Section A.

Title of Project: The Effects of Nutrient Loading on Nutrient Limitation in Great Lakes Coastal Ecosystems

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Abstract: Great Lakes coastlines are made up of complex mosaics of habitats like wetland–stream–lake interfaces. These interfaces are characterized by a high degree of spatial heterogeneity that may facilitate the co-occurrence of biogeochemical processes that are favored under incompatible environmental conditions. We measured rates of N_2 fixation and denitrification, along with nutrient limitation in 10 locations along 5 wetland-stream-lake interfaces in Lakes Superior and Huron. In streams and lakes, N_2 fixation occurred primarily on macrophytes, while in wetlands, N_2 fixation occurred primarily in sediment or detritus. In all three habitats, denitrification occurred exclusively in sediments or decaying macrophytes. The highest rates of N_2 fixation occurred in transect points where no limitation or N limitation of algae biomass was observed using nutrient diffusing substrates. In contrast, the highest denitrification rates occurred across the N, P, N:P, and no limitation treatments. This demonstrates that coastal wetlands cannot simply be thought of as nutrient sinks where N is removed via denitrification, but that N_2 fixation inputs occur and may play an important a role in determining how much N is removed. Our findings also show that spatial heterogeneity within coastal wetland ecosystems is key to maintaining this diversity in nutrient cycling. Anything that alters habitat physical complexity will influence how nutrients are stored and transported. Therefore, from a restoration and conservation perspective, it is important to maintain and restore spatial heterogeneity in these ecosystems to preserve their function in complex biogeochemical cycling.

Keywords: coastal, nitrogen, phosphorus, spatial heterogeneity, nutrient

Executive Summary (2-4 paragraphs)

Great Lakes coastlines support complex mosaics of habitats, such as wetlands, streams, estuaries, dunes, and more. In these coastal mosaics of habitats is where upland runoff of nutrients, like nitrogen and phosphorus, meet the Great Lakes. These coastal ecosystems can help manage water quality by acting as sponges of nutrients. Wetlands can absorb high levels of nutrients that run off from land, such as nitrogen or phosphorus, and prevent them from entering major bodies of water, such as lakes and rivers. Unfortunately, human activities post-European settlement have destroyed more than half of the wetlands once present in the Great Lakes region, and many remaining wetlands are degraded. Excess nutrient loads, or inputs, from Great Lakes rivers and streams could alter the nutrient processing functions in coastal wetlands. Understanding these functions and how they vary across coastal ecosystem complexes is key to managing and restoring healthy Great Lakes habitats.

To evaluate how nitrogen moves through coastal ecosystem complexes we measured rates of N_2 fixation and denitrification across 5 wetland-stream-lake interfaces in Lakes Superior and Huron, along with nutrient limitation. N_2 fixation is the chemical processes by which nitrogen is made available to plants for growth; denitrification is the process by which nitrogen is removed from the water environment and released to the atmosphere. We found that in streams and lakes, N_2 fixation occurred primarily on macrophytes, while in wetlands, N_2 fixation occurred primarily in sediment or detritus. In all three habitats, denitrification occurred exclusively in sediments or decaying macrophytes. This demonstrates that spatial heterogeneity of habitat within coastal wetland ecosystems is key to maintaining diversity in nutrient cycling. Anything that alters habitat physical complexity will influence how nutrients are stored and transported. Therefore, from a restoration and conservation perspective, it is important to maintain and restore spatial heterogeneity in these ecosystems to preserve their function in complex biogeochemical cycling.

Section B.

Introduction

Coastal ecosystems are dynamic mosaics of wetlands, streams, and lakes that are vital for nutrient cycling, nutrient retention, and fish and wildlife habitat, but they are threatened by human activities such as land use change and eutrophication[1,2]. In the Great Lakes region, more than 50% of coastal wetlands have been lost since European settlement and widespread nutrient loading in the lower Great Lakes region is becoming more prevalent[3,4]. Nutrient loading may fundamentally alter the ecological dynamics of aquatic habitats by changing the patterns of nutrient limitation across wetland-stream-lake interfaces. Spatial gradients in nutrient limitation may promote the co-occurrence of a variety of biogeochemical processes – particularly N_2 fixation and denitrification, which have long been thought to be mutually exclusive in freshwater ecosystems. N_2 fixation is the microbial conversion of N_2 gas into an input of biologically available N, while denitrification is the microbial conversion of nitrate into N_2 gas. N_2 fixation is favored when nitrate concentrations are low because the process has significant energy costs to the organism. In contrast, denitrification requires higher concentrations of nitrate, as well as high organic matter and anoxic conditions. Thus, spatial gradients of nutrient availability and limitation in wetland–stream–lake interfaces could control the flux of N_2 in these coastal regions.

<u>Objectives/Hypotheses:</u> **<u>Objective 1</u>:** Evaluate how spatial complexity across a wetland-stream-lake interface controls the net N_2 flux. **Hypothesis 1:** Spatial heterogeneity of the wetland-stream-lake interface will lead to spatial variability in nutrient limitation. **Hypothesis 2:** Spatial variability in nutrient limitation will facilitate the co-occurrence of N_2 fixation and denitrification across wetland-stream-lake interfaces. **Hypothesis 3:** Spatial patterns of nutrients, oxygen, organic matter, and temperature will predict the occurrence of these processes. **<u>Objective 2</u>:** Evaluate rates of N_2 fixation and denitrification in response to N and P enrichment. **Hypothesis 4:** Increased N concentrations will decrease rates of N_2 fixation and increase denitrification and increased P concentrations will increase rates of N_2 fixation only.

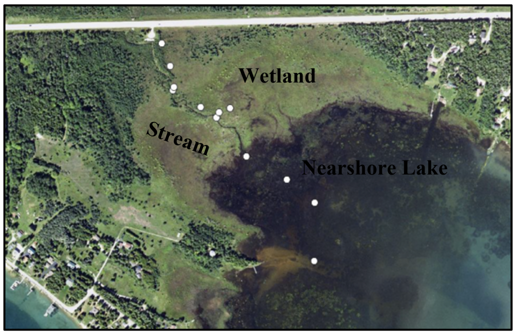

Figure 1. ArcGIS image of the Mackinaw Bay transect in northern Lake Huron. Each white circle represents one transect point.

Project Narrative: To evaluate our first objective, a comparative study was conducted across 5 wetland-stream-lake interfaces on Lakes Huron and Superior, selected to span a gradient of nutrient loading and human impact. Saganing and Wildfowl Bay interfaces were sampled in summer 2020 and Nara, Sioux, and Mackinac Bay interfaces were previously sampled in summer 2018 and 2019. Unfortunately, we were not able to resample these sites in 2020 because of a shortened field season due to the COVID-19 pandemic. In each interface, a transect of 10-15 points was determined based on habitat variation (Fig. 1). At each point, rates of N_2 fixation and denitrification were measured using the acetylene reduction and block methods in static chambers[5]. Canopy cover, temperature, dissolved oxygen, depth, and dissolved N, P, and C concentrations were also measured at each point along the transect.

Figure 2. Image of NDS taken from a transect after a 2-week deployment.

To evaluate nutrient limitation of primary producers, nutrient diffusing substrata (NDS) were deployed at each transect point for 14 days (Fig. 2). At each point, there were 16 total NDS composed of 4 control, 4 N, 4 P, and 4 N+P replicates. To create the NDS, N was added as $NaNO_3$, and P was added as NaH_2PO_4 and the N+P treatment contained both in a 9.85:1 mass ratio. Algal growth occurred on a porous porcelain disc that was placed on top of the NDS. Algal growth was quantified after collection using Chlorophyll-a analysis. Nutrient limitation was then determined using a two-way ANOVA[6] (Table 1).

Table 1. Nutrient limitation data collected from NDS for 4 of the 5 transects. Transect points with N effect are colored blue, P effect yellow, and N:P effect green. No nutrient limitation is colored gray. N = nitrogen and P = phosphorus.

Year	Transect Name	Transect Number	N effect	P effect	Interaction NxP
2019	Nara	1	blue	gray	green
		2	blue	gray	gray
		3	blue	gray	gray
		4	blue	yellow	gray
		6	blue	yellow	green
		7	gray	gray	gray
		11	blue	yellow	green
	Sioux	1	blue	gray	gray
		2	gray	gray	gray
		3	blue	gray	gray
		4	gray	gray	gray
		5	gray	gray	gray
		6	blue	gray	gray
		7	blue	gray	gray
		8	gray	gray	gray
2020	Wildfowl	1	gray	gray	gray
		3	blue	gray	gray
		4	blue	gray	gray
		5	blue	gray	gray
		6	blue	yellow	green
		7	gray	gray	gray
		10	blue	gray	gray
	Saganing	1	gray	gray	green
		2	gray	gray	gray
		4	gray	gray	gray
		5	gray	gray	gray
		6	gray	gray	gray
		7	gray	gray	gray
		8	gray	gray	gray
		9	blue	yellow	gray

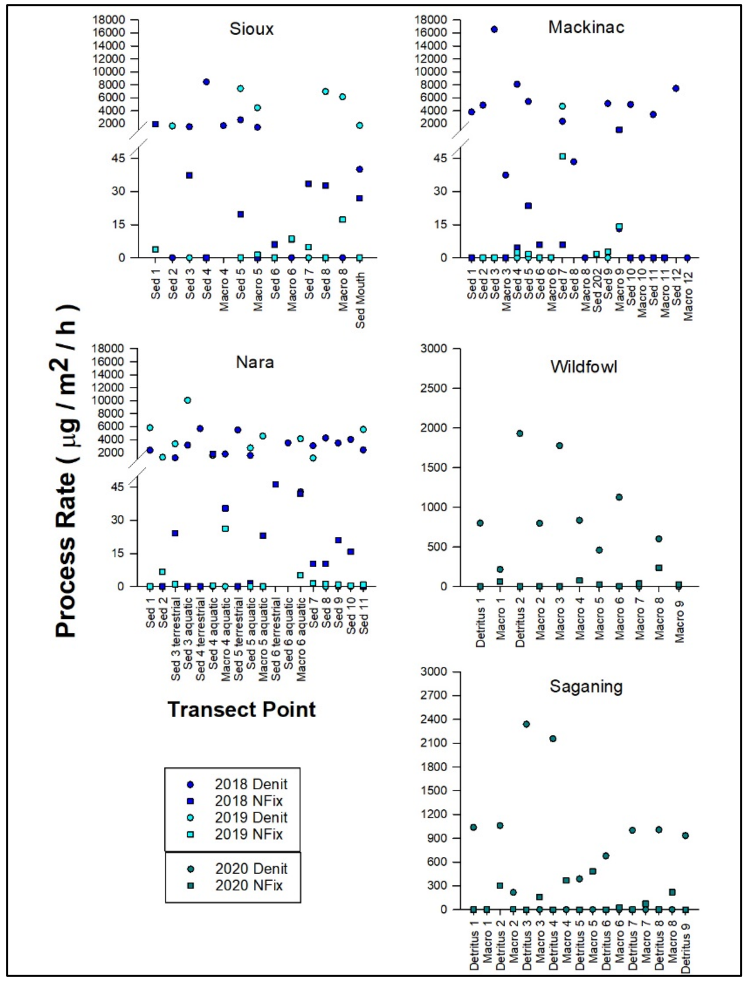

Figure 3. Graph of N_2 fixation and denitrification rates (µg / m^2 / h) for all 5 interfaces. Each year is represented by a different color. N_2 fixation is depicted by squares and denitrification is depicted be circles. Note the Y axis for Wildfowl and Saganing is 6X lower than the other 3 transects.

We found that the spatial heterogeneity of the wetland-stream-lake interfaces did lead to spatial variability in nutrient limitation determined using NDS (Table 1.) At the Nara, Wildfowl, and Saganing transects, we observed a range of nutrient limitation responses, with N limitation, P limitation and co-limitation of N and P at different points along the transects. In contrast, at the Sioux transect, only N limitation was observed at 4 sites, while 4 sites showed no nutrient limitation. No nutrient limitation data is available from the Mackinac transect because most NDS were lost due to high-water levels and storms.

We also found that N_2 fixation and denitrification co-occurred across wetland-stream-lake interfaces. In streams and lakes, N_2 fixation occurred primarily on macrophytes, while in wetlands, N_2 fixation occurred primarily in sediment or detritus. In all three habitats, denitrification occurred exclusively in sediments and decaying macrophytes (Fig. 3). Transect points that had no nutrient limitation displayed the highest rates of N_2 fixation, with those points that had N limitation displaying the second highest N_2 fixation rates. Denitrification rates did not appear to differ with nutrient limitation status. Future data analysis will include model selection to determine which environmental characteristics, including nutrient limitation, most accurately predict the occurrence of N_2 fixation and denitrification.

To evaluate the second objective, a nutrient enrichment experiment was conducted in microcosms in 4 blocks in the Nara Nature Preserve in Houghton, MI to account for spatial variation (Fig. 4). N and P were added to each microcosm in liquid form to encompass a range of N:P from 1.12 to 33.70. Nutrient enrichment concentrations were based on previous measurements of N and P in the region. N was added with a $^{15}NO_3^-$ tracer. Due to the shortened field season resulting from the COVID-19 pandemic, enrichments were made on 1 Sept 2020 and the experiment was terminated two weeks later. After two weeks, 2 small cores were collected from each microcosm to trace nitrate concentrations accumulated in the sediment over the incubation period with $^{15}NO_3^-$ analysis, while a third small core was collected for $^{30}N_2$ incubations to evaluate if N_2 fixation was occurring in the enriched cores. Following initial core collection, 2 additional large cores were collected from each microcosm to be used in a flow through incubation system for isotope tracing of $^{15}NO_3^-$ to assess denitrification rates using Membrane Inlet Mass Spectrometry. To date, all the samples collected during this experiment have been analyzed but have not yet completed data analysis so there are no results to share at this time.

Figure 4. Image of core taken from a microcosm in the Nara Nature Preserve for in lab flow through incubation experiments. Photo Credit: Sarah Atkinson at Michigan Technological

<u>Research/Management Implications</u>: The watershed surveys that form the core of this project clearly demonstrate that N_2 fixation and denitrification do co-occur across wetland-stream-lake interfaces, with rates differing by substrate within wetlands. Nutrient limitation also varies within habitats (e.g. a single wetland point, or stream) and may help facilitate the occurrence of these processes across interfaces, by creating conditions more suitable for a higher rate of a process like we initially see with N_2 fixation. This means that losses via denitrification must be considered relative to inputs from N_2 fixation to accurately understand the role the wetlands play in nutrient uptake and load mitigation because not as much N will be removed as we may think looking at denitrification rates alone. This study also shows that spatial heterogeneity within coastal wetland ecosystems is key to maintaining this diversity in nutrient cycling. Anything that may reduce physical habitat or biodiversity complexity, such as invasive species like *Phragmites*, will alter the way that wetlands cycle, store, and transport nutrients. Therefore, from a restoration and conservation perspective, it is important to maintain and restore spatial heterogeneity in these ecosystems to preserve their function in complex biogeochemical cycling.

Potential Applications, Benefits, and Impacts: In management, this project could be used in future applications to inform wetland restoration designs like those being conducted by groups like H2Ohio in Lake Erie to include spatial complexity in the designs. It could also inform managers on what locations should be prioritized for ecosystem restoration, like those coastal wetlands that are losing habitat spatial heterogeneity. Local watershed management groups could also use this study to help prioritize sites for restoration, which could help them save funding and time and have a higher likelihood of success which would result in more public buy-in into the restoration. Plus, this study could help with pre-project planning stages by informing managers and technicians on what data should be collected ahead of time to better inform their studies of complex coastal ecosystems.

Research Outputs or Products: Initial results from this project were presented as an oral presentation at the 2021 meeting for the International Association for Great Lakes Research, part of an MTU Biological Sciences Department Seminar, and a guest seminar at University of Nevada - Reno. The ideas of this project were also used in a promotional video for recruitment by Michigan Technological University (https://www.youtube.com/watch?v=tMI6vgkLVss). Michigan Sea Grant promoted the project through a blog post (https://www.michiganseagrant.org/blog/2021/07/21/getting-their-feet-wet-michigan-tech-researchers-investigate-wetland-nutrient-cycles/). **Partners:** For site access: Eric Waara at the City of Houghton, Dr. Matthew Cooper at Northland College, Les Cheneaux Watershed Council, Saginaw Basin Land Conservancy, Michigan EGLE, and Michigan DNR. Dr. Silvia Newell and Dr. Mark McCarthy at Wright State University for training on core flow-through incubation systems. Nick Hendrickson and Scott Meneguzzo in the MTU machine lab that helped create our flow-through incubation system.

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