43.5758	-91.2334	41.4	18	0.176	2178	0.41	92.3
FS-334	Mississippi Pool 8 at C	118	S007-222	7/29/13	43.5758	-91.2344	52.8	44.2	0.102	1969	0.4	78.3
FS-311	Mississippi Pool 8 at C	118	S007-222	6/20/13	43.5766	-91.2341	12.7	29.3	0.107	1544	0.62	29.0
FS-209	Mississippi Pool 8 at F	122	S007-556	8/15/12	43.6025	-91.2686	72.3	18.1	0.0711	9187	2.29	187.6
P-14	Mississippi River abov	108	S007-163	9/1/11	47.2379	-93.7196	163.2	1.09	0.053	7964	6.43	41.4

FS-354	Mississippi River abov	108	S007-163	8/13/13	47.2376	-93.7187	132.7	1.18	0.0532	7052	5.76	37.4
FS-58	Mississippi River abov	108	S007-163	8/28/12	47.2386	-93.7197	0.0	1.19	0.0806	8636	9.08	32.0
FS-57	Mississippi River belo	103	S006-923	8/28/12	47.2551	-93.6342	0.0	10.3	0.134	4225	1.2	91.3
P-15	Mississippi River belo	103	S006-923	9/1/11	47.2547	-93.6344	100.2	3.65	0.035	8667	6.07	52.2
FS-355	Mississippi River belo	103	S006-923	8/13/13	47.2553	-93.634	78.3	10.2	0.0819	10479	8.98	47.1
FS-313	Monongalia	46	34-0158-01-203	6/23/13	45.3334	-94.9293	50.0	34.7	0.0941	6028	19.44	6.4
FS-340	Monongalia	46	34-0158-02-203	7/31/13	45.3331	-94.9292	87.9	33.6	0.122	5530	22.1	4.7
FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.3332	-94.9292	154.4	34.6	0.242	5436	26.42	3.7
P-42	Monongalia (Middle F	45.5	34-0158-01-201	9/20/11	45.3481	-94.9509	5.7	16.51	0.042	46471	14.76	455.4
FS-77	Monongalia (near hw	46	34-0158-02-204	7/26/12	45.3331	-94.9268	121.3	21.7	1.37	4953	18.66	4.6
FS-75	Mortenson	44	34-0150-02-201	7/24/12	45.3	-94.9062	0.0	< 0.5	0.103	9071	12.09	25.0
FS-176	North Geneva	29	24-0015-00-209	7/24/12	43.7876	-93.271	0.0	15.6	1.54	2212	13.45	1.5
FS-132	Ox Hide	35	31-0106-00-203	9/7/12	47.335	-93.2134	10.5	26.4	0.042	14936	14.43	52.7
FS-198	Ox Hide	35	31-0106-00-203	9/7/12	47.335	-93.2134	0.6	26.4	0.0751	8743	24.51	10.0
FS-350	Ox Hide	35	31-0106-00-203	8/14/13	47.3351	-93.2132	0.0	25.9	0.119	3889	12.12	4.9
FS-344	Padua	82	73-0277-00-202	8/6/13	45.6231	-95.0187	9.5	< 0.5	0.0806	4520	12.61	6.2
P-29	Padua	82	73-0277-00-203	9/13/11	45.6202	-95.0192	3.4	0.76	0.13	4927	20.15	4.2
FS-220	Padua	82	73-0277-00-202	8/7/12	45.623	-95.0186	0.0	0.86	0.23	2291	9.77	2.3
FS-92	Partridge	119	S007-443	9/12/12	47.5207	-92.1909	4.1	36.3	0.0741	29463	5.87	571.7
P-13	Partridge	119	S007-443	8/31/11	47.5212	-92.1899	65.9	10.39	0.075	11026	1.44	464.3
FS-331	Partridge	119	S007-443	7/24/13	47.5212	-92.1904	60.5	14.6	0.112	10082	1.68	325.0
FS-366	Partridge	119	S007-443	9/3/13	47.5213	-92.19	47.7	34.2	0.057	7671	1.79	178.1
FS-365	Partridge	119	S007-443	9/3/13	47.5212	-92.1901	76.7	34.1	0.0393	9179	2.5	168.6
FS-301	Partridge	119	S007-443	5/28/13	47.5213	-92.1903	0.0	14.8	0.125	9491	3.94	104.3
FS-302	Partridge	121	S007-513	5/30/13	47.5153	-92.1894	0.0	43.1	0.0624	24784	6.27	378.8
FS-364	Partridge	121	S007-513	8/30/13	47.5138	-92.1894	105.7			28890	8.19	369.5
FS-332	Partridge	121	S007-513	7/24/13	47.5137	-92.1894	79.6	54.4	0.102	20512	8.34	187.1

FS-316	Partridge	121	S007-513	6/28/13	47.5137	-92.1899	0.0	24.9	0.098	6291	2.6	77.8
FS-55	Pelkey	55	49-0030-00-202	8/26/12	45.9962	-94.2273	0.0	3.42	0.0522	30642	17.32	168.8
P-10	Pike	104	S006-927	8/30/11	47.7325	-92.3468	43.0	8.31	0.063	15572	10.9	80.0
FS-91	Pike	104	S006-927	9/11/12	47.7327	-92.3473	3.5	14.2	0.0656	6565	4.72	41.4
FS-190	Pine	18	15-0149-00-205	8/28/12	47.6841	-95.5414	114.9	14.7	0.368	4477	7.08	12.2
FS-84	Pleasant	13	11-0383-00-207	8/10/12	46.9228	-94.4874	0.0	< 0.5	0.0218	7065	23.99	6.8
P-27	Pleasant	13	11-0383-00-206	9/9/11	46.928	-94.4757	28.6	0.49		5331	30.37	3.0
FS-215	Popple	101	S006-188	9/11/12	47.7254	-94.0817	36.3	< 0.5	0.0269	2971	14.42	2.4
FS-196	Prairie	115	S007-209	9/3/12	47.2519	-93.4884	44.6	9.63	0.0709	15071	10.51	78.4
FS-82	Rabbit	20	18-0093-02-204	8/8/12	46.5313	-93.9285	0.0	15.3	0.22	10903	11.79	36.7
P-28	Raymond	83	73-0285-00-203	9/12/11	45.629	-95.0234	68.6	0.82	0.094	3922	10.06	6.2
FS-343	Raymond	83	73-0285-00-203	8/6/13	45.629	-95.0233	61.4	1.92	0.0903	3270	7.59	6.1
FS-53	Raymond	83	73-0285-00-203	8/2/12	45.6286	-95.0225	61.1	< 0.5	0.0787	1905	4.79	3.8
FS-56	Rice	19	18-0053-00-203	8/27/12	46.3389	-93.8915	19.4	< 0.5	0.0259	83421	31.88	558.1
FS-376	Rice	19	18-0053-00-203	9/11/13	46.3394	-93.8918	46.5	< 0.5	0.0451	65261	33.36	329.7
P-69	Rice	19	18-0053-00-203	9/27/11	46.3394	-93.8913	43.0	0.23	0.021	50389	35.55	185.8
FS-304	Rice	19	18-0053-00-203	6/10/13	46.3387	-93.8906	5.7	< 0.5	0.0236	48287	33.61	183.1
FS-324	Rice	19	18-0053-00-203	7/15/13	46.3392	-93.8918	56.7	< 0.5	0.11	44704	33.18	160.3
FS-181	Rice	66	66-0048-00-203	7/27/12	44.3332	-93.4734	0.0	5.22	0.777	3829	21.67	2.4
FS-345	Rice	80	73-0196-00-216	8/7/13	45.3865	-94.6313	0.0	6.85	2.08	2012	14.83	1.1
FS-184	Rice	80	73-0196-00-216	7/30/12	45.3864	-94.6309	0.0	2.58	2.97	1523	15.03	0.6
FS-179	Rice	84	74-0001-00-201	7/25/12	44.0842	-93.0737	0.0	3.84	0.217	4152	19.07	3.2
FS-199	Rice	102	S006-208	9/5/12	47.6742	-93.6547	75.4	1.57	0.0552	3273	10.88	4.0
FS-231	Rice	2	02-0008-00-206	8/17/12	45.1604	-93.121	0.0	3.6	0.145	2159	7.98	2.6
P-11	Sand	97	S003-249	8/30/11	47.6348	-92.4235	14.4	7.69	0.046	22677	17.49	93.5
FS-90	Sand	97	S003-249	9/11/12	47.6351	-92.4234	2.9	15.9	0.152	7287	9.68	21.4
FS-321	Sandy-1	76	69-0730-00-203	7/9/13	47.6255	-92.5885	0.0	122	0.189	36502	29.51	124.9

FS-306	Sandy-1	76	69-0730-00-203	6/11/13	47.6255	-92.5884	0.0	11	0.0918	35357	28.53	122.3
FS-251	Sandy-1	76	69-0730-00-203	9/21/12	47.6254	-92.5886	3.8	3.05	0.123	35905	33.08	105.5
FS-382	Sandy-1	76	69-0730-00-203	9/17/13	47.6255	-92.5885	0.0	67.9	0.135	26645	32.28	61.2
FS-320	Sandy-2	76	69-0730-00-204	7/9/13	47.6188	-92.5936	0.0	118	3.08	19749	15.43	83.3
FS-348	Sandy-2	76	69-0730-00-204	8/13/13	47.6186	-92.5934	0.0	123	0.305	13216	8.23	81.6
FS-381	Sandy-2	76	69-0730-00-204	9/17/13	47.6187	-92.5931	0.0	126	0.0342	16172	11.67	79.2
FS-305	Sandy-2	76	69-0730-00-204	6/11/13	47.6187	-92.5937	0.0	135	1.08	19094	22.23	50.4
FS-380	Sandy-2	76	69-0730-00-204	9/17/13	47.6187	-92.5939	0.6	126	0.0342	17868	22.7	43.3
FS-349	Sandy-3	76	69-0730-00-205	8/13/13	47.6191	-92.5898	0.0	122	0.0697	14897	20.46	34.6
P-24	Second	17	15-0091-00-201	9/7/11	47.8255	-95.3635	37.3	0.87	0.139	3813	25.67	1.9
FS-105	Second	17	15-0091-00-202	6/27/12	47.8258	-95.3637	48.4	0.74	0.119	2527	33.3	0.6
FS-310	Second	117	S007-220	6/14/13	47.5205	-92.1925	57.6	316	0.0927	31190	4.22	946.8
FS-384	Second	117	S007-220	9/19/13	47.5204	-92.1925	27.7		0.104	22634	3.42	657.3
FS-303	Second	117	S007-220	5/30/13	47.5204	-92.1925	0.0	303	0.0991	13086	2.2	388.6
FS-323	Second	117	S007-220	7/11/13	47.5204	-92.1925	76.4	405	0.067	10036	2.91	166.9
FS-351	Second	117	S007-220	8/15/13	47.5205	-92.1925	66.8	838	0.0447	7088	1.84	148.0
FS-197	Snowball	36	31-0108-00-202	9/4/12	47.3355	-93.244	0.0	8.4	0.0936	4213	6	13.2
FS-347	Snowball	36	31-0108-00-202	8/12/13	47.3356	-93.2439	0.0	8.2	0.097	1136	1.19	7.4
FS-177	South Geneva	30	24-0015-02-208	7/24/12	43.7709	-93.2851	0.0	14.1	3.19	1618	16.71	0.6
P-16	St. Louis	106	S006-929	9/1/11	47.4015	-92.3773	0.0	24.5	0.025	1488	0.1	240.3
FS-69	St. Louis	114	S007-208	9/7/12	47.4671	-91.9279	0.0	1.33	0.181	11429	27.16	14.8
P-17	St. Louis	114	S007-208	9/1/11	47.4668	-91.9355	68.6	1.23	0.04	9654	30.4	9.3
FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.6545	-92.2739	0.0	16	0.0445	6169	1.73	122.0
FS-330	St. Louis Estuary	120	S007-444	7/22/13	46.6518	-92.2372	11.8	6.71	0.0901	5817	1.55	124.3
FS-315	St. Louis Estuary	120	S007-444	6/24/13	46.6516	-92.2373	0.0	8.1	0.147	6056	1.68	122.0
FS-300	St. Louis Estuary	120	S007-444	5/27/13	46.6515	-92.2376	0.0	9.4	0.0713	4499	1.26	97.2
FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.6518	-92.2372	31.2			4761	1.4	95.5

FS-67	St. Louis Estuary Poke	105	S006-928	9/5/12	46.6859	-92.1606	0.0	9.97	0.112	14015	3.66	241.1
FS-341	Stella	54	47-0068-00-205	8/1/13	45.066	-94.4339	57.6	24.7	0.0884	1786	1.35	15.1
P-30	Stella	54	47-0068-00-203	9/14/11	45.0659	-94.4339	31.6	7.59	0.08	2159	2.88	8.8
FS-188	Stella	54	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	18.1	1.79	1257	2.34	4.0
FS-224	Stone Lake	68	69-0046-00-201	9/19/12	47.5039	-91.8857	21.0	3.26	0.0533	5225	18.87	5.1
FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	1.62	0.0659	2505	0.65	69.6
FS-61	Swan	34	31-0067-02-206	8/30/12	47.2888	-93.2127	12.4	12.5	0.332	5827	22.71	5.0
FS-62	Swan	34	31-0067-02-206	8/30/12	47.289	-93.2124	3.8	14	0.221	4821	22.53	3.5
FS-125	Tamarac	56	56-0192-00-203	8/19/12	46.3637	-95.5714	0.0	2.33	0.0768	21908	18.41	82.3
FS-356	Trout	41	31-0216-00-212	8/14/13	47.2591	-93.3942	0.0	39.1	0.103	11992	12.59	40.7
FS-219	Trout	41	31-0216-00-212	9/13/12	47.2592	-93.3942	0.0	38.6	0.117	12535	15	35.9
FS-93	Turpela	71	69-0427-00-201	9/12/12	47.4613	-92.2371	1.0	3.3	0.115	6979	31.08	4.9
FS-183	Unnamed	50	34-0611-00-201	7/30/12	45.2675	-94.865	64.9	16.8	0.15	2157	5.61	4.0
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.286	2311	6.48	3.8
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	2193	8.1	2.6
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	1946	13.8	1.1
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	6.42	0.065	1689	12.6	0.9
FS-383	Upper Panasa	37	31-0111-00-204	9/18/13	47.3059	-93.2676	0.0	33.6	0.0399	19148	2.86	590.3
FS-59	Upper Panasa	37	31-0111-00-202	8/29/12	47.306	-93.2652	0.0	29.6	0.126	895	0.43	15.8
FS-139	Welby family farm	93	86-0231-00-202	9/21/12	45.3592	-94.0782	17.2	< 0.5	0.118	7267	30.76	5.3
FS-228	West battle	57	56-0239-00-204	8/15/12	46.2906	-95.6049	144.8	4.03	0.189	3108	17.37	2.1
FS-186	Westport	63	61-0029-00-204	8/1/12	45.6897	-95.217	0.0	7.11	1.79	4917	20.15	4.2
FS-346	Westport	63	61-0029-00-205	8/8/13	45.7042	-95.203	6.7	6.3	0.205	3262	19.66	2.0
FS-65	Wild Rice	11	09-0023-00-202	9/4/12	46.6712	-92.6055	0.0	< 0.5	0.083	13650	28.82	19.4
P-36	Wild Rice Reservoir	70	69-0371-00-204	9/16/11	46.9098	-92.1636	17.2	1.13	0.023	5555	3.75	39.5
FS-68	Wolf	69	69-0143-00-101	9/6/12	47.2564	-91.963	8.9	2.01	0.119	9526	17.19	18.0
P-19	Wolf	69	69-0143-00-202	9/2/11	47.2586	-91.9618	128.8	1.54	0.139	8240	25.1	8.7

MPCA Field Survey Data with CPSC
(Sorted by Water Body - Only Lowest CPSC)

LacCore_field_ID	Site_name	UniqID	DNRStateID	Date	Lat	Long	WRavestemM2	WRpresent	SO4mg_L	TSmgL	SedFeμgg	SedTOCpct	CPSC120
P-34	Anka	26	21-0353-00-201	9/16/11	46.0769	-95.7292	25.9	YES	2.23	0.671	1485	23.57	0.3
FS-134	Bass	43	31-0576-00-207	9/18/12	47.2844	-93.6276	64.0	YES	1.01	0.0664	3740	26.12	1.8
FS-85	Bean	8	03-0411-00-201	8/21/12	46.9337	-95.8706	0.0	NO	85	16	1967	11.85	1.4
FS-87	Bee	60	60-0192-00-202	8/23/12	47.6527	-96.0504	39.8	YES	11	0.67	3054	13.62	2.7
FS-193	Big Mud	79	71-0085-00-201	8/30/12	45.4529	-93.7418	14.3	YES	< 0.5	0.0308	12943	18.63	29.5
FS-216	Big Sucker	39	31-0124-00-203	9/12/12	47.3919	-93.2658	3.8	YES	7.78	0.145	3559	21.45	2.1
FS-204	Big Swan	86	77-0023-00-207	8/10/12	45.8795	-94.742	133.7	YES	5.49	0.0914	1731	5.94	2.4
P-12	Birch	67	69-0003-00-205	8/30/11	47.7357	-91.9428	68.6	YES	3.58	0.104	12431	26.8	17.7
FS-52	Blaamyhre	48	34-0345-00-203	8/1/12	45.364	-95.186	102.2	YES	0.62	0.078	3517	9.33	5.5
FS-214	Bowstring	116	S007-219	9/11/12	47.7024	-94.0608	69.7	YES	1.34	0.256	1974	24.34	0.6
FS-126	Bray	58	56-0472-00-202	8/20/12	46.4518	-95.8783	7.6	YES	1.65	0.072	3937	21.95	2.5
FS-63	Caribou	72	69-0489-00-206	9/3/12	46.8913	-92.3135	0.0	NO	1.21	0.0938	13791	29.44	19.3
FS-109	Carlos Avery Pool 9	4	02-0504-00-202	7/3/12	45.3192	-93.0611	52.8	YES	< 0.5	< 0.011	14736	12.51	61.0
FS-339	Christina	28	21-0375-00-315	7/31/13	46.0734	-95.7567	0.6	YES	14.6	1.93	1741	8.96	1.5
FS-314	Clearwater	96	S002-121	6/24/13	47.9372	-95.6907	0.6	YES	28	0.0664	3946	2.68	30.6
FS-88	Clearwater	98	S004-204	8/24/12	47.5174	-95.3904	148.3	YES	2.04	0.0488	9874	22.17	14.2
P-31	Cloquet	52	38-0539-00-201	9/14/11	47.4313	-91.4844	74.4	YES	0.81	0.024	4252	6.58	12.1
FS-128	Cromwell	14	14-0103-00-201	8/22/12	46.9651	-96.3171	0.0	NO	41.2	1.22	2948	2.85	16.2
FS-322	Dark	77	69-0790-00-202	7/10/13	47.6389	-92.7781	3.2	YES	175	0.131	2480	1.48	25.5
P-44	Dead Fish	12	09-0051-00-202	9/20/11	46.7451	-92.6863	48.7	YES	0.3	0.056	9685	16.6	19.4
FS-378	Duck Lake WMA	22	18-0178-00-202	9/12/13	46.7521	-93.8851	113.0	YES	< 0.5	0.0251	12151	26.57	17.1
FS-86	Eighteen	61	60-0199-00-202	8/22/12	47.6397	-96.0607	40.1	YES	4.29	0.164	1860	3.1	6.1
FS-137	Elk	15	15-0010-00-204	9/19/12	47.1952	-95.2249	42.7	YES	< 0.5	0.0936	6334	10.07	15.6
FS-95	Embarrass	73	69-0496-00-203	9/14/12	47.5334	-92.2979	0.0	NO	18.8	0.0298	21847	1.89	1248.9
FS-76	Field	45	34-0151-00-201	7/25/12	45.2964	-94.9058	0.0	NO	< 0.5	0.0687	7586	8.68	26.3

FS-195	Fisher	78	70-0087-00-201	8/31/12	44.7942	-93.4061	20.7	YES	6.85	0.136	11140	5.76	90.1
P-52	Flowage	1	01-0061-00-206	9/22/11	46.6895	-93.338	123.1	YES	0.56	0.018	4302	21.79	2.9
FS-194	Gilchrist	91	86-0064-00-201	8/31/12	45.2309	-93.824	0.0	NO	6.98	0.355	3117	20.81	1.7
FS-51	Glesne Slough	49	34-0353-00-201	7/31/12	45.3514	-95.1887	99.6	YES	< 0.5	0.061	7983	3.01	103.2
FS-104	Gourd	10	04-0253-00-201	6/27/12	47.8121	-94.965	0.0	NO	0.27		1776	36.87	0.3
P-20	Gull	9	04-0120-00-203	9/6/11	47.6559	-94.6944	15.6	YES	0.78	0.103	1608	5.08	2.5
FS-130	Hay	33	31-0037-00-202	9/6/12	47.2874	-93.102	141.0	YES	31.7	0.0738	13154	5.79	123.3
FS-221	Hay Creek Flowage	59	58-0005-00-202	9/17/12	46.0894	-92.4104	97.7	YES	1.95	0.119	9456	22.05	13.2
P-1	Height of Land	5	03-0195-00-209	8/22/11	46.9129	-95.6095	62.9	YES	0.24	0.053	1298	1.76	6.0
FS-131	Hinken	113	S007-207	9/5/12	47.7271	-93.9923	46.8	YES	< 0.5	0.0876	2960	4.53	9.4
FS-185	Hoffs Slough	85	76-0103-00-201	8/1/12	45.3255	-95.7059	0.0	NO	273	0.0343	3512	0.75	112.3
FS-218	Holman	42	31-0227-00-202	9/13/12	47.3005	-93.3445	0.0	NO	24.2	1.01	3035	29.74	1.0
FS-182	Hunt	65	66-0047-00-208	7/27/12	44.3275	-93.4443	0.0	NO	17.1	0.0729	2412	1.21	30.8
FS-191	Ina	27	21-0355-00-202	8/29/12	46.0715	-95.7281	30.2	YES	7.08	0.274	2216	9.09	2.3
P-5	Itasca	16	15-0016-00-208	8/25/11	47.2381	-95.2065	45.8	YES	0.26	0.056	1355	7.4	1.2
FS-207	Kelly Lake	64	66-0015-00-204	8/13/12	44.3542	-93.3743	0.0	NO	1.92	0.0927	4387	27.33	2.3
P-55	Lady Slipper	53	42-0020-00-204	9/22/11	44.5702	-95.6274	0.0	NO	107.71	14.84	2814	2.09	21.5
FS-180	Lily	90	81-0067-00-202	7/26/12	44.1947	-93.647	38.2	YES	< 0.5	0.0295	5095	28.07	3.0
FS-54	Little Birch	87	77-0089-00-207	8/3/12	45.7779	-94.7978	70.0	YES	7.4	0.0353	1794	6.02	2.6
P-4	Little Flat	6	03-0217-00-201	8/24/11	46.9981	-95.6641	83.1	YES	0.22	0.011	7479	33.13	5.2
FS-250	Little Rice	75	69-0612-00-201	9/20/12	47.7086	-92.4389	29.3	YES	1.03	0.0293	9488	26.45	10.7
P-3	Little Round	7	03-0302-00-202	8/24/11	46.9759	-95.7404	57.2	YES	0.46	0.032	1689	20.91	0.5
FS-223	Little Sucker	40	31-0126-00-202	9/14/12	47.3765	-93.246	0.0	NO	13.7	0.534	6297	16.56	8.5
FS-202	Long Prairie	110	S007-204	8/9/12	46.0072	-95.2634	13.4	YES	7.71	0.0793	2897	2.85	15.7
FS-200	Louisa	94	86-0282-00-205	8/8/12	45.2998	-94.258	0.0	NO	7.04	0.192	7824	8.76	27.6
FS-226	Louise	25	21-0094-00-202	8/14/12	45.9331	-95.4148	46.5	YES	4.09	0.0746	1833	0.83	28.5
FS-357	Lower Panasa	38	31-0112-00-204	8/15/13	47.3026	-93.2561	0.0	NO	28.5	1.26	2347	2.42	12.7
P-26	Lower Rice	109	S007-164	9/8/11	47.3817	-95.4926	120.1	YES	0.55	0.07	2364	6.76	3.8
P-25	Lower Rice	107	S006-985	9/8/11	47.3793	-95.4834	114.4	YES	1.02	0.097	2337	17.76	1.2
FS-377	Mahnomen	21	18-0126-02-201	9/11/13	46.4986	-93.9956	0.0	NO	21.1	0.0283	16540	7.47	141.1
P-63	Maloney	88	79-0001-00-201	9/29/11	44.2243	-91.9328	148.7	YES	1.83	0.01	10269	4.24	111.2

FS-187	McCormic	81	73-0273-00-203	8/2/12	45.722	-94.9121	8.9	YES	1.54	0.144	1512	1.1	14.0
FS-229	Mill Pond	23	21-0034-00-202	8/16/12	46.0716	-95.2218	102.2	YES	7.16	0.109	5143	7.86	14.0
FS-225	Miltona	24	21-0083-00-205	8/13/12	46.0496	-95.4217	0.0	NO	4.11	0.0694	2624	1.77	22.9
FS-129	Mink	92	86-0229-00-207	8/23/12	45.2767	-94.0299	0.0	NO	1.22	0.182	4247	13.63	5.0
FS-80	Mission	95	S001-646	8/6/12	45.8623	-93.0011	87.5	YES	0.62	0.0485	9231	4.83	77.5
FS-83	Mississippi Crow Wing	111	S007-205	8/8/12	46.4386	-94.1251	0.0	NO	3.13	0.127	13451	3.88	207.8
FS-210	Mississippi Pool 4/Robinson Lake	89	79-0005-02-202	8/16/12	44.3593	-91.9881	35.3	YES	15.7	0.07	6450	1.16	214.5
FS-312	Mississippi Pool 5 / Spring	123	S007-660	6/21/13	44.2018	-91.8444	35.7	YES	28.3	0.0844	3563	0.67	132.2
FS-311	Mississippi Pool 8 at Genoa	118	S007-222	6/20/13	43.5766	-91.2341	12.7	YES	29.3	0.107	1544	0.62	29.0
FS-209	Mississippi Pool 8 at Reno Bottoms	122	S007-556	8/15/12	43.6025	-91.2686	72.3	YES	18.1	0.0711	9187	2.29	187.6
FS-58	Mississippi River above Clay Boswe	108	S007-163	8/28/12	47.2386	-93.7197	0.0	NO	1.19	0.0806	8636	9.08	32.0
FS-355	Mississippi River below Clay Boswe	103	S006-923	8/13/13	47.2553	-93.634	78.3	YES	10.2	0.0819	10479	8.98	47.1
FS-379	Monongalia	46	34-0158-02-203	9/13/13	45.3332	-94.9292	154.4	YES	34.6	0.242	5436	26.42	3.7
P-42	Monongalia (Middle Fork Crow R)	45.5	34-0158-01-201	9/20/11	45.3481	-94.9509	5.7	YES	16.51	0.042	46471	14.76	455.4
FS-75	Mortenson	44	34-0150-02-201	7/24/12	45.3	-94.9062	0.0	NO	< 0.5	0.103	9071	12.09	25.0
FS-176	North Geneva	29	24-0015-00-209	7/24/12	43.7876	-93.271	0.0	NO	15.6	1.54	2212	13.45	1.5
FS-350	Ox Hide	35	31-0106-00-203	8/14/13	47.3351	-93.2132	0.0	NO	25.9	0.119	3889	12.12	4.9
FS-220	Padua	82	73-0277-00-202	8/7/12	45.623	-95.0186	0.0	NO	0.86	0.23	2291	9.77	2.3
FS-301	Partridge	119	S007-443	5/28/13	47.5213	-92.1903	0.0	NO	14.8	0.125	9491	3.94	104.3
FS-316	Partridge	121	S007-513	6/28/13	47.5137	-92.1899	0.0	NO	24.9	0.098	6291	2.6	77.8
FS-55	Pelkey	55	49-0030-00-202	8/26/12	45.9962	-94.2273	0.0	NO	3.42	0.0522	30642	17.32	168.8
FS-91	Pike	104	S006-927	9/11/12	47.7327	-92.3473	3.5	YES	14.2	0.0656	6565	4.72	41.4
FS-190	Pine	18	15-0149-00-205	8/28/12	47.6841	-95.5414	114.9	YES	14.7	0.368	4477	7.08	12.2
P-27	Pleasant	13	11-0383-00-206	9/9/11	46.928	-94.4757	28.6	YES	0.49		5331	30.37	3.0
FS-215	Popple	101	S006-188	9/11/12	47.7254	-94.0817	36.3	YES	< 0.5	0.0269	2971	14.42	2.4
FS-196	Prairie	115	S007-209	9/3/12	47.2519	-93.4884	44.6	YES	9.63	0.0709	15071	10.51	78.4
FS-82	Rabbit	20	18-0093-02-204	8/8/12	46.5313	-93.9285	0.0	NO	15.3	0.22	10903	11.79	36.7
FS-53	Raymond	83	73-0285-00-203	8/2/12	45.6286	-95.0225	61.1	YES	< 0.5	0.0787	1905	4.79	3.8
FS-324	Rice	19	18-0053-00-203	7/15/13	46.3392	-93.8918	56.7	YES	< 0.5	0.11	44704	33.18	160.3
FS-199	Rice	102	S006-208	9/5/12	47.6742	-93.6547	75.4	YES	1.57	0.0552	3273	10.88	4.0
FS-179	Rice	84	74-0001-00-201	7/25/12	44.0842	-93.0737	0.0	NO	3.84	0.217	4152	19.07	3.2

FS-181	Rice	66	66-0048-00-203	7/27/12	44.3332	-93.4734	0.0	NO	5.22	0.777	3829	21.67	2.4
FS-184	Rice	80	73-0196-00-216	7/30/12	45.3864	-94.6309	0.0	NO	2.58	2.97	1523	15.03	0.6
FS-231	Rice	2	02-0008-00-206	8/17/12	45.1604	-93.121	0.0	NO	3.6	0.145	2159	7.98	2.6
FS-349	Sandy-3	76	69-0730-00-205	8/13/13	47.6191	-92.5898	0.0	NO	122	0.0697	14897	20.46	34.6
FS-351	Second	117	S007-220	8/15/13	47.5205	-92.1925	66.8	YES	838	0.0447	7088	1.84	148.0
FS-105	Second	17	15-0091-00-202	6/27/12	47.8258	-95.3637	48.4	YES	0.74	0.119	2527	33.3	0.6
FS-347	Snowball	36	31-0108-00-202	8/12/13	47.3356	-93.2439	0.0	NO	8.2	0.097	1136	1.19	7.4
FS-177	South Geneva	30	24-0015-02-208	7/24/12	43.7709	-93.2851	0.0	NO	14.1	3.19	1618	16.71	0.6
P-16	St. Louis	106	S006-929	9/1/11	47.4015	-92.3773	0.0	NO	24.5	0.025	1488	0.1	240.3
P-17	St. Louis	114	S007-208	9/1/11	47.4668	-91.9355	68.6	YES	1.23	0.04	9654	30.4	9.3
FS-66	St. Louis Estuary	112	S007-206	9/5/12	46.6545	-92.2739	0.0	NO	16	0.0445	6169	1.73	122.0
FS-363	St. Louis Estuary	120	S007-444	8/26/13	46.6518	-92.2372	31.2	YES			4761	1.4	95.5
FS-67	St. Louis Estuary Pokegama Bay	105	S006-928	9/5/12	46.6859	-92.1606	0.0	NO	9.97	0.112	14015	3.66	241.1
FS-188	Stella	54	47-0068-00-204	8/27/12	45.0683	-94.4334	0.3	YES	18.1	1.79	1257	2.34	4.0
FS-224	Stone Lake	68	69-0046-00-201	9/19/12	47.5039	-91.8857	21.0	YES	3.26	0.0533	5225	18.87	5.1
FS-94	Sturgeon	100	S004-870	9/13/12	47.656	-92.9315	37.9	YES	1.62	0.0659	2505	0.65	69.6
FS-62	Swan	34	31-0067-02-206	8/30/12	47.289	-93.2124	3.8	YES	14	0.221	4821	22.53	3.5
FS-125	Tamarac	56	56-0192-00-203	8/19/12	46.3637	-95.5714	0.0	NO	2.33	0.0768	21908	18.41	82.3
FS-219	Trout	41	31-0216-00-212	9/13/12	47.2592	-93.3942	0.0	NO	38.6	0.117	12535	15	35.9
FS-93	Turpela	71	69-0427-00-201	9/12/12	47.4613	-92.2371	1.0	YES	3.3	0.115	6979	31.08	4.9
P-57	Unnamed	50	34-0611-00-201	9/23/11	45.2675	-94.865	74.4	YES	6.42	0.065	1689	12.6	0.9
FS-59	Upper Panasa	37	31-0111-00-202	8/29/12	47.306	-93.2652	0.0	NO	29.6	0.126	895	0.43	15.8
FS-139	Welby family farm	93	86-0231-00-202	9/21/12	45.3592	-94.0782	17.2	YES	< 0.5	0.118	7267	30.76	5.3
FS-228	West battle	57	56-0239-00-204	8/15/12	46.2906	-95.6049	144.8	YES	4.03	0.189	3108	17.37	2.1
FS-346	Westport	63	61-0029-00-205	8/8/13	45.7042	-95.203	6.7	YES	6.3	0.205	3262	19.66	2.0
FS-65	Wild Rice	11	09-0023-00-202	9/4/12	46.6712	-92.6055	0.0	NO	< 0.5	0.083	13650	28.82	19.4
P-36	Wild Rice Reservoir	70	69-0371-00-204	9/16/11	46.9098	-92.1636	17.2	YES	1.13	0.023	5555	3.75	39.5
P-19	Wolf	69	69-0143-00-202	9/2/11	47.2586	-91.9618	128.8	YES	1.54	0.139	8240	25.1	8.7