2016 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Assessing Effectiveness of Wetland Restorations for Improved Water Quality
PROJECT MANAGER: Jacques Finlay
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04u

APPROPRIATION AMOUNT: $420,000
AMOUNT SPENT: $420,000
AMOUNT REMAINING: $0

Sound bite of Project Outcomes and Results
Wetland restorations are vital for achieving water quality improvement in Minnesota. Assessment of common wetland restoration techniques across 58 wetlands demonstrated the importance of removing accumulated sediment, time since restoration, and hydrology to restoration outcomes. Benefits to native plants and nutrient removal are likely worth the extra cost of sediment removal, but continued management is necessary to maintain them following restoration.

Overall Project Outcome and Results

Wetland restorations are vital for enhancing habitat and protecting against growing threats from eutrophication to Minnesota's drinking and recreational waters. Using comparisons of standard wetland restoration practices with those that also removed accumulated sediment, we examined outcomes of restorations across gradients of wetland size, age, and hydrology. Our goal was to investigate the effects of 1) excavating accumulated eroded sediment, 2) time since restoration, and 3) hydrology, on the ability to store and remove nutrient input from the watershed over time, and on the abundance and diversity of native and invasive vegetation in restored wetlands. We studied 58 restored agricultural wetlands, collecting over 1000 water, 800 soil, and 258 plant samples over three years. Substantial water quality improvements resulted from both standard and sediment removal treatments. Excavation reduced total nitrogen (N) and total phosphorus (P) in soil and surface water, although the strength of effects varied substantially by nutrient and wetland type. In general, soil and water nutrient content increased with wetland age since restoration, suggesting that wetlands effectively stored incoming nutrients. Restored wetlands overall had a high capacity to remove nitrate under a wide range of temperature, age, size and geomorphic conditions, resulting in extremely low concentrations of nitrate. Permanent N removal via denitrification did not differ between excavated and standard restoration practices, but seasonally flooded wetlands had significantly higher denitrification rates than semi-permanent basins that dry out much less frequently. N removal by denitrification increased steadily following restoration, indicating improved capacity for nitrate reduction in older wetlands. In contrast to nitrogen, seasonal flooding promoted mobilization of inorganic phosphorus to surface waters, suggesting effects of long term enrichment of phosphorus in watershed soils. Vegetation accounted for a substantial portion of N and P stored in wetland basins during the growing season, with invasive hybrid cattail containing over 70% of the N and P stored in plant biomass. Following restoration, excavated wetlands had significantly lower hybrid cattail cover and higher native species cover compared to wetlands restored without sediment removal. However, rapid expansion by hybrid cattail offset vegetation benefits of sediment removal within eight years following restoration. Our study demonstrated that sediment excavation promotes native species and at the same time, reduces nutrient availability and improves water quality in restored agricultural wetlands. Environmental factors such as basin inundation patterns and time since restoration influence the ability of wetlands to perform key services. Eutrophication is a growing threat to Minnesota's drinking and recreational waters, and our work showed that agricultural wetland restorations can substantially reduce the risk of eutrophication. Benefits of wetland restoration can be maximized by removing accumulated sediment during restoration and managing invasive species in the years following restoration.

Project Results Use and Dissemination

Information from this project has been used and disseminated in diverse ways during the three year project. Results from the project have been presented at national, regional, and state meetings and events including; the Society for Freshwater Sciences annual conference (May 2019), the Society for Wetland Sciences annual conference (May 2019), the Minnesota Chapter of the Wildlife Society annual conference (February 2017 and February 2018), the joint meeting of the Upper Midwest Invasive Species Conference and North American Invasive Species Management Association (October 2018), and a meeting of Minnesota private lands managers and conservation specialists including The Nature Conservancy, the US Fish and Wildlife Service, Minnesota Department of Natural Resources, Minnesota Land Trust, Ducks Unlimited, and the U.S. Department of Agriculture (June 2018). We have shared our research with local entities including the University of Minnesota's Shared Water, Shared Responsibility: Engaging Minnesota's Communities, Students, & Policy-Makers event (March 2017), the Water Resources Science Spring Research Symposium (January 2018), the Pomme de Terre Watershed Task Force (May 2018), Restoration Evaluation Specialists at the Minnesota DNR Division of Ecological and Water Resources (March 2018), and The Nature Conservancy (August 2018), and we continue to reach out to other stakeholders and land management groups to share the results of our research. Furthermore, the results from this research have been shared regularly with the US Fish and Wildlife Service Private Lands Office and restoration specialists working with landowners across the state. We have submitted one manuscript addressing the effects of sediment excavation on plant communities to the journal *Restoration Ecology* (submitted July 2018) and another manuscript to the journal *Wetlands*. In addition, two more manuscripts are in preparation, and others are planned. Copies of the manuscripts will be provided upon publication. Finally, we have developed a set of interactive tools to start conversations about wetlands with children and adults. Using visual aids, hands-on activities, and informational handouts, we were able to reach hundreds of people in the summer of 2018 at the West Ottertail County Fair and the Fergus Falls Aqua Chautauqua, by focusing on exploration and discovery in our backyard wetlands. Our activities and handouts are still being used by environmental and K-12 educators in the Ottertail Public School District. Appendix 2 provides examples of our outreach materials.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2016 Work Plan Final Report

Date of Report: August 16, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Assessing Effectiveness of Wetland Restorations for Improved Water Quality

Project Manager: Jacques Finlay

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Location: Becker County, Big Stone County, Douglas County, Grant County, Lac Qui Parle County, Stevens County, Mahnomen County, Otter Tail County, Pope County, Swift County, Todd County.

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$420,000**
	Amount Spent:	**$420,000**
	Balance:	**$0**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04u

Appropriation Language:
$420,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to quantify the environmental benefits of sediment removal and native plant communities in wetland restorations by measuring resulting reductions in nitrogen and phosphorus delivery to groundwater and surface water. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Assessing wetland restorations for improved water quality

II. PROJECT STATEMENT:

Wetland restoration is a priority for improving environmental services in Minnesota, but the methods to achieve the widest benefit for the least cost are unclear. A key unanswered question is whether wetland restoration practices designed to improve waterfowl habitat by restoring native plant communities also provide benefits to water quality. This question is of considerable concern, since restoration and management practices are costly and may limit the ability of managers to accept additional wetland restoration projects. We will assess the benefits of two specific restoration and management activities - sediment removal and native plant management - by quantifying and comparing the amount of nitrogen (N) and phosphorus (P) that are stored in wetland basins and removed from surface waters as they percolate through wetlands into the ground water supply.

Between 1850 and 1980, about 80% of Minnesota's prairie pothole wetlands were drained for agriculture. Temporary and seasonal (hereafter seasonal) wetlands were particularly vulnerable because they are local depressions that do not remain wet throughout the entire growing season, but fill and slowly drain into the groundwater supply following rainfall events. These seasonal wetlands are thought to be particularly valuable habitat for breeding and rearing waterfowl, flood water retention, and excess nutrient storage and removal. Over time, these wetland basins fill with topsoil eroded from the surrounding landscape, burying native plant seeds in the relic wetland soils. Eroded topsoil accumulation decreases the volume of water held in wetland basins and the duration of time that the water can be held on the landscape. Furthermore, the accumulated sediment may alter the availability of nitrogen (N) and phosphorus (P) in the soil which can influence the rates of microbial activity and subsequent nutrient burial or removal.

One strategy used to restore agricultural wetlands is to excavate the accumulated sediment – exposing buried, relic wetland soils – prior to restoring the water supply. After sediment excavation native wetland plants quickly re-establish, but invasive hybrid cattail (*Typha x glauca*) and reed canary grass (*Phalaris arundinacea*) regularly invade the restored basins and outcompete native vegetation. These invasive plants form dense monocultures that provide very little food and cover for waterfowl. In addition, the root systems of native and invasive plants can be drastically different from one another. Native plants tend to have robust root systems with many fine roots, while cattail and reed canary grass have shallow root systems with fewer, larger roots. The robust root systems of native plant species may provide increased surface area and habitat for microbially mediated nitrogen removal. Our goal is to understand how water quality is influenced by wetland restoration practices and the resulting plant communities.

Through the Partners for Fish and Wildlife Program, the United States Fish and Wildlife Service (USFWS) is engaged in an adaptive management project characterized by a series of wetland restorations with and without accumulated sediment removal. The goal of sediment excavation is to restore the original hydrologic regime of the basin, increase water storage potential, and expose wetland soils and the associated native plant community, thereby increasing the probability of restoring high quality waterfowl habitat and increasing nutrient removal from surface and groundwater. We will survey approximately 50 wetlands between 0.1 and 3 acres in size that were restored by the USFWS in the last 5 years in western Minnesota. Accumulated sediment was excavated and removed in half of the wetlands. We will measure how much N and P is stored in the wetland basin and removed from surface water as it percolates into the groundwater supply. We will calculate differences in nutrient removal in wetlands with and without sediment excavation. In addition, we will examine whether native wetland plants increase the rate of nitrogen removal compared to invasive plants. This project will provide valuable quantitative information that will directly influence wetland restoration and management decisions in Minnesota.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of December 28, 2016:

Our activities focused on sampling for detailed measurement of soil physical and chemical characteristics, collection of samples for dissolved nutrient concentrations and water flux, and testing methods for measurement of below-ground biomass and denitrification. We collected over 150 soil cores across all of our sites. Soil sampling was initially slowed by technical difficulties with sediment collection, but we solved these issues through use of an alternate design for soil core sampling and by use of tools available at the National Lacustrine Coring Facility at the University of Minnesota. We have measured wet and dry bulk density, organic carbon, carbonate, and inorganic content at discrete intervals on all of the soil cores and are currently drying and sieving soil samples in preparation for further analyses. We collected our first set of surface water samples in the summer of 2016. We collected soil temperature profiles to measure surface-groundwater exchange to develop methods to measure shallow groundwater input and outflow from wetlands. We installed five piezometers at a series of wetlands to examine water table fluctuations and compare dissolved nutrient concentration in water entering and leaving the wetlands. Finally, we collected soil pore water samples at two wetlands while testing a new method for determining in-situ denitrification rate. The denitrification data are currently being analyzed, and the dissolved nutrient analyses will begin in February 2017. We hired two undergraduate students from the University of Minnesota, one undergraduate from St. Thomas University, and one recent graduate from Carleton College as field and lab assistants to help process and analyze soil and water samples. In addition, these students have been essential members of our team, often taking the initiative to solve problems as they arise. We have also relied heavily on the junior scientist who has helped to coordinate laboratory activities.

Amendment Request (12/30/2016):

1) We request a shift in site location for the wetland basin located in Lyon county. Initially, we believed that this basin would be representative of a restored seasonal wetland in the prairie pothole region, but upon restoration, we found that the basin did not hold water as well as anticipated. We have identified an alternative wetland in Stevens county that was restored at approximately the same time as the Lyon county site and is holding water well. In addition, this site is located in the middle of our existing study area, helping to improve our sampling by decreasing travel time between sites.
2) We request authorization to shift funds from “Equipment/Tools/Supplies” to “Other expenses” in order to incorporate an additional method for analysis of denitrification and to cover costs related to sending soil samples to the University of Minnesota Soils Lab. We propose to make use of recent improvements to the soil “peeper” method to measure in situ denitrification rates, a technique that provides additional information and complements the method originally proposed in several ways. First, this method accounts for site specific variation in soil physical and chemical properties such as density and porosity. In addition, it incorporates effects of living plants roots on soil properties. Finally, this method allows us to measure temperature and nutrient availability in soil pore waters at the same time as we are measuring denitrification, thus improving our ability to analyses controls of denitrification and increasing the number of wetlands in which we will be sampling soil pore water.

   The second reason for the requested re-allocation is to pay for soil and water analyses outside of our lab. We had originally planned to measure pH and phosphorus content of soils in our lab, but have found that University of Minnesota Soils Lab is can make these measurements at greater efficiently and a lower cost per sample than we are able to. Similarly, we had planned to measure soluble reactive phosphorus concentration in our lab, but having the analyses done at the St. Croix Watershed Research Station will allow us to process a larger number of samples at lower cost per sample. To make these changes (i.e. fabricate soil water peepers for denitrification assays, increase the resolution of our measurements, and access specialized equipment), we are requesting the shift of funds from “Equipment/Tools/Supplies” to “Other expenses”.
3) We request authorization to add a research technician to the Personnel section of the budget. The large number of samples and measurements we are collecting requires extensive time at the field sites as well as in the lab. Due to the difficulty in scheduling part-time student assistants, we need someone that can

conduct field sampling with the graduate student on a full-time basis during the May through November field season, and can help process samples in a timely fashion. One academic year of graduate student support would be reallocated to this position. The graduate student will be working continuously on this project but we will seek other sources of support such as teaching assistantships and funding from the University of Minnesota in order to provide additional full time support for the project from the research technician. [Amendment Approved by LCCMR 1/04/2017].

Project Status as of June 01, 2017:

Our research since January 2017 has focused on sample preparation and analysis for samples collected in 2016, and preparation for field work. We ground soils to a fine powder and analyzed the samples for total nitrogen content. We submitted a subset of soil samples to the University of Minnesota Soils lab for pH testing and subsequent Bray-Olsen phosphorus analysis. We measured dissolved organic carbon, total dissolved nitrogen, and total dissolved phosphorus in surface water samples collected during summer of 2016. We also measured nitrate, ammonium, and soluble reactive phosphorus concentrations in surface and soil pore water samples. Finally, we explored the data in preliminary analyses of differences between wetlands with and without sediment excavation, including other likely sources of variation in our model design.

Other aspects of our work included preparing for the 2017 field season and connecting with policy makers and state agency officials in a poster session at the University of Minnesota for the Minnesota Governor's Year of Water Action event.

Project Status as of December 30, 2017:

Since June 2017, our research has focused on field work designed to measure the interaction between surface and groundwater supplies, surface water nutrient content, nutrient storage in plant biomass, and nitrogen removal rates. In order to better understand interactions with groundwater supply, we deployed water-level loggers at 10 sites to record hourly changes in water level across multiple wetlands. To characterize early-season nutrient content in our wetland basins, we collected surface water samples across all of our wetlands before July 10. We also collected monthly surface water samples from a subset of sites characterize changes in nutrient concentrations throughout the growing season. Whenever possible, we collected surface water samples in conjunction with soil and water for denitrification assays, which were used to estimate nitrogen removal rates. We estimated denitrification rates across 30 wetlands before July 01. At an additional 12 wetlands, we measured denitrification rates at monthly intervals. Finally, we measured the standing biomass of emergent vegetation at two times; during peak above-ground biomass, and again as plants began to go dormant in the fall. We sampled below-ground biomass during peak above-ground biomass. Both above- and below-ground biomass samples are currently being ground for further analyses.

We decided to sample five additional sites located in Ottertail (two sites), Grant (one site), and Pope (two sites) counties. These wetlands were either immediately adjacent to existing sites or they were very near existing sites. In all cases the wetlands add statistical power to the study and improve our sample size while remaining within the geographical range dictated by our study design. In order to measure soil nutrient storage, we collected and processed soil cores from each site using the same methods employed in 2016. We have measured wet and dry bulk density, organic matter content, carbonate content, and inorganic matter at discrete intervals in all soil cores. We have dried and sieved the samples, and are currently in the process of grinding the samples to a fine powder in preparation for total nitrogen analysis. Sediment samples collected in conjunction with the denitrification portion of the study have also been dried and sieved, and are currently being ground further analysis.

Our outreach activities included presenting preliminary results to our collaborators at the United States Fish and Wildlife Service (Region 3) and providing a research poster for display at the Fergus Falls Wetland Management District Office. Additional posters are planned for wetland management district offices located across the study area.

Project Status as of June 30, 2018:

Between January and June 2018, we continued to enhance understanding of how soil, water, and vegetation influence nutrient movement through geographically isolated wetlands by continuing to process samples collected in 2017 and beginning our 2018 field season in May of this year. We measured nutrient concentrations in surface and ground water collected in 2017 and found patterns similar, though slightly different from those recorded in 2016. We measured nutrient concentrations in vegetation samples collected in 2017, exposing interesting trends that have prompted additional sampling in the summer of 2018. Finally, we are currently processing samples for suspended particulate matter, which will help us identify the nutrient stored as algae and bacteria in the water column. In May, we began the 2018 field season by collecting spring water samples for dissolved nutrient content and examining denitrification potential at 30 wetlands. Beginning in late June, we transitioned into nitrification assays which help elucidate nitrate sources and sinks, and the fate of ammonium in restored wetlands.

We have been engaged in multiple outreach activities since January 2018, including presentations to land management groups and outreach to children and families. We presented our preliminary findings to multiple groups including the Pomme de Terre Watershed Task Force, groups and individuals at the Minnesota DNR, the United States Fish and Wildlife Service, and at the Minnesota Chapter of The Wildlife Society annual meeting.

In an effort to engage in public outreach toward youth and families, we are working with the Prairie Wetlands Learning Center (Fergus Falls, MN) and the University of Minnesota Extension office to participate in multiple outreach events focused in Fergus Falls and the surrounding area. In particular, we participated in the second annual Aqua Chautauqua, a street festival celebrating our state's water resources. We are working with our collaborators to participate in the West Ottertail county fair and in development of additional outreach activities.

Project Status as of December 28, 2018:

From June to December 2018, we completed denitrification potential and nitrification potential assays for 30 wetland basins. We also completed detailed vegetation surveys at 24 wetlands, evenly divided between business as usual and sediment excavated treatments, and ranging in age from 3 to 9 years post-restoration, in order to elucidate how time and excavation influence vegetation communities and the dominance of invasive species. In August, we collected vegetation biomass from a known area and developed percent cover-biomass relationships for approximately 10 of the most abundant wetland plant species found in our study wetlands. These curves will be combined with vegetation survey data and nutrient content data to estimate the quantity of nutrients stored in plant biomass in our study wetlands. Together, the vegetation survey and nutrient data will help elucidate how excavation in wetland restoration impacts the prevalence and dominance of invasive species over time, and the amount of nutrient being stored in plant biomass. We are currently measuring nutrient concentrations in water and vegetation samples collected throughout the field season. We will examine the data and compare to 2016 and 2017 results beginning in early 2019.

Our data shows that sediment excavation in wetland restoration has the potential to remove substantial quantities of nutrients from wetland basins. Soils and surface waters contain significantly lower nitrogen and phosphorus concentrations in excavated wetlands compared to business as usual controls. We found that denitrification potential was limited by nitrate availability, but when sufficient nitrate was made available denitrification potential was very high. These results suggest that any available nitrate in our wetlands is quickly removed by denitrifying bacteria, thus decreasing the nitrogen available for export. Our preliminary results from the vegetation survey illustrate how excavation during wetland restoration appears to substantially suppress the invasive plant community (e.g., hybrid cattail and reed canary grass) in the first 4-8 years following restoration. However, our data suggests that after year 7 or 8, there is no detectable reduction in invasive plant cover compared to business as usual controls.

We have been engaged in outreach activities designed for the public and for land managers. In July, we collaborated with the Prairie Wetlands Learning Center, operated by the USFWS in Fergus Falls, MN, to present educational content at a display table at the West Ottertail County Fair. We developed a variety of activities for adults and kids to help illustrate the value of wetlands and communicate how our research will improve our understanding of how wetlands function. In addition, we provided coloring sheets and activity pages designed to help kids explore their local wetlands and the animals and plants that live there. In October, we presented the preliminary results from our vegetation survey at the Upper Midwest Invasive Species Conference which includes city, county, state, tribal, and federal land and natural resource managers. In addition, we shared our preliminary results with the USFWS and the Nature Conservancy (TNS).

Amendment Request (12/28/2018):

1) We request authorization to shift funds from "Personnel" ($23,000) and "Printing" ($1500) to "Equipment/Tools/Supplies" ($14000), "Travel" ($3500), and "Other Expenses" ($7000). Overall, activity 1 budget will increase by $1000 and Activity 3 budget will decrease by $1000, while allowing us to meet our stated objectives for all activities. This shift will allow us to cover costs of additional soil analyses at the University of Minnesota Soils Lab and surface water analyses at the St. Croix Watershed Research Station. We collected significantly more surface water samples than anticipated in order to assess how seasonality and rainfall events influence water quality in the study wetlands. The additional sampling required that the crew to split into two groups at times. Likewise, late season sampling and vegetation sampling went well into September and October, necessitating unforeseen travel costs. In addition, we were invited to present our research in a special session focused on restoration at the Upper Midwest Invasive Species Conference in October of this year, a cost we had not planned for. We were able to share a substantial portion of our vegetation survey results with land managers, city officials, county officials, and watershed management district personnel from across the state and region. We made several connections with representatives looking to expand their current wetland restoration activities and looking for guidance about best practices. We were also encouraged to contact a number of individuals when our results analysis is complete because they are interested in the other aspects of the project. We will use the additional funds in "Travel" to attend another conference for the Minnesota Chapter of the Wildlife Society, which provides a valuable way to reach Minnesota's natural resource managers. We believe it is essential to present at this conference, since it is an opportunity to disseminate our research results with several private, tribal, state, and federal land management groups operating in Minnesota. We anticipate similar feedback as we encountered at the Upper Midwest Invasive Species Conference as many groups have a vested interest in stopping the spread of aggressive exotic species since invasive species degrade habitat and severely impair ecosystem service provisions. Travel funds will also be used for sampling in spring 2019. Additional "Equipment/Tools/Supplies" and "Other Expenses" funds will allow us to create more outreach activities for the 2019 Aqua Chautauqua (which will we participate in again) and provide the resources required to complete measurements and sample analyses.
2) Based on this request, we ask for retroactive approval of the movement of funds to "Travel", described above, to apply to expenses incurred in summer/fall 2018. Travel costs for research at our sites (which are dispersed widely in 11 counties) in 2018 exceeded our original budget estimates from 2016, and resulted in minor overspending within that category. However, this has allowed data collection to characterize contrasting water quality responses in seasonal and semipermanent wetlands restored via sediment removal or standard restoration practices. [Amendment Approved by LCCMR 2/01/2019].

Overall Project Outcomes and Results:

Wetland restorations are vital for enhancing habitat and protecting against growing threats from eutrophication to Minnesota's drinking and recreational waters. Using comparisons of standard wetland restoration practices with those that also removed accumulated sediment, we examined outcomes of restorations across gradients of wetland size, age, and hydrology. Our goal was to investigate the effects of 1) excavating accumulated eroded sediment, 2) time since restoration, and 3) hydrology, on the ability to store and remove nutrient input from the watershed over time, and on the abundance and diversity of native and invasive vegetation in restored wetlands. We studied 58 restored agricultural wetlands, collecting over 1000 water, 800 soil, and 258 plant samples over three years. Substantial water quality improvements resulted from both standard and sediment removal treatments. Excavation reduced total nitrogen (N) and total phosphorus (P) in soil and surface water, although the strength of effects varied substantially by nutrient and wetland type. In general, soil and water nutrient content increased with wetland age since restoration, suggesting that wetlands effectively stored incoming nutrients. Restored wetlands overall had a high capacity to remove nitrate under a wide range of temperature, age, size and geomorphic conditions, resulting in extremely low concentrations of nitrate. Permanent N removal via denitrification did not differ between excavated and standard restoration practices, but seasonally flooded wetlands had significantly higher denitrification rates than semi-permanent basins that dry out much less frequently. N removal by denitrification increased steadily following restoration, indicating improved capacity for nitrate reduction in older wetlands. In contrast to nitrogen, seasonal flooding promoted mobilization of inorganic phosphorus to surface waters, suggesting effects of long term enrichment of phosphorus in watershed soils. Vegetation accounted for a substantial portion of N and P stored in wetland basins during the growing season, with invasive hybrid cattail containing over 70% of the N and P stored in plant biomass. Following restoration, excavated wetlands had significantly lower hybrid cattail cover and higher native species cover compared to wetlands restored without sediment removal. However, rapid expansion by hybrid cattail offset vegetation benefits of sediment removal within eight years following restoration. Our study demonstrated that sediment excavation promotes native species and at the same time, reduces nutrient availability and improves water quality in restored agricultural wetlands. Environmental factors such as basin inundation patterns and time since restoration influence the ability of wetlands to perform key services. Eutrophication is a growing threat to Minnesota's drinking and recreational waters, and our work showed that agricultural wetland restorations can substantially reduce the risk of eutrophication. Benefits of wetland restoration can be maximized by removing accumulated sediment during restoration and managing invasive species in the years following restoration.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Quantifying rates of nitrogen removal from groundwater in restored wetlands

Description: Comparing nitrogen storage and removal in restored wetlands with and without accumulated sediment requires an understanding of the movement of nutrient between different pools. In order to evaluate nitrogen dynamics, we will consider four major processes and the associated factors that influence nitrogen retention in wetlands:

A. Nitrogen storage in wetland soils and sediments,
B. Permanent nitrogen removal via denitrification,
C. Nitrogen uptake by emergent macrophytes, and
D. Nitrogen transport from ponded surface water to the shallow aquifer.

To measure nitrogen storage in soils and sediments, we will use elemental analysis to quantify nitrogen and carbon content of accumulated sediment and wetland soils. These values will be scaled to nutrient per volume of soil using measurements of soil bulk density. The nutrient content of soil can be used to calculate the quantity of nutrient contained in accumulated sediments removed during wetland restoration and the potential for nutrient storage in wetland basins.

Permanent nitrogen removal will be measured using assays designed to quantify denitrification rates and the factors that regulate nitrogen removal. We will compare measurements of denitrification potential and soil characteristics that are known to influence denitrification rates at sites with and without accumulated sediment. We will pair denitrification rates with measurements of the duration of saturation made using shallow groundwater wells to quantify the role of sediment removal on permanent nitrogen removal in restored wetlands throughout the growing season.

To examine temporary nutrient storage in emergent macrophytes, we will measure nitrogen assimilation and fate in plant tissues. We will quantify temporary nitrogen storage in aboveground and belowground biomass using elemental analysis of nitrogen and carbon. We will pair these measurements with traditional plant surveys and productivity measurements to calculate the nutrient storage by plants per unit area. In particular, we will compare the nutrient storage capacity of invasive plant species, including cattail (*Typha species*) and reed canary grass (*Phalaris arundinacea*) with native plant species including sedges, rushes, and forbs.

Nitrogen transport to shallow groundwater will be quantified by comparing measurements of nutrient concentration in surface and groundwater at periods of groundwater recharge. Ammonium and nitrate concentrations in water samples will be measured using the phenol hypochlorite and cadmium reduction methods, respectively. Dissolved nutrient concentrations will be paired with sediment temperature profiles to estimate the quantity of nutrient flowing from surface to shallow groundwater supplies in wetland basins. We will compare nutrient export from wetlands in basins with and without accumulated sediment and basins with and without native wetland plant communities.

Summary Budget Information for Activity 1: **ENRTF Budget: $ 304,000**
Amount Spent: $ 304,000
Balance: $ 0

Outcome	**Completion Date**
1. Measure nutrient concentrations in the accumulated sediment and soils from wetland basins with sediment removed, and evaluate nitrogen retention.	June 2018
2. Measure denitrification in wetlands with and without accumulated sediments.	June 2019
3. Collect emergent macrophyte samples for nutrient analysis, and analyze nutrient content in aboveground and belowground biomass.	June 2019
4. Install instrumentation to measure nutrient transfer from surface to shallow groundwater.	December 2017
5. Compare surface water and groundwater nitrogen concentrations at wetlands with and without accumulated sediments and native plant communities.	June 2019

Activity Status as of December 30, 2016:

Our research in Activity 1 focused on collecting soil samples in order to examine nutrient storage in sediments. We collected 218 soil samples from 53 sites this fall. Analyses of chemical and physical characteristics are proceeding. The soil sampler we had intended to use was ineffective so we developed and tested a new sampler, which was better designed to capture intact soil cores. We evaluated methods for sectioning and splitting cores and found that the Lacustrine Core Facility (LacCore), located at the University of Minnesota, has the appropriate equipment for splitting frozen soil cores. Subsequently, we measured wet and dry bulk density at three discrete intervals (0-5 cm, 10-15 cm, and 20-25 cm) along the length of each soil core. In addition, we measured the soil organic matter and carbon, carbonate, and inorganic fractions of the cores. Each full soil core was divided into subsections by five centimeter intervals as outlined above. The individual subsections are currently being dried and sieved in preparation for further analysis.

We collected preliminary soil samples to develop a final sampling design for root biomass analyses. The root samples are being prepared for analysis to determine the number of samples required to accurately measure root biomass at our study sites. Sampling for above-ground biomass will begin next field season, following methods testing in the spring.

Surface water samples were collected at three discrete locations in each of our wetlands in order to quantify dissolved nutrient concentrations. The samples were immediately filtered and frozen. We also collected soil pore water samples at two wetlands in conjunction with the denitrification study. In February, we will begin dissolved nutrient analyses on these samples.

We assessed an alternative method for measuring denitrification that will allow us to examine actual denitrification rates in-situ. We made preliminary measurements at two wetlands, and are in the process of analyzing the data.

To examine the rate of exchange between surface and shallow groundwater supplies, we measured temperatures at multiple depths and locations in two wetlands. These soil temperature profiles were consolidated and analyzed the magnitude and direction of water flux was estimated by solving a one-dimensional heat flux model. We are currently exploring the preliminary data and analyses are ongoing. In addition, we have installed five piezometers at a series of three wetlands in an effort to assess fluctuations in the water table and differences in dissolved nutrient content entering and leaving each wetland.

Activity Status as of June 01, 2017:

To achieve outcomes of Activity 1, we focused our efforts on completing soil nutrient and water quality analyses for samples taken in 2016. We dried, sieved, and ground soils to a fine powder in order to analyze total nitrogen content. We used an elemental analyzer to measure total nitrogen content and corroborate total carbon estimates made earlier using less precise methods. In surface water samples, we measured dissolved organic carbon and total dissolved nitrogen. Acidified samples were analyzed on a Shimadzu autoanalyzer, which can simultaneously measure dissolved organic carbon and total dissolved nitrogen. This data was used in conjunction with measures of dissolved inorganic nitrogen to calculate dissolved organic nitrogen, providing more information about the form and relative availability of dissolved nitrogen in these wetlands. We measured dissolved inorganic nitrogen as nitrate and ammonium in surface and soil pore water samples using a SmartChem autoanalyzer. This portion of the research was done in collaboration with the Science Museum of Minnesota and the St. Croix Watershed Research Station.

Activity Status as of December 30, 2017:

We addressed nearly all aspects of our Activity 1 research goals over the 2017 field season. To address outcome 1, we collected soil cores from 5 additional wetlands. We processed the soils for wet and dry bulk density, organic matter content, carbonate content, and inorganic content at discrete intervals in all 15 soil cores. We have dried and sieved the samples, and are currently in the process of grinding the samples to a fine powder in preparation for total nitrogen analysis. Likewise, sediment samples collected in conjunction with the denitrification portion of the study (outcome 2) have also been dried and sieved, and are currently being ground further analysis.

We estimated denitrification rates across 30 wetlands before July, and at monthly intervals at 12 wetlands (outcome 2). Whenever possible, we collected surface water samples (outcome 5) in conjunction with soil and water for denitrification assays.

Surface water samples were collected across all sites before July 10, while surface water was sampled at monthly intervals at 12 wetlands in order to track seasonal changes in nutrient availability (outcome 5). In addition, we collected shallow-groundwater samples from piezometers put in place during fall of 2016. However, 3 of the 5 piezometers were damaged by wildlife and were not functional. We pulled these piezometers and altered the remaining units to make them less conspicuous. Currently, most of the surface water samples have been acidified and we have measured dissolved organic carbon and total dissolved nitrogen, but this work will continue throughout the winter. Measurement of dissolved inorganic nitrogen content is scheduled to begin in mid-December and conclude before February 1st, 2018.

In addition to characterizing surface water nutrient content, we deployed water-level loggers at 10 sites to track hourly fluctuations in water level across multiple wetlands (outcome 4). Loggers were deployed on June 01 and retrieved on November 01 after 1 inch of ice formed across most of the wetlands. Upon retrieval, we performed regular maintenance on the T-post support by driving posts further into the sediment to prevent ice-sheer over the winter. In two cases, we re-located the posts so that they would provide a less desirable point around-which muskrats could build their huts.

Finally, we addressed outcome 3 by collecting standing biomass of emergent vegetation at two times; during peak above-ground biomass, and again as plants began to go dormant in the fall. All samples were dried and weighed, and are currently being ground for total carbon and total nitrogen analysis. In addition to above-ground biomass, we sampled below-ground biomass during peak plant growth. Below-ground biomass was carefully sorted from detritus, and samples were dried, weighed, and are currently being ground to a fine powder for further analysis.

Activity Status as of June 30, 2018:

We addressed multiple aspects of Activity 1 between December 2017 and June 30, 2018. Specifically, we measured soil nitrogen and carbon content in soils collected in 2017, adding to our understanding of spatial heterogeneity of surficial soil nutrient content across multiple sites and over multiple years. Analyses of the interannual and spatial variability in soil nutrient content will be analyzed later this year following the end of field research.

We enhanced our understanding of wetland responses to nitrate flux by examining denitrification potential in 9 new wetlands and repeating the assay in 21 wetlands that were surveyed in 2017. Denitrification assays were performed between May 14 and June 21, 2018, ensuring that they were representative of wetland response to spring thaw which was set back due to an April snow storm and re-freeze. We maintained a balanced design by choosing 15 *Business As Usual* (BAU) and 15 *Excavated* (EXC) sites. Among the BAU sites, five wetlands were classified as having semipermanent hydrology. Likewise, five of the EXC sites had semipermanent hydrology. Further understanding of nitrogen cycling is being examined using nitrification assays, which may elucidate one possible source of nitrate for denitrification. These assays began in mid June and will be conducted through July.

Surface and ground water dissolved nitrogen, phosphorus, and organic carbon content were measured for samples collected in 2017. In addition, we collected spring surface and ground water samples across all basins between May 14 and June 14. A second round of surface and ground water samples are being collected for a subset of sites between June 21 and July 19. These samples will help us identify seasonal shifts in dissolved nutrient content moving from spring snowmelt to summer. Particulate nitrogen and carbon were collected for spring 2018 samples. We are currently preparing 2017 samples for particulate nitrogen and carbon analysis.

Emergent macrophyte biomass samples were weighed, subsampled, and ground to a fine powder using a Wiley Mill with a #40 mesh. Samples were prepared for analyses for carbon and nitrogen content via Costech CN analyzer following standard protocols. Initial results were promising and have informed additional collection of biomass samples at more sites in the 2018 field season. In addition, we are conducting vegetation surveys designed to provide accurate estimates of plant diversity and cover, which will include a biomass collection component.

Activity Status as of December 28, 2018:

Analyses of data collected over the past two years shows that sediment excavation during wetland restoration significantly decreased total nitrogen in wetland soils and surface waters (Figures 1.1 and 1.2). Surface water total dissolved nitrogen (TDN; Figure 1.2A) and dissolved organic nitrogen (DON; Figure 1.2B) concentrations were significantly lower at excavated wetlands in 2016 and 2017 compared to business as usual wetlands (Table 1.1). A more thorough investigation, now ongoing, is needed to understand patterns and interannual variability in inorganic nitrogen concentrations (e.g., ammonium and nitrate). However, initial results indicate much lower levels of inorganic nitrogen in wetlands than in larger streams, and in particularly very low concentrations of nitrate, suggesting a high capacity to remove excess nitrogen in runoff from the surrounding landscape.

In our study, soil nitrogen content did not differ significantly between seasonal and semipermanent wetlands (Figure 1.1). This result suggests that despite completely drying during most years, seasonal wetlands have statistically similar soil nitrogen content as wetlands that completely dry out approximately once every 10 years (i.e. semipermanent wetlands). The patterns in wetland type and soil nitrogen content were reflected by surface water TDN and DON concentrations (Figure 1.2), suggesting that there is a very large pool of organic nitrogen stored in soils.

Analyses of data collected so far indicates little sensitivity of denitrification in response to wetland hydrology or restoration method. Denitrification was also relatively insensitive to seasonal changes in temperature, and denitrifiers were primarily limited by concentrations of nitrate, suggesting a large capacity to remove any nitrate that enters these geographically isolated wetlands, even in recently excavated wetlands. We are in the process of analyzing the results from the denitrification assays performed in June to assess annual variation in denitrification potential. We are preparing to analyze surface water samples from the 2018 sampling effort.

Preliminary analysis of the vegetation survey data from 24 wetlands (12 excavated, 12 business as usual), suggests that invasive species cover is significantly lower in excavated wetlands compared to business as usual controls (Figure 1.3A; p=0.0105). However, we found that invasive hybrid cattail (*Typha x glauca*) and reed canary grass (*Phalaris arundinacea*) are able to quickly establish and spread after restoration (p=0.0017), excluding native species at excavated sites and undoing effects of excavation after 8 years. We found that native species richness decreased significantly over time (p=0.0363) as invasive species became more dominant (Figure 1.3B). This result reinforces the conclusion that invasive species negatively influences overall community richness and, by extension, resilience.

Despite the competitive advantage of invasive species, excavation improved overall community diversity and evenness metrics (p=0.0202 and p=0.0166, respectively), an effect which did not appear to change significantly over time. These results suggest that while excavation provides an initial advantage to native species, we can expect less diverse communities as hybrid cattail and reed canary grass spread following restoration, especially in the absence of management activities designed to control the establishment and proliferation of aggressive invasive species. However, our data also suggests that *invasive species management in the first 4 years following restoration may be essential for maintaining a robust and diverse community of native species*.

Soil

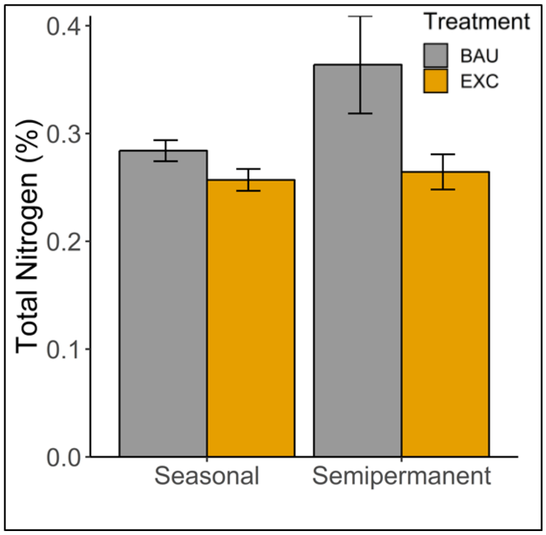


Figure 1.1: Soil total nitrogen (percent) in seasonal and semipermanent wetlands. Excavated sites had significantly lower total nitrogen (p=0.00409), but seasonal and semipermanent wetlands did not differ significantly (p=0.236).

Surface Water: 2016

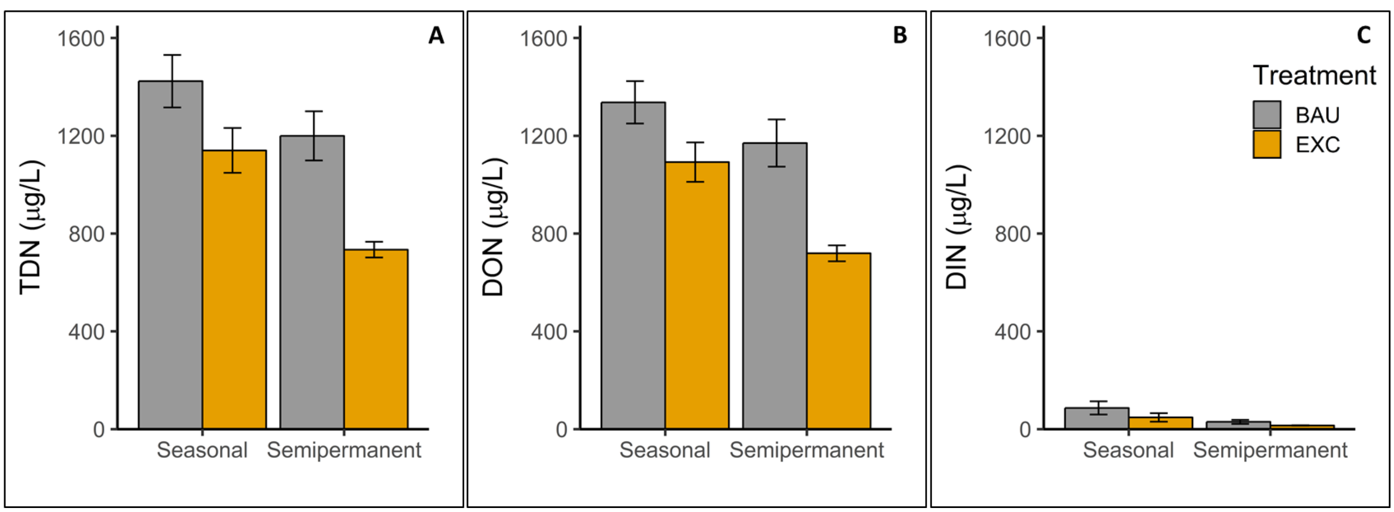


Figure 1.2: Surface water, A) total dissolved nitrogen (TDN), B) dissolved organic nitrogen (DON), and C) dissolved inorganic nitrogen (DIN) concentration (micrograms per liter) in seasonal and semipermanent wetlands in the 2016 growing season.

Table 1.1: Statistical results from ANOVAs for surface water dissolved nutrient concentrations. Total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), total dissolved phosphorus (TDP), dissolved organic phosphorus (DOP), and soluble reactive phosphorus (SRP). Treatment represents the difference between excavated and business as usual wetlands. Wetland Type represents the difference between seasonal and semipermanent wetlands.

	2016			***2017***		
	Treatment	*Wetland Type*	*Treatment X Wetland Type*	*Treatment*	*Wetland Type*	*Treatment X Wetland Type*
TDN (μg/L)	**0.006573 ****	0.05175 ·	0.2530	0.0559 ·	**0.01458 ***	0.7646
DON (μg/L)	**0.01288 ***	0.07409	0.3122	**0.02316 ***	**0.01202 ***	0.8405
DIN (μg/L)	0.0876	**0.03851 ***	0.9112	0.8649	0.1059	0.5702
TDP (μg/L)	**0.000234 ****	**< 0.0001 ****	0.7230	**0.007025 ****	**0.0002931 ****	0.6363
DOP (μg/L)	**0.02095 ***	**0.004044 ****	0.502225	**0.006462 ****	**0.0003652 ****	0.3134
SRP (μg/L)	**0.002195 ****	**0.0000127 ****	0.7267	**0.04909 ***	**0.002087 ****	0.6746

****** Significant p values < 0.01
***** Significant p values < 0.05
· Marginally significant p values

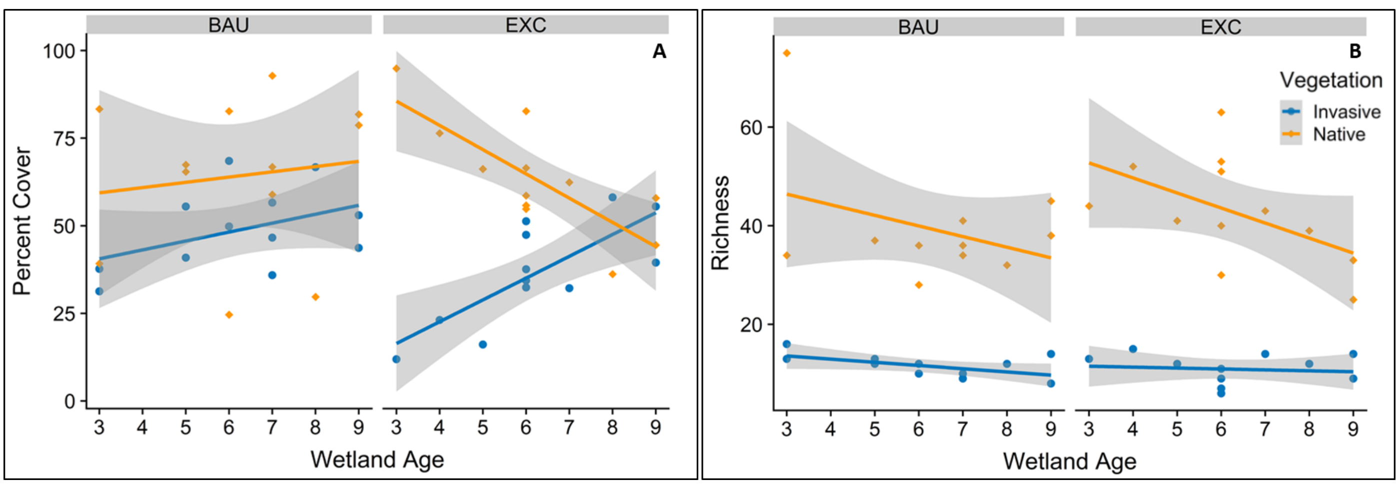


Figure 1.3: Vegetation survey percent cover (A) and species richness (B) over time since restoration (wetland age). Business as usual (BAU) and excavation (EXC) treatments are shown in each graph on the left and right panels, respectively. Invasive species data are shown in blue and native species are shown in gold. Grey shading indicates the 95% confidence interval.

Final Report Summary:

We examined the effects of sediment excavation (EXC) versus business as usual restoration that did not include excavation (BAU), wetland age since restoration, and hydroperiod (seasonal versus semi-permanent) on nutrient storage and removal in wetlands across west central Minnesota. We included wetland hydroperiod, because there appeared to be important differences in vegetation, water clarity, and water residence time among wetlands that dry periodically throughout the growing season (seasonal hydroperiod) and basins that dry out once every ten or twenty years (semi-permanent hydroperiod). In general soils at EXC wetlands had lower nitrogen (N) content (*P*=0.035), but higher rates of N accumulation over time (*P*=0.042; Appendix 1, Table 1). Excavated and BAU basins had different soil N dynamics over time (*P*=0.023), such that EXC basins tended to accumulate N, while BAU basins showed a small reduction in soil N as they aged (Figure 1.4). Statistical analysis revealed EXC wetland soil N content increases at approximately 0.018% or 180 ppm per year, while soils in BAU basins had a very small reduction (0.0015% or 15 ppm) in N content per year. Furthermore, nutrient availability decreased deeper in the soil profile (Appendix 1, Table 1). This suggests that N may be accumulating at different rates in EXC and BAU wetland soils. Collection of additional soil profiles could be used to generate site-specific N accumulation estimates which could allow more detailed evaluation of restoration treatments, hydroperiods, and time and better understanding of how different patterns in nutrient accumulation over time in EXC and BAU basins influence overall nutrient storage.

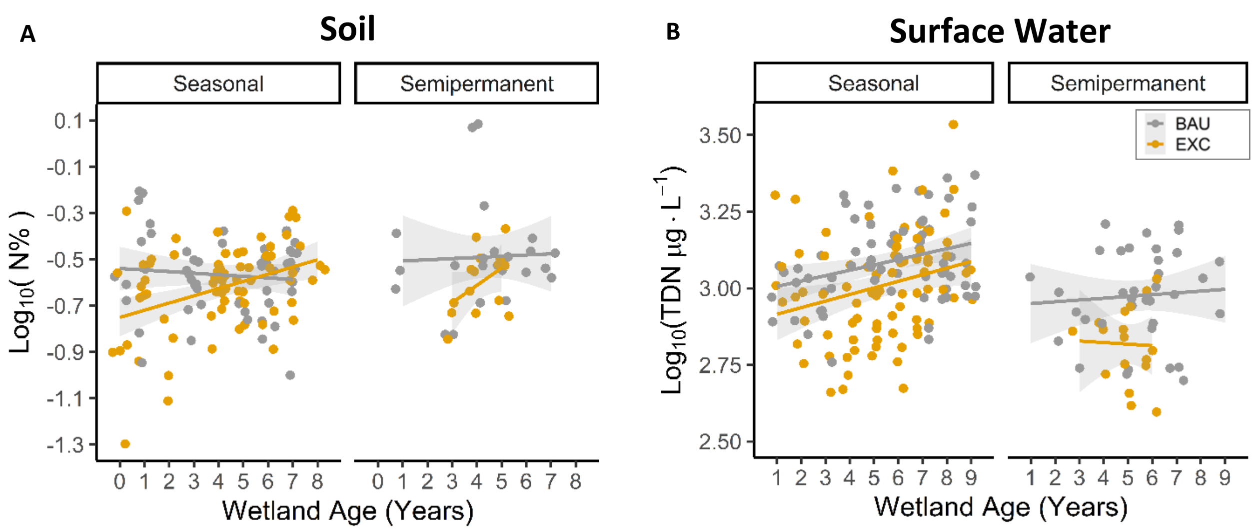


Figure 1.4: Soil percent nitrogen (A), and surface water total dissolved nitrogen (B) over time since restoration (wetland age). Seasonal and semipermanent hydroperiods are shown in each graph on the left and right panels, respectively. BAU wetlands are shown in grey and EXC wetlands are shown in gold. Grey shading indicates the 95% confidence interval of trend lines. All response data were Log transformed. Soil nitrogen content (left) was higher at BAU basins (Treatment, *P*=0.035) and increased over time (Wetland Age, *P*=0.042), but the trend of increasing nitrogen over time was driven by EXC sites, not by BAU wetlands (Treatment X Age interaction, *P*=0.023). Surface water total dissolved nitrogen (TDN, right) was significantly higher in BAU wetlands (Treatment, *P*=0.016), seasonally inundated basins (Hydroperiod, *P*<0.001), and increased over time (Wetland Age, *P*=0.0026).

Comparisons of spring surface water quality measurements between 2016 and 2018 revealed significantly lower dissolved N concentrations at EXC basins compared to BAU sites, and substantial increases in dissolved N over time (Appendix 1, Table 2). Total dissolved N (TDN), dissolved organic N (DON), and dissolved inorganic N (DIN) concentrations were all significantly lower at EXC wetlands compared to BAU basins (Appendix 1, Table 2). All dissolved N forms increased with wetland age (Appendix 1, Table 2), regardless of hydroperiod or excavation status (Figure 1.4) but these increases were small. Seasonally inundated wetlands had significantly higher TDN and DON concentrations compared to semi-permanent basins (*P*=0.00039 and 0.00018, respectively). Together, these results suggest that 1) Excavated wetlands have lower overall water column nitrogen, but 2) regardless of restoration technique, water column N concentrations increased at the same rate over time, and 3) seasonally inundated basins tend to have higher dissolved N availability than semipermanent basins. Overall, dissolved N in wetland basins was present at much lower levels comparted to agricultural runoff, with nitrate in particular present at extremely low concentrations.

Regardless of treatment, hydroperiod, or age since restoration, dissolved nitrogen was present at low levels that were nearly exclusively in organic form (Figure 1.5). This indicates strong demand for nitrate from assimilation and denitrification for all conditions examined, including cool spring and fall conditions. **This suggests that restored wetlands were strong sinks for inorganic nitrogen, consistent with denitrification**

results. We did not observe clear relationships between soil N content and surface water TDN patterns (Appendix 1, Figure 2) perhaps because TDN was relatively low, and dominated by organic N. However, N has many possible forms, sources, and fates, making it difficult to assess the extent to which surface water N dynamics reflect possible N storage in soils versus permanent nitrogen removal via denitrification. While surface water N concentration may reflect soil nutrient content, the extent of nutrient storage over time remains unclear because nitrogen is both assimilated by vegetation and removed via denitrification (see below). Future work should attempt to form a complete N budget for a small number of restored agricultural wetlands representing both BAU and EXC treatments and seasonal and semi-permanent hydroperiods. These analysis could help better understand the long term fate of nitrogen in wetlands, including potential production of the greenhouse gas N_2O, which can be a by-product of denitrification.

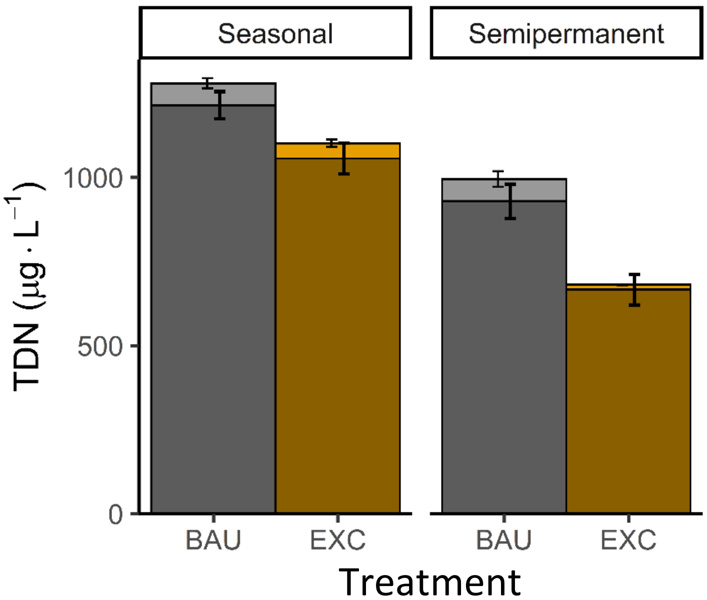


Figure 1.5: Surface water total dissolved nitrogen (TDN) concentration in micrograms per liter, as dissolved inorganic nitrogen (light colors, top) and dissolved organic nitrogen (dark colors, bottom). Seasonal wetland hydroperiod is shown on left and semipermanent on right with BAU wetlands in grey bars and EXC wetlands in gold. Error bars represent one standard error, with inorganic N errors represented by thin lines and organic N errors in bolded lines.

In addition to annual water quality monitoring, we gathered surface water data that directly demonstrate the value of restored wetlands for nitrogen remediation directly receiving agricultural runoff at short time scales. For example, in one semi-permanent basin we identified a drainage ditch flowing into the wetland from a row-crop agricultural field. Following major rainfall events in 2018, we sampled the drainage ditch inlet and we collected water samples along the likely flow path of water moving through the basin. Samples were collected the day after rainfall events and two to three days following rainfall events. *The wetland reduced nitrate-nitrogen (NO_3-N) concentrations by an average of 42.5 mg NO_3-N·L^{-1} (toxicity standard set by EPA is 10 mg NO_3-N·L^{-1}) to less than 0.005 mg NO_3-N·L^{-1} near the constructed spillway.* This illustrates the impact that restored geographically isolated wetlands can have in remediating agricultural runoff. *Strategically placed wetland restorations can protect surface and groundwater quality for down gradient communities*.

At a subset of 38 restored agricultural wetlands, we quantified potential nitrogen removal (e.g., denitrification) rates between 2017 and 2018, assessing the role of sediment excavation, hydroperiod, and wetland age since restoration on potential denitrification rates. Denitrification potential is the ability of the soils to remove nitrate under optimal conditions (e.g., unlimited NO_3^--N availability), and is a better predictor of a wetland's ability to process nitrate during periods of high availability in the spring and periodically throughout the growing season (i.e., after runoff events). Across all wetlands, we measured a mean potential denitrification rate of 18.62 mg N_2O-N · $m^{-2}hr^{-1}$. Excavation did not significantly influence potential denitrification rates, either positively or negatively (P=0.14). However, seasonally flooded wetlands removed N at a faster rate (22.1 mg N_2O-N · $m^{-2}hr^{-1}$) than basins with semi-permanent hydroperiods (11.5 mg N_2O-N · $m^{-2}hr^{-1}$; P=0.0039), and denitrification rates increased substantially with wetland age (P<0.0001; Figure 1.6). Functionally, seasonally flooded wetlands can process incoming nitrate at nearly twice the rate of semi-permanent wetlands, with mean daily denitrification rates of 530.39 and 275.65 mg N·m^{-2}, respectively. Seasonally flooded wetlands likely have higher background nitrate availability and a more robust community of microbes equipped to process N because of alternating oxygenated and anoxic conditions when soils are dry and wet, respectively. Older wetlands (more than five years old) had a greater capacity to remove nitrogen than younger wetlands (zero to five years post restoration), removing up to 132.37 mg N·m^{-2} more in older basins than in younger ones. Age-based differences in nitrogen removal are likely attributable to differences in organic carbon and nitrogen availability in soils and surface waters, resulting from decomposition of plant material.

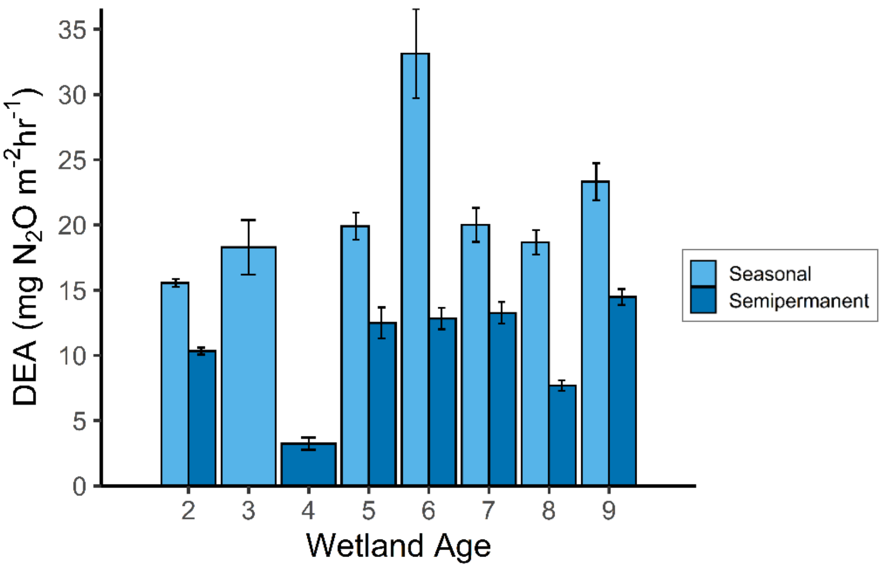


Figure 1.6: Denitrification potential rate (DEA) across all wetlands measured, over time (wetland age). Seasonal hydroperiod is shown in light blue, semipermanent in dark blue, ± SE. Seasonal wetlands had higher denitrification rates compared to semipermanent basins (P=0.0039). Older basins also had higher rates (P<0.0001).

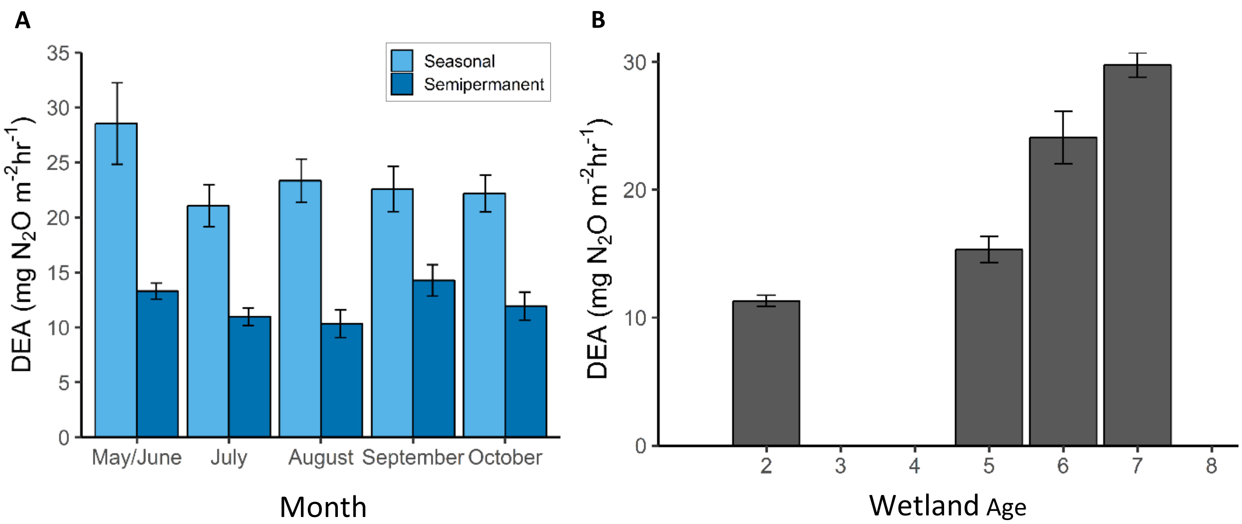


Figure 1.7: Denitrification potential rate (DEA) among 12 basins sampled monthly in 2017. Left panel (A) shows mean monthly rates for seasonal (light blue) and semipermanent (dark blue) hydroperiods. Right panel (B) shows mean rates by wetland age since restoration. Bars indicate standard error. Seasonal wetlands had higher denitrification rates compared to semipermanent basins (P=0.0031). Older basins also had higher rates (P=0.0001).

In 2017, we measured potential nitrogen removal rates for five consecutive months (June to October) at 12 wetlands in order to capture seasonal variability in potential denitrification rates. Potential nitrogen removal rates were highest in May and June, but the difference between denitrification rates across months was only marginally significant (P=0.07). As in the multi-year denitrification data set, seasonally flooded wetlands had higher potential nitrogen removal rates than semi-permanent basins (P=0.0031), and rates increased significantly as wetlands aged (P<0.0001; Figure 1.7). The differences in seasonal and semi-permanent wetland denitrification rates are likely rooted in a diverse microbial community equipped to respond to shifts between oxic and anoxic conditions suited to the many nitrogen transformations required for nitrification and denitrification, respectively. Although denitrification rates were higher in seasonally flooded basins, water is likely to have a longer residence time in wetlands with semi-permanent hydrology. As a result, nitrate concentrations should be similar at the time of export to downgradient systems, regardless of hydroperiod. *This suggests that semi-permanent wetlands with complex shorelines (e.g., large perimeter to surface area ratio) are likely to be particularly well-designed to capture and retain the large quantities of nitrogen runoff while rapidly quickly processing and removing nitrate. Furthermore, older, established wetlands should not be drained, since nitrogen removal rates are higher in older wetlands.*

We considered vegetation dynamics and nitrogen assimilation in EXC and BAU wetlands by constructing species specific cover-biomass relationships for 31 species commonly found in Minnesota's restored agricultural wetlands. We combined data from vegetation surveys performed at a subset of 24 wetlands (12 EXC and 12 BAU) with cover-biomass relationships to assess how much nutrient was stored within invasive and native species biomass at EXC and BAU sites. Results from our vegetation surveys revealed that excavation significantly improved plant biodiversity, by reducing the initial abundance of aggressive invasive species including hybrid cattail and reed canary grass. However, biodiversity benefits were lost within eight years following restoration (Figure 1.3). This sub-study suggests that *early intervention and invasive species management will likely maintain improved diversity and habitat quality, which should help waterfowl that tend to avoid dense monotypic stands of vegetation when nesting and rearing young*.

Excavated sites had lower plant biomass and assimilated N, but both metrics increased significantly as wetlands aged and plant communities reestablished following restoration. BAU wetlands had only marginally higher biomass than EXC basins (mean 4463 and 3308 $g \cdot m^{-2}$, respectively; P=0.073) but substantially more N per unit area (mean 53.5 and 36.6 $g\ N \cdot m^{-2}$, respectively; P=0.013). Older wetlands had significantly higher biomass (P=0.0022) and assimilated N (P=0.0023) than basins that were restored more recently (Figure 1.8), a trend that was driven by invasive species (P<0.0001 for biomass and N), particularly by hybrid cattail and reed canary grass. Meanwhile, native species biomass and assimilated N remained relatively unchanged over time. *On average, invasive species accounted for 85% (mean 3421 g·m^{-2}) of plant biomass and 86% (mean 39.7 g N·m^{-2}) of macrophyte assimilated N across all surveyed wetlands*. While some native species had higher N content per unit biomass than invasive species, aggressive invasive species, like hybrid cattail, had higher biomass per unit area than native species. Since native species productivity is particularly hampered when in direct competition with invasive species, it is unclear whether native species could achieve similar nutrient retention as their invasive counterparts. *Future studies should assess the efficacy of annual or semiannual invasive species harvest and removal as a management strategy, which may drive down overall nutrient availability to a level that is more favorable for native species*.

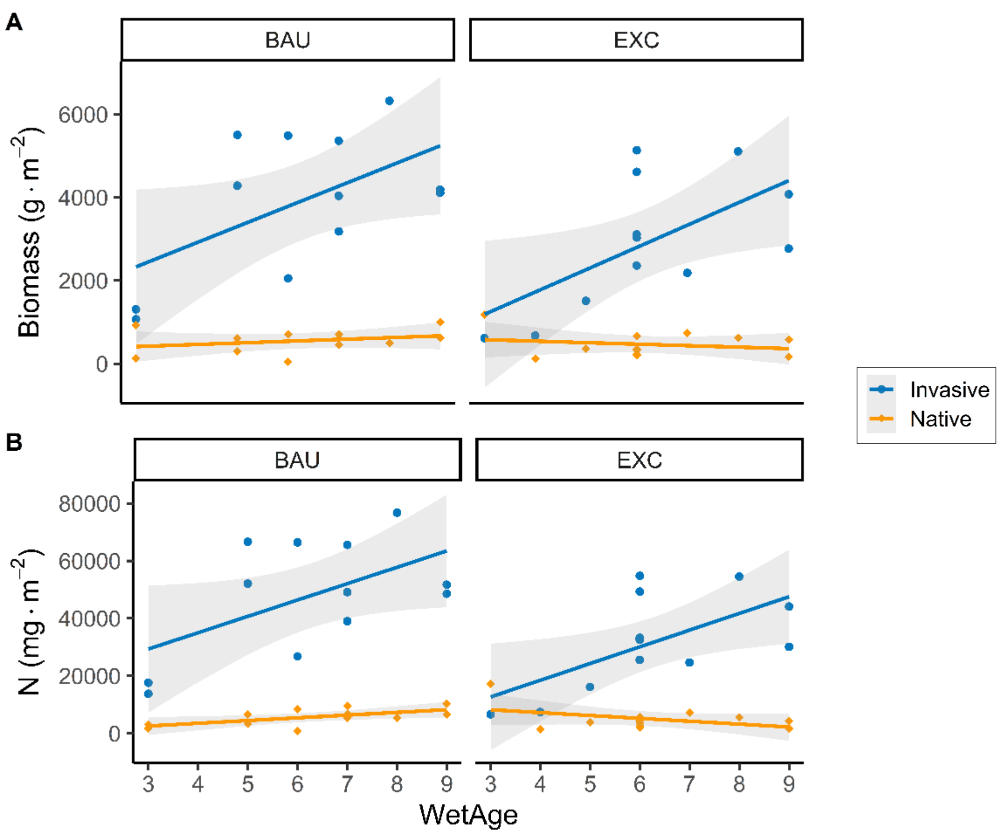


Figure 1.8: Plant biomass (A) and nitrogen (B) per square meter, as invasive species (blue) and native species (gold) over wetlands age three to nine years post-restoration (x-axis) in BAU and EXC wetlands (left and right, respectively). Grey shading indicates the 95% confidence interval. Native species biomass had significantly different trends between EXC and BAU wetlands (P=0.044), such that biomass increased over time at BAU basins and decreased over time at EXC basins. Invasive species biomass increased significantly over time (P=0.0036) and was marginally lower at Excavated wetlands compared to BAU basins (P=0.099). The trend of increasing invasive species biomass was driven by hybrid cattail and reed canary grass (P<0.001, not pictured). Invasive species N content in biomass was significantly lower in EXC basins (P=0.04) and increased with wetland age (P=0.0054). Native species N was influenced by the interaction between excavation and wetland age (P=0.013), such that nutrient content increased over time at BAU sites and decreased over time at EXC sites.

Last, we examined surface and groundwater interactions using a one-dimensional advection model informed by soil temperature gradients. This method was intended to identify where and when groundwater exchange occurs in wetland basins. We found that this technique was not reliable in shallow wetland water bodies with clay-rich substrate. Instead, we collected continuous water level data in 11 wetland basins, which we will analyze with evapotranspiration and rainfall estimates to model surface and groundwater exchange. Work on this aspect is ongoing and results will be shared when they are available.

Scaling-Up

In order to put our storage and removal rates into context, we compared our estimates for plant N assimilation, soil N storage, and denitrification with areal estimates of agricultural N runoff in Minnesota. We assumed that for a typical agricultural wetland the contributing watershed is dominated by row crop agriculture, however in our study a large portion of the contributing watersheds to all of our basins was enrolled in the conservation reserve program (CRP). Estimates for N runoff from a typical agricultural field are quite variable. For example, the University of Minnesota Extension suggests a N application rate of 18.35 g $N \cdot m^{-2}$ for corn and literature values indicate approximately 67% (here, 12.30 g $N \cdot m^{-2}$) of applied N fertilizer is exported without being assimilated by crops (Ruan and Johnson 1999), resulting in an estimated N export rate of approximately 12.30 g $N \cdot m^{-2} yr^{-1}$ from agricultural fields. The University of Minnesota Discovery Farms reports lower N runoff from Minnesota agricultural fields at 2.73 g $N \cdot m^{-2}$ in the 2017 growing season. We chose to use the discovery farm estimates, since this reflects real field conditions in a year that overlapped our study. In our study, the average wetland basin drains a watershed 31.2 times the wetted area of the wetland (median 8.0, range 3.1 to 478.5, SD 74.7). Accounting for the average ratio of watershed-to-wetland area, *we estimate that the contributing watersheds of typical agricultural wetlands in our study area could export approximately 85.13 g $N \cdot m^{-2} yr^{-1}$ to recipient basins, if the uplands were actively being cultivated*. We used this estimate of N export to examine how restored agricultural wetlands can improve water quality in Minnesota.

We found that restored agricultural wetlands are providing essential nitrogen storage and removal functions by accumulating nutrients in a variety of different pools including vegetation and soils while actively removing nitrogen from the surface water. Our measurements suggest that in BAU wetlands, roughly 47.8 and 5.7 g $N \cdot m^{-2} yr^{-1}$ are assimilated by invasive and native species, respectively. At EXC basins, approximately 31.6 and 5.0 g $N \cdot m^{-2} yr^{-1}$ are assimilated by invasive and native species. Based on estimates of N loading from an average agricultural watershed in Minnesota, we suggest that restored *BAU wetlands can assimilate approximately 62.8% of potential agricultural N runoff in plant tissues* (56.1 and 6.7% for invasive and native species, respectively). At *EXC wetlands, about 43% of the potential agricultural N load could be assimilated by plant tissues* (37.1 and 5.9% for invasive and native species, respectively). Potential nitrogen removal rates did not differ significantly between BAU and EXC wetlands, despite higher availability of soil N, which is typically a good indicator of denitrification rates and potentials. However, if we assume that wetlands have the potential to remove N at the mean potential denitrification rate of 18.62 mg $N_2O\text{-}N \cdot m^{-2} hr^{-1}$ (446.87 mg $N_2O\text{-}N \cdot m^{-2} d^{-1}$) between May and October (six months), then *we estimate the typical restored agricultural wetland can permanently remove about 81.55 g $N \cdot m^{-2}$ via denitrification during a six month growing season, or 95.8% of potential agricultural N load entering the basin*.

While it is challenging to calculate real N storage in soils over time without multiple measurements from a single site over time, we used a space for time substitution, estimating the annual change in total N in the top 30 cm of the soil profile by combining data from 55 wetlands that ranged in age from 0 to 8 years post restoration. We estimated that BAU wetlands lose N at approximately 15 $ppm \cdot yr^{-1}$ while soils in EXC wetlands accumulate N at a rate of about 180 $ppm \cdot yr^{-1}$. We translated our volumetric estimates of soil N storage to areal estimates, accounting for differences in soil bulk density at BAU and EXC basins. Our calculations show that BAU wetlands lose approximately 5.7 g $N \cdot m^{-2} yr^{-1}$ and soils in EXC basins accumulate roughly 70.5 g $N \cdot m^{-2} yr^{-1}$. We would expect soil N to accumulate over time as was the case in EXC basins. Estimated reductions in BAU soil N over time could result from higher rates of N assimilation by plants, removal by denitrification (but see below), translocation into the water column, or percolation into surrounding or underlying soils. It is potentially worth noting that the rate of N loss in BAU soils is roughly equivalent to the annual assimilation of N in native plant species. It remains unclear when or if soils in EXC basins become saturated with N, as appears to be the case in BAU wetland soils. Overall, *our estimates of N storage and removal suggest that restoring small geographically isolated agricultural wetlands provides important water quality benefits from assimilation, storage, and ultimately, permanent removal of N in runoff*.

ACTIVITY 2: Quantify phosphorus capture and burial in wetland basins

Description: Comparing phosphorus storage and removal in restored wetlands with and without accumulated sediment requires an understanding of the movement of nutrient between different pools. In order to evaluate phosphorus dynamics, we will consider 3 major processes and the associated factors that influence phosphorus retention in wetlands:

A. Phosphorus storage in wetland soils and sediments,
B. Phosphorus uptake by emergent macrophytes, and
C. Phosphorus transport from ponded surface water to the shallow aquifer.

We will use total and particulate phosphorus analyses to quantify phosphorus content in accumulated sediment and wetland soils. These values will be scaled to nutrient per volume of soil using measurements of soil bulk density. The nutrient content of soil can be used to calculate the quantity of nutrient contained in accumulated sediments removed during wetland restoration and the potential for nutrient storage in wetland basins.

To examine temporary nutrient storage in emergent macrophytes, we will measure phosphorus assimilation and fate in plant tissues. We will quantify temporary phosphorus storage in aboveground and belowground biomass using total and particulate phosphorus analyses. We will pair these measurements with traditional plant surveys and productivity measurements to calculate the nutrient storage by plants per unit area. In particular, we will compare the nutrient storage capacity of invasive with native plant species.

Phosphorus transport to shallow groundwater will be quantified by comparing measurements of nutrient concentration in surface and groundwater at periods of groundwater recharge. Soluble reactive phosphorus concentrations in water samples will be measured using the ascorbic acid method. Dissolved nutrient concentrations will be paired with sediment temperature profiles to estimate the quantity of nutrient flowing from surface to shallow groundwater supplies in wetland basins. We will compare nutrient export from wetlands in basins with and without accumulated sediment and basins with and without native wetland plant communities.

Summary Budget Information for Activity 2: **ENRTF Budget: $ 115,000**
Amount Spent: $ 115,000
Balance: - $ 0

Outcome	**Completion Date**
1. Measure nutrient concentrations in the accumulated sediment and soils from wetland basins with sediment removed, and evaluate phosphorus retention.	June 2017
2. Collect emergent macrophyte samples for nutrient analysis, and analyze nutrient content in aboveground and belowground biomass.	June 2019
3. Compare surface water and groundwater phosphorus concentration at wetlands with and without accumulated sediments and native plant communities.	June 2019

Activity Status as of December 30, 2016:

We focused on collecting soil samples surface water samples, and preliminary samples of below-ground plant biomass. Sample collection was as described in Activity 1. Preparation of samples for nitrogen and phosphorus analyses is similar, but the analyses are different. We are in the process of drying and sieving soil samples. Analyses for total and plant available phosphorus in soil samples will proceed this winter. Soil samples will then be analyzed for pH and plant available phosphorus at the University of Minnesota Soils Lab, and a subsample of sieved bulk soil will be ground into a fine powder for total phosphorus analysis.

Activity Status as of June 01, 2017:

We addressed soil phosphorus storage and surface water phosphorus content by preparing soils for phosphorus analysis and examining dissolved phosphorus species in surface water samples taken in 2016. We dried and sieved soils in order to prepare the samples for testing at the University of Minnesota Soils Lab. We submitted a subset of soil samples to the University of Minnesota Soils lab for pH testing and Bray-Olsen phosphorus analysis, traditional methods for measuring bioavailable soil phosphorus. These two methods are designed for soils with slightly different pH ranges and thus require a fairly accurate measure of soil pH prior to determining which method is most appropriate.

Through our collaboration with the Science Museum of Minnesota and the St. Croix Watershed Research Station, we measured dissolved inorganic phosphorus as soluble reactive phosphorus in surface and soil pore water samples using a SmartChem autoanalyzer. Later, we measured total phosphorous in surface water samples using benchtop colorimetric methods. Total phosphorus data was used in conjunction with the inorganic phosphorus data to calculate dissolved organic phosphorus.

Activity Status as of December 30, 2017:

To achieve Activity 2 outcomes, we focused on collecting surface water, vegetation, and additional soils samples for phosphorus content analysis. We collected soil cores from five additional wetlands. Three of the wetlands are immediately adjacent to existing sites, while two wetlands are located very near existing sites and helped to add more sites to our Pope county dataset. In all cases the wetlands add statistical power to the study and improve our sample size while remaining within the geographical range dictated by our study design. We divided dried and sieved soils from all subsections and cores, and we are planning to measure soil pH and Bray-Olsen phosphorus over the winter months. Likewise, sediment samples collected in conjunction with the denitrification portion of the study have been dried and sieved, and are awaiting further analysis.

Surface water samples were collected from all sites between May 30 and July 10, in order to compare nutrient content in wetlands early in the growing season, when groundwater recharge is particularly high. We also took surface water samples once per month at 12 wetlands in order to track seasonal changes in nutrient availability (outcome 3). In addition, we collected shallow-groundwater samples from piezometers put in place during fall of 2016. Measurement of soluble reactive phosphorus content is scheduled to begin in mid-December and conclude before February, while total dissolved phosphorous is scheduled to begin later in the winter.

Finally, we addressed outcome 2 by collecting standing biomass of emergent vegetation at two times; during peak above-ground biomass, and again as plants began to go dormant in the fall. All samples were dried and weighed, and are currently being ground for total phosphorus analysis. In addition to above-ground biomass, we sampled below-ground biomass during peak plant growth. Below-ground biomass was carefully sorted from detritus, and samples were dried, weighed, and are currently being ground to a fine powder for further analysis.

Activity Status as of June 30, 2018:

Surface water samples were collected between May 14 and June 14. A second round of surface water samples is being collected for a subset of sites between June 21 and July 19 to assess the seasonal shifts in surface water nutrient content. We collected shallow-groundwater samples in June 2018 from piezometers put in place during fall of 2016. We will collect groundwater samples periodically throughout the summer. Particulate phosphorus from surface water was collected for 2018 spring samples and is currently being processed from spring 2017.

We measured soil phosphorus content in soils collected in 2017. Analyses of the interannual and spatial variability in soil phosphorus content will be conducted later this year following the end of field research.

Phosphorus content was measured in emergent macrophyte biomass collected in 2017. Biomass samples were collected during peak above-ground biomass, and again as plants began to go dormant in the fall. All samples were dried, weighed, and ground to a fine powder using a Wiley Mill for subsequent total phosphorus analysis. Data analysis will continue following completion of the 2018 field season.

Characterization of hydrologic response to climate variation in restored wetlands is ongoing. We deployed water level loggers for a second year at a select group of sites located in Ottertail, Grant, and Douglas counties. The loggers will continue to measure water level every hour from May through the fall. We are using data available online to help construct weather events across the focal watersheds and are working towards building a watershed model to explain what proportion of surface water level fluctuation can be explained by evapotranspiration versus groundwater interaction.

Activity Status as of December 28, 2018:

Our analysis of soil and surface waters thus far has revealed important information regarding the value of excavation. Specifically, we found that *excavating accumulated sediment during wetland restoration removed significant quantities of bioavailable (Bray-Olsen) phosphorus from the soil* (Figure 2.1). We also found that wetlands with different hydrological conditions (Seasonal and Semipermanent) had different magnitudes of response to excavation. Seasonal wetlands tended to have more phosphorus in soils, and excavation did not remove as much phosphorus from the bioavailable pool compared to semipermanent wetlands, despite similar amounts of overall sediment removal. Semipermanent wetlands had less bioavailable phosphorus in the soils and had a much larger response to excavation (Figure 2.1). These results may indicate that seasonal wetlands are better at storing phosphorus in forms that are not easily bioavailable compared to semipermanent wetlands, but more research is needed to understand why a larger pool of bioavailable phosphorus remains in excavated seasonal wetlands.

Soil and surface water phosphorus availability had similar trends. Total dissolved phosphorus (TDP), dissolved organic phosphorus (DOP), and soluble reactive phosphorus (SRP) concentrations were all significantly lower at excavated sites than business as usual sites (Figure 2.2, Table 1.1). Seasonal wetlands had much higher TDP and SRP concentrations, indicating that the majority of phosphorus dissolved in the water column was in the inorganic (soluble reactive) form. Meanwhile, semipermanent wetlands had a larger proportion of organic phosphorus in the water column, which is less bioavailable than the inorganic form. These results may reflect higher stability of phosphorus on the landscape due to assimilation by vegetation and sorption to periodically oxygenated soils in seasonal wetlands. Alternatively, these results may be telling us more about semipermanent wetlands. Perennially low oxygen availability near the sediment-water interface in semipermanent wetlands could result in the consistent movement of inorganic phosphorus out of wetlands and into the surrounding uplands and groundwater, which could lead to lower phosphorus availability in soils and surface water. Understanding why phosphorus concentrations are lower in semipermanent wetlands and higher in seasonal wetlands can help us better understand whether seasonal or semipermanent wetlands are better equipped to store phosphorus on the landscape and away from our waterways where it can contribute to eutrophication.

We are preparing to analyze surface water dissolved phosphorus concentrations from this summer's sampling effort, and we are nearly finished analyzing total dissolved phosphorus in surface waters. We have submitted soil samples for total phosphorus analysis at the University of Minnesota Research Analytical Lab located St. Paul, MN. These data will help us further interpret results of bioavailable P analyses and decipher which wetlands are best situated to capture and store phosphorus on the landscape, thus decreasing the risk of further eutrophication in down-gradient lakes and streams.

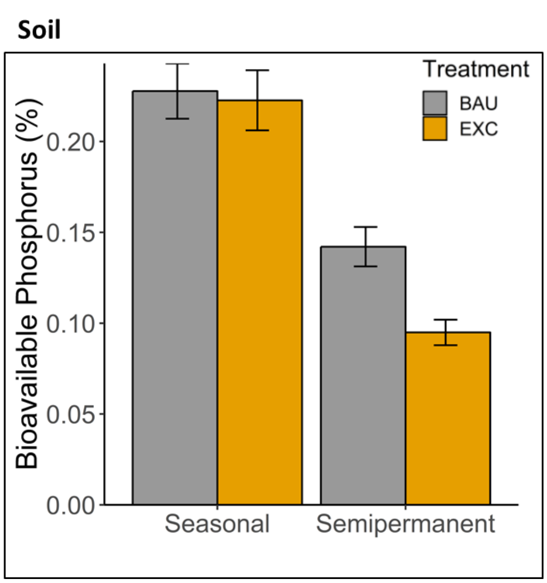


Figure 2.1: Soil bioavailable (Bray-Olsen) phosphorus (percent) in seasonal and semipermanent wetlands. Excavated sites had significantly lower phosphorus ($p=0.0409$), and semipermanent wetlands had significantly less phosphorus than seasonal wetlands ($p<0.001$).

Surface Water: 2016

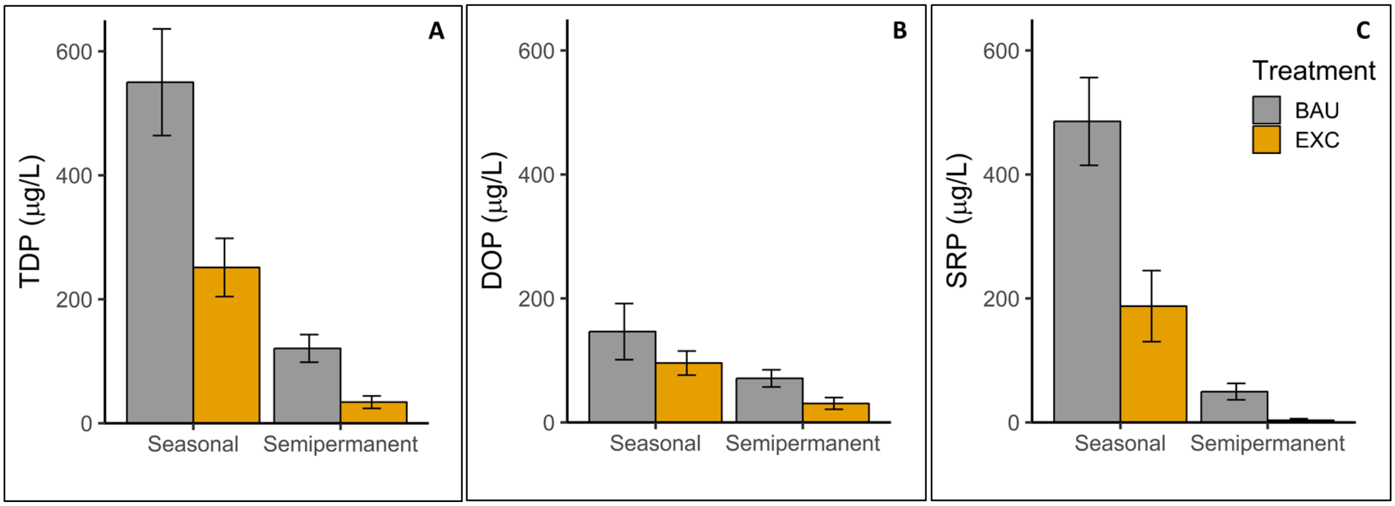


Figure 2.2: Surface water, A) total dissolved phosphorus (TDP), B) dissolved organic phosphorus (DOP), and C) soluble reactive phosphorus (SRP) concentration (micrograms per liter) in seasonal and semipermanent wetlands in the 2016 growing season.

Final Report Summary:

Minnesota waters remain threatened by phosphorus (P) pollution. Permanent P storage is a critical service provided by wetlands. Agricultural wetland restoration may play a key role in P remediation by capturing and storing phosphorus in soils and vegetation prior to export to larger, flow-through systems. For example, in one wetland we identified a culvert carrying water from a drainage ditch adjacent to a large agricultural field. On four dates we sampled where the culvert delivered water into the wetland and we sampled at three points along the likely flow path of water through the basin. The wetland reduced total dissolved P concentrations from 318 µg $P \cdot L^{-1}$ at the inlet to 166 µg $P \cdot L^{-1}$ near the constructed spillway. The ability of wetlands to capture and store P in soils and plant biomass are topics of considerable importance since, unlike nitrogen, P cannot be permanently removed by microbial processes, burial (or recycling) are the only effective long-term solutions for P pollution.

Soil bioavailable P concentrations were significantly influenced by wetland hydroperiod and age since restoration, but not by excavation (Appendix 1, Table 1). In a previous report where we did not include wetland age in our soil models, we found that soil P availability was lower in EXC wetlands and in semipermanent basins (Figure 2.1) compared to BAU and seasonal wetlands, respectively. When we added wetland age to the model, we found that semipermanent basins still had lower overall P availability, but wetland age was a more important predictor of soil P availability than excavation (Figure 2.3). Overall, wetlands accumulated P as they aged, such that older wetlands had significantly higher soil P availability and there did not appear to be a saturation point over the age range represented (zero to eight years post-restoration). Seasonal wetlands had larger pools of available P and they generally had higher soil P availability with increasing age; meanwhile soil P availability was lower in semipermanent basins and did not appear to increase as wetlands aged (Figure 2.3), but statistical analyses did not reveal a meaningful differences between P availability in BAU and EXC wetlands as they aged (Appendix Table 1, P=0.52). Instead, statistical analyses revealed significant overall increases in soil P availability over time (P=0.024) and higher P content in seasonally inundated basins (P=0.0087). Predictive analysis revealed that wetlands generally had approximately 0.77 ppm more bioavailable P each year, regardless of hydroperiod or treatment. However, seasonally inundated basins had about 3.18 ppm higher P availability than semipermanent wetlands.

We performed analyses for total soil P on a subset of soils due to substantial analysis expense and limited sample availability. Despite small sample size, we identified trends in total soil P content, including significantly lower total P at EXC sites (P=0.011, Figure 2.4). Our results also show trends that total soil P accumulates over time at EXC sites but may not accumulate at BAU sites (Figure 2.4), though wetland age did not significantly influence total P in the subset of soils we analyzed (P=0.14). In order to fully assess the interaction between wetland age and hydroperiod on bioavailable P, we would need an additional collection of soil core samples from all of the study wetlands. Additional soil cores would also provide the material required to fully assess how total soil P changes over time in BAU and EXC basins.

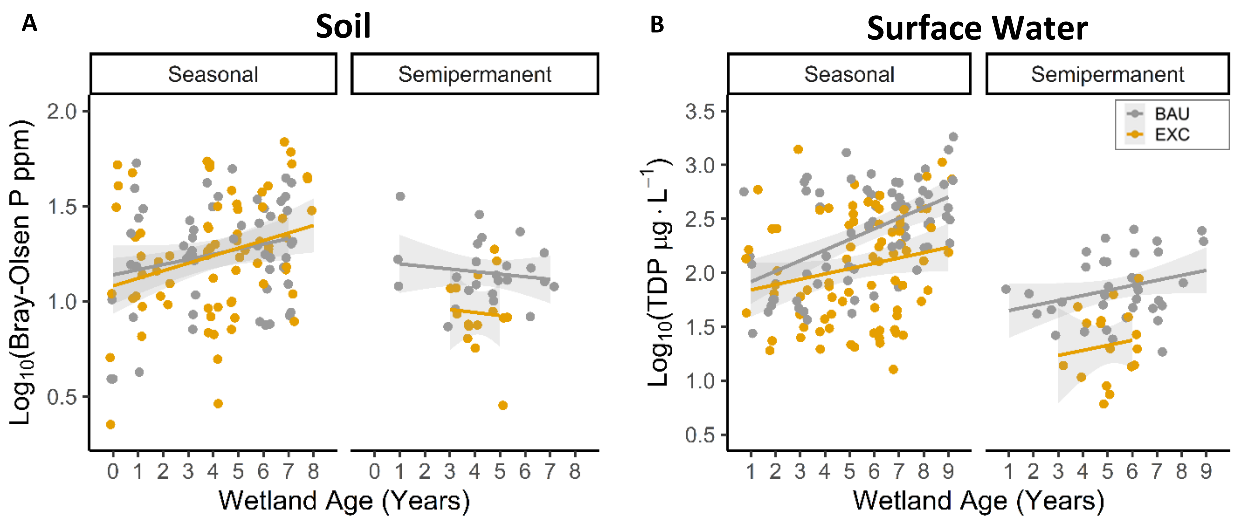


Figure 2.3: Soil phosphorus (A), and surface water total dissolved phosphorus (B) over time since restoration (wetland age). Seasonal and semipermanent hydroperiods are shown in each graph on the left and right panels, respectively. BAU wetlands are shown in grey and EXC wetlands are shown in gold. Grey shading indicates the 95% confidence interval of trend lines. All response data were log transformed. Soil phosphorus content (left) was higher at seasonally flooded basins (Hydroperiod, P=0.0087) and increased over time (Wetland Age, P=0.024). Surface water total dissolved phosphorus (TDP, right) was significantly higher in BAU wetlands (Treatment, P=0.0019), seasonally inundated basins (Hydroperiod, P<0.001), and increased over time (Wetland Age, P<0.001).

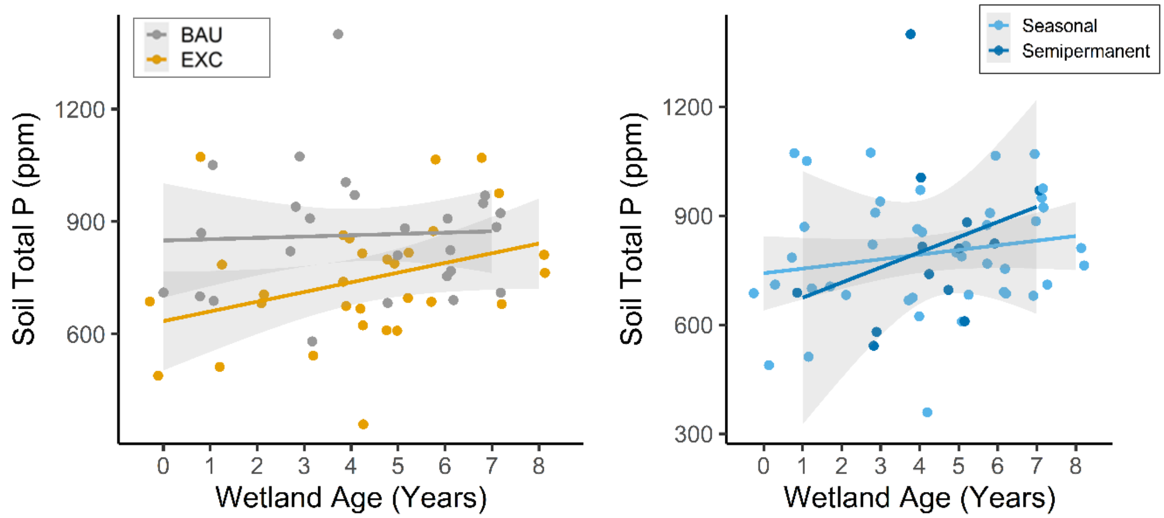


Figure 2.4: Soil total phosphorus over time since restoration (wetland age). BAU wetlands are shown in grey and EXC wetlands are shown in gold in the left panel. Differences in hydroperiod (right) were not statistically significant (P=0.89) for Seasonal (light blue) or semipermanent (dark blue) hydroperiods. Grey shading indicates the 95% confidence interval of trend lines. Total soil phosphorus content was higher at BAU basins (P=0.011). Wetland age did not have a significant influence on soil total phosphorus (P=0.14).

Surface water phosphorus content reflected differences in soil nutrient availability (Figure 2.3; 2.5), such that seasonal wetlands had higher overall P concentrations than semipermanent basins. However, surface water quality also showed significantly lower total dissolved P (TDP) and soluble reactive P (SRP) at EXC wetlands compared to BAU basins (Appendix 1, Table 2), a trend that was not statistically significant in the soil dataset.

Total dissolved P and SRP concentrations were also demonstrably higher in older wetlands, indicating that wetlands accumulate P as they age (Figure 2.6). This suggests that excavation significantly reduces overall P availability in wetland basins and these wetlands capture and store additional P input from the surrounding landscape as they age. However, full verification of this trend requires a small follow-up study to elucidate whether reductions in dissolved P at EXC wetlands reflect meaningful differences in soil P. Similar trends in surface water and soil P availability have been observed elsewhere in agricultural watersheds (Sharpley et al. 1996). While we found a strong link between soil and surface water P, it remains unclear why our soils models did not reveal a significant excavation effect, but the surface waters were significantly influenced by excavation. A future study may find more meaningful results using P fractionation methods that can quantify the form that P is stored in, which appears important in determining P fate.

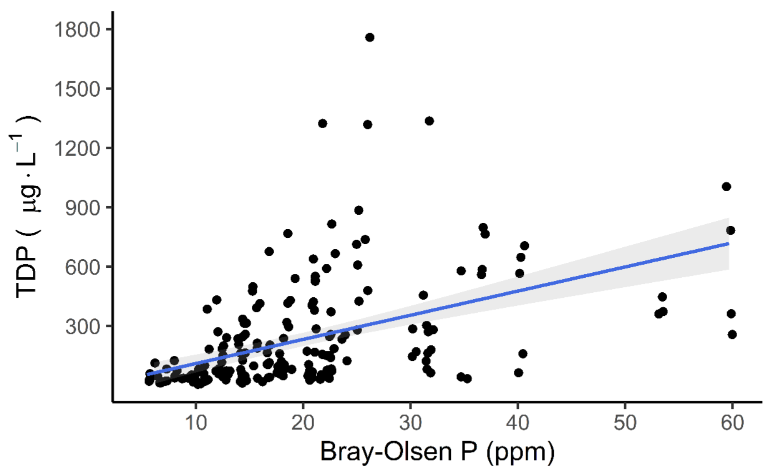


Figure 2.5: Relationship between soil phosphorus availability and TDP in the water column for all sites. Line indicates single linear regression fit.

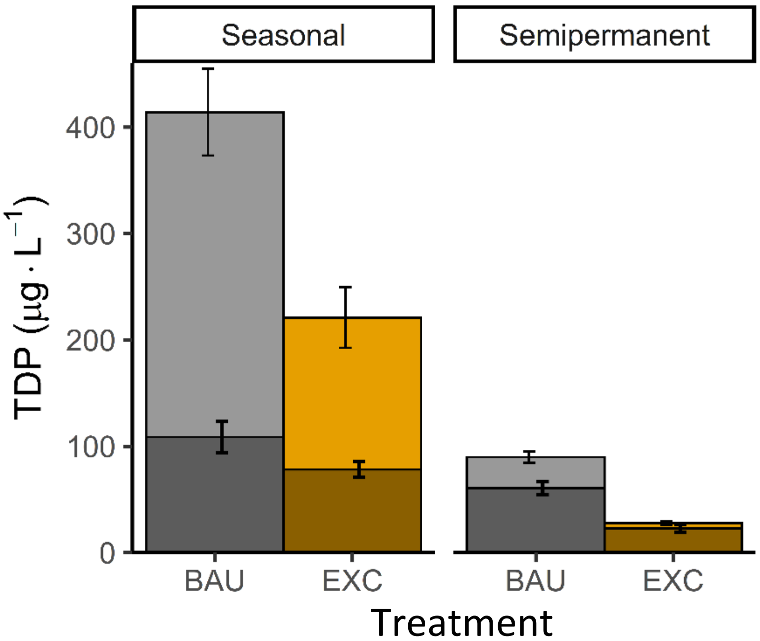


Figure 2.6: Surface water total dissolved phosphorus (TDP) concentration in micrograms per liter, as dissolved inorganic phosphorus (light colors, top) and dissolved organic phosphorus (dark colors, bottom). Seasonal wetland hydroperiod is shown on left and semipermanent on right with BAU wetlands in grey bars and EXC wetlands in gold. Error bars represent one standard error, with inorganic errors represented by thin

Vegetation assimilated more P at BAU wetlands compared to EXC basins. Similar to the N results, we found that BAU wetlands had significantly higher plant P content per unit area than EXC wetlands (Figure 2.7). This trend was driven by higher biomass accumulated by invasive species, particularly hybrid cattail, which constituted over 73% of P stored in vegetation. On average, invasive species in BAU wetlands contained about 6.9 g $P{\cdot}m^{-2}$ while at EXC sites invasives stored approximately 4.3 g $P{\cdot}m^{-2}$. This translates to approximately 28 and 17 kg $P{\cdot}acre^{-1}$ at BAU and EXC sites, respectively. Since P is largely removed from systems via physical methods, future studies should assess the efficacy of annual or semiannual invasive species harvest and removal to drive down overall P availability to a level that is more favorable for native rather than invasive species.

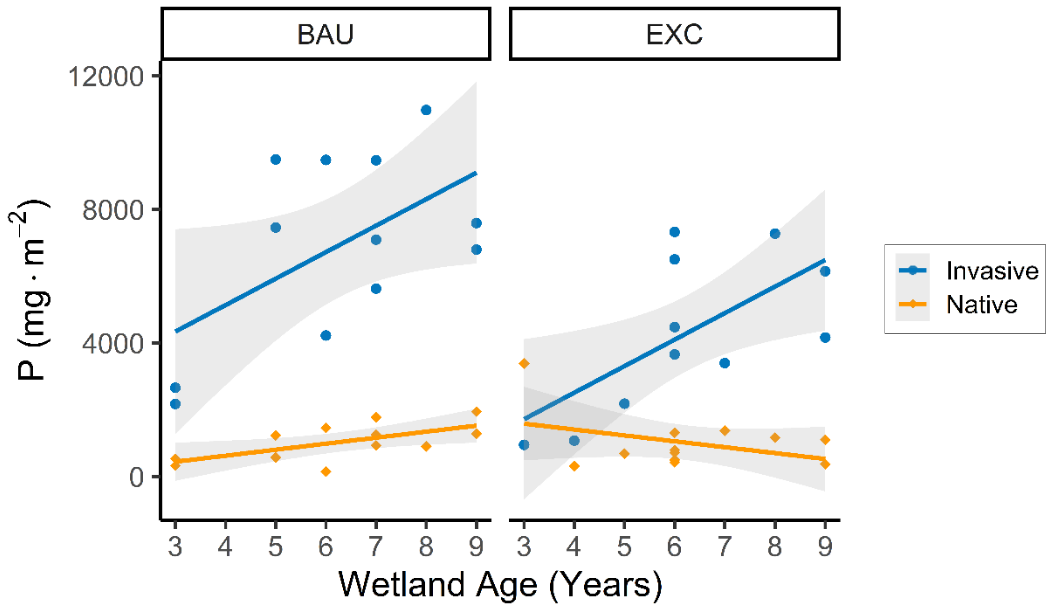


Figure 2.7: Plant phosphorus (P) per square meter, as invasive species (blue) and native species (gold) over wetlands age three to nine years post-restoration (x-axis) in BAU and EXC wetlands (left and right, respectively). Grey shading indicates the 95% confidence interval. Invasive species P content was significantly lower in EXC basins (P=0.016) and increased with wetland age (P=0.0046). Native species P was influenced by the interaction between excavation and wetland age (P=0.017), such that nutrient content increased over time at BAU sites and decreased over time at EXC.

Scaling-Up

As with N, we put our P assimilation and storage measurements into a broader context of agricultural wetland restoration and ecosystem services. We used estimates of P runoff from University of Minnesota Discovery Farms 2016 annual report (0.074 g P·m^{-2}yr^{-1}). We scaled the 2016 estimate for annual agricultural P runoff for average wetland-to-watershed area for our study, *resulting in an estimated delivery of 2.31 g P·m^{-2}yr^{-1} from contributing watersheds of typical agricultural wetlands in our study area*. We used this estimate of P export to examine how restored agricultural wetlands can improve water quality in Minnesota by assimilating and storing P. Our measurements suggest that at BAU wetlands, roughly 6.9 and 1.0 g P·m^{-2}yr^{-1} are assimilated annually by invasive and native species, respectively; At EXC basins approximately 4.3 and 1.0 g P·m^{-2}yr^{-1} are assimilated by invasive and native species. Based on estimates of P loading from an average agricultural watershed in Minnesota, we suggest that restored *wetlands can assimilate more than 100% of potential agricultural P runoff in plant tissues, regardless of BAU or EXC treatment*. Unlike N, P is not readily removed from systems; rather, it must be stored permanently either in soils, organic matter, continually recycled by plants, or it is exported down gradient. We considered soil P storage by estimating the annual change in bioavailable P (e.g., Bray-Olsen P) in the top 30 cm of the soil profile by combining data from 55 wetlands that ranged in age from 0 to 8 years post restoration. We estimated that restored agricultural wetland soils accumulate approximately 0.30 g P·m^{-2}yr^{-1}, regardless of restoration technique. Bioavailable P accumulation in soils only accounts for roughly 13% of annual agricultural P loading to wetlands, suggesting that P is likely being stored in some other soil P pool such as organically or minerally bound P. Alternatively, P may be transported down gradient to surface waters. Further investigations into the form of P in soils and how that changes over time are required in order to identify the fate of P entering agricultural wetland basins.

ACTIVITY 3: Dissemination of results

Description: We will report our results to the public through several avenues. We will participate in outreach events at the West Central Research and Outreach Center in Morris, MN. In addition, we will create a fact sheet for distribution through the University of Minnesota Extension service. In addition, we will submit manuscripts reporting our results to high impact peer reviewed journals. Finally, we will provide a comprehensive report of our results and analyses to the LCCMR.

Summary Budget Information for Activity 3:	**ENRTF Budget:**	**$ 1,000**
	Amount Spent:	**$ 1,000**
	Balance:	**$ 0**

Outcome	**Completion Date**
1. West Central Research and Outreach Center presentation.	June 2019
2. Manuscript preparation for publication in notable peer reviewed journals, at least 1 informational flyer, and a final report of results.	June 2019
3. Poster presentations by undergraduate students.	May 2019

Activity Status as of December 30, 2016: No progress has been made on this activity.

Activity Status as of June 01, 2017:

We explored the data for preliminary trends and differences between wetlands with and without sediment excavation, including wetland type (seasonal versus semipermanent) as an additional explanatory variable in our model design. We shared our preliminary results with policy makers and representatives from a handful of Minnesota agencies in a poster session at the University of Minnesota. The "Shared Water, Shared Responsibility: Engaging Minnesota's Communities, Students, & Policy-Makers" event was held at the Hubert H. Humphrey School of Public Affairs on Thursday, March 23rd as part of Governor Dayton's Year of Water Action (https://www.wrc.umn.edu/news-events/yowa/yowa-student-event).

Activity Status as of December 30, 2017:

Our outreach activities have been focused on sharing our preliminary results with our partners at the United States Fish and Wildlife Service, Minnesota Private Lands Office. These land managers work cooperatively with private land owners to restore agricultural wetlands across the state of Minnesota and work closely with other land management groups including Pheasants Forever and the Minnesota Department of Natural Resources. We have also printed a poster for display at the Fergus Falls Wetland Management District Office, where land owners and the public can view the results of our research. The poster summarizes our preliminary results and will be one of many that we hope to produce as the project continues. Future posters will be distributed to the Minnesota Private Lands Office and other wetland management district offices located across the study area.

Activity Status as of June 30, 2018:

We have been engaged in multiple outreach activities since January 2018, including research updates and presentations to land management groups across the state and educational outreach to children and families. We presented preliminary results at The Wildlife Society of Minnesota annual meeting, where representatives from natural resource management groups from across the state meet to share information, ideas, and research results. The land managers at the conference showed great interest in our research and encouraged us to continue sharing our results whenever possible. In response, we shared our preliminary findings to the Pomme de Terre Watershed Task Force, a collaboration of representatives from soil and water conservation districts, Board of Water and Soil Resources, Minnesota DNR, and Minnesota Department of Agriculture all working under the One Watershed One Plan model of watershed management. In addition, we met with representatives from the Minnesota DNR on two separate occasions. First, we met with Restoration Evaluation Specialists at the Minnesota DNR Division of Ecological and Water Resources. Separately, we participated in a meeting of wetland researchers from across the state at the Mini Minnesota Wetland Research Round-up hosted at the Minnesota DNR headquarters on May 3. Finally, on June 14th, we presented our preliminary results in a meeting of Minnesota private lands managers and conservation specialists including The Nature Conservancy, the US Fish and Wildlife Service, Minnesota Department of Natural Resources, Minnesota Land Trust, Ducks Unlimited, US Department of Agriculture, and more groups.

We have also focused on outreach to children and families. Working with the Prairie Wetlands Learning Center (Fergus Falls, MN) and the University of Minnesota Extension office, we participated in the second annual Aqua Chautauqua (June 23, 2018), a street festival celebrating Minnesota's water resources. Our learning station provided the opportunity for children and adults to conduct their own experiments demonstrating how wetlands remove and neutralize pollutants to keep our water clean. We inspired questions and exploration using demonstrations and games to illustrate how wetlands clean water and participate in water storage on the landscape. In addition, we provided handouts with ideas for fun and easy summer activities for kids and adults to learn more about wetlands. Informal feedback from the event suggested that many parents and even adults without young children were excited to try these activities at home and explore their local aquatic habitats. We had between 50 and 100 people visit our station during the 3-hour event. More outreach events and activities are planned for July and August.

Activity Status as of December, 2018:

During summer 2018, we engaged in community outreach geared towards children, families, and teachers. Specifically, we worked with the West Ottertail County Fair and the US Fish and Wildlife Service to set up a wetlands learning station at the West Ottertail County Fair. We selected the outreach activities that were the most effective at the Aqua Chautauqua event and invested more time and energy in perfecting those activities, including creating larger displays and generating more coloring pages and backyard science pages. We were pleased to receive foot traffic from children and families, but also from elderly residents, and residents without children who were interested in wetland restoration and conservation efforts. Our displays generated conversations with residents from across West Central Minnesota (e.g., Ottertail, Grant, and Pope counties), and illustrated how much Minnesotans care about wetland and shoreline restoration.

After the county fair, a local elementary school teacher asked to use our outreach materials in the classroom stating, "I have been searching for a way to teach my kids about how wetlands clean water, these activities are perfect and really show concepts that are difficult to explain". In a different interaction a parent told us, "The kids love these things! I guess I need to make the kids a wetland board for their birthdays". Through our experiences at Aqua Chautauqua and collaboration with the US Fish and Wildlife Service, we identified a few strategies that help engage kids and families with the wetlands they see every day and we hope to use what we have learned to generate new outreach activities for future events.

Figure 3.1: Image from the West Ottertail County Fair. We made wetland boards (left) which are a spin on plinko boards, where marbles are released through a matrix of pegs of varying densities. In this case, the marbles represented water and the pegs represented wetlands on the landscape. Wetland boards were used to demonstrate how wetlands retain water on the landscape. We used a simple filtration experiment (center) to demonstrate how holding water on the landscape longer helps to clean out pollutants. Finally, we had activity and coloring pages available for kids and families to take home and explore the wetlands in their own backyard. By the end of the fair we had distributed more than 500 activity and coloring sheets, and interacted with hundreds of people.

Final Report Summary:

We developed a set of interactive tools to start conversations about wetlands with children and adults. Using visual aids, hands-on activities, and activity handouts we were able to reach hundreds of people in the summer of 2018 at the West Ottertail County Fair and the Fergus Falls Aqua Chautauqua by focusing on exploration and discovery in our backyard wetlands. These activities were designed, beta tested, and implemented with help from two of the eight undergraduate students and one masters student who have worked on this project. Our activities and handouts are still being used by environmental and K-12 educators in the Ottertail Public School District. Please see Appendix 2 for examples of outreach materials developed by our team and others.

V. DISSEMINATION:

Description: We will submit our results for publication in peer-reviewed journals, and we will present our work at state meetings (e.g., Minnesota Chapter of the Wildlife Society, Minnesota Water Resources Conference) frequented by natural resource management officials. Through our close collaboration with the United States Fish and Wildlife Service, we will inform current management practices in Minnesota. In addition, we will foster relationships with the Minnesota Department of Natural Resources and the Minnesota Pollution Control Agency. We will work with the West Central Research and Outreach Center, affiliated with the University of Minnesota, to present our results to the public and provide informational flyers about wetlands in water quality. Finally, we will provide a comprehensive report of results and analyses from this project to the LCCMR by June 2019.

Status as of December 30, 2016:
In December 2016, we presented preliminary results and observations to biologists from wetland management districts participating in this research project. Most of the biologists were from the U.S. Fish and Wildlife Service, but there were also representatives from Minnesota Department of Natural Resources and Pheasants Forever. The meeting was designed to evaluate the status of the project and discuss directions for further inquiry. In order to prepare public outreach opportunities in the following years, we have contacted a biology professor (name would be good to provide) at the University of Minnesota, Morris, to schedule a seminar outlining our research and preliminary findings.

Status as of June 01, 2017:
We shared our preliminary results with policy makers and representatives from a handful of Minnesota agencies in a poster session at the University of Minnesota. The "Shared Water, Shared Responsibility: Engaging Minnesota's Communities, Students, & Policy-Makers" event was held at the Hubert H. Humphrey School of Public Affairs on Thursday, March 23rd as part of Governor Dayton's Year of Water Action (https://www.wrc.umn.edu/news-events/yowa/yowa-student-event). Several representatives from Minnesota Department of Agriculture, Minnesota Department of Pollution Control, and Minnesota Department of Natural Resources and other state agencies were present at the event.

Status as of December 26, 2017:
Our outreach activities have been focused on sharing our preliminary results with our partners at the United States Fish and Wildlife Service, Minnesota Private Lands Office. These land managers work cooperatively with private land owners to restore agricultural wetlands across the state of Minnesota and work closely with other land management groups including Pheasants Forever and the Minnesota Department of Natural Resources. We have also printed a poster for display at the Fergus Falls Wetland Management District Office, where land owners and the public can view the results of our research. The poster summarizes our preliminary results and will be one of many that we hope to produce as the project continues. Future posters will be distributed to the Minnesota Private Lands Office and other wetland management district offices located across the study area.

Status as of June 30, 2018:
We shared preliminary results with Restoration Evaluation Specialists at the Minnesota DNR Division of Ecological and Water Resources and with wetland researchers from across the state at the Mini Minnesota Wetland Research Round-up hosted at the Minnesota DNR headquarters on May 3. On June 14th, we presented our preliminary results in a meeting of Minnesota private lands managers and conservation specialists including The Nature Conservancy, the US Fish and Wildlife Service, Minnesota Department of Natural Resources, Minnesota Land Trust, Ducks Unlimited, US Department of Agriculture, and more groups. Finally, on June 23rd, we participated in the Fergus Falls Aqua Chautauqua, a street festival celebrating water. Our learning station

provided the opportunity for children and adults to conduct their own experiments demonstrating how wetlands remove and neutralize pollutants to keep our water clean.

Status as of December 28, 2018:

We reported our preliminary results from the vegetation surveys at 24 wetlands (12 Business As Usual, 12 Excavated) at the joint meeting of the North American Invasive Species Management Association and the Upper Midwest Invasive Species Conference in Rochester, MN. The audience included city, county, state, and tribal representatives from Minnesota, Iowa, Missouri, Nebraska, Wisconsin, and Michigan. We also presented our preliminary results to The Nature Conservancy (TNC) who are involved in wetland restorations in central and west central Minnesota. Specifically, TNC is hoping to find ways to identify high priority wetlands for restoration or wetlands that are likely to respond well to restoration activities.

We presented our preliminary findings to our collaborators at the US Fish and Wildlife Service as well, and are continuing our close collaboration with them, while establishing new collaborations with the Minnesota DNR wetland research group. Our public outreach activities at the West Ottertail County Fair were also done in collaboration with the US Fish and Wildlife Service. This outreach was well received by adults and children because we focused on engaging activities and eye-catching visual aids.

Final Report Summary:

Results from this project have been presented at national, regional, and state meetings and events including; the Society for Freshwater Sciences annual conference (May 2019), the Society for Wetland Sciences annual conference (May 2019), the Minnesota Chapter of the Wildlife Society annual conference (February 2017 and February 2018), the joint meeting of the Upper Midwest Invasive Species Conference and North American Invasive Species Management Association (October 2018), and a meeting of Minnesota private lands managers and conservation specialists including The Nature Conservancy, the US Fish and Wildlife Service, Minnesota Department of Natural Resources, Minnesota Land Trust, Ducks Unlimited, and the U.S. Department of Agriculture (June 2018). We also shared our research with local entities including, the University of Minnesota's Shared Water, Shared Responsibility: Engaging Minnesota's Communities, Students, & Policy-Makers event (March 2017), the Water Resources Science Spring Research Symposium (January 2018), the Pomme de Terre Watershed Task Force (May 2018), Restoration Evaluation Specialists at the Minnesota DNR Division of Ecological and Water Resources (March 2018), and The Nature Conservancy (August 2018), and we continue to share results with stakeholders and land management groups . Furthermore, the results from this research have been shared regularly with the US Fish and Wildlife Service Private Lands Office and restoration specialists working with landowners across the state. We have submitted one manuscript addressing the effects of sediment excavation on plant communities to the journal *Restoration Ecology* (submitted July 2018) and another manuscript to the journal *Wetlands*. In addition, at least two more manuscripts are in preparation. Copies of the manuscripts will be provided upon publication.

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:	$ 290,000	• Project Coordinator, 4% time for 3 years, 75% salary, 25% benefits [$21,000]. • Project Collaborator, 4% time for 3 years, 75% salary, 25% benefits [$23,000]. • Graduate Research Assistant, 50% time for 1.72 years, 51% salary, 49% benefits during academic year; 85% salary, 15% benefits during summer [$81,500]. • 3 Undergraduate Students, 44% time for 3 years, 100% salary, 0% benefits [$73,000].

		• Lab Technician for 3 years, 23% time, 78% salary, 22% benefits [$21,000]. • Junior Scientist, 25% time for 3 years, 78% salary, 22% benefits [$33,000]. • Lab Technician for 1 year, 80% time for 1 year, 78% salary, 22% benefits [$36,000].
Equipment/Tools/Supplies:	$ 51,000	• Sample collection and preservation supplies including polycarbonate tubes and caps, survey stakes, polycarbonate bottles, capsule and glass fiber filters, geopump, sieves, waders, etcetera [$12,500]. • Sample preparation supplies including laboratory tape, 60 mL syringes, aluminum weighing dishes, centrifuge tubes, grinders for soils, and chemicals for nutrient extractions and analyses [$9,500]. • Supplies to construct and deploy piezometers, temperature sensors, and water level meters including buckets, garden hose fittings, PVC tubing and fittings, sign posts, bentonite clay, fiberglass cloth, hole digger and auger et cetera [$3,000]. • Field denitrification assay supplies including incubation bottles and caps, pipettes, portable scale, and shaker table [$11,700]. • Sample dryer for soils and vegetation [$4,000]. • 20 water level loggers ($300 each) [$6,000]. • 64 temperature loggers ($60 each) [$3,800]. • Outreach materials including materials for making interactive activities and demonstrations [$500].
Printing:	$ 500	• Printing posters and educational brochures for outreach activities [$500].
Travel Expenses in MN:	$ 26,500	• University of Minnesota vehicle rental (3 months, $775 per month for 3 years) and fuel reimbursement for travel to and from Fergus Falls wetland management district and select sites (approx. 500 miles per week, 12 weeks per year, 3 years, $0.23 per mile) [approx. $11,120]. • Travel reimbursement for fuel used while traveling to and from the Fergus Falls, Morris, and Detroit Lakes wetland management offices (approx. 360 miles per week, 18 weeks per year, 3 years, $0.575 per mile) [approx. $14,560]. • Camping fees while working out of the Morris wetland management office ($15 per night, 2 sites per night, 8 nights per year, 3 years) [approx. $720].
Other:	$ 52,000	• Sample analysis processing fees; plant tissue and soil elemental analysis ($6/sample, 300-400 samples total), dissolved organic carbon analysis ($2.59/sample, 1000-1300 samples total), dissolved phosphorus analysis ($11.40 /sample, 1300-1400 samples total), particulate phosphorus analysis ($11.40 10.50/sample, 200-300 samples total), and dissolved inorganic nitrogen analysis ($12.24/sample, 1000-1100 samples total) [$45,000].
TOTAL ENRTF BUDGET:	**$420,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 8.5 FTE

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
University of Minnesota (In-kind services)	$187,000	$20,953.48	Indirect costs, lab space, electricity, et cetera.
TOTAL OTHER FUNDS:	**$187,000**	**$20,953.48**	

VII. PROJECT STRATEGY:

A. Project Partners:

Project Partners Receiving Funds:

- Dr. Jacques Finlay (Project Manager; University of Minnesota): $397,000 for to oversee data acquisition, sample processing, and overall project management.
- Dr. James Cotner (Collaborator; University of Minnesota): $23,000 for sample processing.

Project Partners Not Receiving Funds:

- Sheldon Myerchin (Collaborator; USFWS): coordinating in-kind support from USFWS.
- Shawn Papon (Collaborator; USFWS)
- Dr. Chip Small (Collaborator; University of St. Thomas): providing logistical and technical support for denitrification assays and access to membrane inlet mass spectrometer.

B. Project Impact and Long-term Strategy: This research will inform land management decisions by providing valuable data about how wetland restoration practices and invasive plant species influence water quality in the state of Minnesota. Specifically, the USFWS will use the data to inform on-the-ground restoration and management decisions. In addition, we will take the initiative to share our results with Minnesota state agencies whenever possible, and public presentations of our results will be designed to reach as many of Minnesota's natural resource management professionals as possible.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	$ Amount
University of Minnesota	June 1, 2015 – October 31, 2015	$8,100
United States Fish and Wildlife Service (In-kind support)	June 1, 2015 – August 31, 2015	$3,445
United States Fish and Wildlife Services (wetland restorations: values are based on historical average restoration cost per acre, and assuming each restoration is approximately an acre in size).	June 1, 2011 – August 31, 2015	$37,500

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS:

A. Parcel List: N/A

B. Acquisition/Restoration Information: N/A

IX. VISUAL COMPONENT or MAP(S): See attached figure.

X. RESEARCH ADDENDUM: See attached research addendum.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than December 30, 2016; June 30, 2017; December 30, 2017; June 30, 2018; and December 30, 2018. A final report and associated products will be submitted between June 30 and August 15, 2019.

Final Attachment A (Budget Sheet): Budget Detail for M.L. 2016 Environment and Natural Resources Trust Fund Projects

M.L. 2016 Project Budget

Project Title: Assessing Effectiveness of Wetland Restorations for Improved Water Quality

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04u

Project Manager: Jacques Finlay

Organization: University of Minnesota

M.L. 2016 ENRTF Appropriation: $420,000

Project Length and Completion Date: 3 Years, June 30, 2019

Date of Report: September 30, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	**Revised Activity 1 Budget (1/1/2019)**	**Amount Spent**	**Activity 1 Balance**	**Revised Activity 2 Budget (1/1/2019)**	**Amount Spent**	**Activity 2 Balance**	**Revised Activity 3 Budget (1/1/2019)**	**Amount Spent**	**Activity 3 Balance**	**Revised Total Budget (1/1/2019)**	**TOTAL BALANCE**
BUDGET ITEM	Quantifying rates of nitrogen removal from restored wetlands			Quantify phosphorus capture and burial in wetland basins			Dissemination of results				
Personnel (Wages and Benefits)	$213,000	$213,000	$0	$77,000	$77,000	$0	$0	$0	$0	$290,000	$0
Jacques Finlay, Project Manager: $21,000 (75% salary, 25% benefits): 4% FTE each year for 3 years.											
James Cotner, Project Collaborator: $23,000 (75% salary, 25% benefits): 4% FTE each year for 3 years.											
1 Graduate Research Assistant: $81,500 (51% salary, 49% benefits during academic year; 85% salary, 15% benefits during summer): 50% FTE each year for 1.72 years.											
3 Undergraduate Research Assistant: $73,000 (100% salary, 0% benefits): 44% FTE each year for 3 years.											
1 Laboratory Technician: $21,000 (78% salary, 22% benefits): 23% FTE each year for 3 years.											
1 Junior Scientist: $33,000 (78% salary, 22% benefits): 25% FTE each year for 3 years.											
1 Laboratory Technician: $36,000 (78% salary, 22% benefits): 80% FTE each year for 1 year. (12/30/2016)											
Equipment/Tools/Supplies	$44,000	$44,000	$0	$6,500	$6,500	$0	$500	$500	$0	$51,000	$0
Sample Collection and Preservation Supplies. Samples include soils (e.g., 100 polycarbonate plastic core tubes and caps, 2 sieves, plastic bags), vegetation (e.g., 1 - 200 meter field measuring tape, 15 survey stakes, 2 hedge shears, 1 shovel, plastic bags), surface and ground water (e.g., 3000 - 60 mL polycarbonate bottles, 300 capsule filters, 1000 glass fiber filters, 20 packs water resistant labels, 1 geopump for collecting water from piezometers, dry ice for storage and transport), and personal protective gear for field assistants (e.g., 3 pair of waders) [$12500 total].											
Sample Preparation Supplies including laboratory tape (15 rolls), 60 mL syringes (200 syringes), aluminum weighing dishes (5000 dishes), coffee grinder for soil and vegetation (~~3-~~ 10 grinders), 50 mL centrifuge tubes (500 tubes), chemicals for ammonium, nitrate, and phosphorus analyses and extractions, et cetera [$9500 total].											

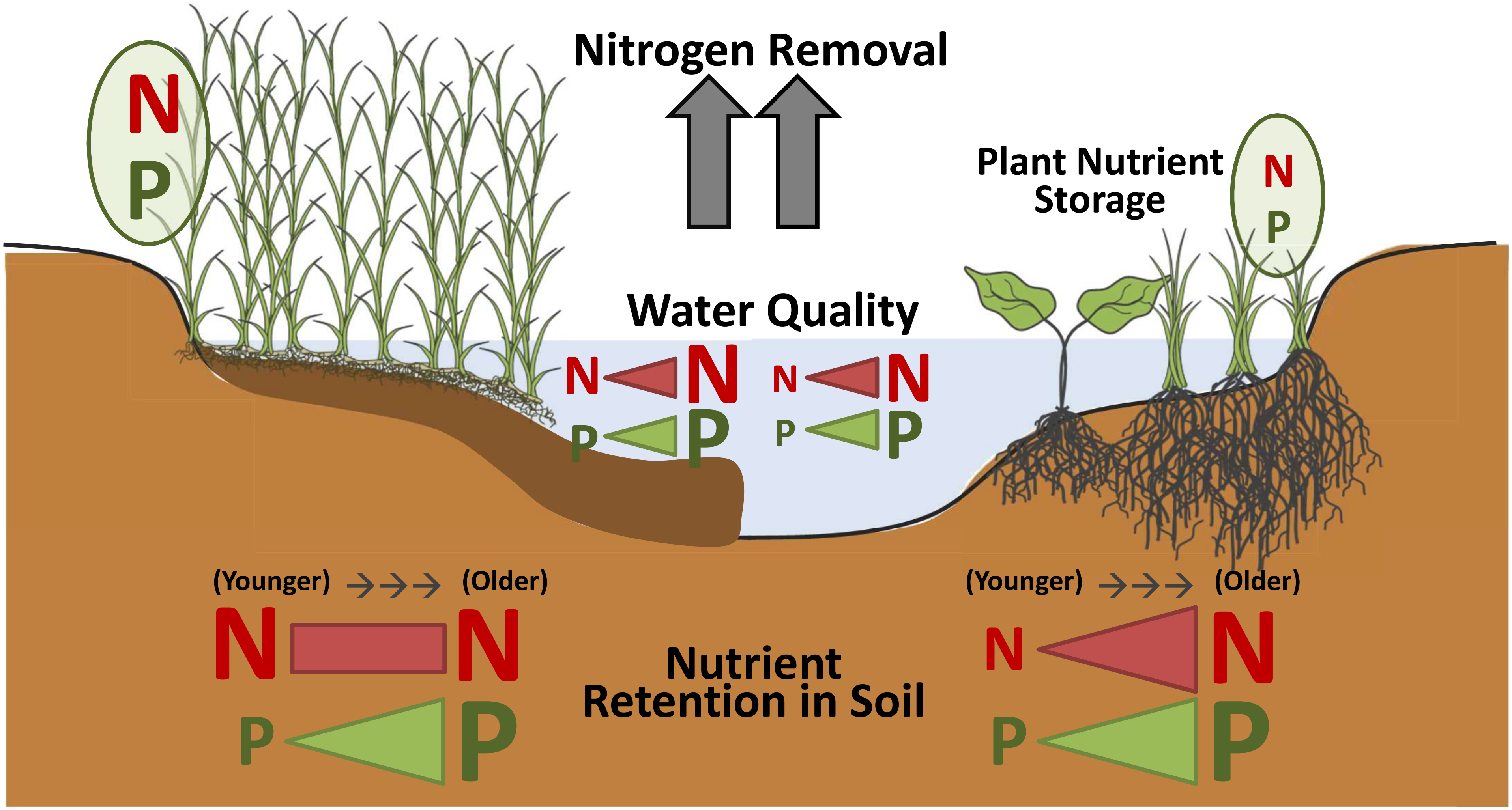
Hydrology Monitoring Meter Construction, Housings, and Deployment Supplies including, PVC tubing and fittings (for 20 water level loggers), sign posts (30 posts), bentonite clay (approx. 50 lbs.), sand (approx. 100 lbs.), fiberglass cloth), 1 hole digger, 1 hole digger auger, et cetera. [$3000 total].											
Field Denitrification Assay Supplies including incubation bottles (96 @ $476/48 bottles) and caps (100 @ $144/100 caps), pipettes (2 @ $280/pipette), vials with septa and caps, serum vial crimpers/decrimpers, PVC for soil peepers ($55.5/peeper, 38 peepers), Membrane for peepers ($ 29.76/foot, 63 feet) [$11700 total].											
20 Water Level Loggers ($300 each; $6000 total)											
64 Temperature Loggers ($60 each; $3800 total)											
Sample Dryer for soils and vegetation [1 unit; $4000].											
Outreach materials including materials for making interactive activities and demonstrations [$500] (12/28/2018)											
Printing	$0	$0	$0	$0	$0	$0	$500	$500	$0	$500	$0
Posters for presentations of research results ($80 per poster, 1 graduate poster per year, 3 years) [$240 total].											
Educational brochures and outreach activities (activity handouts at $100 per 200) [$260 total].											
Travel expenses in Minnesota	$17,500	$17,500	$0	$9,000	$9,000	$0	$0	$0	$0	$26,500	$0
University vehicle rental (3 months, $775 per month for 3 years) and fuel reimbursement for travel to wetland management office and select sites (approx. 500 miles per week, 12 weeks per year, 3 years, $0.23 per mile) [$11,120 total].											
Travel reimbursement for personal vehicle use by graduate student and field technicians (approx. 360 miles per week, 18 weeks per year, 3 years, $0.575 per mile) and conference expenses for outreach in Minnesota (Conference fees and hotel lodging) [$14,560 total].											
Camping Fees for Sites in the Morris Wetland Management District ($15 per night, 2 sites per night, 8 nights per year, 3 years) [$720 total].											
Other	$29,500	$29,500	$0	$22,500	$22,500	$0	$0	$0	$0	$52,000	$0
Sample processing and analyses including plant tissue and soil elemental analysis ($6/sample, 300-400 samples total), dissolved organic carbon ($2.59/sample, 1000-1300 samples total), dissolved phosphorus ($6.25/sample, 1300-1400 samples total), extractable phosphorus ($10.50/sample, 300-500 samples total), and dissolved inorganic nitrogen analysis ($12.24/sample, 1000-1100 samples total), Processing Soil Cores ($6.95/foot, 159 feet), Machine Shop Building Peepers ($250/peeper, 40 peepers) [$45,000 total].											
COLUMN TOTAL	**$304,000**	**$304,000**	**$0**	**$115,000**	**$115,000**	**$0**	**$1,000**	**$1,000**	**$0**	**$420,000**	**$0**

Business As Usual (BAU)

- ✓ More Invasive Species
- ✓ Plant Nutrient Storage: N and P Higher
- ✓ Soil Nutrient Content: N Higher, P Equal to EXC
- ✓ Soil P Increasing Over Time
- ✓ Water Quality: N and P Higher, Increasing Over Time
- ✓ N Removal Equal to EXC

Sediment Excavation (EXC)

- ✓ Fewer Invasive Species
- ✓ Plant Nutrient Storage: N and P Lower
- ✓ Soil Nutrient Content: Lower N, P Equal to BAU
- ✓ Soil N and P Increasing Over Time
- ✓ Water Quality: N and P Lower, Increasing Over Time
- ✓ N Removal Equal to BAU



Appendix 1

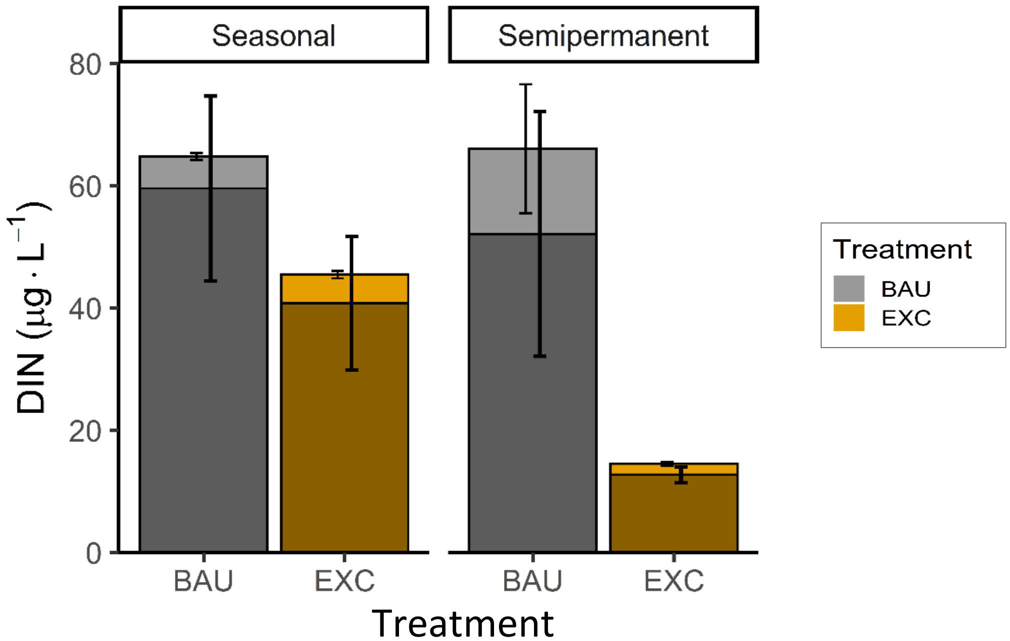


Figure 1. Surface water dissolved inorganic nitrogen (DIN) concentration in micrograms per liter, as nitrate (NO_3^--N; light colors, top) and ammonium (NH_4^+-N; dark colors, bottom). Seasonal wetland hydroperiod is shown on left and semipermanent on right with BAU wetlands in grey bars and EXC wetlands in gold. Error bars represent one standard error, with nitrate errors represented by thin lines and ammonium errors in bold lines.

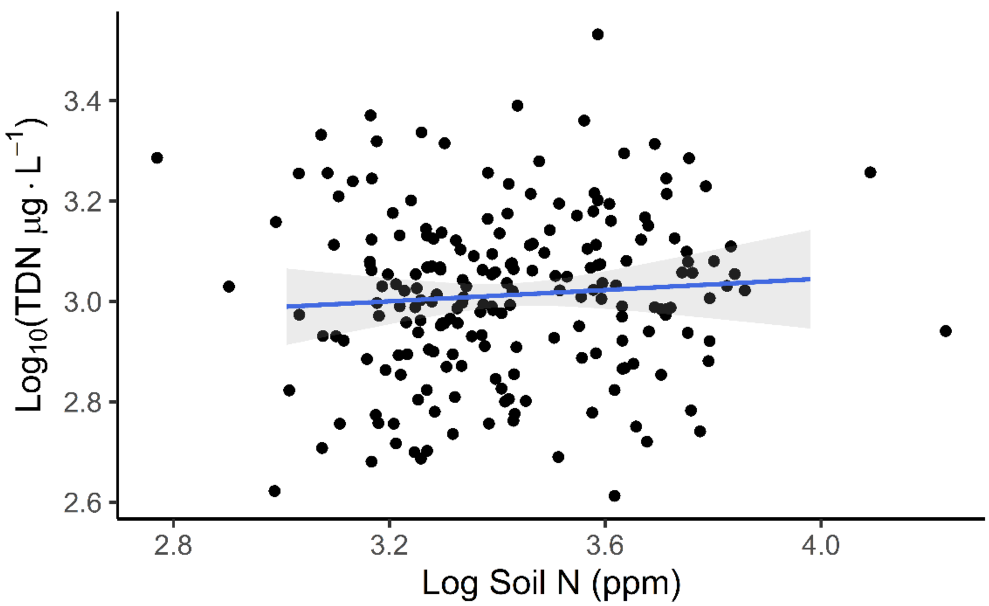


Figure 2. Relationship between surface sediment (i.e. “soil”) nitrogen and total dissolved nitrogen in the overlying water.

Appendix 1

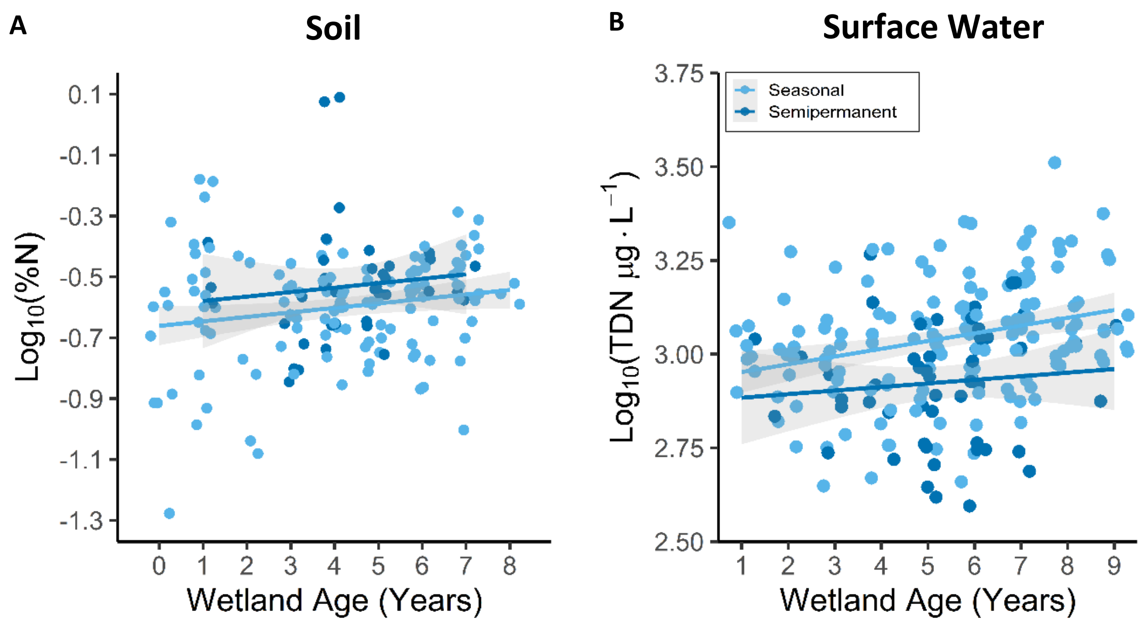


Figure 3. Soil total nitrogen (A, left) and surface water total dissolved nitrogen (TDN; B, right) concentration in micrograms per liter. Seasonal wetland hydroperiod in light blue, semipermanent hydroperiod in dark blue. Grey shading indicates the 95% confidence interval of trend lines.

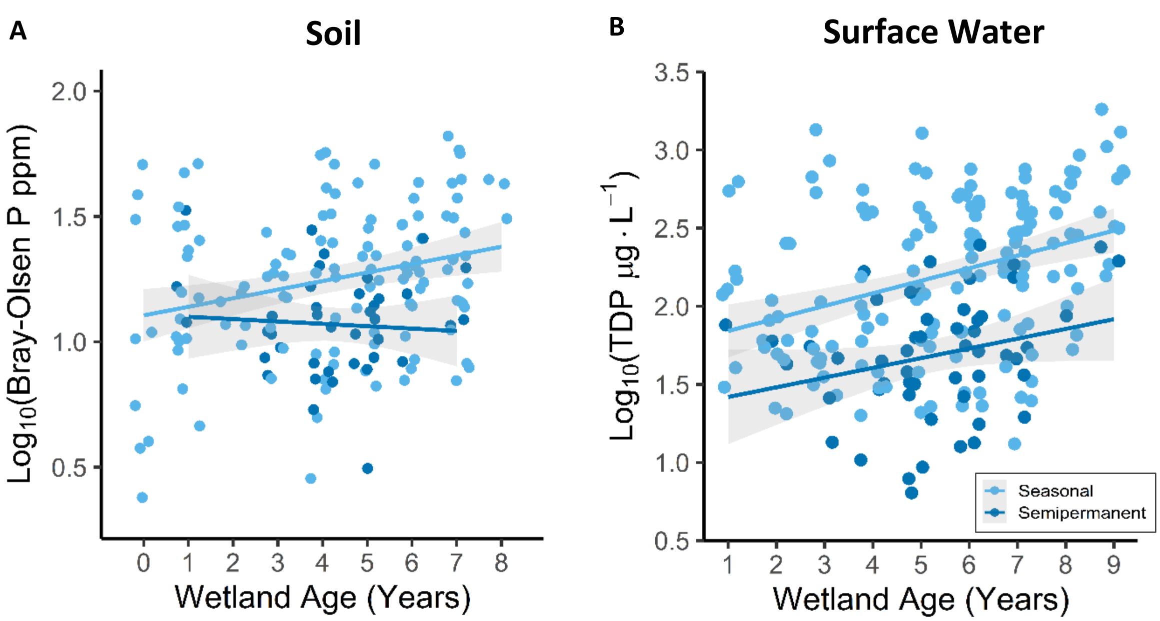


Figure 4. Soil Bray-Olsen phosphorus (A, left) and surface water total dissolved phosphorus (TDP; B, right) concentration in micrograms per liter. Seasonal wetland hydroperiod in light blue, semipermanent hydroperiod in dark blue. Grey shading indicates the 95% confidence interval of trend lines.

Appendix 1

Table 1: Statistical results from ANOVAs for soil physical and chemical properties. Treatment represents the difference between excavated and business as usual wetlands. Hydroperiod represents the difference between seasonal and semipermanent wetlands. Core Section represents changes from the soil surface to deeper in the soil profile.

	Treatment	***Hydroperiod***	***Wetland Age***	***Treatment : Hydroperiod***	***Treatment : Age***	***Core Section***
Bulk Density (g/cm^3)	0.52	0.55	0.20	0.74	**0.042 ***	**< 0.0001 ****
CaCO3 (%)	**< 0.0001 ****	0.54	**0.030 ***	**0.030 ***	0.76	0.34
Organic Matter (%)	0.10	0.48	0.39	0.86	*0.063 ·*	**< 0.0001 ****
Organic Carbon (%)	0.20	0.17	0.32	0.56	**0.019 ***	**< 0.0001 ****
Total Nitrogen (%)	**0.035 ***	0.16	**0.042 ***	0.56	**0.023 ***	**< 0.0001 ****
Bray-Olsen P (ppm)	0.40	**0.0087 ****	**0.024 ***	0.13	0.52	**< 0.0001 ****
pH	**0.00098 ****	0.87	0.51	0.28	0.95	0.21

****** Significant p values < 0.01
***** Significant p values < 0.05
· Marginally significant p values

Table 2: Statistical results from ANOVAs for surface water dissolved nutrient concentrations across all sites and years (2016-2018). Total dissolved nitrogen (TDN), dissolved organic nitrogen (DON), dissolved inorganic nitrogen (DIN), total dissolved phosphorus (TDP), dissolved organic phosphorus (DOP), and soluble reactive phosphorus (SRP). Treatment represents the difference between excavated and business as usual wetlands. Hydroperiod represents the difference between seasonal and semipermanent wetlands.

	Treatment	***Hydroperiod***	***Wetland Age***	***Treatment : Hydroperiod***	***Treatment : Wetland Age***
TDN (µg/L)	**0.016 ***	**0.00039 ****	**0.0026 ****	0.37	0.69
DON (µg/L)	**0.023 ***	**0.00018 ****	**0.0045 ****	0.48	0.57
DIN (µg/L)	**0.023 ***	0.30	**0.0019 ****	0.17	0.43
NH_4^+-N (ug/L)	**0.028 ***	0.40	**0.0016 ****	0.19	0.42
NO_3^--N (ug/L)	0.059 ·	**0.011 ***	**0.018 ***	0.54	0.67
TDP (µg/L)	**0.0019 ****	**0.00000035 ****	**0.00047 ****	0.46	0.45
DOP (µg/L)	0.096	**0.025 ***	0.55	0.098	0.62
SRP (µg/L)	**0.0066 ****	**0.000023 ****	**0.00020 ****	0.77	0.91

****** Significant p values < 0.01
***** Significant p values < 0.05
· Marginally significant p values

Plinko Experiment

It takes time to clean pollutants from our water and the longer water stays in the landscape the more nutrients we can remove from the water. In this experiment you will see how adding wetlands on the landscape and how adding vegetation inside our wetlands helps to slow down water and increase the amount of time that it spends on the landscape.

What You Will Need:

- 1 large plinko board with 2 lanes
  - Lane 1: Few nails
  - Lane 2: Many nails
- Marbles
- Starting gate
- Sheet of Paper and Writing Utensil
- Optional: Stopwatch

Step 1

Line up a row of marbles across both lanes of the starting gate.

Get your stopwatch ready and keep an eye on how fast the marbles will move from the top to the bottom of the plinko board.

Remove the starting gate!

Observe!

Step 2

How long did it take for the marbles to get to the bottom in the lane with only a few nails?

How long did it take for the marbles to get to the bottom in the lane with many nails?

What can this tell us about how water moves through a landscape? Through a wetland?

Summary

The marbles represent water on the landscape or in a wetland. The nails represent either wetlands on the landscape OR vegetation within a wetland.

Many wetlands on the landscape help to slow down water and increase the likelihood of removing nutrients from the water. In addition, the water is more likely to move to the groundwater where it makes its way into wells and eventually into our homes.

More vegetation inside a wetland helps to slow down the movement of water and decrease the power it has to erode away soils.

Wetland Waters Activity

It takes time to clean pollutants from our water. In this experiment you will see how time and the right conditions clean the water we drink, the water we swim in, and the water where we catch fish!

What You Will Need:

- 1, 8 Ounce (or larger) Clear Glass Jar with Cap
- 2 Tablespoons Activated Carbon (see pet stores)
- Optional: Tea Bag or Filter Media Bag (see pet stores)
- Red Food Dye
- Sheet of Paper and Writing Utensil
- Water
- Time

Step 1

Add water to the jar. The jar should have at least 6 ounces of water in it, but you can add more if you have a larger jar. Make sure to leave some room in the jar for adding the carbon.

Add just enough food dye to tint the water red (Hint. For 8 ounces of water, 2 drops of food dye is perfect).

Cap the jar and mix it up or watch how the food dye moves through the water. Can you describe what is happening?

Step 2

Add the activated carbon to a mesh bag or tea bag if you have one.

Close the bag securely and rinse out all the little bits of carbon powder.

Add the activated carbon to the jar with red water.

Note the date and time on a sheet of paper, then take some quick notes. What do you see? What color is the water? Can you take a picture?

Step 3

Wait a few hours … play outside in the sprinkler or go fishing.

When you return, note the date and time, then take some more notes. How has the color of the water changed?

Wait overnight or for a whole day.

When you return, note the date and time, then take some more notes. How has the color of the water changed?

You should see that the water turns from red to pink to clear over the course of a few days. When it rains, water runs off of fields, lawns, and streets and collects in wetlands. The microbes and plants in the wetland help to clean pollutants from our water before the water makes its way to lakes, streams, the groundwater that we drink!

Step 4

What other things might change how clean we can make the water? Try putting the water in the refrigerator, or in the sun. Maybe see how other colors change your results!

Soil Erosion Experiment

This might look like a simple experiment but it will definitely show how vegetation prevents soil erosion and keeps water clean!

What you'll need:

- 6 empty 1 Liter bottles
- 1 piece of ply wood (30cm x 30cm x 2cm thick)
- Wood glue
- Scissors and Stanley knife
- String
- Soil from the garden and compost
- 4 Seedlings
- Mulch (bark chips, dead leaves and sticks)
- Water

Step 1
Prepare three of the coke bottles by cutting a rectangular hole roughly 7cm x 25cm along the side of the bottle (Hint: use a permanent marker to mark out the piece you want to cut away).

Step 2
Stick the bottles to the wood with the wood glue making sure that the necks of the three bottles protrude a little over the edge of the board.

Fill the first bottle with plain garden soil and the other two with a soil and compost mixture. Press down firmly to compact it.

Step 3

Leave the first bottle as is.

Cover the top of the soil in the second bottle with your mulch (bark chips, dead leaves and sticks etc).

Plant your seedlings in the third bottle. Make sure you plant them tightly together and press down firmly to compact the soil.

Step 4

Cut the other three bottles in half, horizontally and keep the bottom halves.

Make two small holes opposite each other, nearest the cut side of the bottle.

Cut three pieces of string, roughly 25cm long and insert each end into the holes. Tie a knot on the ends to secure them. This will form a “bucket” to collect the water.

Hang them over the necks of each of the three bottles on the board.

Step 5

Slowly pour equal amounts of water into each of the bottles. Pour the water in at the end furthest from the neck of the bottle.

Take note of the colour of the water collecting in the cups! The water in the first cut is really dirty, the water from the second and third cups are much cleaner which shows that both mulch as well as the root structure of plants assist in preventing soil erosion.

Do this every day for a week or two and notice how the soil erodes away in the first container while the plants hold the soil in place in the last container. It's natures glue, so let's look after our plants and while we're about it … plant some more.

For More Information Visit: https://www.lifeisagarden.co.za/soil-erosion-experiment/#.U3uAtV

Nye Labs .com

HOME DEMO NO. 6

The Name's Pond, James Pond

When you look at a pond, it's hard to see what's underwater. The surface reflects light like a mirror. Here's a way to scratch the surface of a pond. You can build your own waterscope to gaze on creatures and features under the water. .

What you need:

1. Juice can
2. Plastic wrap
3. Rubber bands
4. Packing tape

What you do:

1. Remove both ends of your juice can. Sometimes they pull off; sometimes you need a can opener. Check the cans. If any sharp edges stick up, use a hammer to tap and bend them down. Cover the edges with plastic tape to keep from getting cut.
2. Tear off a piece of plastic wrap. Stretch the wrap tightly over one of the can's openings and hold it tight with a rubber band.
3. To use your waterscope, gently set the plastic-wrapped end into the water and look through the other end. Now you can see through the mirror made by the pond's surface.

Extra! Extra!

You can make two juice can viewers and tape them together for binocular pond viewing.

Muy Importante!

Remember your ABC's of safety: always be careful. Don't fall face first. Bring an adult with you.

Appendix 2: Custom Coloring Page