

## **M.L. 2017, Chp. 96, Sec. 2, Subd. 03f Project Abstract**

For the Period Ending June 30, 2021

**PROJECT TITLE:** Assessment of Microbes for Improving Wild Rice Restoration

**PROJECT MANAGER:** Chan Lan Chun

**AFFILIATION:** University of Minnesota Duluth

**MAILING ADDRESS:** 1405 University Drive SCiv 221

**CITY/STATE/ZIP:** Duluth/MN/55812

**PHONE:** (218) 726-6449 / (218) 788-2613

**E-MAIL:** chun0157@d.umn.edu

**WEBSITE:** <https://sites.google.com/d.umn.edu/chunlab/welcome>

**FUNDING SOURCE:** Environment and Natural Resources Trust Fund

**LEGAL CITATION:** M.L. 2017, Chp. 96, Sec. 2, Subd. 03f

**APPROPRIATION AMOUNT:** \$ 334,000

**AMOUNT SPENT:** \$ 333,222

**AMOUNT REMAINING:** \$ 778

### **Sound bite of Project Outcomes and Results**

The project improved our understanding of microbial and nutrient associations with self-sustaining wild rice wetlands. This information will be useful to develop management strategies for wild rice restoration success, which will improve long-term protection of native species and aquatic biodiversity, and support management of Minnesota's culturally and ecologically important natural resource.

### **Overall Project Outcome and Results**

Wild rice (*Zizania palustris*), a native emergent aquatic plant, has a multitude of ecological functions and high cultural and economic value in Minnesota. Wild rice was historically abundant in northern Minnesota but its abundance and distribution have been reduced due to various factors. There have been collaborative efforts to restore wild rice wetlands for improved wildlife habitat and increased opportunities for wild rice harvest. Despite ongoing efforts, restoration has been met with mixed success. Much research was conducted on surface water and sediment chemistry that is conducive to wild rice growth. However, one ecological component of the wild rice ecosystem that remains under-explored is microbial communities that are involved in processing key nutrients. This project characterized microbial communities associated with wild rice wetland. Wild rice and coexisting plant samples were collected from 7 wild rice wetlands along with water and sediment. High-throughput DNA sequencing analyses indicated that wild rice-associated microbial communities were distinct from those found in water and sediment. Moreover, the influence of surface/porewater chemistry and nutrients on the microbial communities were evaluated. The project outcomes will allow the restoration partners to understand why restoration efforts are successful or not and can be immediately transferable to restoration managers for the development of applicable restoration practices. Likewise, if beneficial microbial groups associated with self-sustainable wild rice beds were identified from this project, the methods to encourage their abundance and functions for wild rice growth are needed through seeding with inoculants derived from successful wild rice stands and sediment amendments. If coexisting or invasive species alter nutrients and microbial community structure unfavorable for wild rice fitness by imposing some degree of selective pressures, targeted species control is essential prior to current restoration efforts. This will be useful to for the partners to develop effective management strategies for wild restoration goals.

### **Project Results Use and Dissemination**

The project findings have been disseminated via reports to LCCMR, publications, and regional and national presentations at conferences. We held four meetings with wild rice managers and the project partners for field sampling plan, project progress, consultation and outcomes. The project findings were shared with the public through the [university's news article](#), public outreach activities (e.g. Lake Superior Youth Symposium), and

[student stories](#). Moreover, microbial DNA sequences of environmental samples collected from wild rice wetlands were archived at National Center for Biotechnology Information.



## Environment and Natural Resources Trust Fund (ENRTF) M.L. 2017 LCCMR Work Plan Final Report

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**Date of Submission:** August 15, 2021

**Date of Next Status Update Report:** Final Report

**Date of Work Plan Approval:** 06/07/2017

**Project Completion Date:** June 30, 2021

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**PROJECT TITLE:** Assessment of Microbes for Improving Wild Rice Restoration

**Project Manager:** Chan Lan Chun

**Organization:** University of Minnesota Duluth

**Mailing Address:** 1405 University Drive SCiv 221

**City/State/Zip Code:** Duluth/MN/55812

**Telephone Number:** (218) 726-6449 / (218) 788-2613

**Email Address:** chun0157@d.umn.edu

**Web Address:** <https://sites.google.com/d.umn.edu/chunlab/welcome>

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**Location:** Northeastern

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**Total ENRTF Project Budget:**

**ENRTF Appropriation:** \$334,000

**Amount Spent:** \$333,222

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**Balance:** \$778

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**Legal Citation:** M.L. 2017, Chp. 96, Sec. 2, Subd. 03f as extended by M.L. 2020, First Special Session, Chp. 4, Sec. 2

**Appropriation Language:**

\$334,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota, Natural Resources Research Institute, to evaluate the microbial communities and nutrients associated with wild rice and competing vegetation, with the goal of enhancing restoration success to increase the abundance of wild rice. This appropriation is available until June 30, 2020, by which time the project must be completed and final products delivered.

M.L. 2020 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2021]

## **I. PROJECT TITLE:** Assessment of Microbes for Improving Wild Rice Restoration

### **II. PROJECT STATEMENT:**

Wild rice (*Zizania palustris*) is an ecologically and culturally important plant in Minnesota and its state grain. Wild rice was historically abundant in northern Minnesota but its abundance and distribution have been reduced due to environmental contaminants, habitat destruction, physical disturbance, and establishment of competitive or invasive plant species. In recent years, there have been collaborative efforts to restore wild rice wetlands by reducing competitive or invasive plant cover, seeding, and other means; this results in improved wildlife habitat and increased opportunities for wild rice harvest. Even with many ongoing restoration efforts, however, post-restoration monitoring is not common. When such monitoring does occur, the evaluation is primarily based on wild rice results, which may not be sufficient to understand other factors influencing restoration success. We hypothesize that changes in sediment microbial communities and nutrients in wild rice beds are key determinants in the re-establishment of self-sustaining wild rice populations. Microbes are known as primary mediators of plants' growth, adaptation, and competitive success. Particularly, plant-microbe interactions in the rhizosphere are directly associated with nutrient uptake and disease/stress tolerance, resulting in changes in plant community composition and ecology. Such associations, which are not well-characterized, can be important to restoration success and provide a promising opportunity to develop targeted control of competitive or invasive plants such as pickerel weed, arrowheads, and narrow-leaf cattails. The overall goal of this project is to improve restoration activities that increase the abundance and distribution self-sustaining wild rice beds by assessing re-establishment of wild rice stands in comparison with competitive or invasive plants species, and evaluating changes in sediment microbial communities and nutrients in wild rice beds. This project will examine total microbial (both fungal and bacterial) communities and nutrients in the rhizosphere of both wild rice and competitive or invasive plants in well-established wild rice wetlands as well as pre-restoration and post-restoration sites. The results of this work will identify microbial and nutrient associations in self-sustaining wild rice wetlands and apply the information to develop a management strategy to promote restoration success in the St. Louis River estuary and wild rice lakes in Minnesota.

### **III. OVERALL PROJECT STATUS UPDATES:**

#### **Project Status as of January 1, 2018:** January 23, 2018

Starting on July 1, 2017 through present most work was performed with Activity 1 and initial analyses were conducted for Activity 2. The study sites were selected based on the presence of self-sustaining wild rice, past restoration sites with and without success, and current restoration sites through the consultation with the project partners in MNDNR, 1854 Treat Authority, and Fond du Lac Natural Resources. In late July and early August seven potential sample sites in three counties were visited to assess sampling conditions and select our initial sample sites. The seven sites were Kingsbury Bay and Rask Bay in the St. Louis River estuary (SLRE) in St. Louis County, Kettle Lake in Carlton County, Sandy Flowage in Aitkin County, Norway Point and Skibo Landing on the upper St. Louis River in St. Louis County, and Big Rice Lake in St. Louis County. Among them, the field sampling were conducted in six sampling sites from August to October, 2017. At each site, vegetation data were collected to assess establishment of wild rice stands in comparison with competitive or invasive plants species and water, sediment, and plant samples were sampled. Field samples were processed and water quality and nutrient analyses are currently in progress. In Activity 2, DNA samples have been extracted for microbial community analysis. Microscopic examination of root anatomy, spatial distribution of microbes on root, and elemental partitioning such as iron plaques is currently undertaken using scanning electron microscopy.

**Project Status as of July 1, 2018:** July 25, 2018

Since the field sampling at late summer-early fall (first season sampling) in 2017, water quality and nutrient analyses of the samples were completed, and vegetative composition analysis was performed. Overall, water quality and vegetation composition data are relatively site-dependent and species-area curves show adequate sample size. Based on our review on field sampling protocol and operation procedures in 2017 and have developed sampling plans in relation to wild rice lifecycle during May-October in 2018. The field work has been conducted in the same field sites (3 self-sustaining wild rice sites; reference sites, 2 managed wild rice stands, and 2 sites dominated by competing cattails or pickerel weed). Kettle Lake, Carlton County was added as a self-sustaining wild rice site for Lake type. This season sampling focuses on high producing and pristine reference sites to understand relationship between wild rice life cycles and sediment microbiome as baseline study. In Activity 2, nucleic acids from samples of the first season have been submitted to the University of Minnesota Genomic Center for sequencing to examine microbial community in water, sediment, and plants' roots. Microscopic examination of root anatomy and elemental partitioning such as iron plaques is performed using scanning electron microscopy. We are working on fluorescence in situ hybridization method to examine the spatial distribution and relative abundance of the bacteria-root.

**Project Status as of January 1, 2019:** January 3, 2019

We completed the second season sampling for both vegetation and environmental samples (water, sediment, and plants) based on the sampling plan and operation procedures developed from the previous reporting period. Our research team and partners' reviews on our first season sampling brought up discussion on categories of study sites. The terminology/categories of the study sites were updated based upon wild rice presence and density, and so now we are referring to sites with little or no rice as "wild rice restoration sites", and sites with wild rice present nearly every year as "self-sustaining wild rice". In the list below we've also split sites into river and lake sites. In some cases (especially Kingsbury Bay), wild rice density is so sparse that no wild rice has been found in our vegetation samples. Portions of Kingsbury Bay were originally scheduled for restoration dredging in 2018, but that work was delayed until 2019. In 2018 we sampled two sites that weren't sampled in 2017: Kettle Lake in Carlton County, and Skibo on the upper St. Louis River in St. Louis County. That brings our total number of sites sampled to eight:

**Categories**

Self-sustaining wild rice river sites:

Self-sustaining wild rice lake site:

Wild rice restoration river sites (sparse or no rice):

Wild rice restoration lake site (sparse rice):

**Study Sites**

Sandy Flowage, Aitkin County

Norway Point (upper St. Louis River), St. Louis County

Skibo (upper St. Louis River), St. Louis County

Kettle Lake, Carlton County

Rask Bay (St. Louis River estuary), St. Louis County

Kingsbury Bay (St. Louis River estuary), St. Louis County

Grassy Bay (St. Louis River estuary), St. Louis County

Big Rice Lake, St. Louis County

This season sampling was designed to examine changes on sediment nutrients and microbial communities in relation to wild rice life cycles and vegetation. The sampling were conducted 2-3 times at each study sites from June to October, 2018. Currently, chemical and nutrient analyses for the second season samples are in progress. In Activity 2, DNA sequencing of first season samples (~200 samples) were completed and bioinformatics analysis has been conducted to examine microbial community in water, sediment, and plants' roots. Simultaneously, we are isolating DNA from the second season samples (over 400 samples) and plan to submit

DNA to the University of Minnesota Genomic Center for sequencing in February, 2019. In Activity 3, our initial results were presented at Minnesota Water Resources Conference in October, 2018 for data dissemination to public and tribal agencies, academic community, and environmental industries.

**Project Status as of July 1, 2019:** July 19, 2019

Overall, the project moves forward with anticipated project timeline. In Activity 1, vegetative composition analysis was performed and water quality and nutrient analyses (biophysical data) of the samples collected during the summer in 2018 were completed. In total, over 200 plots were analyzed for wild rice density and vegetation composition and approximately 300 water and sediment samples were analyzed for biophysical data. Based on the results from year 1 and 2 and the consultation with project partners, the sampling plan for year 3 was developed. Activity 2, microbial community composition analysis for 2017 samples were completed and In Activity 3, we disseminated our project findings on water quality, vegetation data, and microbiome in wild rice wetlands through presentations in regional and national conference, project meeting with research partners and wild rice managers and outreach activities in Lake Superior Youth Symposium.

**Project Status as of January 1, 2020:** January 10, 2020

This summer the third-year field work was completed for both vegetation and environmental samples (water, sediment, and plants), which mean we complete all field samplings planned for the project. This season's sample collection was designed based on the results of 2017-2018 field events: one sampling event for each study site to observe inter-annual variations by comparing three-year data sets and intensive life-stage sampling for self-sustaining wild rice beds (Skybo and Norway Point) to identify key environmental and microbial determinants for healthy wild rice populations. Currently wild rice density and the vegetation composition data have compiled with the statistical analyses. Water quality and nutrient analysis of Year 3 samples have been analyzed and DNA extraction and next generation sequencing are in progress. In Activity 3, we are preparing two manuscripts on water chemistry associated with wild rice habitat and microbial assembly in wild rice wetlands. We plan to disseminate our findings to the project partners and stakeholders in June 2020.

**Amendment Request: January 17, 2020**

We are requesting funds be shifted from the supplies budget line to personnel.

- The Professional/Technical/Service Contract budget would be reduced by \$7,547 to a revised budget of \$17,249
- The Supplies budget would increase by \$7,547 to a revised budget of \$36,127

These changes are being requested because additional nutrient analyses are needed to accomplish Activity 1, Outcome #3. Specifically, we found total nitrogen is one of the key determinants on water chemistry and microbiome in wild rice wetlands in Minnesota with the project's progress. Based on the findings, it is valuable to conduct additional nutrient analysis on our environmental samples, which requires additional supplies for chemicals and analytical vials. We completed all field samplings (Activity 1 Outcome # 1 and 2) proposed for the project using the University's boats and canoe for the sites without wild rice and airboat service (Professional Service) for wild rice habitats.

**Amendment Approved by LCCMR 2/27/2020**

**Project extended to June 30, 2021 by LCCMR 6/18/20** as a result of M.L. 2020, First Special Session, Chp. 4, Sec. 2, legislative extension criteria being met.

**Project Status as of July 1, 2020:**

As we completed field sampling campaigns and biophysical data collection along with vegetation data in Activity 1, we compiled all data and conducted statistical analysis to identify correlation and key drivers of water quality with vegetation composition and restoration activities in the wild rice wetlands of this study. Currently we are working on the manuscript of key findings of Activity 1. We have made good progress on microbial community and nutrient analysis in Activity 2. We completed bacterial community analysis in macro-environment (water and sediment) and micro-environment (rhizosphere and root) and have analyzed bacterial communities associated with nitrogen nutrient and key biogeochemical cycles in the wetlands. This analysis will be completed by early January. Our research progress on fungal community analysis and qPCR analysis of nitrogen cycling genes have been delayed on hold during March-June 2020 due to the COVID-19 pandemic (e.g. the University's lab hibernation and the State's "Stay Home" order). We have resumed the analysis and will complete project activities and report to the extended project timeline (June 30, 2021).

**Amendment Request: 29 September 2020**

We are requesting funds be shifted from travel budget line to personnel.

- The travel expenses Minnesota for activity 3 would be reduced by \$1,002 to a revised budget of \$1,286.
- The personnel budget in Activity 2 and 3 would increase by \$425 and \$577 to a revised budget of \$142,928 and \$13,315, respectively.

These changes are being requested because in-person travels for project dissemination/public outreach are expected to be limited due to the pandemic. We have worked on data analysis of fungal community in wild rice wetland (Activity 2 Outcome #2 and #4) and plan to develop materials and fact sheets for dissemination and outreach activity through virtual conferences and meetings (Activity 3) so we request to move the travel fund to the personnel category.

**Project Status as of January 1, 2021: 22 February 2021**

The project is approximately 95% complete now. Activity 1's data analysis was completed, and a manuscript is ready for submission. Currently we are focusing on data analysis and project outcome dissemination for activity 2 and 3. Two additional manuscripts are in preparation and oral and poster presentations are planned in the Annual Meeting of Society of Wetland Scientists in June.

**Overall Project Outcomes and Results: 16 August 2021**

Wild rice (*Zizania palustris*), a native emergent aquatic plant, has a multitude of ecological functions and high cultural and economic value in Minnesota. Wild rice was historically abundant in northern Minnesota but its abundance and distribution have been reduced due to various factors. There have been collaborative efforts to restore wild rice wetlands for improved wildlife habitat and increased opportunities for wild rice harvest. Despite ongoing efforts, restoration has been met with mixed success. Much research was conducted on surface water and sediment chemistry that is conducive to wild rice growth. However, one ecological component of the wild rice ecosystem that remains under-explored is microbial communities that are involved in processing key nutrients. This project characterized microbial communities associated with wild rice wetland. Wild rice and coexisting plant samples were collected from 7 wild rice wetlands along with water and sediment. High-throughput DNA sequencing analyses indicated that wild rice-associated microbial communities were distinct from those found in water and sediment. Moreover, the influence of surface/porewater chemistry and nutrients on the microbial communities were evaluated. The project outcomes will allow the restoration partners to understand why restoration efforts are successful or not and can be immediately transferable to restoration managers for the development of applicable restoration practices. Likewise, if beneficial microbial groups

associated with self-sustainable wild rice beds were identified from this project, the methods to encourage their abundance and functions for wild rice growth are needed through seeding with inoculants derived from successful wild rice stands and sediment amendments. If coexisting or invasive species alter nutrients and microbial community structure unfavorable for wild rice fitness by imposing some degree of selective pressures, targeted species control is essential prior to current restoration efforts. This will be useful to for the partners to develop effective management strategies for wild restoration goals.

#### **IV. PROJECT ACTIVITIES AND OUTCOMES:**

##### **ACTIVITY 1: Collect vegetation data and sediment samples**

**Description:** In this project, sampling site will be selected based on the presence of self-sustaining wild rice, past restoration sites with and without success, and current restoration sites. Examples of potential sampling sites include areas with self-sustaining wild rice (such as northwest portions of Rask Bay in St. Louis River estuary; Kettle Lake in Carlton County, and St. Louis River at Norway Point and/or Skibo landing in St. Louis County) and restoration sites (such as North Bay, south portions of Rask Bay, and Radio Tower Bay in St. Louis River estuary; and Big Rice Lake in St. Louis County). At each sampling site, we will collect vegetation data to assess establishment of wild rice stands in comparison with competitive or invasive plants species and conduct plant, soil, and water sampling to examine total microbial (both fungal and bacterial) communities and nutrients in the rhizosphere of both wild rice and competitive or invasive plants. Airboat or canoe will be used to access sampling sites since it is an essential and effective way to access wetland with emergent plants without damaging wild rice plants. Key information will include the density of wild rice and presence of coexisting or competing emergent, floating-leaf, and submerged plants. We will choose pairs of sample sites where we can sample natural or managed wild rice stands and stands of competing or invasive plants in the same or similar wetlands. Vegetation in each stand (wild rice or competitors) will be sampled with sets of small plots (0.5 or 1 m<sup>2</sup>); sampling the first year will be designed to determine adequate sample size using a species-area curve. Within each plot, density of wild rice will be determined by counting stems of wild rice and all floating-leaf and emergent species within the plot. The overall cover of all submerged aquatic plants (e.g. sparse, patchy, or dense) will be recorded in field form. All plants within the plots will be identified to species if possible, or to the lowest taxonomic level possible for plants that are not flowering or fruiting and therefore cannot be identified to species. Plant nomenclature will follow the current MNTaxa lists available online at the Minnesota Department of Natural Resources website.

Concurrently, sediment samples associated with wild rice and other coexisting or competing plants will be collected from the sites. Typically, plant root and sediment samples will be collected by using a soil core sampler, which is hammered into the sediment. The cores from field sites will be placed in sterile Whirlpak bags or PTFE bottles and kept in a cooler until transported to the laboratory. Roots are collected from the soil core to harvest three soil fractions: bulk soil (the soil remaining after picking out the roots from the core), rhizosphere (the soil adhering to the roots), and rhizoplane (the washed roots). The rhizoplane fraction will be collected from homogenization of the roots washed with sterile phosphate saline solution twice. Each fraction is weighed and the subsamples will be stored as it is at -20 °C for DNA extraction. To determine the influence of microbial communities in water column on rhizosphere microbial community of wild rice and coexisting plants, we will collect overlying water and sediment porewater near the sampling plots. We will monitor physicochemical parameters including water temperature, pH, dissolved oxygen, conductivity, and redox potential at sampling site and collect water samples for chemical and microbiological analyses. The water samples are collected in two collection bottles, one with acid preservatives and the other without acid preservatives. Acid bottles will be



tested for total N (nitrate + nitrite), total phosphorus, cations and non-acid bottles for nitrite, soluble phosphorus, ammonia, total suspended solid, other anions, and microbial community analyses. For microbial community analyses, the water samples will be filtered through 0.22 µm, polyethane sulfonate filters to trap bacterial microorganisms.

**Summary Budget Information for Activity 1:**

**ENRTF Budget: \$ 115,634**  
**Amount Spent: \$ 115,634**  
**Balance: \$ 0**

Outcome	Completion Date
1. Monitoring and compilation of vegetation data from sampling sites	November 2019
2. Collection of sediment associated with wild rice and other coexisting plants	November 2019
3. Field water quality measurement and sampling	November 2019
4. Evaluate restoration success based on wild rice and vegetation data	March 2020

**Activity 1 Status as of January 1, 2018:** January 23, 2018

The eight sites were initially selected based current information on the presence of self-sustaining wild rice, past restoration sites with and without success, and current restoration sites along with recommendations of the wild rice managers and the project partners. The sites include Kingsbury Bay, Rask Bay, Grassy Point in the St. Louis River estuary (SLRE) in St. Louis County, Kettle Lake in Carlton County, Sandy Flowage in Aitkin County, Norway Point and Skibo Landing on the upper St. Louis River in St. Louis County, and Big Rice Lake in St. Louis County shown in Figure 1.1. The field sites were visited to assess sampling conditions and select our initial sample sites. Among them, we conducted vegetative sampling in five of these sites and water, sediment, and plant sampling in six of these sites from August to October of 2017. Sites were selected to represent a range of self-sustaining wild rice stands, managed wild rice stands, and stands dominated by competing cattails or pickerel weed (Table 1.1).



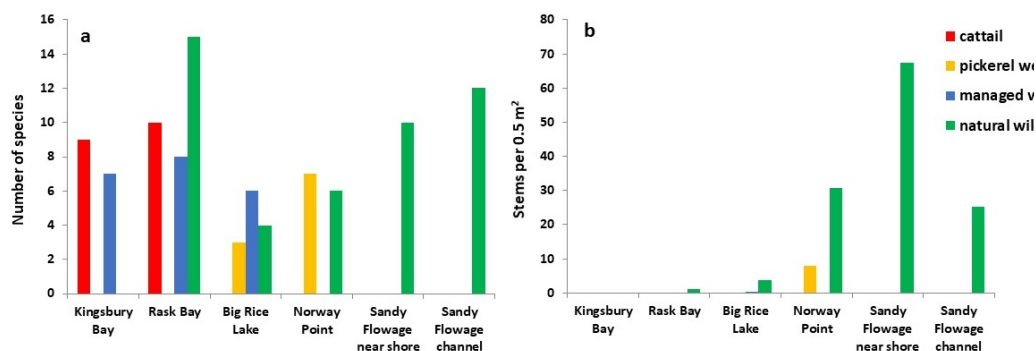
**Figure 1.1.** Sampling sites in Northeastern Minnesota

Within each site we attempted to delineate 5 m x 20 m sample areas in one or two areas representing the range of treatments (self-sustaining wild rice, managed wild rice, competing plants). There was a total of 13 sample areas delineated in the five sample sites. Within each sample area, we randomly selected 3 sediment sample points, and then attempted to sample two vegetation plots within 3 meters of the sediment sample points within each sample area. 6 vegetation samples (1 m<sup>2</sup> sample plots), and 6 counts of wild rice density in half the sample plot (0.5 m<sup>2</sup>) were targeted to provide a measure of wild rice density consistent with sampling methods recommended by wild rice managers. Vegetation samples were lists of plant species present in each 1 m<sup>2</sup> sample plot. Density of wild rice was measured by counting the number of wild rice stems in one half of the m<sup>2</sup> sample plot. We also measured water depths and Secchi depths at each plot.

**Table 1.1.** Numbers of vegetation plots sampled in each field site during late summer season of 2017

Sites sampled:	Total plots sampled	Self-sustaining wild rice	Managed wild rice	Competing cattails	Competing pickerel weed	Extra in bulrush along shore
Kingsbury Bay (SLRE), St. Louis Co.	18		12	6		
Rask Bay (SLRE), St. Louis County	18	6	6	6		
Big Rice Lake, St. Louis County	18		6		12	
Norway Point, St. Louis County	11	5			6	
Sandy Flowage, Aitkin County	13	12				1
<b>Totals</b>	<b>78</b>	<b>23</b>	<b>24</b>	<b>12</b>	<b>18</b>	<b>1</b>

There were about 40 aquatic and emergent plant species present in 78 sample plots. The self-sustaining wild rice stands had from 4 to 15 species present (average 9.4 species), the managed wild rice stands had from 5 to 9 species present (average 7 species), the pickerel weed stands had 3 to 7 species (average 5), and the cattail stands had 9 to 10 species (average 9.5). Figure 1.2 for a summary of species diversity by sample site. Mean density of wild rice in self-sustaining wild rice ranged from 1.2 to 67.3 stems per 0.5 m<sup>2</sup> (average 25.7); in managed wild rice the mean density ranged from 0 to 0.2, in competing pickerel weed had mean density from 0 to 8 stems of wild rice, and there were no wild rice stems in the competing cattails. Both the managed and self-sustaining wild rice stands in Rask Bay had been severely browsed by Canada geese, so that only a few inches of stem were standing above the water surface (all the flowering parts had been removed). Currently, vegetation composition analysis is in progress to evaluate species-area curves, which allow us to determine adequate sample size.



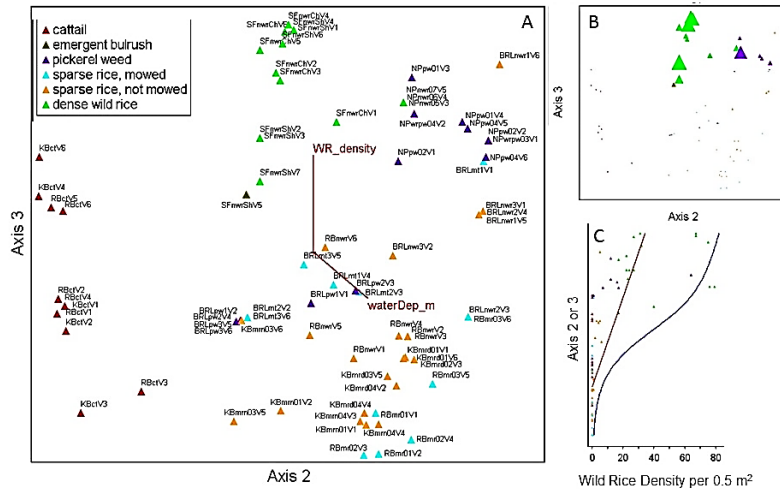
**Figure 1.2.** Mean species diversity (a) and mean wild rice density (b) in sampling sites in 2017

Concurrently, wild rice and other coexisting or competing plants and their associated water and sediment samples were collected from the sites. During field sampling, physicochemical parameters (e.g. water temperature, pH, dissolved oxygen, conductivity) were measured. Generally, physicochemical properties of the water samples are relatively similar among sampling sites with circumneutral pH (6.6-8.1), low conductivity (40~200 μS/cm) and moderate dissolved oxygen concentration (4-6 mg/L). Each sample (plant, sediment, surface water) was processed using aforementioned procedures for chemical and microbiological analyses. Water samples including surface water and porewater have been analyzed for total N (nitrate + nitrite), total phosphorus, cations and non-acid bottles for nitrite, soluble phosphorus, ammonia, and other anions. Figure 1.3

represents the composition of cations and anions in surface water (a) and pore water (b). Even though the dissolved solid concentrations are low, chloride (Cl<sup>-</sup>), magnesium (Mg<sup>2+</sup>), and calcium (Ca<sup>2+</sup>) are major ions in both surface and porewater, which reflect this region's geological condition. Generally, the water in our study sites are low in nutrient constituents such as nitrate, phosphate, and sulfate. The analysis to determine the composition and speciation of nutrients in porewater samples and sediment is in progress. Sediment and plant materials were processed and stored in -20 °C for Activity 2.

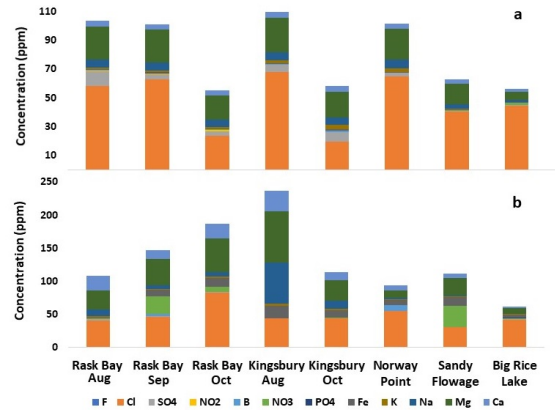
Early spring samples will be collected in the same study sites in 2018. Both vegetation and water quality data will be evaluated to determine adequate sample size, which can be reflect to the next sampling plan.

**Activity 1 Status as of July 1, 2018: July 25, 2018**



**Figure 1.4.** Bray-Curtis ordination analysis (A) of vegetation composition (78 plots and 40 taxa) by sample types described in Table 1. B is another version of plot A with wild rice density per 0.5 m<sup>2</sup> which is represented by symbol size (larger symbol indicates greater wild rice density). Plot C represents species-area curves as a function of wild rice density.

density (described in Table 1) and water depth. Based on statistic correlation analysis (Kendall's tau coefficient=0.6 and Pearson's *r* =0.65) with component, axis 3, species composition is positively correlated with wild rice density.

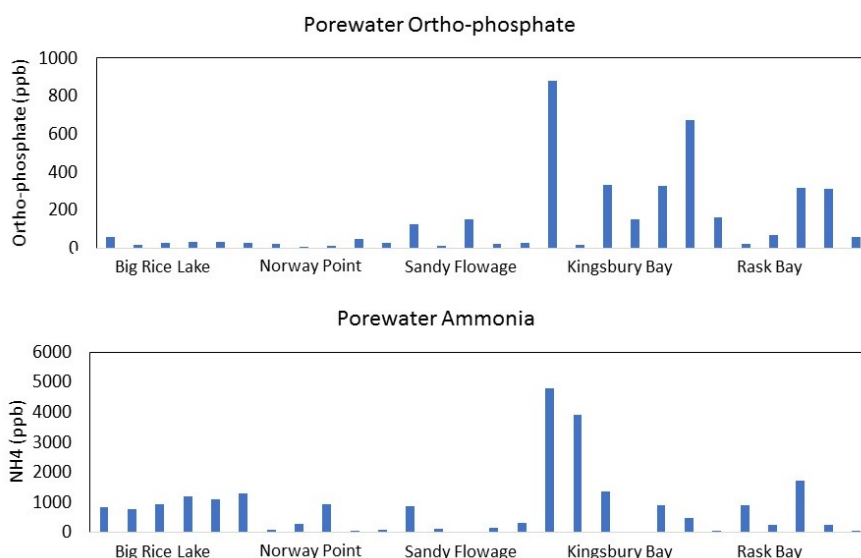


**Figure 1.3.** Common anions and cations in surface water (a) and porewater (b) collected from wild rice beds in 2017. Anions and cations were determined using an ion chromatography and atomic absorption spectroscopy, respectively.

Since the last status report, we have performed vegetative composition analyses and water quality and nutrient analyses and for the samples collected in 2017 and both vegetation and water quality data were used to determine adequate sample size, which reflected to the sampling plan for the second year. As for vegetation composition analysis, we evaluated species-area curves and correlation between species composition and wild rice density through ordination analyses. Figure 1.4 is Bray-Curtis ordination analysis of vegetation composition analysis. Generally, the ordination analyses show that the similarity in vegetative composition depends on the characteristics of sampling types (habitat types) in relation to wild rice

We also completed nutrient analyses in porewater samples collected during the first season. Figure 1.5 shows the orthophosphate and ammonia/ammonium concentration in porewater samples. Overall, nutrient concentration in porewater were low in wild rice beds (Norway Point and Sandy Flowage; natural wild rice stand and Big Rice Lake as managed wild rice beds), while they were much higher (5-10 fold) in St. Louis estuary (Kingsbury Bay and Rask Bay). The trend is similar to other cationic and anionic species (Figure 3B). Presumably, this may be related with the degree of human activities. Such correlation will be statistically analyzed.

Generally, both vegetation and water quality data indicate that the sample sizes were adequate. We reviewed the field sampling protocols and sample processing methods conducted late summer in 2017 and developed the field sampling plan for 2018 as the second year's sampling. Overall, we follow the first season's sampling protocols in the same sampling location. In 2018, we have developed sampling plan focusing changes in microbial community and water quality at the different life stage of wild rice and completing plants. In order to examine the relationship, we plan to collect water, sediment and plant roots throughout floating leaf (June), aerial shoots (July), flowering (late July and early August) and ripening (Late August and September) at self-sustaining wild rice beds. These sites include Sandy Flowage, Kettle Lake (new site this season) and headwater of St. Louis River; Skibo (new site this season). Additionally, we have conducted vegetation data collection and field sampling twice (late June and late July/early August) for the other managed sites (Big Rice Lake and St. Louis River estuary sites). Currently we have been actively conducting the field sampling this summer.



**Figure 1.5.** Nutrient concentrations (orthophosphate; top and ammonia; bottom) in porewater samples collected in 2017

**Activity 1 Status as of January 1, 2019:** January 3, 2019

In summer of 2018 vegetation was sampled twice at all but one site, early in the summer (mid-June to early July) and when rice was in flower or forming seeds (late July to late August). Kettle Lake was sampled in late June, but was inaccessible with a canoe in August, due to low water levels in the lake. In early summer we sampled 103 vegetation plots (one square meter), and counted density of wild rice in the 0.5 m<sup>2</sup> half plot closest to the boat at 8 sites; and in late July through August we sampled 105 vegetation plots in seven sites, along with counting density of wild rice again. We chose to sample twice this year to find out if there were early-blooming plant species that would be missed when sampling in late July and August. Only a few early species were found and many species were still too immature for identification in June. Based on our finding, we determined that early season vegetation sampling is not needed in 2019. Following recommendations of research partners, the summary categories were refined to reflect the density and presence of wild rice, so now we have five categories for plot summaries: two with wild rice present, and three lacking wild rice (Table 1.6). We split the

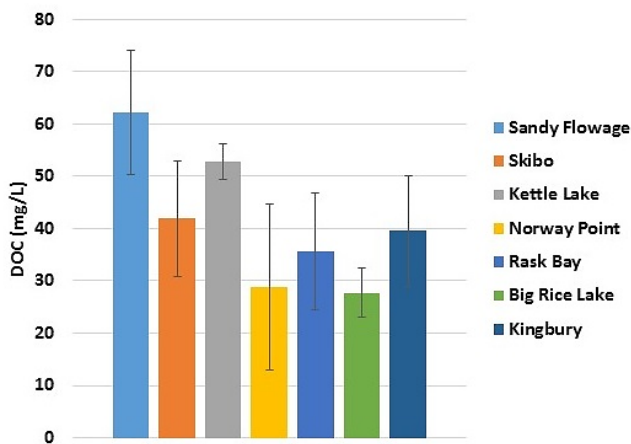
two with rice present based on density of wild rice, so plots with at least 30 stems per half square meter are called dense self-sustaining wild rice, and plots with less than 30 stems per half square meter are called sparse wild rice (some of these may be self-sustaining). For the plots at restoration sites lacking any wild rice this year, we split these into three categories based on dominant plants: cattails dominant, pickerel weed dominant, and water lily or water celery or algae dominant. Unknown or uncertain plants were collected and pressed in the summer, and we're still working on keying a few more plants. Plant data will be entered and summarized in early 2019.

**Table 1.5** Vegetation plots sampled during June-September, 2018

Sample Sites:	SAMPLE AREA TYPES					
	total vegetation plots sampled	dense self-sustaining wild rice, at least 30 stems/0.5m <sup>2</sup>	sparse wild rice, < 30 stems/0.5 m <sup>2</sup>	no rice, cattails dominant	no rice, pickerel weed dominant	no rice, water lily/water celery/or algae dominant
Big Rice Lake	36		24		12	
Kingsbury Bay (SLRE)	36			12		24
Rask Bay (SLRE)	37		18	12		7
Grassy Point (SLRE)	9			6		3
Sandy Flowage	24	14	10			
Norway Point	26		26			
Skibo	28	10	18			
Kettle Lake	12	6		6		
<b>Totals</b>	<b>208</b>	<b>30</b>	<b>96</b>	<b>36</b>	<b>12</b>	<b>34</b>

Concurrently, wild rice and other coexisting or competing plants and their associated water and sediment samples were collected from the sites. Over 600 environmental samples (plant, sediment, surface water) were collected and processed using aforementioned procedures for chemical and microbiological analyses. Physicochemical parameters (e.g. water temperature, pH, dissolved oxygen, conductivity) were measured in the field. Generally, physiochemical properties of the water samples are comparable with those in 2017: circumneutral pH (6.6-8.1), low conductivity (60~500  $\mu$ S/cm) and moderate dissolved oxygen concentration (3-6 mg/L).

Chemical and nutrient analyses for porewater and surface water samples collected in 2018 are in progress: major cations/anions, nutrients (Total P, Ortho-P, Total N, NH<sub>4</sub>-N, and NO<sub>2</sub>/NO<sub>3</sub>-N), and dissolved organic carbon (DOC). As a snapshot, Figure 1.6 shows DOC concentration in surface water collected in 2018. The concentration ranges from 5-80 mg/L which depends on sampling sites. DOC concentration also appears to be greater in self-sustaining wild rice sites (Kettle Lake, Skibo, and Sand River Flowage), and the sites with the lowest DOC concentrations (Norway Point, Big Rice Lake) have

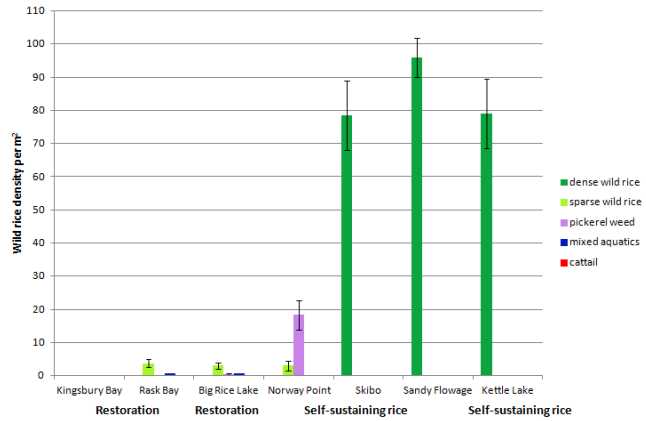


**Figure 1.6** Dissolved organic carbon (DOC) concentration in pore water collected in 2018

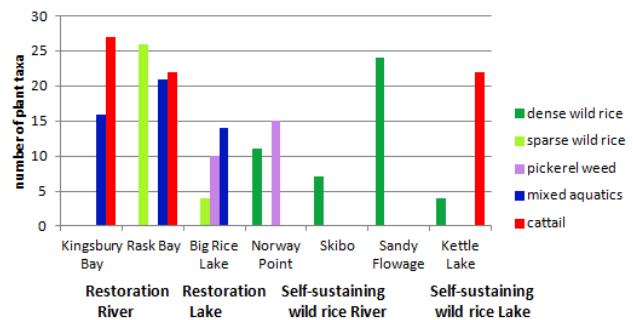
the highest cover of competing pickerel weed. These preliminary observations warrant further statistical analysis. All analyses will be completed along with statistical analysis in early 2019.

**Activity 1 Status as of July 1, 2019:** July 18, 2019

We completed vegetative composition analysis and water quality and nutrient analyses (biophysical data) of the samples collected during the summer in 2018. In total, over 200 plots (.5 square meter) were analyzed for wild rice density and vegetation composition. Figure 1.7 shows mean wild rice densities at seven sites in mid-June to early July floating-leaf stage. We define “dense rice” as average density over 30 stalks per square meter, and “sparse rice” as average density with less than 30 stalks per square meter. Averages were calculated over 6 vegetation sample plots. In a few cases where rice was very patchy, we counted density in 6 extra plots (12 total). We conducted vegetation data collection when rice was in flower or forming seeds (late July to late August) in 2018 and wild rice density in in flower or forming seeds was comparable with that during early summer. As for vegetation composition, there were about 30 aquatic and emergent plant species present in the sampling plots. The self-sustaining wild rice stands had species present, the managed wild rice stands had from 5 to 9 species present (average 7 species), the pickerel weed stands had 3 to 7 species (average 5), and the cattail stands had 9 to 10 species (average 9.5). Generally, species diversity and richness vary by sites and habitat categories.

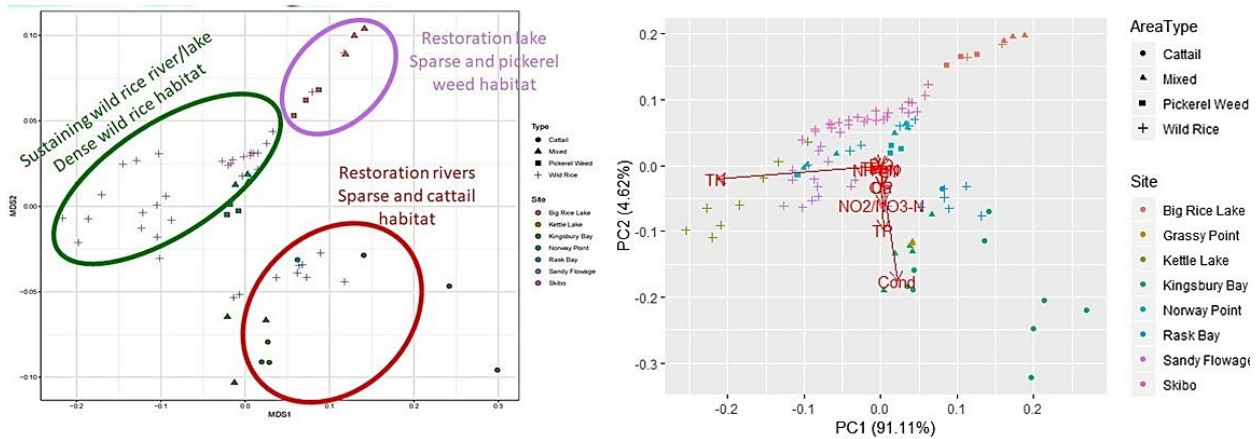


**Figure 1.7** Mean wild rice density in sampling sites early in the summer (mid-June to early July)



**Figure 1.8** Mean species richness at each sites in 2017-2018

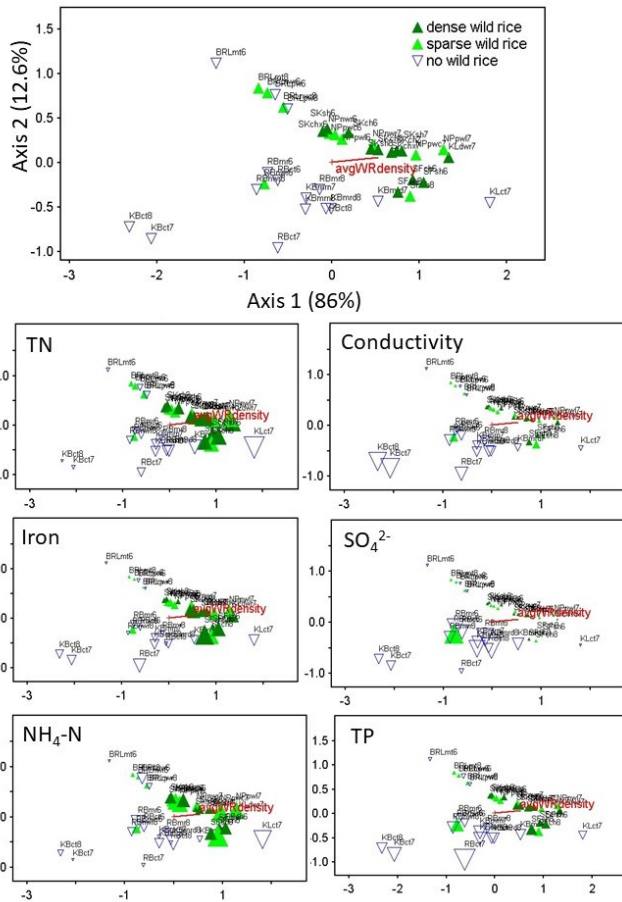
Physiochemical properties of surface water collected in 2018 are similar to those in 2017: circumneutral pH (6.6-8.1), low conductivity (40~600 mS/cm) and moderate dissolved oxygen concentration (4-6 mg/L). Major ions are Cl<sup>-</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup>, reflecting this region’s geological condition. Low levels of nutrients were observed: Low levels of nutrients: TN (400-1400 ppb), NH<sub>4</sub><sup>+</sup> (10-40 ppb), NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> (3-100 ppb), TP (8-80 ppb) and ortho-P (2-40 ppb). We conducted multivariate ordination analyses for all samples (~350 samples) collected so far to examine water quality characteristics associated with wild rice habitats. We observed that there is a habitat quality-biophysics concentration gradient. Biophysical composition (water quality parameters and nutrient levels) in sustaining wild rice habitat is dissimilar from restoration sites with pickerel weed or cattail. In particular, self-sustainingly wild rice habitats have higher total nitrogen and low conductivity (Figure 1.9). The use of compositional dissimilarity measures with multivariate analyses avoids problems that could arise if habitats in geographic proximity are assumed to have the same biotic potential.



**Figure 1.9** Nonmetric multidimensional scaling ordination and principal component analysis of biophysical data with wild rice density (Sample size: ~350)

**Activity 1 Status as of January 1, 2020:** January 10, 2020

As all vegetation and water chemistry analyses of 2017-2018 samples are completed, we performed statistical analysis to evaluate the relationships between water chemistry and vegetation habitats. Analyses relating sampling sites (independent variables; n=39), vegetation habitat (independent variables, n=3), water chemistry parameters (dependent variables; n=13; pH, conductivity, major ions (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Cl<sup>-</sup>, F<sup>-</sup>, total dissolve Fe, and SO<sub>4</sub><sup>2-</sup>), N species (total N, NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup>) and P species (TP and ortho-P) were conducted. Water chemistry characteristics are strongly correlated with wild rice density (Figure 1.9), indicating certain water chemistry requires or is conducive for wild rice population. The plots of individual water chemistry parameter in Figure 1.10 show the degree of correlation of each parameter with wild rice density. While total nitrogen (TN), iron,



**Figure 1.10** Nonmetric multidimensional scaling ordination of water chemistry (13 variables) in wild rice habitats (dense wild rice: > 30 stems/m<sup>2</sup>, sparse wild rice: 1-30 stems/m<sup>2</sup>, and no wild rice). Symbol size in individual water chemistry parameter indicates relative concentration level (The bigger symbol size has the greater concentration).

samples are nearly 90% complete. All data of three-year environmental samples will be compiled and statistical analysis will be performed.

**Activity 1 Status as of July 1, 2020:** August 10, 2020

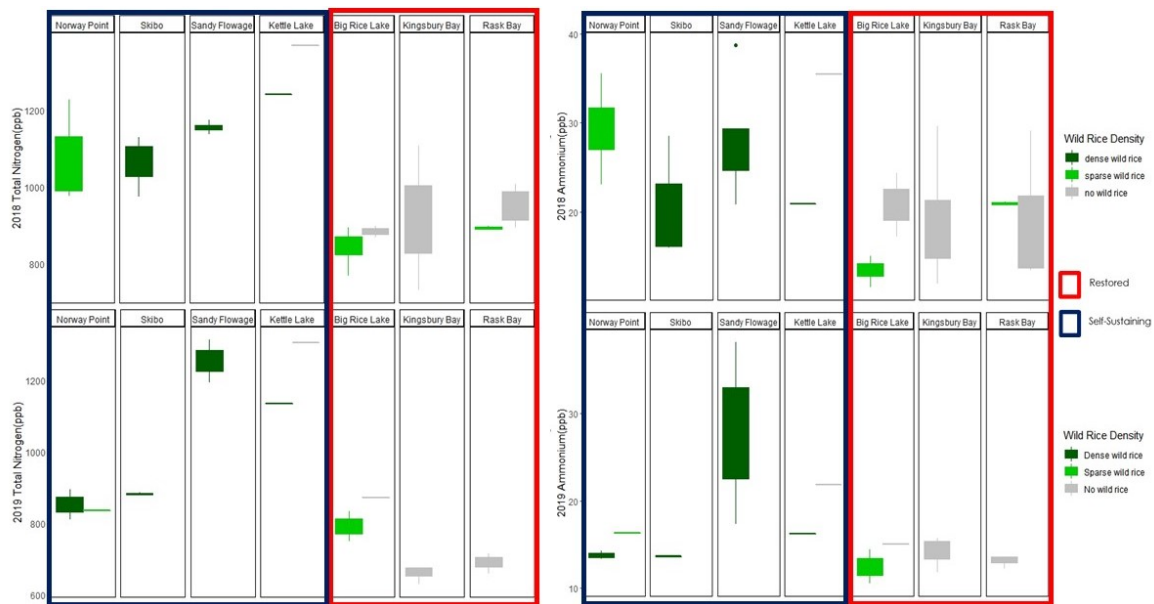
We completed field sampling campaigns and biophysical data analysis along with vegetation data in Activity 1. All data is compiled and statistically analyzed. Major findings include 1) Surface water chemistry strongly correlated with wild rice density than porewater when we evaluated 21 biophysical parameters. Particularly, TN and ammonia in surface water appear to be significant factors for wild rice presence and density while conductivity and TP were negatively associated with wild rice density. 2) TN and ammonia in surface water appear to be significant factors for wild rice presence and density. Despite geographical variation, we observed narrow concentration ranges of TN (800-1300 ppb) and ammonia (10-40 ppb) in dense wild rice sites over the

and ammonia (NH<sub>4</sub>-N) in surface water has strong or moderate positive correlation with wild rice density, conductivity, sulfate, and total phosphorus (TP) has strong negative correlation. Particularly, the level of TN has the strongest positive correlation (Kendall's tau=0.94; 0: no relationship and 1: perfect relationship). Major cations such as calcium and magnesium have similar relationship as conductivity. DO, pH, temperature, chloride, nitrate/nitrite have a weak or no correlation with wild rice.

The Year 3 field work was completed for both vegetation and environmental samples (water, sediment, and plants) this summer and fall. Based on the results of the previous years, the field sample collection was designed to 1) observe inter-annual variations through one sampling event for all study site so that we can compare comparing three-year data sets for both self-sustaining wetland and restoration sites and 2) to identify key environmental and microbial determinants for healthy wild rice populations through intensive life-stage sampling for self-sustaining wild rice beds (Skybo and Norway Point). Currently we are compiling all vegetation data including wild rice density and vegetation assembly in the study site. Generally, wild rice densities (stems/m<sup>2</sup>) in the study sites in 2019 were greater than those in the past two years. The water chemistry and nutrient analyses of surface and porewater



season (June-October) shown in Figure 1.11. 3) Big Rice Lake, which is a current wild rice restoration site by mechanical mowing for pickerel weed, has characteristic water chemistry such as low N and P nutrient level, conductivity and iron concentration in compared to those in other sites. We also observed wild rice density increased over the year of restoration activity. We plan to evaluate vegetation and biophysical data of Big Rice Lake over three years of restoration. These data sets have been used in Activity 2 for their correlation with microbial community structures.



**Figure 1.12** Ranges of total nitrogen and ammonium levels in all study sites (2018-2019) with wild rice density (dense wild rice: > 30 stems/m<sup>2</sup>, sparse wild rice: 1-30 stems/m<sup>2</sup>, and no wild rice).

**Activity 1 Status as of January 1, 2021:** February 23, 2021

The activity 1 was completed as we reported previously.

**Final Report Summary:** August 16, 2021

Water chemistry varied amongst all the sites, but commonalities were found between areas that support significant wild rice populations. We categorized the sites as dense, sparse, and no wild rice based the density of stems in square meter. Among 21 water chemistry parameters, total nitrogen has strong positive correlation with wild rice density, indicating total nitrogen is major influencing factor when compare to other nutrient. Surface water nutrients get transferred and absorbed into the plants and sediment by microbes. High nitrogen concentrations in the surface water can be transferred to the plant depending on how much is available and how much leaches out. The 2018 data provides evidence that total nitrogen is influencing wild rice wetlands particularly Sandy Flowage (SF), Kettle Lake (KL), Norway Point (NP), and Skibo (SK) where the density of wild rice was over 30 stems/m<sup>2</sup>. When compared to total nitrogen, ammonia correlates well at NP and SK but at BRL NH<sub>4</sub><sup>+</sup> is higher in areas where there is no wild rice. The findings show that the nitrogen cycle plays a critical role in the development of wild rice.

In contrast, conductivity, sulfate, chloride and total phosphorus were associated with sites with no wild rice. Presumably, this is related to contaminant input from runoffs. KB and RB had exceptionally high sulfate and conductivity measures and produced no wild rice at a most of the sampling locations. Not only does wild rice prefer areas with low conductivity and low sulfate, but pickerel weed appears to be present in similar conditions. This sheds light onto why specifically pickerelweed may be succeeding in areas of historically high wild rice density. Cattails and other salt tolerant macrophytes are able to thrive in areas (e.g. KB and RB) due to their tolerance to road salts and other human made pollutants.

#### **ACTIVITY 2: Identification of microbial communities and nutrient associated with wild rice**

**Description:** We will characterize total microbial (both fungal and bacterial) communities and the level of nutrients (both macro- and micronutrients) in three fractions: bulk soil, rhizosphere soil, and the rhizoplane fraction of each sample. Total microbial communities of subset samples will be determined using culture-independent methods (amplicon-based sequencing) and microscopic analyses (fluorescence microscopy and scanning electron microscopy). Genomic DNA of microbial cells from three fractions of bulk soil, rhizospheric soil, and rhizoplane of each sample and water samples will be extracted using the Powersoil™ DNA isolation kit and will be used for Next Generation, Illumina sequencing, of total microbial community populations. DNA sequence analysis of triplicate samples of each fraction will be done using two independent primer sets targeting archaeal, bacterial and fungal populations. For archaeal and bacterial community analysis, 16S rRNA will be amplified by polymerase chain reaction (PCR) using barcoded primers. The internal transcribed spacer region will be used for amplification of eukaryotes, broadly, with a focus on microbial eukaryotic lineages for fungi. The amplicons from individual samples will be pooled together, and the multiplexed amplicons will be paired-end sequenced on an Illumina/Solexa Sequencer at the University of Minnesota Genomics Center. We will also perform total metagenomic analyses on a few selected wild rice samples to obtain functional information about microbial genes present in samples. The DNA sequence data obtained in our studies will be sorted, trimmed, verified and aligned to a taxonomic database for assessment of phylogenetic diversity using the mothur and QIIME software programs. The taxonomic signature of microorganisms in each sample will be compared within and across samples at all time points and statistically analyzed. Comparisons of bacterial constituents of environmental samples, between sites and years, will be determined by examining the numbers and types of operational taxonomic units. Species diversity, and richness, evenness, and rarefaction analysis will also be determined. Furthermore, to determine the influence of microbial communities of water column on rhizosphere microbiome of wild rice and other coexisting plants, all water and bulk soil samples collected are treated as a source and compared against a sink community of rhizosphere and rhizoplane of plant root. The relative contribution will be determined using the SourceTracker.

In addition to metagenomic analyses, high resolution microscopies will be used to examine root anatomy, spatial distribution of microbes on root, and elemental partitioning such as iron plaques. Scanning electron microscopy (SEM) will be used for high resolution imaging and microanalytical platform of rhizosphere of wild rice. Initially, the root samples will be observed using cryo-SEM system after flash-freezing them using liquid nitrogen. Furthermore, the spatial distribution and relative abundance of the bacteria-root environment will be investigated using a combination of species specific fluorescent stains, non-specific fluorescent stains, and fluorescence in situ hybridization probes for differentiating the bacteria associated with the plant root from root itself. Taken together, this activity will contribute to the overall understanding of microbial biodiversity and ecology of wild rice and competing plants. The comparison of rhizosphere microbiome and nutrients between wild rice and competitive or invasive aquatic plants will identify the contribution of plant-microbe interactions to restoration success as well as improve management strategies accordingly.

**Summary Budget Information for Activity 2:****ENRTF Budget: \$ 203,265****Amount Spent: \$ 203,265****Balance: \$ 0**

<b>Outcome</b>	<b>Completion Date</b>
1. Sample processing and DNA extraction	December 2019
2. Identify rhizosphere microbial communities associated with emergent aquatic plants using DNA sequencing	May 2020
3. Determine the level of nutrients and genes relating microbial nutrient cycling	December 2019
4. Correlation analysis of microbial communities/nutrient with restoration success	March 2020

**Activity 2 Status as of January 1, 2018:** January 23, 2018

Each plant sample collected in Activity 1 was processed into bulk soil, rhizosphere soil, and the rhizoplane fraction to characterize total microbial (both fungal and bacterial) communities in the fractions. All fractional samples were stored at -20 °C and the extraction of their genomic DNA using the Powersoil™ DNA isolation kit has been performed for amplicon-based sequencing and quantitative PCR analysis. Surface water samples were also filtered through 0.22 µm, polyethane sulfonate filters to trap bacterial microorganisms. Once DNA extraction is completed, the samples will be submitted to UM Genomic center for sequencing analysis. Concurrently, microscopic examination of root anatomy, spatial distribution of microbes on root, and elemental partitioning such as iron plaques is currently undertaken using scanning electron microscopy.

**Activity 2 Status as of July 1, 2018:** July 25, 2018

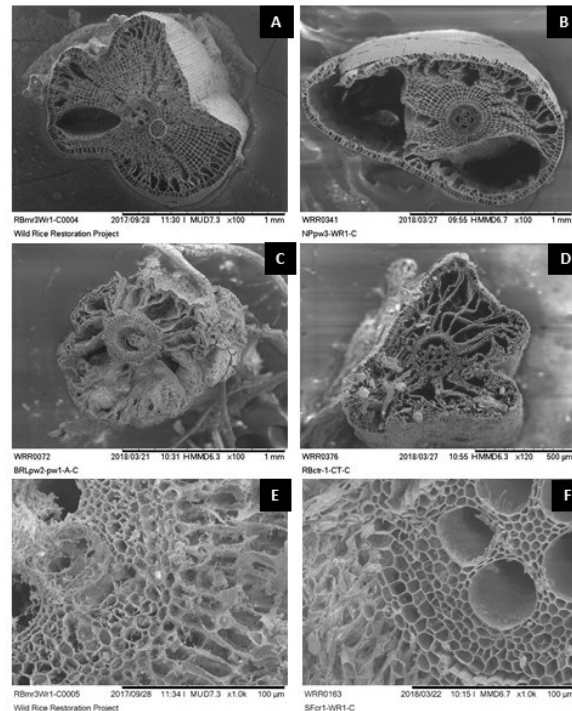
The genomic DNA (~200 samples) of water, sediment, rhizosphere, and rhizoplane collected in the first season were submitted to UM Genomic center for sequencing analysis (both bacterial and fungal communities). The sequence data is expected to be available for the analysis late August. We have been working on quantitative PCR analysis (qPCR) to examine population density of nutrient utilizing microbial community (nitrification, denitrification, and ammonification using the same genomic DNA and the analysis is in progress.

Microscopic examination of root anatomy and elemental partitioning such as iron plaques is performed. Fresh samples were aseptically sectioned by 0.25-1 cm for cryo-SEM. Some sectioned roots were preserved using 2.5 % glutaraldehyde for electron microscopy and 4% paraformaldehyde for fluorescence microscopy. Cleaned roots were used to examine root anatomy for each plant shown. Figure 2.1 shows SEM images of cross-sectioned wild rice roots and competing plants, pickerel weed and cattail. Wild rice roots examined from three sites (Big Rice Lake, Sandy Flowage, and Norway point) demonstrated similar anatomical characteristics in comparison to pickerel weed and cattail. If we describe from outer layer to inside, a band of sclerenchyma, hard woody cells (1–3 cells in thickness) was present adjacent to the hypodermis. Aerenchyma, a soft plant tissue containing air spaces were observed throughout the cortex of the majority of samples and the size and distribution of air spaces vary among wild rice roots collected from the same location. Four to five xylem tubules were observed in the vascular cylinder of all cross-sectioned samples. Inner structure, xylem to endodermis and aerechyma of wild rice are major distinctive features from those of pickerel weed and cattail. Concurrently, we

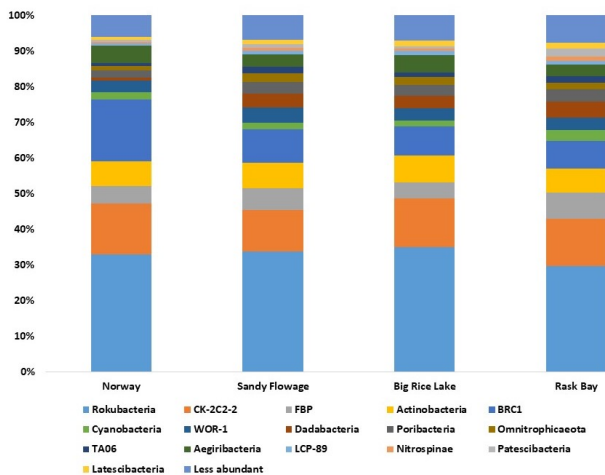
are working on fluorescence in situ hybridization method to examine the spatial distribution and relative abundance of bacteria and fungi on the roots.

**Activity 2 Status as of January 1, 2019:** January 3, 2019

DNA sequencing of the genomic DNA (~200 samples) of water, sediment, rhizosphere, and rhizoplane collected in the first season was completed for bacterial communities. Currently we are working on sequencing data analysis (bioinformatics). Initial analyses indicate bacterial communities in the sediment and rhizosphere samples were diverse for both abundance and evenness based on diversity indices such as Shannon index (6-8) and Simpson's index (near 1) of diversity. Overall, the bacterial community composition of wild rice rhizosphere was diversely comprised of the phyla of Rokubacteria, CK-2C2-2, FBP, BRC1, and Actinobacteria up to 80 % (Figure 2.2). Most of them except for the phylum Actinobacteria are candidate bacterial phyla whose



**Figure 2.1.** Scanning electron micrograph images of wild rice (A and B; low magnification and E and F; high magnification of aerechyma and cortex), pickerel weed (C) and invasive cattail (D).



**Figure 2.2.** Most abundant bacterial phyla (averaged) associated with wild rice rhizospheres (n=33) by the location.

members have not been cultivated and only discovered by genome sequencing. Many of these candidate phyla associated with wild rice rhizosphere are presumably involved in biogeochemical carbon, nitrogen, and sulfur cycling such as denitrification, nitrate reduction to ammonia, ammonia oxidation and sulfate reduction/oxidation, and interconnected with other microbial communities and plants for exchange of resources. The phylum Actinobacteria are commonly found as plant commensals. We will thoroughly examine the specific microbial communities associated wild rice and other coexisting plants along with water and sediments.

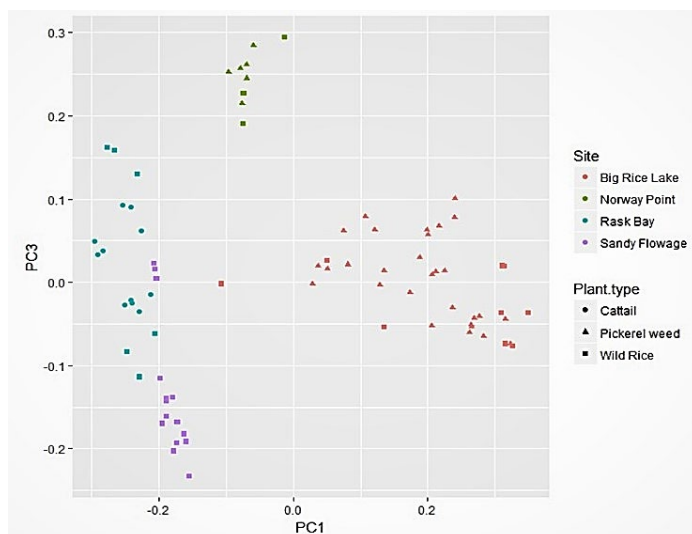
Even though overall relative composition of microbial communities associated with wild rice rhizosphere are similar, the structure and composition of bacterial communities appears to be dependent on the sites (Figure 2.2). The principal coordinate analysis also support the site specificity of rhizosphere bacterial communities as they are clustered by the study sites (Figure 2.3). Particularly, the samples collected from Big Rice Lake have distinctive rhizosphere bacterial communities from those in other sites. Potential correlation of the rhizosphere bacterial communities with physiochemical characteristics and vegetation composition will be analyzed. Additionally, we are extracting DNA from the samples collected in 2018 and the genomic samples will be submitted to the UMN genomic center in early 2019.

We have also worked on quantitative PCR analysis (qPCR) to examine population density of nutrient utilizing microbial community (nitrogen fixation, nitrification, denitrification, and ammonification using the same genomic DNA and the analysis is in progress.

In addition to their population density, we have developed a method to quantify nitrogen fixation (microbial conversion of atmospheric nitrogen gas to ammonia or nitrate) activities in rhizosphere sediment and plant roots. The method will be applied for samples which we will collect in 2019.

#### Activity 2 Status as of July 1, 2019:

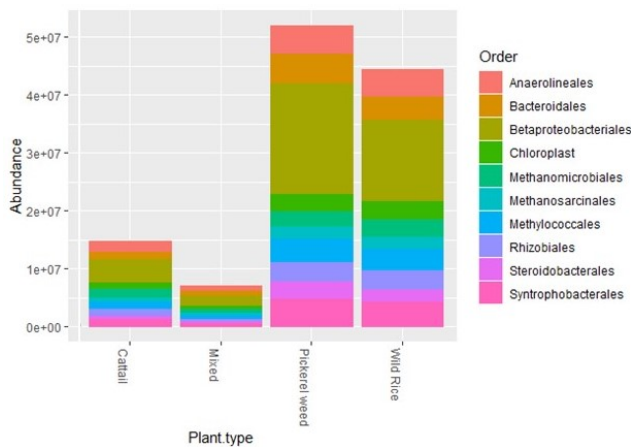
We continue to analyze microbial community compositions and structure in the samples collected in 2017. A mean of 2,500 operation taxonomic unit (OTUs) were identified among the samples. Expectedly, both species richness and evenness of microbial communities are great in all samples and across the sites as wetland samples based on diversity index (total observed taxa, Shannon and Simpson index).



**Figure 2.3** Principal coordinate analysis of bacterial communities associated with wild rice and coexisting plants in wild rice wetlands based Bray-Curtis distance. The samples were collected late summer in 2017.

Figure 2.4 shows most abundant 10 orders which appear in at least 75% of sediment and root samples by plant types. The bacterial community composition was diversely comprised of members of the orders, *Betaproteobacteriales*, *Steriodobacteriales*, and *Rhizobiales* who play roles in nitrogen cycling such as nitrogen fixation, ammonia oxidation and *Methanomicrobiales*, *Methanosarcinales* and *Methanosarcinales* who generate and oxidize methane. Generally, microorganisms responsible for biogeochemical (largely carbon and nitrogen) cycling and wetland functions are dominant. Microbial communities are significantly different by sites (geographic proximity) but wild rice habitats appear to associate with distinct bacterial communities. Wild rice and pickerel weed have greater similarity in microbial community assembly.

We completed genomic DNA isolation for the samples collected in 2018 and submitted ~ 800 samples of two self-sustaining wild rice sites, Skybo and Norway point and one restoration site, Big Rice Lake to DNA sequencing analysis. The selection of these sites is based on similarity of bacterial community between wild rice and pickerel weed and MNDNR's observation on displacement of wild rice by pickerel week in wild rice lakes and wetlands



**Figure 2.4** Most abundant 10 bacterial orders in sediment and root samples by plant types

across the state including Big Rice Lake. Relationships between biophysical data with microbial community composition (order level) will be examined. Currently we are collecting year 3 samples to examine inter-annual variations and to quantify nitrogen fixation (microbial conversion of atmospheric nitrogen gas to ammonia or nitrate) activities in rhizosphere sediment and plant roots for microbial community.

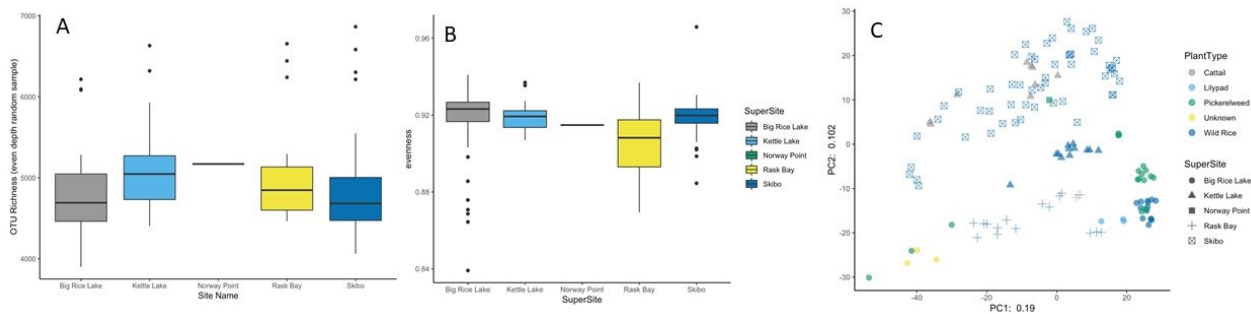
**Activity 2 Status as of January 1, 2020:**

January 16, 2020

We completed the bacterial community analysis of the samples collected in 2017 and 2018 using a next-generation sequencing

and have currently evaluated the relationship of bacterial assembly and structure with wild rice density and vegetation type. Generally, microbial community composition and structure differ by geographic proximity and/or vegetation habitat and while microbial richness and evenness are similar across the samples (Figure 2.5). When we compare microbial communities within the sampling site such as Kettle Lake, bacterial composition appears to be influenced by vegetation habitat (type) vice versa. We continue to determine the influence of microbial communities of water column on rhizosphere microbiome of wild rice and other coexisting plants using statistical analyses. Additionally, the analysis of functional genes responsible for nitrogen cycling is in progress as TN is one of key correlators/determinants for wild rice density.

As the samples collection was completed for the third (last) season, genomic DNA isolation from the samples is actively in progress. We plan to complete the isolation by this month (January) and submit them to the UM Genomic center for sequencing analysis. The sequence data is expected to be available for the analysis late March and the analysis will be complete at the end of the project along with quantitative PCR analysis (qPCR) to examine population density of nutrient utilizing microbial community (nitrification, denitrification, and ammonification using the same genomic DNA.



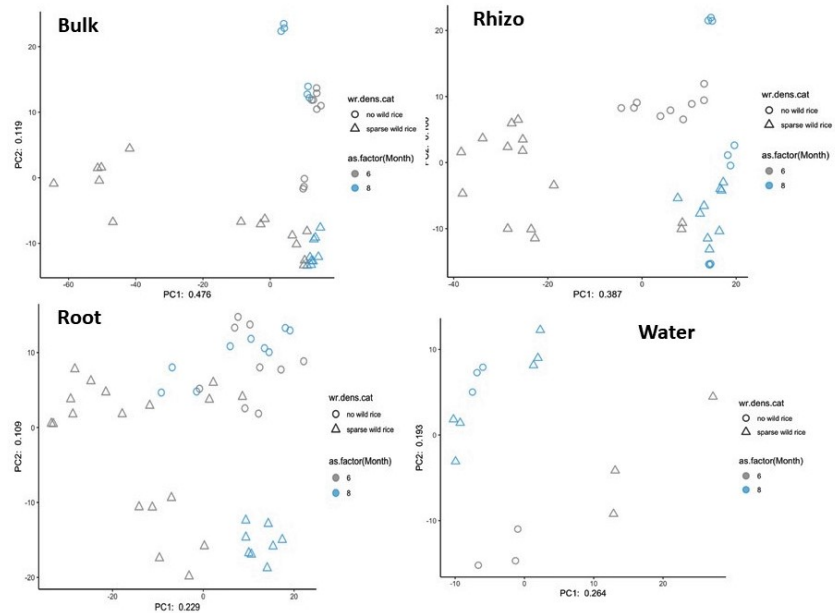
**Figure 2.5** Microbial community richness (A) and evenness (B) by study sites. Principal coordination analysis (C) to observe the extent of change in community composition or degree of community differentiation by geographic proximity and vegetation habitat in relation to a complex-gradient of wild rice wetlands in 2018.

**Activity 2 Status as of July 1, 2020:** August 10, 2020

All DNA extraction and raw sequencing data collection for 3-year study were completed and bioinformatics and statistical analysis have been conducted. As our data sets are large (over 800 samples) and have very diverse bacterial community (Shannon's diversity index: 0.7-0.9), we have analyzed data sets in sub-categories based on initial analysis: 1) microbial community in macro-environment (surface water and bulk sediment) of all study sites with water chemistry and vegetation data set, 2) microbial community in micro-environment (rhizosphere and root) as a function of wild rice life stage in self-sustaining wild rice sites (Skibo and Noway Point), 3) fungal communities associated with

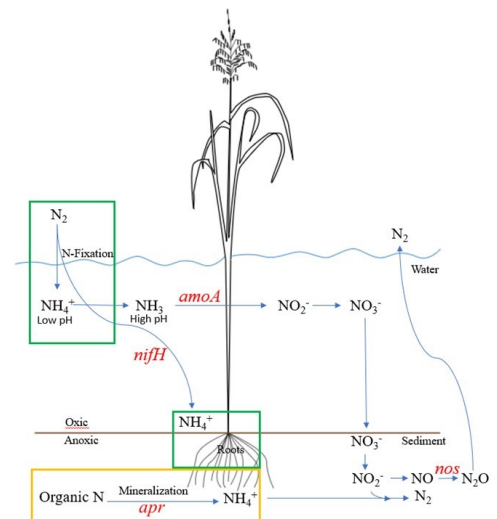
wild rice in self-sustaining wild rice sites (Skibo and Noway Point), 4) the effect of restoration activities on microbial community and water chemistry in Big Rice Lake, and 5) biogeochemical cycles (N, S, Fe, and C) for wild rice in comparison to pickerel weed, a native plant displaced wild rice. The analysis 1) and 2) are approximately 80% completed. Generally, macro-environment samples and water samples (water and bulk sediment) appears to be geographically dependent and associated with water chemistry, particularly TN and sulfate level. In contrast, microbial communities in micro-

environmental samples such rhizosphere and root samples have closely associated with sampling area and vegetation types. We also observed temporal differences (June vs August) in microbial community structures within the sample types for all sample types. For example, Figure 2.6 shows microbial community structure in Big Rice Lake by sample types and sampling month. Generally, samples are separate by sampling month for all sampling types, indicating dissimilarity in samples collected in between June and August. The analysis 3), 4) and 5) are currently in progress.

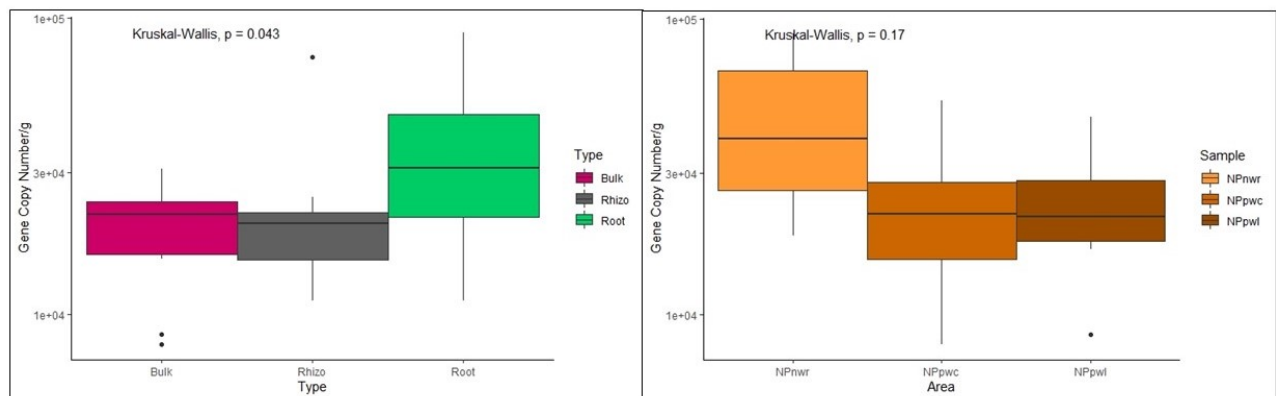


**Figure 2.6** Principle component analysis of microbial community structure in Big Rice Lake as a function of wild rice density and sampling month (June vs August)

In addition to DNA sequencing analysis, we made progress on qPCR analysis to examine population density of nutrient utilizing microbial community (e.g. nitrogen fixation, nitrification, denitrification, and mineralization) using the same genomic DNA. Figure 2.7 show key nitrogen cycling process associated with wild rice bed and N functional genes (red) chosen to understand what N-transforming processes are conducive to nitrogen available and the conditions favorable to wild rice fitness. We anticipate that saprophytic microbes in bulk sediment will be major drivers on nitrogen balance for wild rice early in the life stages, while nitrogen fixing in the rhizosphere and root will be drivers throughout the season. Currently we completed the analysis of nitrogen fixation (*nifH* gene) and other gene analysis is in progress. Figure 2.8 shows the range of abundance of nitrogen fixation function gene in different sample type and area with different plants in Skibo and Norway Point. Root samples shows statistically greater abundance of *nifH* gene in comparison to bulk and rhizosphere sediment, indicating both wild rice and pickerel weed host nitrogen fixing microbes in the root. Additionally, sampling areas where wild rice is dominant plant species have the greater *nifH* gene than those with pickerel weed. Given the nitrogen fixation that allows for the assimilation of nitrogen into plants for biosynthesis, the association of nitrogen fixing bacteria with wild rice may have implications to wild rice fitness and the development of wild rice restoration methods.



**Figure 2.7** Key nitrogen cycling associated with wild rice bed and N-utilizing function genes analyzed in this study.



**Figure 2.8.** Box plots of abundance of nitrogen fixing (*nifH*) functional gene in different types of nitrogen pool in Skibo and Norway Point (left) and in sampling areas with different plant types in Norway Point (right). NPnwr is the sampling area with dense wild rice and NPpwc and NPpwl are the sampling areas dominated with pickerel weed.

**Activity 2 Status as of January 1, 2021:** February 23, 2021

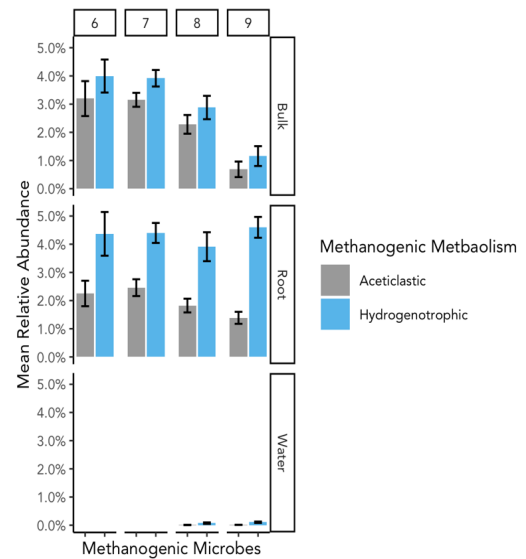
We made progress in identifying microbes involved in the biogeochemical cycling of N, S, Fe and CH<sub>4</sub> by analyzing a subset of wild rice samples taken from Skibo in greater detail. Respiratory and fermentative metabolisms based on elements other than oxygen are unevenly distributed between wild rice roots, the



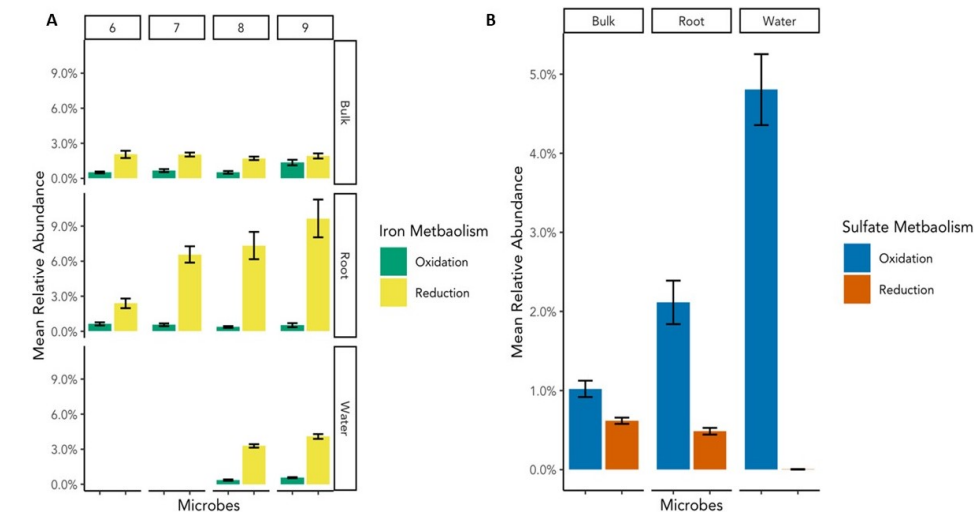
sediment, and the surrounding water. We hypothesized that changes in the rate of radial oxygen loss from the roots of wild rice over the season would effect changes in the dominant microbial metabolism within each cycle.

The roots of wild rice plants consistently host a larger proportion of hydrogenotrophic methanogens when compared to both sediments and water samples from each site. Root samples were composed of a mean 5% methanogenic species at the peak of their abundance in September (Figure 2.9). Unlike acetoclastic methanogens which are vulnerable to competition from sulfate, nitrate, and iron metabolizing bacteria, hydrogenotrophic methanogens rely only on the presence of hydrogen and carbon dioxide gas in the environment to produce methane.

Microbes with iron reduction pathways were most abundant in root samples when compared to bulk sediment and water. The relative abundance of microbes associated with iron oxidation and reduction reach near equal proportions in late summer in the sediment, while in both water and root samples, iron reducing microbes were more than 3 times as common than iron oxidizing microbes (Figure 2.10A). The total relative abundance within samples reached a mean of 9% in root samples by September. The most abundant iron reducing bacteria were identified as *Rhodofera*, *Geobacter*, *Geothrix*, and *Anaeromyxobacter*. These results suggest that iron reducing microbes play a role in forming the protective iron oxide plaque on the roots of wetland plants and may have additional beneficial roles



**Figure 2.9.** Relative abundance of bacteria and archaea capable of producing methane through either the metabolism of Acetate or of CO<sub>2</sub> and H<sub>2</sub>. Bars represent 95% confidence intervals.



**Figure 2.10.** Mean relative abundance of prokaryotes capable of reducing or oxidizing iron (A) and sulfur (B). Bars represent 95% confidence intervals.

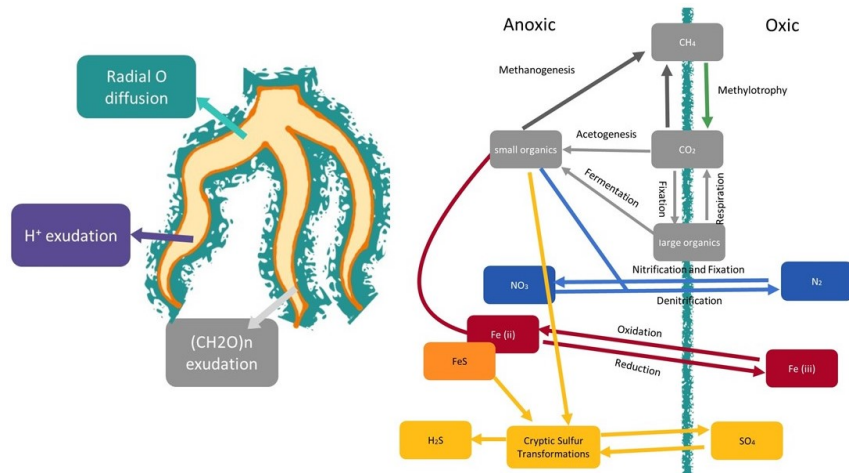
through interactions with other bacteria and archaea. Bacteria capable of sulfate oxidation were more common than those capable of sulfate reduction in all sample types (Figure 2.10B). Sulfate oxidizing species were most common in water samples (mean 5% relative abundance) and accounted for a relative abundance of 1-2.5% per samples in root and sediment samples. Sulfate oxidizing species were often photobacteria and

had stable proportions within samples over the season. The most common OTUs were identified as *Rhodofera*, *Aquabacterium*, *Sulfuritalea*, *Rhodobacter*, and *Chlorobium*.

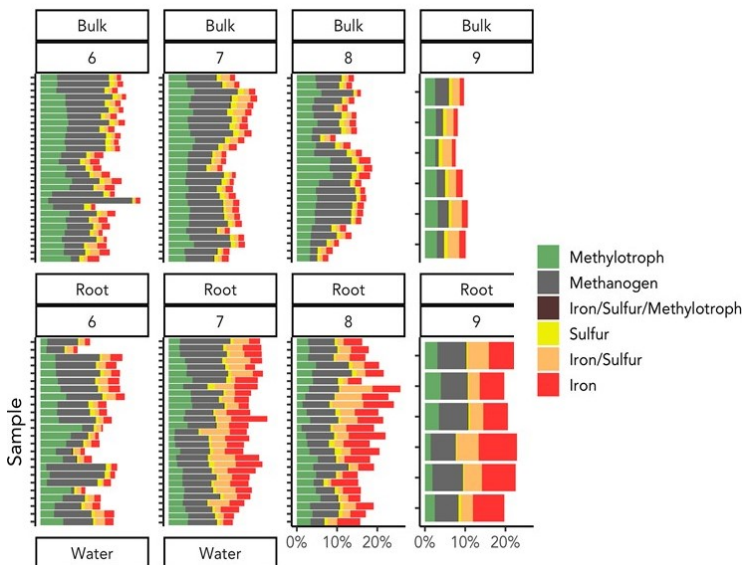
Additionally, we have made progress on fungal community analysis. The fungal communities in water, sediment, and plant root have been analyzed with seasonality, biogeochemical data, and plant types in Big Rice Lake and Norway Point. We also analyzed genomic evidence on the presence of potential fungal pathogens in wild rice wetland. This will be the last activity for this project.

**Final Report Summary:** August 15, 2021

We examined microbial communities in sediment and dynamic interactions between sediment and root zone. Plant roots extend the oxic/anoxic interface deeper into the wetland sediment and there are more microbial roles due to redox gradient (Figure 2.11). Particularly, we studied how microbes involve in and change elemental cycling in the root in comparison to those in sediment. Due to microbial interaction with plant roots and the creation of microniches, many microbes participate in



**Figure 2.11** Dynamic biogeochemical processes in the oxic/anoxic root zone of wild rice



**Figure 2.11** Relative abundance of microbes associated for iron, sulfur, and methane/organic cycles in the self-sustaining wild rice wetland (Skibo site)

more than one cycle and competition for small organic compounds creates dynamic connections among biogeochemical cycles, amplified by mutualistic relationships. Thus, we created a taxonomy library of microbes associated for iron, sulfur, and methane/organics cycles (Figure 2.12). We compared relative abundance of microbes associated with those cycles in sediment and root as a function of month. The number in each plot represent the month samples collected. First, we observed seasonal variation on microbial communities in sediment and root. For example, methanogens which is shown in gray decrease with time in sediment. We also found that the wild rice root hosts hydrogen-utilizing methanogens, iron-reducers and sulfur oxidizers selectively. The abundance

of iron reducers in root generally increases with time which are likely related to life stage of the plant.

As total nitrogen has strong correlation with wild rice density, we examined what nitrogen-transforming processes are conducive to nitrogen availability and the condition favorable to wild rice fitness. As ammonia is the preferable nitrogen source for wild rice, we examined nitrogen processes which are sources or sinks for ammonia. Nitrogen fixation and mineralization are sources while ammonia oxidation and denitrification are sinks. Through DNA sequencing and qPCR, we found microbes with nitrogen fixing capability were abundant in sediment and root over denitrification species. This trend was consistent throughout season or life stage of wild rice: floating leaf to seed production. We are looking into a way to quantify saprophytic microbes who does mineralization. Overall, nitrogen fixing bacteria in the rhizosphere and root appear to be one of drivers on the nitrogen balance for wild rice throughout the season.

**ACTIVITY 3: Project data dissemination and public outreach**

**Description:** Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. A fact sheet that summarizes our findings will also be distributed to wild rice restoration managers at state, tribal, and federal agencies. Several manuscripts will be written and submitted for publication in peer-reviewed journals. Results will be presented at state and national wildlife and ecology conferences. All publications resulting from this project will be made available through Open Access journal websites.

In addition, we will develop educational materials and opportunities for community discussion about wild rice restoration efforts in collaboration with wild rice managers and research partners at Fond du Lac Natural Resources, 1854 Treaty Authority, St. Louis River Alliance, and the Great Lakes Indian Fish and Wildlife Commission. These activities will provide opportunities to engage school and community groups in small-scale projects, build community support for wild rice restoration efforts. Moreover, these partner and researchers will take the results of our study into consideration as they make management decisions, and will work with us to ensure that our data products and research papers reach a broad audience within their agencies.

**Summary Budget Information for Activity 3:**

**ENRTF Budget: \$ 15,101**  
**Amount Spent: \$ 14,323**  
**Balance: \$ 778**

Outcome	Completion Date
1. Development of educational materials and community discussions	April 2020
2. Dissemination of project data and results via seminars and workshops	June 2020

**Activity 3 Status as of January 1, 2018:** January 23, 2018

A project planning meeting has been conducted with wild rice managers and the project partners. We have working on a pamphlet describing the overview of the project for public and wild rice managers.

**Activity 3 Status as of July 1, 2018:** July 25, 2018

We held a meeting with wild rice managers and the project partners on May 23 to discuss water quality and vegetative data collected in 2017, the information on the current wild rice restoration efforts, and feedback on 2018 sampling plans. We are developing an educational and outreach plan regarding water quality and wild rice restoration efforts in 2018 Lake Superior Youth Symposium on May 16-19, 2018.

**Activity 3 Status as of January 1, 2019:** January 3, 2019

The results of water quality and vegetation data collected in 2017 was presented at Minnesota Water Resources Conference held in Saint Paul, MN on 10/16-10/17, 2018 (Title: Sediment Nutrients Associated with Wild Rice and Competing Plants in Minnesota). Our research team attended Manoomin Collaboration Conference to discuss current research activities and findings on wild rice wetlands and share our research with researchers, students, and tribal partners in Minnesota and Wisconsin. The conference was organized by research team at the University of Minnesota –Twin Cities (Grand Challenges) on Dec 19, 2018. We continue to work with our research partners and wild rice managers for project progress, consultation and field sampling support. One of our original project partners, John Lindgren, currently has a different work assignment and is not currently working on estuary restoration projects, so Melissa Sjolund from MN DNR has joined our project team.

**Activity 3 Status as of July 1, 2019:** July 18, 2019

We disseminated our project findings on water quality, vegetation data, and microbiome in wild rice wetlands through presentations in regional and national conference, project meeting with research partners and wild rice managers and outreach activities. We shared our findings through an oral and two poster presentations at St. Louis River Estuary Summit (March 15-16, 2019) which brings together regional stakeholders including the community, students, scientists, natural resource managers, and educators in Minnesota. It was also presented at 2019 Meeting of Association of Environmental Engineering and Science Professionals (May 14-16, 2019). We continue to work with our research partners and wild rice managers for project progress, consultation and field sampling support. Additionally, we conducted a hand-on activities for middle and high school students during Lake Superior Youth Symposium (an international biannual program in the Great Lakes region) held at the University of Minnesota Duluth. The workshop was “Extraction Escapade: how studying DNA and environmental forensics are important in regards to water resources” where we discuss motivation and results of this project and extracted DNA from sediment in wild rice wetlands and aquatic plant roots.



**Figure 3.1** Photos of outreach activities during “Extraction Escapade” workshop in Lake Superior Youth Symposium

**Activity 3 Status as of January 1, 2020:** January 20, 2019

During this period, we have not had specific activities to disseminate our project findings but focused on the next-step research direction based on our findings. For example, we submitted a proposal regarding sediment chemistry and microbiome transplant for wild rice restoration to Minnesota Sea Grant and the proposal was awarded. We also plan to present our results at St. Louis River Estuary Summit (March 3-4, 2020) which brings together regional stakeholders including the community, students, scientists, natural resource managers, and educators in Minnesota and Society of Freshwater Science Conference (June 7-12, 2020). Additionally, we plan to have project wrap-up meetings with our research partners and wild rice managers.

**Activity 3 Status as of July 1, 2020:** August 10, 2020

We presented our results at St. Louis River Estuary Summit (March 3-4, 2020) which brings together regional stakeholders including the community, students, scientists, natural resource managers, and educators in Minnesota. In the meeting, we also provided our research update to our project partners and wild rice managers. We planned to present our research findings via one oral and two poster presentations in Society of Freshwater Science Conference (June 7-12, 2020) but the meeting were cancelled due to COVID-19 pandemic. We are also preparing several scientific manuscripts for peer-reviewed journals.

**Activity 3 Status as of January 1, 2021:** February 22, 2021

We have been working on scientific manuscripts to disseminate water chemistry and microbial ecology of wild rice wetlands as project outcomes. In addition, we submitted abstracts to the Annual Meeting of Society of Wetland Scientists for oral and poster presentations in June.

**Final Report Summary:** August 15, 2021

We presented final research presentation in the session “Microbes: Small players with big impacts on invasive species management and wetland restoration” at the Annual Meeting of Society of Wetland Scientists (June 1-10, 2021). The presentation included not only scientific findings of the project but also our collaborative effort on integration of the traditional ecological knowledge into the project.

**V. DISSEMINATION:**

**Description:** Findings will be disseminated and archived via reports to LCCMR, peer-reviewed publications, and presentations at conferences. A fact sheet that summarizes our findings will also be distributed to wild rice restoration managers at state, tribal, and federal agencies. Results will be presented at state and national wildlife and ecology conferences. Our activity 3 also include public outreach plans.

**Dissemination Status as of January 1, 2018:** January 20, 2018

**Dissemination Status as of July 1, 2018:** July 25, 2018

We submitted our abstract on findings of the first season’s study to Minnesota Water Resources Conference (Oct 16-17, 2018) for the poster presentation.

**Dissemination Status as of January 1, 2019:** January 3, 2019

The results of water quality and vegetation data collected in 2017 was presented at Minnesota Water Resources Conference held in Saint Paul, MN on 10/16-10/17, 2018 (Title: Sediment Nutrients Associated with Wild Rice and Competing Plants in Minnesota).

**Dissemination Status as of July 1, 2019:** July 18, 2019

We disseminated our project findings on water quality, vegetation data, and microbiome in wild rice wetlands through presentations in regional and national conference, project meeting with research partners and wild rice managers and outreach activities. See the details in activity 3.

**Dissemination Status as of January 1, 2020:** January 16, 2020

We submitted our abstract on findings to St. Louis River Estuary Summit (March 3-4, 2020) and Society of Freshwater Science Conference (June 7-12, 2020) for the poster and oral presentations. See the details in activity 3.

**Status as of July 1, 2020:** August 10, 2020

We disseminated our project findings through presentations in regional conference, project meeting with research partners and wild rice managers. Currently we are preparing 3-4 manuscripts. See the details in activity 3.

**Status as of January 1, 2021:** February 22, 2020

We have finished a manuscript which is ready to submit to Science of the Total Environment and preparing for two additional manuscripts. We plan to present our project finding in the Annual Meeting of Society of Wetland Scientists.

**Final Report Summary:** August 15, 2015

The project findings have been disseminated via reports to LCCMR, publications, and regional and national presentations at conferences. We held four meetings with wild rice managers and the project partners for field sampling plan, project progress, consultation and outcomes. The project findings were shared with the public through the [university's news article](#), public outreach activities (e.g. Lake Superior Youth Symposium), and [student stories](#). Moreover, microbial DNA sequences of environmental samples collected from wild rice wetlands were archived at National Center for Biotechnology Information.

**VI. PROJECT BUDGET SUMMARY:**

**A. Preliminary ENRTF Budget Overview:**

**\*This section represents an overview of the preliminary budget at the start of the project. It will be reconciled with actual expenditures at the time of the final report.**

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 238,844	Chan Lan Chun, Principal Investigator: \$36,043 (66.3% salary, 33.7% benefits); 7.4% FTE each year for 3 years; Randall E. Hicks, co-PI: \$23,476 Co-Investigator (66.3% salary, 33.7% benefits); 3.7% FTE each year for 3 years; George Host, co-PI: \$18,743 (66.3% salary, 33.7% benefits); 4% FTE each year for 3 years; Carol Reschke, co-PI: \$45,669 (72.6% salary, 27.4% benefits); 20% FTE each year for 3 years; Adelle Schumann, Research Technician: \$23,185(72.6% salary, 27.4% benefits); 15% FTE each year for 3 years; Graduate Research Assistant \$91,728 (82.4% salary, 17.6% benefits); 50% FTE each year for 3 years.

Professional/Technical/Service Contracts:	\$ 24,796	<p>\$24,796 is budgeted to use airboat service for wild rice, invasive plants, water and sediment sampling from Natural Resources Department of the Fond du Lac Band of Ojibwe. Airboat is essential for access to wild rice stands without damaging wild rice plants, as well as the most efficient access into cattail stands for sampling invasive emergents. \$40/hour including fuel × 200 hours boating time = \$8,000 for use of airboat; \$30/hr x 2 boat operators x 200 hrs boating time=\$12,000 for boat operators' salary; 10 samplings per yr x 150 mi per sampling 3 yrs x \$0.54 per mi =\$2,430 for FdL truck &amp; airboat trailer mileage to sample sites; \$1,602 field survey lodging (2 rooms at \$89/night x 9 nights) and \$764 per diem for two boat operators (\$51 per day x 2 people x 7.5 days) for overnight travel.</p>
Equipment/Tools/Supplies:	\$ 28,580	<p>\$4,600 is budgeted for plant and sediment sampling supplies for activity 1: Chest waders/boots \$360; field guide to aquatic plants \$100; soil knife \$60; plant press supplies \$110; plastic collecting bags \$100; gaps batteries \$50, sediment core samplers \$250, water collection bottles \$500, and porewater samplers \$ 350, and 2 YSI field water quality probes \$1410x2</p> <p>\$12,000 is budgeted for DNA/RNA extraction kit and molecular biological agents for activity 2: Nucleic acid extraction \$600/kit*12kits for ~1000 samples= \$7,200; PCR reagents \$2/reaction*2000 samples=\$4000; and various molecular markers \$800</p> <p>\$5,700 for activity 1 and \$5,780 for activity 2 are budgeted for chemical and expendable lab supplies: Disposable plasticware and lab supplies (e.g., Petri dishes, etc.) and miscellaneous chemicals (e.g., stains, solvents, antibiotics)</p> <p>Brochure and factsheet production for public dissemination of technical research results for activity 3: \$500</p>

Travel Expenses in MN:	\$ 6,770	In-state sampling: \$4,482: 10 samplings/yr x 150mi/sampling x 3yrs x\$0.54/mi =\$2430 + vehicle rental use \$10/day x 45days=\$450 + field survey lodging \$89/night x 3nights x 2rooms x 3 summers=\$1,602 for activity 1  In-state conference attendance: \$2,288: Registration 2 people: \$750; lodging \$89/night x 3nights x 2rooms=\$1,284; per diem/meals for 3 days \$38.25+\$51+\$38.25=\$127.50 x 2people = \$255) for activity 3
Other: DNA sequencing and chemical analyses	\$ 35,010	\$22,010 is budgeted for Illumina sequencing and supercomputer usage fee: UMN Genomic Center (UMGC): Illumina Sequencing and library preparation ~ \$4670/lane + \$10 library prep/sample: ~800 samples per project = \$8,000 (sample prep) + 3 lanes (\$14,010)  \$13,000 is budgeted for chemical and nutrient analyses: UMD NRRRI Central Analytical Lab: \$65/sample x 200 samples
<b>TOTAL ENRTF BUDGET: \$334,000</b>		

**Explanation of Use of Classified Staff:** not applicable

**Explanation of Capital Expenditures Greater Than \$5,000:** not applicable

**Total Number of Full-time Equivalent (FTE) Directly Funded with this ENRTF Appropriation:** 3.0 FTEs

**Total Number of Full-time Equivalent (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:** not applicable

**B. Other Funds:**

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
State	\$159,766	\$	Indirect fee (53% UMN Modified Total Direct Costs 7/1/17-6/30/18; 54% UMN Modified Total Direct Costs 7/1/18-6/30/20) are provided in-kind.
<b>TOTAL OTHER FUNDS:</b>	<b>\$159,766</b>	<b>\$</b>	

**VII. PROJECT STRATEGY:**

**A. Project Partners:**

Partners receiving ENRTF funding



- Chan Lan Chun, Assistant Professor, UMD, \$36,044: Oversee the project and take lead on plant-microbe interaction and metagenomic analysis
- Randall Hicks, Professor, UMD, \$23,476: Provide expertise on microbial ecology in St. Louis River Estuary (SLRE) wetland
- George Host, Initiative Director - Forest and Land, UMD, \$18,743 and Carol Reschke, Senior Scientist, UMD, \$45,669: provide expertise on distribution of wild rice and other emergent plants in SLRE and inland lakes and streams, plant community sampling and analysis.
- Adelle Schumann, Research Scientist, UMD, \$23,185: Conduct microscopic study of plant-microbe interactions using SEM and fluorescence microscopy
- Graduate student, TBD, \$91,728: Collect plants and sediment and take lead on the analysis of nutrient and metagenomic analyses.

**Partners NOT receiving ENRTF funding**

John Lindgren (MNDNR), Darren Vogt (1854 Treaty Authority), and Tom Howes (Fond du Lac Natural Resources), St. Louis River Alliance who are involved in restoration of wild rice will provide consultation and assistance for site selection, sampling and outreach program.

**B. Project Impact and Long-term Strategy:**

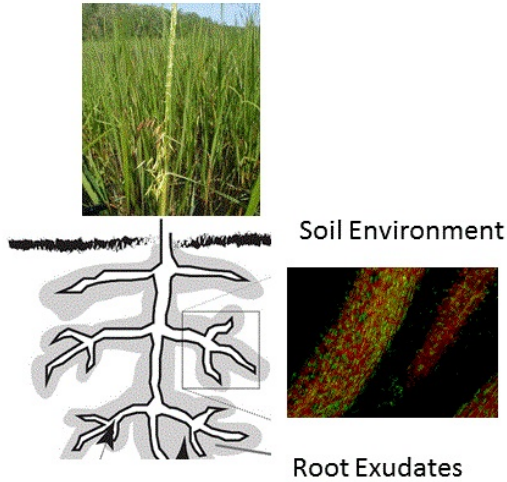
This project will provide key information on the microbial communities and sediment nutrients associated with wild rice and competing vegetation, with the goal of promoting restoration success to increase the abundance of self-sustaining wild rice in the SLRE and inland wild rice habitats in Minnesota. Restoration of wild rice wetlands will improve long-term protection of native species and aquatic biodiversity, and support management of this culturally and ecologically important natural resource in Minnesota.

**VIII. REPORTING REQUIREMENTS:**

- **The project is for 4 years, will begin on July 1, 2017, and end on June 30, 2021.**
- **Periodic project status update reports will be submitted January 1 and July 1 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021.**

**IX. VISUAL COMPONENT or MAP(S):**

**Soil nutrients and microbial communities will be key determinants in the re-establishment of self-sustaining wild rice populations.**



- Wild rice growth, adaptation and competitive success
- Above-ground plant community composition and ecology



**Microbial** association with roots in soil environment

- Nutrient availability and cycling
- Enhanced mutualist (novel symbiosis)
- Disease defense

**Environment and Natural Resources Trust Fund  
M.L. 2017 Final Project Budget**

**Project Title:** Assessment of Microbes for Improving Wild Rice Restoration

**Legal Citation:** M.L. 2017, Chp. 96, Sec. 2, Subd. 03f

**Project Manager:** Chan Lan Chun

**Organization:** University of Minnesota Duluth

**M.L. 2017 ENRTF Appropriation:** \$ 333,222

**Project Length and Completion Date:** 4 years, June 30, 2021

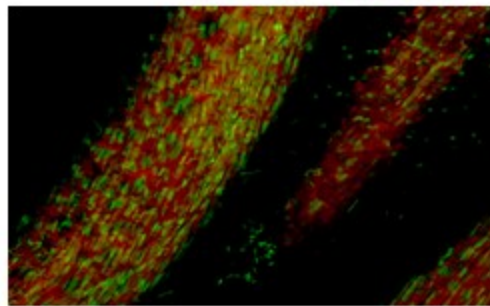
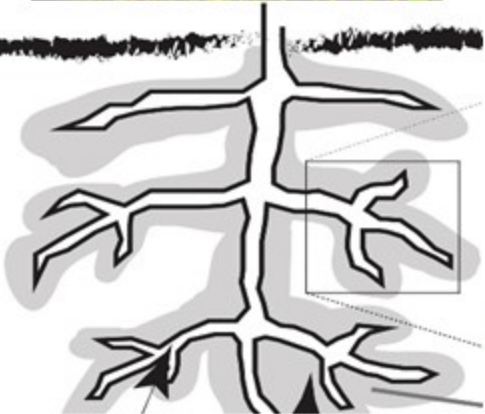
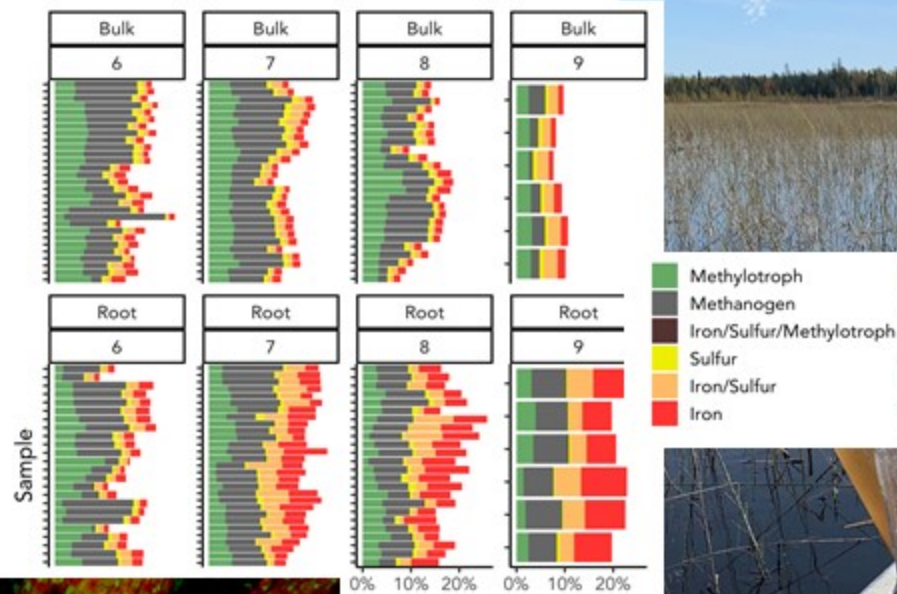
**Date of Report:** August 15, 2021



ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised budget 1-17-20	Amount Spent	Activity 1 Balance	Revised Budget 11-20-20	Amount Spent	Activity 2 Balance	Revised Budget 11-20-20	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
<b>BUDGET ITEM</b>											
<b>Personnel (Wages and Benefits)</b>	<b>\$83,603</b>	<b>\$83,603</b>	<b>\$0</b>	<b>\$142,928</b>	<b>\$142,928</b>	<b>\$0</b>	<b>\$13,315</b>	<b>\$12,537</b>	<b>\$778</b>	<b>\$239,846</b>	<b>\$778</b>
Chan Lan Chun, Principal Investigator: \$36,043(66.3% salary, 33.7% benefits); 7.4% FTE each year for 3 years.											
Randall E. Hicks,co-PI: \$23,476 Co-Investigator (66.3% salary, 33.7% benefits); 3.7% FTE each year for 3 years											
George Host, co-PI: \$18,743 (66.3% salary, 33.7% benefits); 4% FTE each year for 3 years											
Carol Reschke, co-PI: \$45,669 (72.6% salary, 27.4% benefits); 20% FTE each year for 3 years											
Adelle Schumann, Research Technician: \$23,185(72.6% salary, 27.4% benefits); 15% FTE each year for 3 years											
Graduate Research Assistant: \$91,728 (82.4% salary, 17.6% benefits); 50% FTE each year for 2 years											
<b>Professional/Technical/Service Contracts</b>	<b>\$17,249</b>	<b>\$17,249</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$17,249</b>	<b>\$0</b>
\$24,796 \$17,249 (11/17/20) is budgeted to use airboat service for wild rice, invasive plants, water and sediment sampling from Natural Resources Department of the Fond du Lac Band of Ojibwe. Airboat is essential for access to wild rice stands without damaging wild rice plants, as well as the most											
<b>Equipment/Tools/Supplies</b>	<b>\$10,300</b>	<b>\$10,300</b>	<b>\$0</b>	<b>\$25,327</b>	<b>\$25,327</b>	<b>\$0</b>	<b>\$500</b>	<b>\$500</b>	<b>\$0</b>	<b>\$36,127</b>	<b>\$0</b>
\$4,600 is budgeted for plant and sediment sampling supplies for activity 1: Chest waders/boots \$360; field guide to aquatic plants \$100; soil knife \$60; plant press supplies \$110; plastic collecting bags \$100; gas batteries \$50; \$12,000 is budgeted for DNA/RNA extraction kit and molecular biological agents for activity 2: Nucleic acid extraction \$600/kit*12kits for ~1000 samples= \$7,200; PCR reagents \$2/reaction*2000 samples=\$4000; and various molecular \$5,700 for activity 1 and \$5,780 for activity 2 are budgeted for chemical and expendable lab supplies: Disposable plasticware and lab supplies (e.g., Petri dishes, etc.) and miscellaneous chemicals (e.g., stains, solvents, antibiotics).											
Brochure and factsheet production for public dissemination of technical research results for activity 3: \$500											
<b>Travel expenses in Minnesota</b>	<b>\$4,482</b>	<b>\$4,482</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$1,286</b>	<b>\$1,286</b>	<b>\$0</b>	<b>\$5,768</b>	<b>\$0</b>
\$4,482 is budgeted for in-state sampling:10 samplings/yr*150mi/sampling* 3yrs*\$0.54/mi = \$2430 + vehicle rental use \$10/day*45days = \$450 + field camp lodging \$80/night*2nights*2trips*2 summers = \$1,600 for activity 1 \$2,288 is budgeted for in-state conference attendance (Minnesota Water Resources Conference and Annual St. Louise River Summit): Registration 2											
<b>Other</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$35,010</b>	<b>\$35,010</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$0</b>	<b>\$35,010</b>	<b>\$0</b>
\$22,010 is budgeted for Illumina sequencing and supercomputer usage fee: UMN Genomic Center (UMGC): Illumina Sequencing and library preparation.~ \$13,000 is budgeted for chemical and nutrient analyses: UMD NRRI Central Analytical Lab: \$65/sample x 200 samples											
<b>COLUMN TOTAL</b>	<b>\$115,634</b>	<b>\$115,634</b>	<b>\$0</b>	<b>\$203,265</b>	<b>\$203,265</b>	<b>\$0</b>	<b>\$15,101</b>	<b>\$14,323</b>	<b>\$778</b>	<b>\$334,000</b>	<b>\$778</b>

# Understanding microbial and nutrient associations with self-sustaining wild rice wetlands will identify limitation to restoration and promote restoration success.

## Dynamic microbial interactions between sediment and root zone



Microbes (green) on the root

