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MINNESOTA
SEA GRANT COLLEGE PROGRAM
RESEARCH ANNUAL REPORT

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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