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The Influence of Herbivory on Macrophyte Community Structure and Nitrogen Retention in Created Wetlands

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The Influence of Herbivory on Macrophyte Community Structure and Nitrogen Retention in Created Wetlands

By:

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Abstract

Wetlands are frequently created to mitigate the loss of natural wetlands due to commercial expansion. However, wetland ecosystem function is determined by complex abiotic and biotic interactions that are not well understood in natural wetlands, and even less so in created wetlands. This lack of understanding may lead to shortcomings in meeting desired restoration outcomes. Key abiotic drivers in wetlands include hydrology and nutrient availability, while herbivory provides both direct and indirect controls on plant communities and biogeochemical cycling. I hypothesized that by decreasing emergent plant biomass and shifting plant community structure to favor submerged plants, large grazers such as geese would promote cascading impacts on nitrogen immobilization, denitrification, nitrogen fixation and sediment nutrient regeneration. To assess these effects, paired caged and uncaged plots were established in three created emergent freshwater wetlands in Western New York State. At the site with high waterfowl abundance, emergent macrophyte cover was reduced and there was a shift towards submerged plants. More complex impacts were observed at other sites where grazing pressure was lower: grazer exclusion substantially increased emergent growth at one site and had no effect where the dominant macrophyte *Nymphea odorata* covered the water surface. Potential denitrification was lowest in the fall, with significantly higher values where submerged plant cover was higher. While sediment nitrogen fixation was consistently below detection limits, periphyton nitrogen fixation, which was dominated by heterotrophs, was slightly higher in caged plots. Sediment nutrient fluxes exhibited some seasonality, with higher rates in spring than summer or fall. These results suggest that grazers have a significant impact on vascular plant community structure, leading to shifts in nitrogen cycling and a reduction of nitrogen fixation.

Exclusion of grazers may be a management tool to protect wetland plants during the early stages of wetland development.

1.0 Introduction

Freshwater wetland ecosystems provide important ecosystem services and functions that make them one of the most ecologically and economically valuable ecosystems on earth (Costanza et al. 2014). Historically, wetlands have been destroyed or degraded by anthropogenic activities; the Clean Water Act was enacted to require mitigation by creating wetlands to compensate when loss was unavoidable. Wetlands are complex systems and the abiotic and biotic interactions that determine functionality are not fully understood, particularly in created wetlands. While much attention has been paid to abiotic control of wetland ecosystem function, biotic control may be equally important. Herbivores may change plant community structure by significantly decreasing plant biomass, potentially altering nitrogen immobilization by plants, denitrification, nitrogen fixation and regeneration of inorganic nutrients in the sediments (Badzinski et al. 2006; Rodriguez-Villafane et al. 2007; Vaieretti et al. 2013; Steidl et al. 2019). There is a gap in knowledge in how important wetland processes, such as nutrient removal and nitrogen fixation, are enhanced or limited in both natural and created wetlands. In the deeper wetlands that are characteristic of created wetlands, this can change the plant community structure and may favor submerged plants over emergent plants (Weisner et al. 1994). This in turn may impact nutrient cycling by changing redox chemistry, organic matter availability, and periphyton communities, thereby affecting nutrient removal (Weisner et al. 1994; Song et al. 2010; Liao et al. 2012). Wetlands can be designed with certain macrophyte community structures that will directly control the nitrogen cycling processes. Wetlands designed with deeper parts favoring submerged macrophytes and with shallower parts covered by emergent macrophytes may promote nitrogen removal and autotrophic nitrogen fixation in created wetlands (Weisner et al. 1994). Exploring the relationships between macrophyte community structure, abiotic, and

biotic factors is crucial in order to understand herbivore influences on nitrogen cycling in created wetlands. The goal of my research is to determine how herbivory impacts submerged and emergent plant community structure and in turn drives nitrogen fixation and nitrogen removal in created emergent freshwater wetlands.

1.1 Wetland ecosystem services

The unique properties of wetlands as transitional ecosystems lead to unique ecosystems that provide a multitude of ecosystem services such as: flood control (Mitsch & Gosselink 1993), carbon sequestration (Winton and Richardson 2017), habitat for fish and wildlife (Murkin et al. 1997; Lor & Malecki 2006), and nutrient removal and water purification (Tang et al. 2017). A recent (1997-2011) global valuation of gross domestic product for 21 biomes assessed the value of wetlands at \$140,174 $ha^{-1}yr^{-1}$, making wetlands among the most economically and ecologically valuable ecosystems on earth (Costanza et al. 2014).

An important ecosystem service attributed to wetlands is the provision of habitat for wildlife, especially waterfowl. The productivity and species composition of wetlands were thought to be regulated by nutrient availability, i.e. bottom-up interactions. More recently, research has suggested the importance of top-down approaches on how ecosystems and wetlands function (Bakker et al. 2016; Kadlec et al. 2007; Stafford et al. 2012). In freshwater ecosystems in the northeast of the United States, including New York State, are primary grazers, and they therefore play an important role in the plant community structure (Stafford et al. 2018). However, the population size of *Branta canadensis* has been exponentially increasing since 1965 (Figure 1.1). Because these birds consume vast quantities of wetland vegetation, there are potentially significant implications for wetland plant communities, and several interdependent ecosystem functions including nitrogen cycling (Perrow et al. 1997; Lor et al. 2006). The

potential impact of grazers on created wetlands is largely unknown and is the subject of this study.

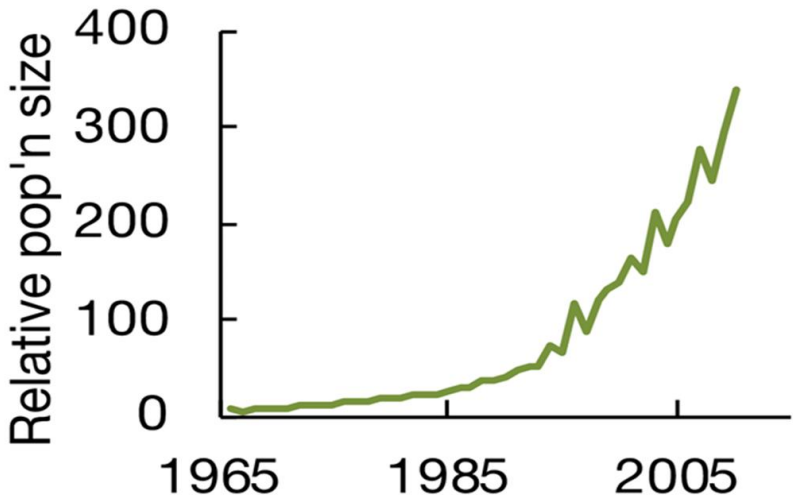

Figure 1.1: Population growth of *Branta canadensis* in NYS. Data from Breeding Bird Survey.

The primary ecosystem service provided by wetlands central to this research is nutrient removal. Wetlands are efficient at removing nutrients, such as nitrogen, from the environment through different, temporary and permanent, pathways such as plant root uptake, microbial immobilization, and denitrification. Denitrification is the primary pathway of nitrogen removal in wetlands (Weisner et al. 1994), whereby nitrate (NO_3^-) and nitrite (NO_2^-) are converted to atmospheric nitrogen (N_2) by microorganisms in the soil and water column under anaerobic conditions. Denitrification is limited by a variety of abiotic conditions such as temperature, oxygen availability, nitrate and nitrite, and labile organic matter (Ballantine et al. 2014; Dieberg et al. 2002; Hanson et al. 1994), which can in turn be influenced by biotic factors such as the plant community structure, which are controlled by herbivores (Perrow et al. 1997; Rodriguez-Villafane et al. 2007; Gyimesi et al. 2011; Winton and Richardson 2017; Stafford et al. 2018). However, wetlands may also have an internal source of nitrogen, through the process of biological nitrogen fixation. Microorganisms utilize the enzyme nitrogenase to catalyze the

conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Biological nitrogen fixation occurs through two primary pathways and is performed by autotrophic (cyanobacteria) or heterotrophic bacteria in the soil, the water column, or in epiphytic communities associated with emergent plants (Rejmankova et al. 2018). While there are many studies focusing on nitrogen fixation in agricultural systems, fewer examine natural plant populations, and fewer still have evaluated both nitrogen fixation and denitrification in created wetlands. The balance between denitrification and nitrogen fixation is unknown in created wetlands.

1.2 Created wetlands

Human activities have caused the loss of natural wetlands, leading to significant investment in wetland creation and restoration in order to replace ecosystem functions and services provided by lost natural wetlands. By 1984, over half of the natural wetlands in the United States had been destroyed or filled because of commercial development, landfills, industrial expansion, and agriculture (USEPA 2008). Policy makers in the United States responded by creating the Wetland 'No Net Loss Act of 1989', under section 404 of the Clean Water Act. This regulation prohibits any development that destroys a natural wetland unless a wetland of equivalent function is created. In April 2008 the Federal Environmental Protection Agency EPA and the US Army Corps of Engineers implemented a new rule that created equivalent standards that apply to all forms of wetland compensation (Hough & Robertson 2017) and requires clear performance standards and procedures, and the use of scientific wetland research.

However, research over the past 30 years suggests that created and natural wetlands do not provide the same ecosystem functions and services. The act of constructing wetlands causes a disturbance to the natural ecosystem and results in altered physical characteristics that recover

over decades. Moreno-Mateos et al. 2012 conducted a meta-analysis of 621 global wetland sites and concluded that biological structure and biogeochemical functioning remain on average 26% and 23% lower, respectively, then natural wetlands, even after decades. Larger wetlands in warmer climates recovered more rapidly than smaller wetlands in colder climates (Moreno-Mateos et al. 2012), thus small, temperate freshwater wetlands, such as the ones in this study, are more likely to underperform relative to their natural counterparts. For example, litter decomposition rate, tissue nutrient concentrations, aboveground biomass production, soil organic carbon, nitrogen, and plant available phosphorus were significantly higher in natural than restored wetlands (Fennessy et al. 2008; Moreno-Mateos et al. 2012; Jessop et al. 2015; Whigham et al. 2019).

Likewise, a study of 30 mitigation wetlands in Illinois demonstrated that biodiversity was inversely proportional to soil organic matter, biomass, decomposition, and potential denitrification rates (Jessop et al. 2015). Such results present a challenge for wetland management since promoting a specific function of a wetland may decreases another function or service. Hydrology may differ significantly between natural and created wetlands, with potential for longer inundation times and greater water depth in created wetlands (Cole and Brooks 2000). Created wetlands are often deeper and more homogenous, which will change the macrophyte communities and the role of grazers, and in turn influence nitrogen cycling processes. Therefore, the functioning in created wetlands will differ from natural wetlands but are relatively poorly understood.

1.3 Role of macrophytes in nutrient availability

Nutrient availability in wetlands is critically important because of its impacts on species composition, productivity, and plant survivorship (Elser et al. 2000). Factors controlling nutrient

availability are site specific, and include atmospheric deposition, nutrient accumulation from flooding and groundwater, nutrient leaching, and denitrification (Venterink et al. 2002). The plant community structure, specifically the submerged aquatic vegetation, can impact nutrient availability in wetlands by influencing phosphorus and nitrogen concentrations. Phosphorus removal from the water column in wetlands is primarily caused by microbial communities attached to the submerged aquatic vegetation (Dierberg et al. 2001). However, nitrogen removal in the water column by microbial communities attached to submerged aquatic vegetation can be minimal (Dierberg et al. 2001).

Nitrogen availability in wetland ecosystems is regulated by two main processes: nitrogen fixation and denitrification (Weisner et al. 1994). Biological denitrification is the primary biological pathway for nitrogen removal in wetlands by reducing nitrates to atmospheric nitrogen or nitrous oxide (Dierberg et al. 2002; Lin et al. 2017; Weisner et al. 1994; Zhang et al. 2016). Denitrification is an anaerobic process restricted to anoxic sediments or micro-environments with low dissolved oxygen (Toet et al. 2003). Denitrification rates can be highly variable and depend on multiple factors including the availability of oxygen, nitrates, pH, temperature, and labile organic matter (Ballantine et al. 2014; Toet et al. 2003). These factors are also influenced by the vegetation communities and structure as well as indirect controls such as herbivory (Winton and Richardson 2017). Figure 1.2 below outlines possible impacts herbivores may have on nutrient cycling in freshwater wetlands. Previous work has focused primarily on rates and controls on this process under anaerobic conditions in the sediment (Hanson et al. 1994; Kallner et al. 2005; Weisner et al. 1994; Zhang et al. 2016); however, others have shown measurable denitrification associated with epiphytic periphyton or in the water column (Bastviken et al. 2005; Song et al. 2011; Toet et al. 2003).

Wetland plants can provide a temporary sink by the uptake of nutrients through their roots (Brix 1994). Venterink et al. 2002 analyzed nitrogen, phosphorus and potassium budgets along biomass gradients in 44 wetlands in Western Europe and found that nitrogen was the major limiting nutrient. Different species of wetland plants can store significant amount of nutrients, which slightly contributes to wetlands ability to act as nitrogen sinks in the environment (Brix 1997). The aquatic macrophytes, *Azolla filiculoides*, *Ceratophyllum demersum*, and *Myriophyllum spicatum* filter and store nutrients efficiently at low nitrogen loading rates (Tang et al. 2017). Therefore, submerged aquatic macrophytes can play an effective role in wastewater polishing (Bastviken 2005; Song et al. 2011; Toet et al. 2003; Zhang et al. 2016). However, when these plants senesce and decompose the nutrients stored will be released back into the environment.

Periphyton, which can be attached to submerged macrophytes or free-floating mats, contain a mixture of algae, heterotrophic microbes, and detritus. Periphyton can provide significant contributions to primary productivity (Dodds et al. 2002). In wetlands and shallow lakes, periphyton can also regulate water column nutrient concentrations (Gaiser et al. 2004; Thomas et al. 2006). Communities of submerged macrophytes and periphyton can significantly increase total nitrogen fixation (Liao and Inglett 2011). Both phosphorus and nitrogen availability interactively control nitrogen fixation in periphyton communities in submerged aquatic vegetation (Liao et al. 2014). Nitrogenase activity may increase with higher phosphorus concentrations and decrease with higher nitrogen concentrations (Inglett et al. 2004, 2009). Although periphyton nitrogen fixation has received little attention in temperate wetland systems, it is considered to have a major role in the nitrogen budget and cycle in the Florida, Everglades' tropical freshwater wetland system (Liao and Inglett 2011). Seasonal flooding patterns are an

important factor that can regulate periphyton productivity (Liao and Inglett 2013). Therefore, seasonal patterns of nitrogen fixation can be established in certain areas with overall higher nitrogen fixation in the wet season (Liao and Inglett 2013).

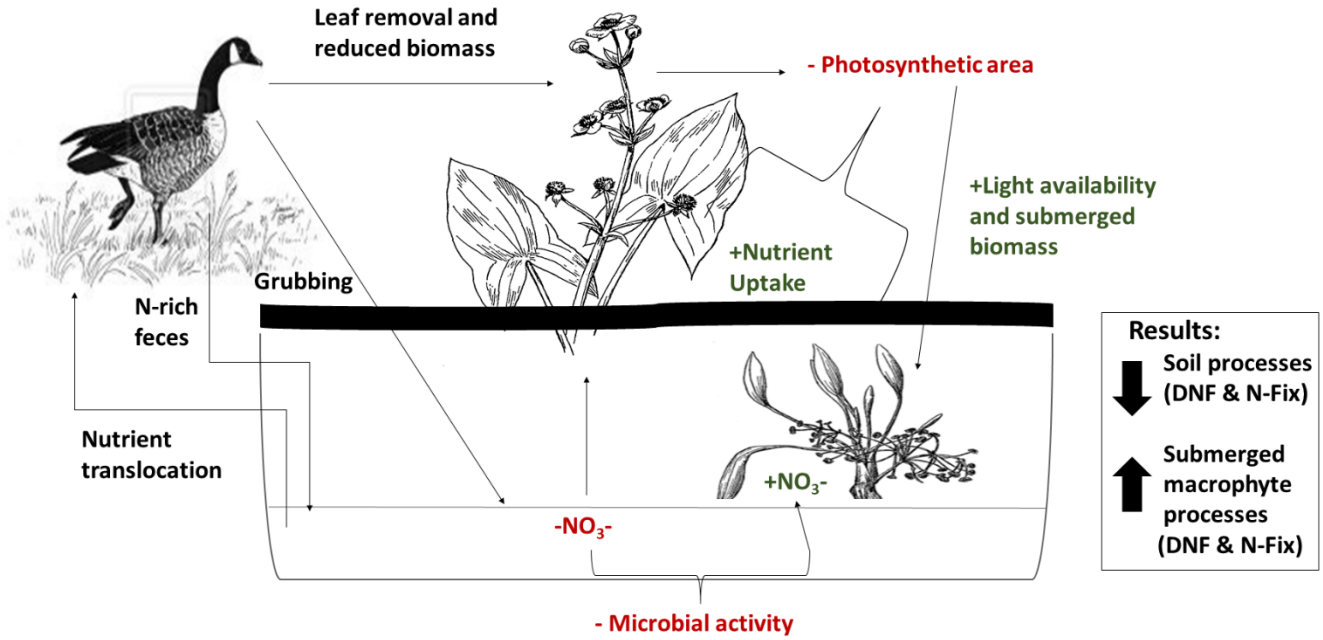

Figure 1.2: Possible interaction between herbivory and nutrient cycling in a freshwater wetland.

1.4 Hydrology influences on herbivory and macrophyte community structure

Hydrology is an important abiotic factor in wetlands that can influence the plant community structure, nutrient availability, and the presence of herbivory. Reduced hydrological variability of aquatic ecosystems (reservoirs or systems with controlled water levels and flooding) can lead to significantly lower species richness and density of waterbirds (Kingsford et al. 2004). Systems with controlled water levels limit opportunities for terrestrial and woody vegetation colonization. Emergent vegetation provides important microhabitats for biota in the aquatic system and provides breeding sites for waterfowl (Kingsford et al. 2004). Variation in water depths within wetland ecosystems can have substantial impacts on nitrogen cycling processes, such as nitrogen fixation and nitrogen removal. Strong water level fluctuations allow

for higher plant biomass and production in shallow lake and wetland ecosystems (Rodriguez-Villafane et al. 2007). The study sites used in this experiment are permanently flooded, however, water levels may be important in wetlands that are seasonally dry.

Hydrology can influence other biotic factors in wetlands such as driving the vegetation communities present and the microbial communities in the periphyton and soils (Dierberg et al., 2001). Submerged macrophytes play a key role in maintaining clear water in shallow lakes (Rodriquez-Villafane et al. 2007). A study observing waterfowl grazing effects in a Mediterranean lake in Spain found that *C. demersum* was more abundant in waterfowl exclusion cages than in the rest of the lake, however, waterfowl grazing did not statistically impact the submerged macrophyte biomass (Rodriquez-Villafane et al. 2007). Because created wetlands often have deeper water levels and longer hydroperiods the role of submerged plants may be more important and not well understood. Wetlands that are designed with deeper water levels and fewer fluctuations in water level will drive the plant community structure to submerged aquatic vegetation and emergent vegetation such as white-water lily (*N. odorata)* (Weisner et al. 1994). Deep and permanent water levels establish anerobic conditions which may cause differences in denitrification rates between created and natural wetlands.

The impacts of hydrologic pulsing and hydrologic variation on nitrogen cycling processes and microbial communities has not been well examined. Denitrification rates in shallow water marshes and vegetated deeper water level marshes may have more sensitivity to hydraulic pulsing events than deeper water level marshes without vegetation (Song et al. 2010). This could be explained by the aerobic conditions in shallow marshes during the dry season and increase in nutrients during flooding events. However, hydrologic pulsing did not cause any significant changes to the microbial communities present in the marshes (Song et al. 2010). Hydrology

certainly impacts certain nitrogen cycling processes but there are many factors such as temperature, plant communities, and substrate that may influence microbial communities.

1.5 Impacts on plant communities

Herbivory can directly influence vegetation community composition and lead to community shifts in terrestrial and aquatic ecosystems. Some studies note that herbivores may remove up to 10x more macrophyte biomass in aquatic ecosystems than in terrestrial ones (Turcotte et al. 2014; Wood et al. 2017) (Table 1.1). Herbivory can be challenging to quantify in aquatic systems because it's heterogeneous spatially and temporally. Waterfowl will congregate in select sites based on season and feeding changes (Badzinski et al. 2006). Therefore, changes in macrophyte biomass may be correlated to seasons in specific locations. Some wetland sites may experience earlier waterfowl grazing in the summer because of climate change, which could result in less tuber biomass in autumn (Gyimesi et al. 2011; Klaassen et al. 2006). High grazing intensity in the summer and autumn can lead to biomass reduction and vegetation composition shifts (Perrow et al. 1997). A modeling approach to plant herbivore interactions concluded that herbivory influences plant competition by giving tall, less palatable vegetation, such as phragmites and cattails, a competitive advantage (Oene et al. 1999). Therefore, herbivores can shift both the quantity and quality of litter accumulation by promoting unpalatable vegetation with lower nutrient content.

Table 1.1 Grazing impacts on wetland vegetation communities, above-ground biomass, and below-ground biomass.

		Impact on		
Ref	Grazer(s)	Vegetation Communities	Above-Ground Biomass	Below-Ground Biomass
1	Mute swans	Vegetation	No impact.	No impact.

	(*Cygnus olor*) Canadian Geese (*Branta canadensis*)	communities remained the same in this exclosure experiment.		
2	Waterfowl	Species composition changed and shifted inside the exclosure as opposed to in the open water of the wetland.	Plant biomass reached a higher value inside the cages than in the lake. However, no significant impact.	Plant biomass reached a higher value inside the cages than in the lake. However, no significant impact.
3	Mute swans (*Cygnus olor*)	Coontail and Eurasian water milfoil were the most common species in the exclosures and in the open water of wetland.	The mute swans did not impact the above-ground biomass of the submerged vegetation.	Mean below-ground biomass was significantly higher in the exclosures than in the control plots.
4	Muskrats (*Ondatra zibethicus*)	Species composition includes cattails (*Typha* spp.), bulrush (*Scirpus* spp.), and water lilies (*Nympaea* spp.).	Muskrats significantly remove cattails for consumption and to create mounds.	No impact.
5	Waterfowl	Grazing pressure in the summer was the most important variable predicting autumn vegetation community composition and biomass.	Large-bodied mute swans caused major impact on aboveground vegetation earlier in the summer (June).	Summer and autumn tuber biomass were significantly reduced by waterfowl grazing.
6	Coot (*Fulica atra)*	Waterfowl significantly decreased macrophyte cover in the summer and macrophyte consumption was 76-fold higher than in the spring.	No impact.	No impact.

1. *Badzinski et al. 2006, 2. Rodriguez-Villafane et al. 2006, 3. Stafford et al. 2012, 4. Kadlec et al. 2006, 5. Gyimesi et al. 2011, 6. Perrow et al. 1997, 7. Kadlec et al. 2007*

However, few studies evaluate impacts on submerged vegetation, and those that have demonstrate contrasting results. Biomass of the emergent, perennial macrophyte, *Lythrum salicaria*, was significantly lower in the presence of below-ground herbivores, such as muskrats and weevils (Notzold et al. 1998). Likewise, the disappearance of submerged macrophytes from two shallow lagoons in Spain was attributed to a combination of waterfowl grazing and the presence of exotic crayfish and fish (Rodrigo et al. 2012). In contrast, grazing by waterfowl in ten shallow lakes in eastern England did not significantly impact submerged macrophyte biomass relative to potential growth rates (Perrow et al. 1997).

Herbivores can also exert a strong influence over vegetation community structure through non-consumptive behavior that can physically alter the wetland system. Large waterfowl and muskrats often consume the root systems and rhizomes of vegetation, which is known as grubbing. Grubbing caused by waterfowl has been linked to soil erosion, loss of organic soil, and increased surface temperatures (Esselink et al. 1999). Muskrats feed by grubbing as well and can be especially disruptive in restored wetlands because they will burrow into banks, berms, and dikes to create their dens (Kadlec et al. 2006). Vegetation surveys in created wetlands indicate grazing selectivity varies seasonally and by site location (Lodge et al. 2016). These impacts on vegetation community structure are likely to, in turn, have strong indirect impacts on wetland ecosystems by modifying the, nutrient availability, and soil processes.

1.6 Impacts on nutrient availability

Herbivory has been noted as a powerful indirect control on nutrient cycling and other biogeochemical processes in wetlands (Winton and Richardson 2017). Herbivory may decrease potential denitrification rates in emergent freshwater wetlands (Lodge 2017). Lodge 2017

observed a potential shift in macrophyte composition because of grazing pressure. I am continuing the work of Lodge 2017 by examining the entire nitrogen cycling, including nitrogen fixation, in order to assess the amount of nitrogen entering the wetlands. There is a lack of research quantifying the impacts of herbivory on nutrient availability in both, created and natural freshwater wetlands. This poses a challenge for designing and creating wetlands because these impacts by herbivores are not understood. In wetlands, large densities of herbivores, particularly waterfowl, can cause an increase in nutrient concentrations through non-consumptive effects, such as trampling and fecal deposition that impact soil processes and plant growth (Bakker et al. 2016; Martinsen et al. 2011). These impacts in freshwater wetlands could be exacerbated in migration stopover sites or sites that provide seasonal habitat for high populations of waterfowl because of the large density of waterfowl populations. Figure 2 highlights potential impacts herbivory may have on nutrient availability and nitrogen cycling in freshwater wetlands. Herbivory in wetlands can lead to major impacts on carbon and nitrogen content of plant tissues, nutrient cycling, and accelerate decomposition of targeted plant leaves and stems (Hunt-Joshi et al. 2004).

The impact herbivores have on nutrient inputs and soil processes may also influence the diversity of microbial communities that drive soil processes, such as anaerobic denitrification. A study looking at the grazing impacts of deer in a floodplain wetland in China concluded grazing promoted nutrient availability that favored bacterial communities (Chen et al. 2013). However, there aren't many studies focusing on freshwater wetlands in temperate regions, so the results and impacts of herbivory may be variable and depend on site-specific differences (See Figures 4 and 5). The relationship between herbivory and submerged macrophytes, which can in turn have

significant impacts on biogeochemical processes in wetlands, needs to be more carefully studied in created wetlands.

The overall goal of the study was to examine the influence of herbivory on plant community composition, nutrient availability, potential denitrification rates, nitrogen fixation rates, and nutrient cycling in created wetlands. A better understanding of the impacts by herbivory can inform the design and management of wetland. The influence of herbivory was evaluated in three created, emergent freshwater wetlands with different prior land use histories. However, the created wetlands had similar hydrology regimes. We hypothesized that: 1) herbivores will selectively graze the emergent macrophytes and thereby promote competitive ability of submerged macrophytes, 2) this shift in macrophyte community structure will influence nitrogen removal by decreasing emergent macrophyte root uptake and soil immobilization, ultimately leading to a decrease in nitrogen removal capacity, and 3) a shift in macrophyte community structure from emergent vegetation to submerged vegetation and periphyton communities will allow for higher light availability and microbial activity in the water column; resulting in higher nitrogen fixation rates.

2.0 Methods

2.1 Site description:

This study was conducted in three created wetlands: High Acres Nature Area (HANA) located in Perinton, NY and two sites on Rochester Institute of Technology's (RIT) campus in Rochester, NY. The HANA site, Area 1 North (A1N) was created in 2009 and is owned and managed by Waste Management of NY, LLC (Appendix A). The wetlands at RIT were created in 2002 (R2) and 2007 (R1) (Appendix B). All sites are permanently flooded emergent, emergent wetlands.

A1N has a complicated land use history. The site was formerly used as a repository for the adjacent gravel mine, prior to three decades left fallow. Antecedent to the gravel industry, the land was likely used for row-crop agriculture. This wetland was created as part of a required mitigation because of the High Acres Landfill expansion in 2007. A1N was created with the intention of providing wildlife habitat, flood control, and pollution and sediment removal/retention. After construction, A1N, was quickly colonized by invasive plants, such as cattails (*Typha latifolia* and *Typha angustifolia*) and purple loosestrife (*Lythrum salicaria*). In 2011, invasive plants were controlled annually by manual cuttings and periodic herbicide application. Manual cuttings continued until 2014. Native species were also planted in 2011 and, the wetland is dominated by broadleaf arrowhead (*Sagittaria latifolia*), white waterlily (*N. odorata*), and pickerel weed (*Pontederia cordata*) (Appendix C).

Both R1 and R2 were constructed on land previously used for row crop agriculture. The surrounding landscape consisted of secondary growth forest and forested wetlands. Neither wetland is actively managed at present and both sites currently have a high density of invasive plants, primarily cattails (*T. latifolia* and *T. angustifolia*) and common reed (*Phragmites*

australis). R1 is dominated by cattails (*T. latifolia* and *T. angustifolia*) and soft stem bulrush (*Schoenoplectus tabernaemontani*) on the shoreline and bladderwort (*Utricularia minor*) in deeper water (Appendix C). R2 is dominated by cattails (*T. latifolia* and *T. angustifolia*) on the shoreline and white waterlily (*N. odorata*) in the open water (Appendix C).

2.2 Experimental design: Herbivore exclusion

In June 2014, as described in Lodge (2017), 4 blocks and 4 pairs of caged (herbivore excluded) and uncaged (open to herbivores) and 1 cage-control plot per block were established in A1N (Lodge 2017). These herbivore exclusion plots have been part of a long-term research study since 2014, which therefore, can have a different impact on the vegetation communities than herbivore exclusion plots more recently established (such as R1 and R2). In all three wetlands, paired plots were placed in blocks of 4 throughout the wetland. The caged, uncaged, and cage-control plots established were 1x1 meter. The caged plots were constructed by wrapping hardware mesh cloth (1.25 cm^2, 1.2 meters tall) around 4-PVC pipes at each corner of the plot. Uncaged plots were marked with PVC pipes only. The cage-control plots were constructed by covering 3 of the 4 sides with hardware cloth to rule out cage effects. Because the plant communities in the cage-control plots were not significantly different than the control plots, we did not use them in this study (Lodge 2017). In May 2018, 4 pairs of caged and uncaged plots were established in R1 and 8 pairs of caged and uncaged plots were established in R2 to evaluate the impact of grazing across multiple sites. R1 and R2 had a different land-use history than A1N in order to compare herbivore exclusion between different wetland sites.

Hydrological conditions were evaluated within the plots by measuring surface water depth at three points in each plot every six weeks between May and September of 2018 and 2019.

Grazing pressure was monitored approximately twice a week at each site. A HANA volunteer, Bruce Cady, helped quantify grazing pressure by conducting a waterfowl observational study throughout 2018 and 2019. The large herbivores that were recorded include Canada geese (*Branta canadensis)*, ducks (*Anas* spp.), muskrats (*Ondatra zibethicus*), and white-tailed deer (*Odocoileus virginianucs*). Wetland study sites were visited approximately two to four times each month for a period of 20 minutes throughout 2018 and 2019, and the time of day alternated between early morning and late afternoon. Number of individual grazers, date, time of day, and environmental conditions were recorded. The number of observations at each site was variable, with substantially more at HANA.

2.3 Vegetation communities

Vegetation data in each plot was collected every six weeks from May 2018 through October 2018 and again from May 2019 through October 2019. Vegetation measurements include total plant cover (total plant cover can be greater than 100% because of differences in plant height and submerged vegetation under the water surface), proportion of total cover (emergent to submerged macrophytes), and large grazer damage. Total area of plant cover and specific species percent cover were calculated.

The density of each plant species was measured by recording the number of stems per species per plot. The heights of the tallest individuals of each species in the plots were recorded. Belowground biomass was measured in September 2018 and September 2019 within each plot.

Samples were collected using 5 cm diameter sediment core to a depth of 20 cm, washed through a sieve (1 mm) to remove all soil and gravel, and the remaining plant material dried in an oven at 60°C, weighed, and biomass per m^2 was calculated (Hunt-Joshi et al. 2004).

At the end of September 2019, emergent macrophytes were collected from caged and uncaged plots at A1N, R1, and R2. The emergent macrophytes were ground using a mortar and pestle and electric coffee mill and analyzed for percent C and N content of the macrophytes was measured using a Perkin Elmer 2400 CHNSO elemental analyzer.

In October of 2019, the relationship between percent cover and biomass was established for the three-dominant submerged macrophytes used for periphytic nitrogen fixation measurements (section 2.6; Appendix G) were harvested by percent cover of 0.25 m x 0.25 m plot. The submerged macrophytes were harvested at 5 linear percent cover ranges (10%, 20%, 30%, 40%, 50%) and there were ten replicates for each submerged macrophyte at each percent cover range. The harvest macrophytes were dried at 60°C to calculate the dry weight and linear regressions were used in combination with the percent cover data from vegetation surveys to determine the amount of nitrogen fixation per dominant submerged macrophyte per plot.

2.4 Soil Characteristics

Soil characteristics, including organic matter content, extractable soil nitrate and ammonium, total and inorganic phosphorus, and total carbon and nitrogen in September 2018. In each plot, two 10 cm deep soil cores were removed from each plot using a tulip bulb corer, placed in zipper top bags and frozen until laboratory analysis.

The first core was dried at 60°C and subdivided for percent organic matter (OM) and elemental analysis. OM was measured using the loss-on-combustion method at 540°C in a

muffle furnace (Heiri et al. 2001). Soil for elemental analysis was ground using a mortar and pestle. Total (TP) and inorganic (IP) phosphorus were measured by weighing two 0.1g subsamples and placing them into glass scintillation vials. Total phosphorus samples were mixed with 0.5 mL of Mg $(NO_3)_2$ and combusted for 2 hours at 540°C. Ten mL 1M HCl was added to both TP and IP samples, which were then placed on a horizontal shaker for 16 hours and allowed to settle for 24 hours. Supernatant liquid was analyzed using the ammonium molybdate method and a Shimadzu 1800 Spectrophotometer (Murphy and Riley 1962). Total soil C and N content was measured using a Perkin Elmer 2400 CHNSO elemental.

The second core was subsampled into paired 5 g wet aliquots. The first was shaken for 1 hour with 50 ml 2M KCl and filtered (0.45 um) into Whirl-pak bags. The other aliquot was dried in a 60°C oven to obtain dry weights of the soil. Extractable nitrate was measured using the vanadium chloride method and a Shimadzu 1800 Spectrophotometer (Doane and Horwáth 2003). Extractable ammonium was analyzed using the phenol-hypochlorite method on a Shimadzu 1800 Spectrophotometer (Solorzano 1969). All samples were run in duplicate.

2.5 Potential Denitrification

Potential soil denitrification was measured using the acetylene block method (Toet et al. 2003; Lodge and Tyler 2020) every six weeks in the Spring, Summer, and Fall of 2018 and 2019. Acetylene (C_2H_2) blocks the final process in microbial (anaerobic) denitrification, the conversion of N_2O to N_2 gas, and the subsequent accumulation of N_2O can be used to estimate potential denitrification. Samples were collected from a subset of 8 pairs (of 16 pairs) of caged and uncaged plots in Area 1 North, all 4 pairs in R1, and 4 pairs (of 8 pairs) in R2, using a tulip bulb

corer (2.5 cm diameter x 10 cm depth), placed in zipper top plastic bags, transported back to the lab and refrigerated overnight.

Two 20 g wet weight sub-samples were weighed; one was placed into a160 mL serum bottle and the other was dried at 60°C to calculate the dry weight. In order to promote optimal microbial conditions 10 mL of N2 sparged (20 min) media was added to each serum bottle (100 mg/kg N using KNO_3, 40 mg/kg dextrose, and 10 mg/kg chloramphenicol) and 10 mL of sparged, nanopure water. The serum bottles were flushed with N2 gas to replicate an anoxic environment (3 cycles, 2 minutes) and acetylene was added to each serum bottle (10% of the headspace). Gas samples (11 mL) were collected initially and then at approximately 30, 60, and 120 min after the initial sample and analyzed using a Shimadzu Greenhouse Gas Analyzer Gas Chromatograph. N_2O data (ppm) was converted to µmoles/gram dry soil, and rates were derived by regression over time.

2.6 Nitrogen Fixation – Soil and Submerged Macrophytes

Nitrogen fixation was measured in soil and in periphyton using the acetylene reduction assay (Yoshida and Ancajas 1970). The same laboratory methods described above for the acetylene block method were used for soil measurements, except that soil was incubated in an anaerobic environment using 60 ml of artificial freshwater media (1.2 g/kg $MgSO_4$, 1.92 g/kg $NaHCO_3$, 80 mg/kg KCl, and 1.2 g/kg $CaSO_4$). Periphyton nitrogen fixation was measured for the dominant submerged macrophyte (highest percent cover) in each pair of caged and uncaged plots during the spring, summer, and fall of 2019 (Inglett et al. 2004; Liao and Inglett 2014). N fixation was measured separately in the light (autotrophic and heterotrophic N fixation) and the dark (heterotrophic N fixation only) by darkening half of the serum vials with aluminum foil.

Five g (wet weight) of submerged plant material was placed into serum bottles with 60 ml artificial freshwater media. Dry plant mass was obtained at the end of the experiment. The plant biomass obtained in section 2.3 was used to estimate areal N fix associated with periphyton. Gas samples were collected at the time intervals and analyzed as above.

2.7 Sediment-water column nutrient fluxes

Sediment-water column fluxes of nitrate, ammonium, phosphate and dissolved inorganic carbon (DIC) were measured in site using benthic chambers in late May and late September 2019. The same subset of cages as used for the denitrification and N fixation were used. Chambers were constructed of 9.5 cm inner diameter by 30 cm tall polycarbonate tubing, capped at one end with a butyle rubber stopper fitted with silicone inflow and outflow tubes. Headspace water was continuously recirculated using a peristaltic pump to prevent stratification. Chambers were inserted into the soil and allowed to equilibrate for 30 min prior to sampling. Water samples were collected from an inline stopcock using a 60 mL syringe at 0, 45, 90, and 135 min (Needham et al. 2011). In the field, each sample was filtered through a Supor 0.45 μm filter into a whirl-pak and frozen until analysis. Dissolved inorganic carbon (DIC) samples were placed directly into 1.5 mL screw cap vials without being filtered. After the first set of measurements in the light was made, the chambers were wrapped in aluminum foil to block light, and a set of dark flux measurements were made at the same time intervals. Light availability was measured using a LiCor 4pi underwater sensor concurrent with benthic flux measurements at the water surface, just below the water surface, and at the sediment interface (Appendix H).

In the laboratory, the ammonium molybdate method was used to measure PO_4^{3-} (Murphy and Riley 1962), the phenol-hypochlorite method using nitroprusside as a catalyst was used to

measure NH_4^+ (Solorzano 1969), and the vanadium chloride method was used to measure NO_3^- (Doane and Horwáth 2003). All samples were measured using a Shimadzu 1800 Spectrophotometer. DIC samples were measured by adding 1 µl of sample to concentrated H2SO4 and then measured using a LI-COR 820 CO2 gas analyzer (Neubauer and Anderson 2003). Solute fluxes were determined by linear regression as a function of time, chamber volume, and sediment surface area (Tyler et al. 2001). DIC flux measurements were expressed in terms of gross primary productivity (GPP), net ecosystem metabolism (NEM), and ecosystem respiration (ER). GPP was calculated by the difference between light and dark measurements. Daily NEM was calculated assuming 10 hr dark and 14 hr light.

2.8 Statistical Analysis

All statistical analyses were completed using JMP 16 Pro statistical software. Each dataset was checked for normality and homogeneity of variance and transformed as needed to meet the assumptions of each statistical test.

Soil characteristics that were measured one time, including organic matter, total phosphorus, organic phosphorus, inorganic phosphorus, total inorganic nitrogen, ammonium, and C:N ratios were analyzed using a full-factorial one-way ANOVA with treatment (caged, uncaged) as a fixed factor.

Site comparisons of total macrophyte cover, total emergent macrophyte cover, and total submerged macrophyte cover using a full-factorial one-way ANOVA to compare these macrophyte covers with treatment (caged, uncaged). Belowground biomass measurements and macrophyte C:N ratios were also analyzed using a full-factorial one-way ANOVA to compare with treatment (caged, uncaged). An additional one-way ANOVA was used to compare different

emergent macrophytes C:N ratios in all wetland study sites with treatment (caged, uncaged) (Appendix E).

Potential denitrification and nitrogen fixation rates were both analyzed using a full-factorial two-way ANOVA for comparisons of both potential denitrification and nitrogen fixation rates with season (spring, summer, and fall), and grazing treatment (caged, uncaged). Water column nutrient flux (daily rate) measurements, consisting of ammonia, phosphorus, nitrate, and dissolved inorganic carbon fluxes, were also analyzed using a full-factorial two-way ANOVA using season (early summer and late summer) and treatment (caged, uncaged) as fixed factors. For all ANOVAs, when significant differences were found a Tukey's HSD post hoc analysis was used to reveal differences among treatments.

3.0 Results

3.1 Hydrology

All wetlands remained flooded throughout the study, with higher water in spring and early summer, and lower in the late summer and fall. R2 had consistently deeper average water depths (2018: 40.6 ± 0.8 cm, 2019: 41.2 ± 0.9 cm; mean ± SE) than both A1N (2018: 37.7 ± 0.5 cm, 2019: 28.6 ± 0.9 cm; mean ±SE) and R1 (2018: 29.7 ± 2.6 cm, 2019: 34.9 ± 2.0 cm; from here forward, all values are mean ± SE; Figure 3.1).

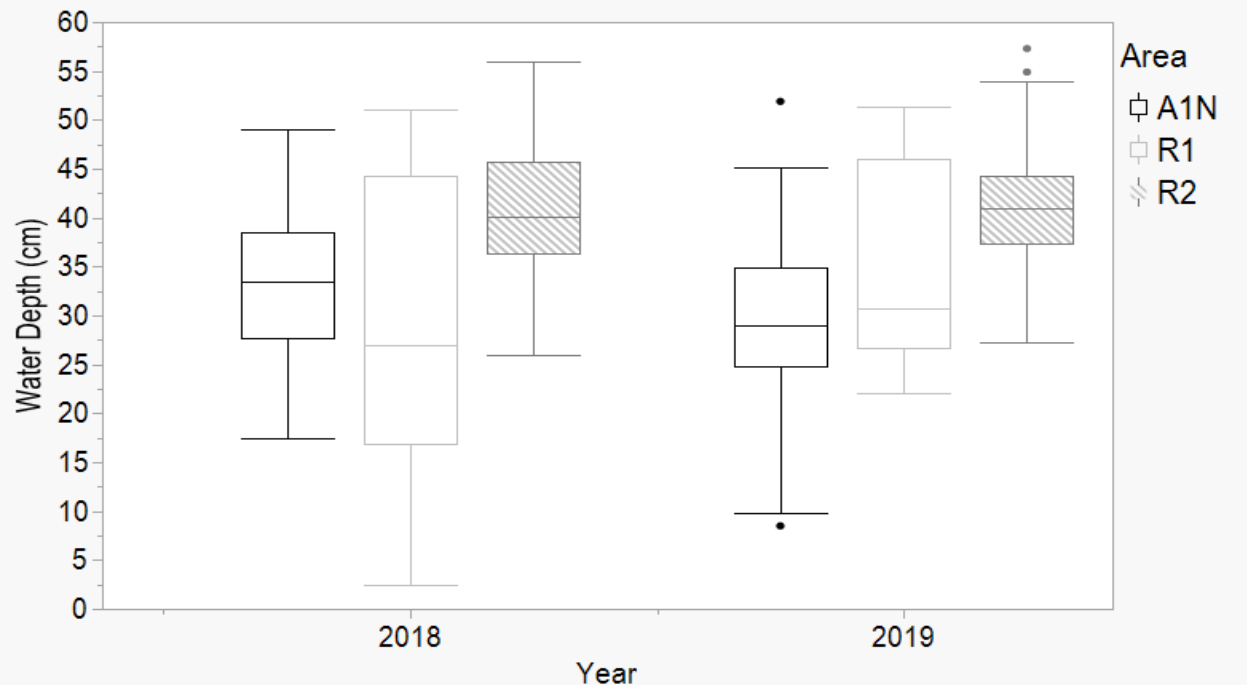

Figure 3.1: Boxplots showing average water depths across the growing season (May – September) in A1N, R1, and R2 for 2018 – 2019.

3.2 Grazing pressure

A1N had the largest number of grazers per unit area throughout all seasons (Figure 3.2), and grazers included a variety of waterfowl, but especially *B. canadensis*. Abundance peaked during the fall migratory season and was lowest in summer. Grazer abundance was substantially

lower at R1 and R2, where *O. virginianus* (R2 only), *B. canadensis* (R2 only), and *O. zibethicus* (R1 and R2) were the only species observed.

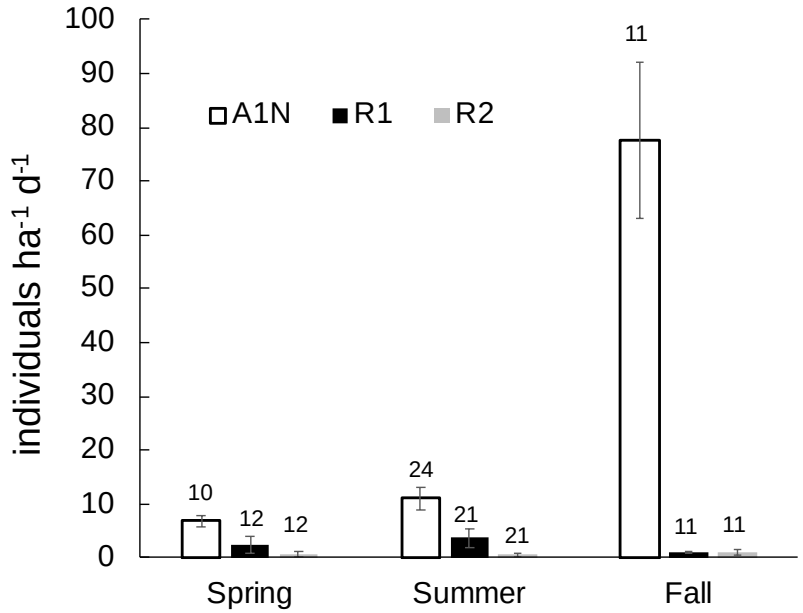

Figure 3.2: Average large grazer counts observed at A1N, R1, and R2 wetlands between June 2018 and October 2019 (spring= March – May, summer= June – Aug, fall= Sept – Nov, winter is excluded; mean ± SE). Values above bars are the total number of counts per season, across both years.

3.3 Soil nutrient characterization

In spite of the shorter time since marsh construction, soil OM was higher at A1N than R1 (15.0 ± 1.9 versus 4.3 ± 1.0 %) or R2 (6.1 ± 1.9 %), which were similar (Table 3.1). Similarly, soil TP (1.2-1.4 times), IP (1.3-1.8 times), and ammonium (6.3-8.2 times) were higher in A1N than R1 and R2 (Table 3.1), with no grazing effect (Table 3.2). Extractable nitrate was very low at all sites. Soil C:N was higher at A1N than R1 or R2, where higher variability was present (Table 3.1). There was a significant increase in both %C and %N in caged plots at A1N, potentially reflecting increased macrophyte production within the cages (Tables 3.1 and 3.2).

Table 3.1. Mean ± SE values for soil chemistry measured in three wetlands in fall 2018.

	A1N		R1		R2	
	Caged	Uncaged	Caged	Uncaged	Caged	Uncaged
OM (%)	15.1±1.5	15.0±0.9	2.6±0.5	4.3±1.0	6.3±1.4	6.1±1.9
TP (µmol/g)	30.3±1.3	27.4±0.84	25.5±1.7	23.8±2.0	18.9±1.8	19.4±1.0
IP (µmol/g)	21.4±0.8	20.0±0.8	15.2±0.5	15.7±0.5	16.1±3.6	11.1±1.0
OP (µmol/g)	8.8±0.9	7.4±0.6	10.3±1.5	8.2±1.6	2.8±3.6	8.2±1.5
NO_3^- (nmol/g	42±2	45±3	45±2	43±1	45±2	42±3
NH_4^+ (µmol/g)	44.1±7.1	42.9±7.6	5.8±3.2	5.2±0.45	5.2±1.4	6.9±2.7
%C	6.5±0.33	5.1±0.36	1.6±0.2	1.9±0.4	2.0±0.4	2.4±0.3
%N	0.39±0.02	0.29±0.02	0.16±0.02	0.17±0.05	0.14±0.03	0.17±0.02
C:N	19.7±0.7	20.8±0.7	11.7±0.6	16.6±4.0	17.9±2.0	16.9±1.2

Table 3.2: Results of one-way ANOVA examining the effect of grazing treatment (caged, uncaged) on soil characteristics. Significant p-values are bolded, p<0.001 are starred (*).

	A1N		R1		R2	
	F	**p**	**F**	**p**	**F**	**p**
OM	$F_{1,31}$=0.4	0.54	$F_{1,8}$=2.6	0.16	$F_{1,8}$=0.0	0.95
TP	$F_{1,31}$=3.3	0.08	$F_{1,8}$=0.4	0.54	$F_{1,8}$=0.0	0.84
IP	$F_{1,31}$=1.3	0.26	$F_{1,8}$=0.5	0.50	$F_{1,8}$=1.8	0.23
NO_3^-	$F_{1,32}$=1.0	0.33	$F_{1,8}$=1.3	0.30	$F_{1,8}$=0.0	0.91
NH_4^+	$F_{1,15}$=0.0	0.983	$F_{1,8}$=0.1	0.83	$F_{1,8}$=0.4	0.56
% C	$F_{1,31}$=8.2	**0.0077***	$F_{1,8}$=0.5	0.50	$F_{1,8}$=0.5	0.51
% N	$F_{1,31}$=11.0	**0.0024***	$F_{1,8}$=0.2	0.6	$F_{1,8}$=1.2	0.31
C:N	$F_{1,29}$=1.7	0.21	$F_{1,8}$=1.4	0.28	$F_{1,8}$=0.2	0.67

3.4 Macrophyte cover

The plant community composition of A1N was characterized by obligate, emergent macrophyte species (Appendix C & F), primarily *S. latifolia*, *S. filiformis*, and *P. cordata,* with high emergent cover relative to the other two sites. Over the course of the study period (2018–2019) submerged macrophytes, specifically *Elodea canadensis* spread throughout the wetland.

The plant community composition of R1 was characterized by submerged macrophytes with obligate, emergent macrophytes, such as *S. tabernaemontani* and *T. latifolia*, surrounding the edges (Appendix C & F). The plant community composition of R2 was characterized primarily by the obligate, emergent macrophyte *N. odorata*. Dominant submerged macrophytes include *L. trisculca* and *U. minor* at both R1 and R2 (Appendix C & F).

At A1N both emergent and submerged cover were significantly enhanced within the cages (p=0.0062 and 0.0267, respectively; Table 3.3; Figure 3.3). At R1, grazer exclusion significantly enhanced submerged macrophyte cover only, especially during the peak cover period in mid-summer (p=0.0059; Table 3.3; Figure 3.3). There was no impact of grazing on either functional group at R2.

Overall, belowground biomass varied between sites and between years within a site, perhaps reflecting the heterogeneous nature of rhizomes of species such as *N. odorata*, especially at R2. Grazing did not significantly reduce belowground biomass at any site (p=0.71; Table 3.3; Figure 3.4), but there was a decrease in uncaged plots in A1N only in fall 2018 (caged= 114.6 ± 25.0, uncaged= 68.9 ± 20.9 g/m2; Figure 3.4).

The C:N of *S. latifolia*, the dominant emergent species at A1N, was slightly higher in caged plots in A1N, but not statistically different (caged=22.8±5.7, uncaged=18.8±1.9; p=0.44; Table 3.3). *N. odorata* C:N was greater than *S.* latifolia, and slightly higher in caged plots, but again not significantly so (R1: caged=33.5±1.8, uncaged=30.4±2.4; R2: caged=33.1±2.5, uncaged= 30.5±2.2; Table 3.3). Emergent macrophytes present in caged and uncaged plots were not always consistent (spatially and temporally variable) and are summarized in Appendix D.

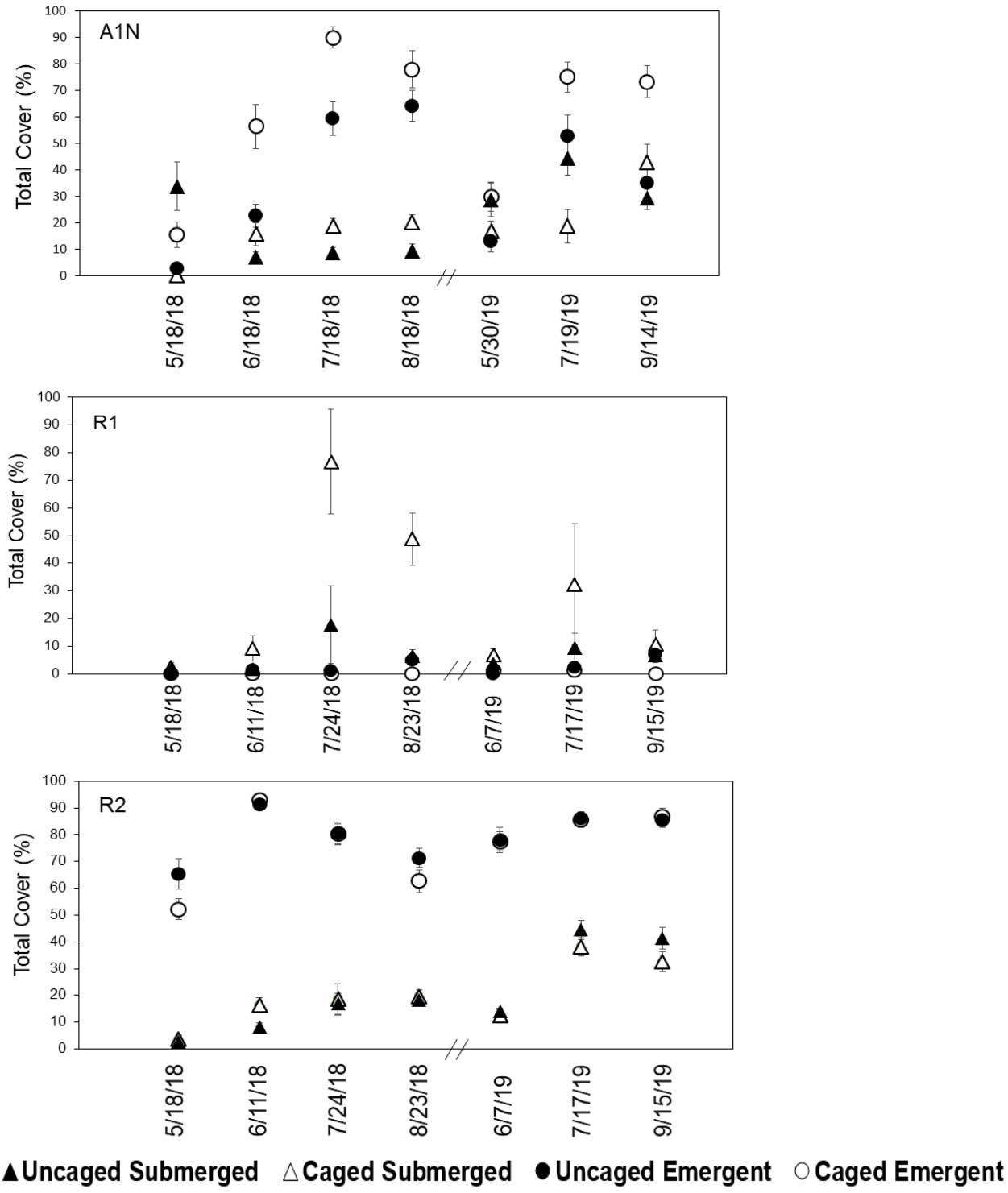

Figure 3.3: Mean ± SE total emergent (circles) and submerged (triangles) macrophyte cover in caged (open) and uncaged (filled) plots in A1N, R1, and R2 during the growing seasons of 2018 – 2019.

Table 3.3. Results of one-way ANOVA examining the relationship between grazing treatment on macrophyte characteristics and aboveground tissue C:N. Significant p-values are bolded, p<0.001 are starred (*).

	A1N		R1		R2	
	F	p	F	p	F	p
Emergent cover	$F_{1,248}$=7.6	**0.0062***	$F_{1,56}$=2.6	0.11	$F_{1,112}$=1.0	0.31
Submerged cover	$F_{1,248}$=5.0	**0.0267***	$F_{1,56}$=8.2	**0.0059***	$F_{1,112}$=0.0	0.96
Belowground biomass	$F_{1,47}$=1.3	0.25	$F_{1,14}$=0.0	0.95	$F_{1,16}$=0.3	0.61
Macrophyte C:N	$F_{1,8}$=0.7	0.44	$F_{1,8}$=1.1	0.34	$F_{1,8}$=0.6	0.47

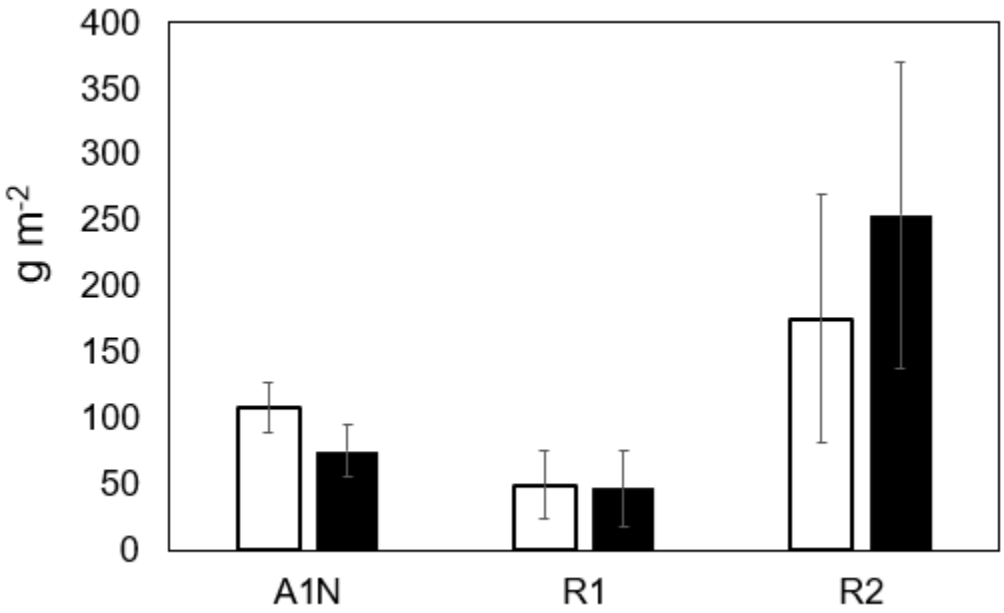

Figure 3.4: Mean ± SE belowground biomass (g m^{-2}) measured in caged (open) and uncaged (filled) plots in A1N, R1, and R2 in fall of 2018 and 2019. * indicates a significant difference between caged and uncaged plots (p<0.05).

3.5 Potential denitrification and nitrogen fixation

Potential denitrification rates significantly varied across the different wetland study sites and were substantially higher in 2018 than 2019 (Figure 3.5). Seasonal variation generally showed higher rates in spring and summer than in fall, but the only significant difference across

season was at A1N (p=0.02; Table 3.4; Figure 3.5). Overall, grazing did not statistically impact potential denitrification (Table 3.4; Figure 3.5).

Nitrogen fixation in the soil was undetectable across all sites and seasons. Periphyton N fixation at R1 was evaluated for *E. canadensis* in spring, and *C. demersum* in summer and fall, reflecting the seasonal shift in submerged macrophyte composition. *U. minor* was used for all seasons at both R1 and R2. was generally low but detectable, varying slightly between species but with no impact of grazing treatment (Figure 3.6). Nitrogen fixation in the light tended to increase over the course of the growing season, with highest rates in the spring and lowest in summer and fall, but this was only a significant pattern at R2 (p=0.0247; Table 3.4). Dark N fixation was not influenced by grazing or season. The contribution of autotrophic N fixers to total N fix ranged from 0 – 75%, with the highest contribution in the fall.

Table 3.4: Results of two-way ANOVA examining the effects of grazing treatment (caged, uncaged) and season (spring, summer, fall) on potential denitrification and epiphytic nitrogen fixation. Significant p-values are bolded, p<0.001 are starred (*). PDNF= potential denitrification, NF= nitrogen fixation, Graz= grazing treatment, Seas= seasons.

		A1N		R1		R2	
		F	p	F	p	F	p
Soil PDNF	Seas	$F_{2,72}$=4.1	**0.0204***	$F_{2,40}$=1.6	0.21	$F_{2,32}$=2.3	0.12
	Graz	$F_{1,72}$=0.3	0.59	$F_{1,40}$=1.1	0.29	$F_{1,32}$=0.0	0.94
	Seas * Graz	$F_{2,72}$=0.3	0.77	$F_{2,40}$=0.1	0.92	$F_{2,32}$=0.2	0.84
Macrophyte NFix (light)	Seas	$F_{2,21}$=0.0	0.97	$F_{2,19}$=3.1	0.08	$F_{2,15}$=5.5	**0.0247***
	Graz	$F_{1,21}$=1.2	0.28	$F_{1,19}$=2.0	0.18	$F_{1,15}$=0.5	0.47
	Seas * Graz	$F_{2,21}$=0.2	0.84	$F_{2,19}$=3.3	0.07	$F_{2,15}$=3.0	0.09
Macrophyte NFix (dark)	Seas	$F_{2,18}$=0.8	0.46	$F_{2,16}$=1.2	0.30	$F_{2,16}$=3.2	0.08
	Graz	$F_{1,18}$=0.1	0.74	$F_{1,16}$= --	----	$F_{1,16}$=0.1	0.80
	Seas * Graz	$F_{2,18}$=0.6	0.55	$F_{2,16}$=0.5	0.49	$F_{2,16}$=1.2	0.34

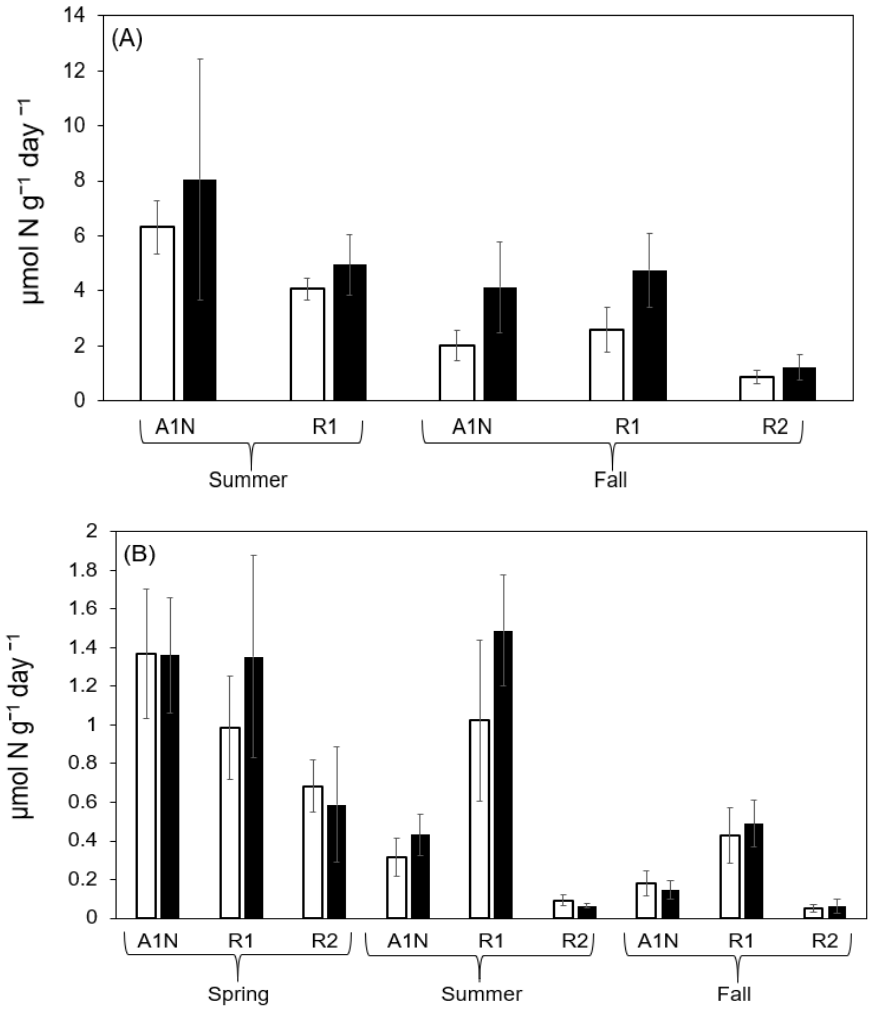

Figure 3.5: Mean ± SE rates of potential denitrification in caged (open) vs uncaged (filled) plots in A1N, R1, and R2 in (A) 2018 and (B) 2019. * indicates a significant difference between caged and uncaged plots ($p<0.05$).

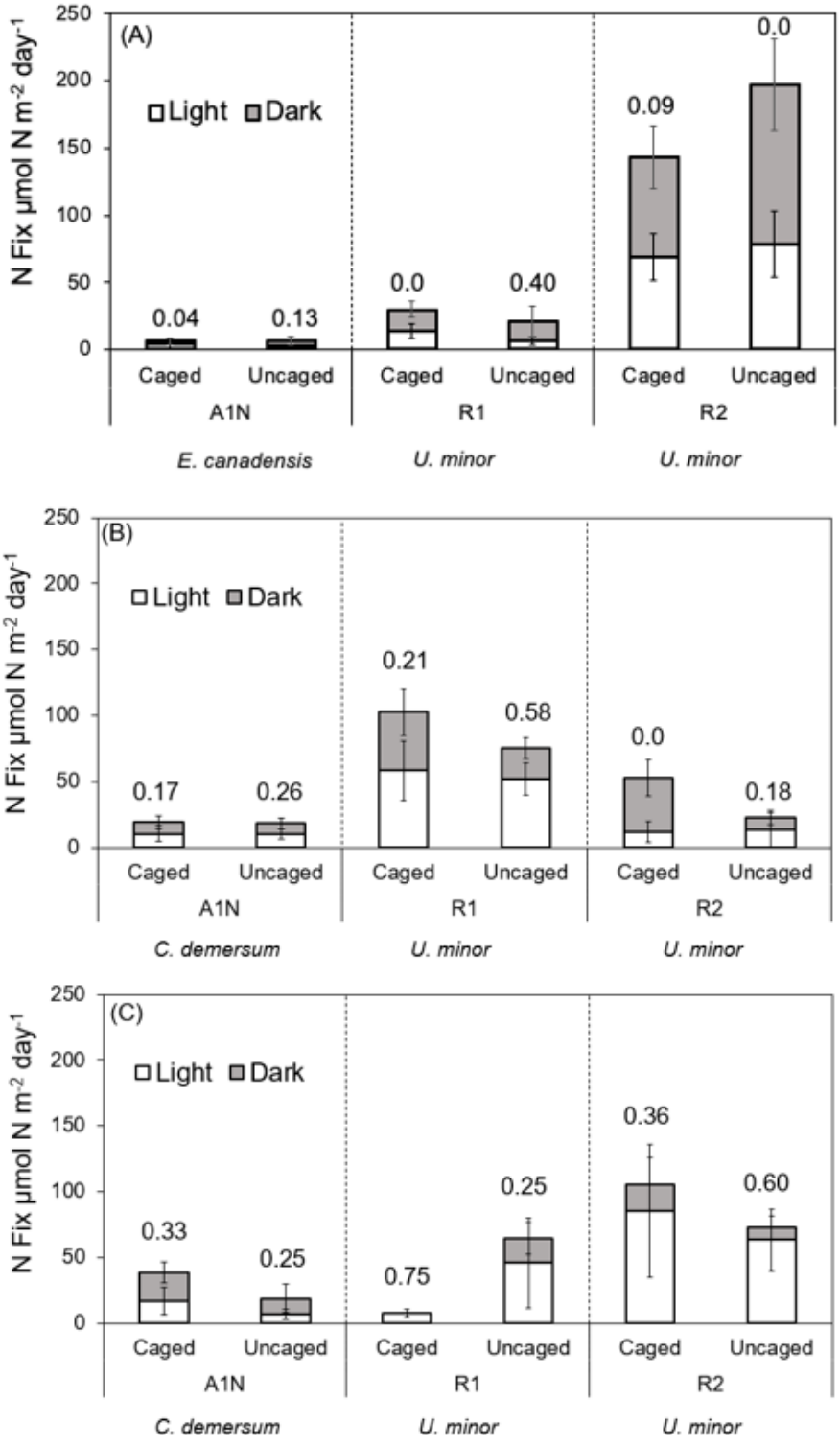

Figure 3.6: Mean ± SE areal periphyton N fixation in the light and dark in caged vs uncaged plots in A1N, R1, and R2 during (A) spring, (B) summer, and (C) fall 2019. Text values above bars indicate the contribution of autotrophic N fixation to the total N fixation in each treatment. * indicates a significant difference between caged and uncaged plots ($p<0.05$).

3.6 Sediment nutrient fluxes

Daily sediment nutrient fluxes were relatively low and highly variable for all nutrients measured and had high variability, leading to no grazing effects for any nutrient (Figure 3.10). Phosphate uptake by sediments was substantially greater in late summer than spring at R1 only (p=0.0152; Table 3.5; Figure 3.7) and nitrate and ammonium release was greater in spring than summer at A1N and R2 (ammonium only). Net ecosystem metabolism (NEM) was also variable but was significantly more autotrophic in caged plots than uncaged plots at A1N, suggesting that the greater macrophyte cover lead to increased CO_2 uptake by the benthos (p=0.047; Table 3.5, Figure 3.7). The sediment at R1 was generally autotrophic and the sediment at R2 was heterotrophic (Figure 3.7D). There were no patterns with grazing or respiration at any site (Figure 3.7E and F). Irradiance measurements were collected prior to sediment nutrient flux measurements in each caged and uncaged plot in A1N, R1, and R2 (Appendix H).

Table 3.5: Results of a two-way ANOVA examining the effects grazing treatment (caged, uncaged), and season (spring, summer) on sediment-water column nutrient fluxes, NEM, ER, and GPP in A1N, R1, and R2. Significant p-values are bolded, p<0.05 are starred (*). NEM= net ecosystem metabolism, ER= ecosystem restoration, GPP= gross primary productivity, Graz= grazing treatment, Seas= seasons.

		A1N		R1		R2	
		F	p	F	p	F	p
PO_4^{3-}	Seas	$F_{1,28}$=0.5	0.48	$F_{1,12}$=8.0	**0.0152***	$F_{1,12}$=0.1	0.72
	Graz	$F_{1,28}$=0.5	0.48	$F_{1,12}$=1.2	0.30	$F_{1,12}$=0.1	0.80
	Seas * Graz	$F_{1,28}$=0.9	0.35	$F_{1,12}$=0.9	0.36	$F_{1,12}$=1.8	0.20
NH_4^+	Seas	$F_{1,28}$=8.6	**0.0067***	$F_{1,12}$=3.4	0.09	$F_{1,12}$=0.9	0.37
	Graz	$F_{1,28}$=0.0	0.89	$F_{1,12}$=0.6	0.47	$F_{1,12}$=1.0	0.33
	Seas * Graz	$F_{1,28}$=0.1	0.74	$F_{1,12}$=0.0	0.87	$F_{1,12}$=2.3	0.15
NO_3^-	Seas	$F_{1,28}$=5.8	**0.0226***	$F_{1,12}$=0.3	0.59	$F_{1,12}$=56.8	**<.0001***
	Graz	$F_{1,28}$=0.1	0.80	$F_{1,12}$=0.6	0.44	$F_{1,12}$=1.6	0.24
	Seas * Graz	$F_{1,28}$=1.2	0.29	$F_{1,12}$=0.1	0.73	$F_{1,12}$=1.3	0.28
NEM	Seas	$F_{1,28}$=0.7	0.40	$F_{1,12}$=0.0	0.84	$F_{1,12}$=0.0	0.94
	Graz	$F_{1,28}$=4.3	**0.0467***	$F_{1,12}$=0.0	0.89	$F_{1,12}$=0.4	0.52
	Seas * Graz	$F_{1,28}$=1.0	0.33	$F_{1,12}$=1.1	0.33	$F_{1,12}$=0.1	0.71
ER	Seas	$F_{1,28}$=0.4	0.55	$F_{1,12}$=0.9	0.36	$F_{1,12}$=0.6	0.45
	Graz	$F_{1,28}$=0.4	0.51	$F_{1,12}$=0.8	0.38	$F_{1,12}$=0.1	0.80
	Seas * Graz	$F_{1,28}$=0.0	0.87	$F_{1,12}$=1.0	0.34	$F_{1,12}$=0.7	0.43
GPP	Seas	$F_{1,28}$=2.1	0.16	$F_{1,12}$=2.3	0.16	$F_{1,12}$=0.5	0.48
	Graz	$F_{1,28}$=0.6	0.46	$F_{1,12}$=1.3	0.27	$F_{1,12}$=0.1	0.73
	Seas * Graz	$F_{1,28}$=0.3	0.58	$F_{1,12}$=0.3	0.58	$F_{1,12}$=0.2	0.64

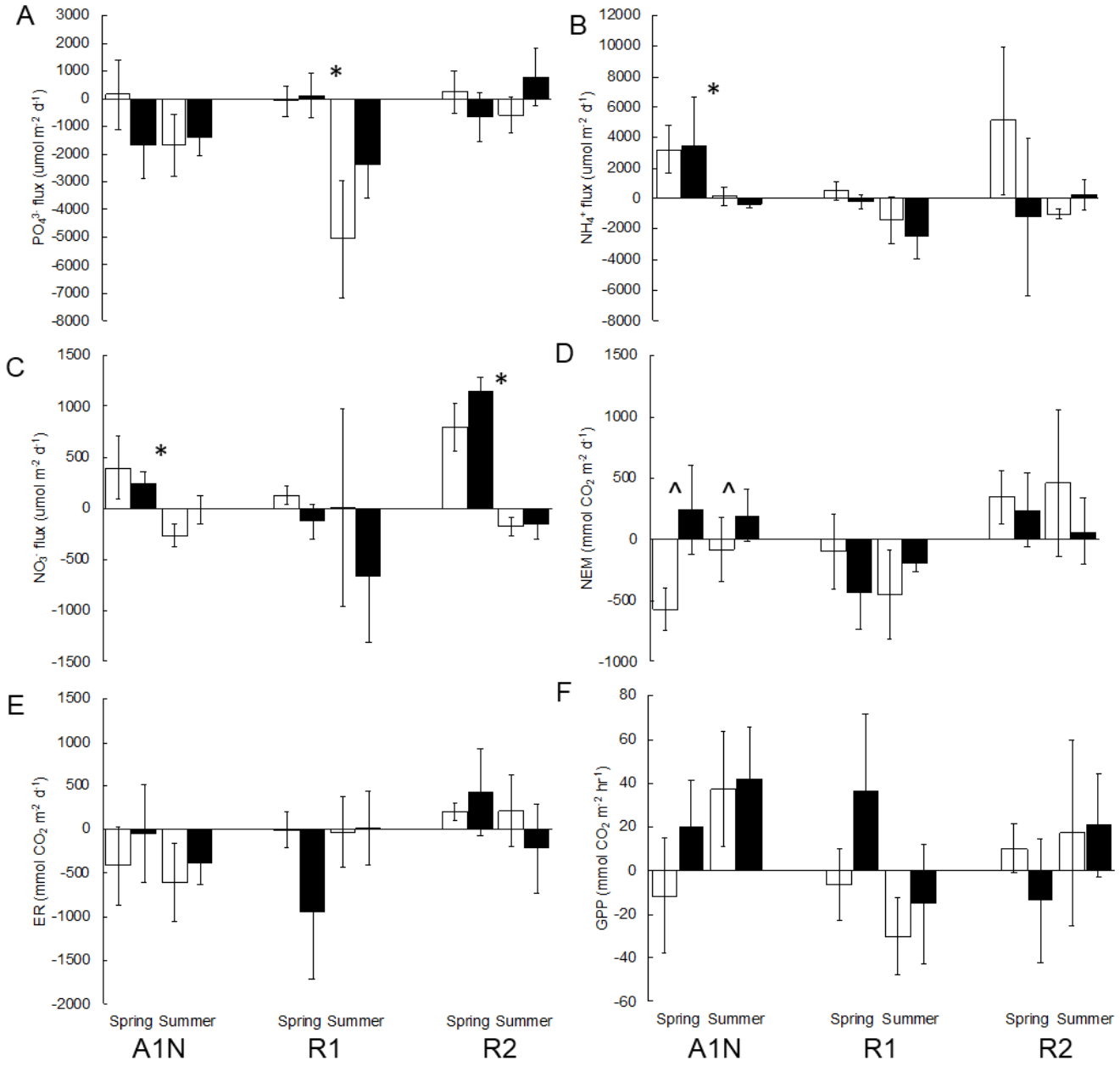

Figure 3.7 Mean ± SE sediment nutrient fluxes: PO_4^{3-} (A), NH_4^+ (B), NO_3^- (C), net ecosystem metabolism (D), ecosystem respiration (E), and gross primary production (F) in caged (open) vs uncaged (filled) plots in A1N, R1, and R2 during spring and late summer in 2019. * indicates a significant difference between caged and uncaged plots ($p<0.05$).

4.0 Discussion

Grazer exclusion had site-specific impacts on plant community structure across the three studied wetlands. These impacts had cascading effects on net ecosystem metabolism and nitrogen cycling at each site. The unique aspects of each site, combined with differences in site history and age, suggest that management of wetlands in the long term must include adaptive plans that consider local use of wetlands by waterfowl.

A1N, R1, and R2 were similar hydrologically, with comparable water depth and the ability to control water level through culverts. Hydrology has important implications for wetland vegetation and biogeochemistry, and hydrology and vegetation models suggest that deeper water may reduce emergent vegetation cover (Poiani and Johnson 1993). Managing the hydrologic regime of constructed wetlands in the context of the surrounding ecosystem and landscape promotes the best ecological outcomes (Zedler 1996). Wetlands constructed to be deeper in the center and shallower on the edges promote submerged vegetation in the center and emergent macrophytes on the edges (Weisner et al. 1994). Emergent cover was present at all sites, but the species present and relative cover of emergent and submerged species varied across sites, likely as a result of the variation in depth. Wetlands A1N and R2 had similar total emergent macrophyte cover, total submerged macrophyte cover, and water depth. A1N had higher diversity in emergent macrophyte species than R2 (Appendix F), perhaps reflecting more recent active management of the site. Wetland R2 was dominated by the floating emergent *N. odorata*, which is dominant in deeper wetlands (Kadlec et al. 2007). In the years following the conclusion of this study, *N. odorata* has expanded in A1N to become the dominant emergent species.

The greater grazer abundance in A1N was reflected clearly in the differences in both emergent and submerged plant cover at the site. The impact of grazers becomes more evident as

the growing season progressed but was apparent even in early spring suggesting that grazing effects across years are substantial and persistent (Lodge and Tyler 2020, Spangler et al. 2021). While in some systems, maximum grazing pressure takes place in early spring (Rodriguez-Villafane 2007), at our site the high grazer abundance in late summer and early fall due to migration patterns. Grazers, such as mallards, wood ducks, Canada goose, green-winged teal, and American black duck prefer smaller (< 8 ha) wetlands with open water (Lemelin et al. 2010). All three study wetlands are below this threshold, but the white-water lily at R2 may act as a deterrent for Canada geese (and other waterfowl). Waterfowl, specifically Canada goose, preferentially to forage broadleaf arrowhead, narrowleaf arrowhead, and pickerel weed over white-water lily (Lemelin et al. 2010), and the dominance of lilies may be a response to high grazing pressure during earlier years of wetland development. However, muskrats, observed in both R1 and R2, selectively forage white water lily (Kadlec et al. 2007).

Grazing impacts to the submerged macrophytes in R1, primarily bladderwort, were possibly minimal because muskrats preferentially forage the *N. odorata*. In addition to the lower grazer populations, another possible contributor to the difference in herbivore impact among sites is that the exclusion has been maintained for several years at A1N (2014-2019) and was only two growing seasons at R1 and R2. Earlier results from A1N suggest that significant impacts of grazers may not show up until after two to three growing seasons (Lodge 2017, Lodge and Tyler 2020).

Studies looking at the impacts of swans on belowground biomass found significant decreases in uncaged plots (Stafford et al., 2012). However, swans are larger grazers than Canada geese and ducks; and forage at deeper depths and consume roots and tubers during winter months (Gyimesi et al. 2011; Stafford et al., 2012). Canada geese primarily consume

emergent macrophytes above the water level, however, they do partake in activities such as grubbing, which can upend roots and tubers (Esselink et al. 1999). We didn't see evidence for grubbing at any of the sites. We only observed a slight impact of grazers on belowground biomass (A1N, 2018), but this was inconsistent. The shallow nature and small number of cores taken for belowground biomass likely contributed to this discrepancy, particularly given the heterogeneous nature of macrophyte roots and rhizomes. Future studies should use a larger number of deeper cores, taking care not to disturb plots.

The observed differences in soil nutrients and organic carbon emphasize the importance of considering prior land use history when constructing wetlands to ensure the desired ecosystem services. The presence of standing water in all wetland study sites promotes anaerobic conditions in the soil and metabolic pathways that define the soil chemistry (Mitch and Gosselink 1993), but legacy nutrients in the soil may persist for many years after construction. Likewise, over time, OM and nutrients will build up in the soil (Moreno-Mateos 2012). Wetland A1N was constructed on gravel substrate, which has been associated with lower nutrient holding capacity, while R1 and R2 were primarily used for row crop agriculture (Johnson 1987). The substantially higher %OM in the younger A1N marsh is therefore surprising. However, agricultural soils, such as those used in construction of R1 and R2, often have altered soil properties, such as compact and homogenized soil (Campbell et al. 2002), which may limit the buildup of organic matter even when the topsoil is replaced following lowering of the surface elevation. The higher soil nutrient concentrations (TP, ammonium) in A1N follow from the higher soil OM. The high emergent biomass in A1N, especially in the caged plots, contributes detritus to the plots, leading to the greater buildup of organic carbon (Spangler et al. 2021). Grazing in freshwater wetlands

increases available carbon to the system when populations of waterfowl are high (Perrow et al. 1997; Rodriguez-Villafane et al. 2007; Stafford et al. 2010).

Longer term studies looking at the impacts of grazing on soil carbon and microbial communities find increased %N and %C when grazing is excluded (Ingram et al. 2008; Jones and Donnelly 2004). Experimental plots in A1N have been part of a longer-term study looking into the different impacts of grazers. The significant impacts grazers had on %N and %C in caged plots in A1N may have not been significant in R1 and R2 because those experimental plots had only been established for less than two years before sampling.

Lower relative carbon availability, which we may see with a decrease in plant biomass, provides less stimulation for microbial processes and results in lower total N removal efficiency (Bachand and Horne 1999; Song et al. 2011; Zhang et al. 2016; Li et al. 2017). Therefore, we may anticipate higher potential denitrification in A1N because of its combined higher organic matter content and more labile carbon source, and a decrease with grazing. However, denitrification rates were highly variable across sites, and in some seasons (summer and fall 2019), rates in R1 were higher. A1N had higher organic matter content, soil and macrophyte C:N ratios, higher soil nutrient levels than R1 and R2. However, the past land use history of R1 and R2 (row crop agriculture) has been shown to contribute towards high potential denitrification rates (Johnson 1987).

Factors such as available labile organic matter, higher percent soil carbon, and soil ammonium concentrations are crucial in the determination of potential denitrification rates (Hanson et al. 1994; Dierberg et al. 2002; Ballantine et al. 2014; Winton and Richardson 2017). Factors such as soil nitrate and oxygen availability did not play an important role in the variation of potential denitrification rates at the different wetland study sites because all wetlands had

similar soil nitrate concentrations (Table 3.2) and were permanently inundated (Figure 3.1). This suggests that other factors play a role in the patterns observed. Emergent macrophyte C:N was only moderately impacted by grazers across species and sites. The individual emergent species that were most common in all wetlands did differ, however, in C:N, with *P. cordata, L. salicaria, T. latifolia, Leersia oryzoides,* and *N. odorata* higher than *S. latifolia* (Appendix F). However, emergent and submerged species tend to differ in C:N, with submerged species having more labile tissue. Denitrifying bacteria have been shown to have higher activity with submerged macrophyte detritus, particularly, *E. canadensis,* than emergent macrophyte detritus (Bastviken et al. 2005). *E. canadensis* was only present in wetland A1N, and the percent cover increased from 2018 to 2019, potentially promoting higher sediment denitrification rates in spring of 2019.

R1 was dominated by submerged macrophytes in the deeper center and emergent macrophytes in the shallower edges only; this combination has been linked to promoting denitrifying processes (Weisner et al. 1994; Tang et al. 2019). Wetland A1N, which experienced some of the highest potential denitrification rates, had a mix of emergent macrophytes such as *S. latifolia*, and submerged macrophytes such as *C.demersum. C. demersum* has been associated with higher potential denitrification rates (Toet et al., 2003; Zhang et al., 2016; Lin et al., 2017; Tang et al., 2017). Wetland R2 is dominated by emergent macrophyte *N. odorata* and submerged macrophyte *U. minor,* both of which may contribute to the low denitrification observed here, due to a lack of efficient oxygen transporters (Steidl et al. 2019).

The overall highest rates of potential denitrification were observed in A1N. In environments with high ammonium and low nitrate concentrations; dissimilatory nitrate reduction to ammonium may be favored over denitrification (Kleinhuizen and Mortazavi 2018). Prior research in this wetland study site found relatively lower potential denitrification rates as

well as lower organic matter content (Lodge 2017), suggesting change over time in the carbon storage and nitrogen removal potential of the wetlands. These carbon inputs likely contribute to the increased potential denitrification in A1N in 2018 (Bachand and Horne 1999; Song et al. 2011; Zhang et al. 2016).

The contribution of internal N fixation to the sites was restricted to periphyton, with unmeasurable levels in the sediments. Relatively, lower light availability and water column phosphorus and nitrate concentrations in all wetland study sites help explain why soil nitrogen fixation rates were very low (Appendix G). Grazing has been shown to significantly reduce nitrogen fixation rates in marshes (Nieuhuis and Groenendijk 1986; Vitousek et al. 2002). However, nitrogen fixation rates in this study were not statistically impacted by grazing treatment, even when the areal rates were taken into account (ie, a biomass specific rate). Nitrogen fixation controls are similar to the controls on potential denitrification, and include both abiotic and biotic factors, such as higher ammonium concentrations, organic carbon availability, and anaerobic conditions (Tyler et al. 2003). Heterotrophic nitrogen fixation can be limited by carbon availability (Welsh et al. 1996; McGlathery et al. 2008; Cole & McGlathery 2012). This can explain why heterotrophic nitrogen fixation in the soil was also below the instrument detector threshold.

However, factors, such as light availability and nitrogen and phosphorus concentrations, can influence autotrophic nitrogen fixation (Capone and Taylor 1977; Reynolds et al. 2015, Welsh et al. 2000). It may be that within cages, the greater shading by increased biomass limits autotrophic N fixation on plant surfaces, so in spite of higher surface area, the overall rates remain consistent with those outside of the cages. In general, R1 and R2 showed greater N fixation, suggesting that the lower nutrient availability at these sites may stimulate internal

production. Further, N fixation was higher on bladderwort than on other submerged species, per gram plant tissue (Appendix G), suggesting that this species may promote N fixation, especially in the light, perhaps because the air bladders maintain the plant at or near the water surface where light is less limiting to autotrophic production.

Sediment-water column nutrient fluxes showed no statistically significant differences between grazing treatments in all three wetland study sites. Fluxes were generally low, corresponding to lower nutrient availability in created wetlands than natural wetlands (Fennessy et al. 2008), but also highly variable. Nutrient fluxes in wetland system often experience a high amount of variability (Fisher and Reddy 2001; Dunne et al. 2010). However, the higher uptake of nitrate and ammonium observed at A1N and R2 in summer relative to spring suggests a potential role of microalgae and microbes during the warmer seasons. Nitrification may be limiting in wetlands with low water column ammonium availability, which could then limit denitrification (Hanson et al. 1994; Toet et al. 2003; Zhang et al. 2016). Lower water column nitrate availability can limit potential denitrification rates and the nitrogen removal potential of all three wetland study sites (Spieles and Mitsch 2000). Nitrate water column fluxes were especially low in all three wetland study sites. The nearly consistent uptake of phosphorus by the sediment reflects the low P content in sediments and excessive demand by microalgae and microbes.

NEM can be regulated by light availability, organic matter content, temperature, and water depth (Giordano et al. 2012). All wetland study sites were similar in temperature, surface light availability, and water depth, but A1N had higher organic matter content (Table 3.2). NEM can vary drastically in different environments and under different conditions. Organic carbon availability and the level of nutrient loading present in a system are both important factors in determining whether NEM is more autotrophic or heterotrophic (Kemp et al. 1997) Marshes,

among other aquatic ecosystems in the northeast, do not exhibit a defined seasonal pattern in NEM (Caffrey 2004). Some aquatic ecosystems in the northeast are balanced or leaning towards being autotrophic while NEM at other sites are heterotrophic (Caffrey 2004). Similar research into the impacts of grazing on NEM in Caribbean meadows found grazing to significantly lower CO_2 uptake by the benthos (Johnson et al. 2019). This suggests a substantial role of surface microalgae in caged plots. Grazers that consume rhizomes and disturb the sediment may consume and disturb surface microalgae. NEM often exhibits large seasonal patterns, with higher values in the summer and lower values in the fall and winter (Caffrey 2004; Souza et al. 2009; Giordano et al. 2012). This study wasn't sufficiently long to capture trends in late fall or winter, but future studies may address this gap.

Although benthic light measurements did not significantly differ between caged and uncaged plots in A1N; they were significantly greater than benthic light measurements in R2, which was heterotrophic (Appendix H). The greater light availability in A1N could allow for an increase in primary production from surface microalgae and greater CO_2 uptake. R1 and R2 had similar benthic light measurements, however, the dominant emergency macrophytes present in R2 may out compete surface microalgae present in the sediments. It is important to note one potential limit of the benthic chamber method is the possibility of disturbing the rhizomes in the sediment and potentially releasing carbon during experimentation.

5.0 Conclusion

Macrophyte community structure has a great impact on nitrogen movement and removal, and grazing as a biotic control is an important factor in indirect control on the N cycle. Large grazers had only moderate impacts on nitrogen cycling processes, however, they significantly impacted macrophyte cover and shifted vegetation structure. The seasonal cycling of nitrogen (both inputs from nitrogen fixation and permanent release from potential denitrification) varied seasonally and across wetland sites, reflecting age, management and land use history (Lodge 2017). The lower carbon and OM content at R1 and R2 may be a symptom of poor soil quality at the outset, leading to a longer trajectory of development (Moreno-Mateos 2012). Wetlands created with high heterogeneity will promote and optimize ecosystem services, such as nitrogen fixation and nitrogen removal (Lodge and Tyler 2020). Waterfowl require a variety of biotic and abiotic factors to thrive; therefore, no specific habitat type can provide all the requirements for a specific species. A complex of wetlands with variability in size, water depth, and vegetation communities is often the goal for wetland waterfowl management (Lor and Malecki 2006). Restoring macrophyte communities to created wetlands and protecting them from grazers is essential in order to allow labile organic matter content and soil carbon to increase (Spangler et al. 2021).

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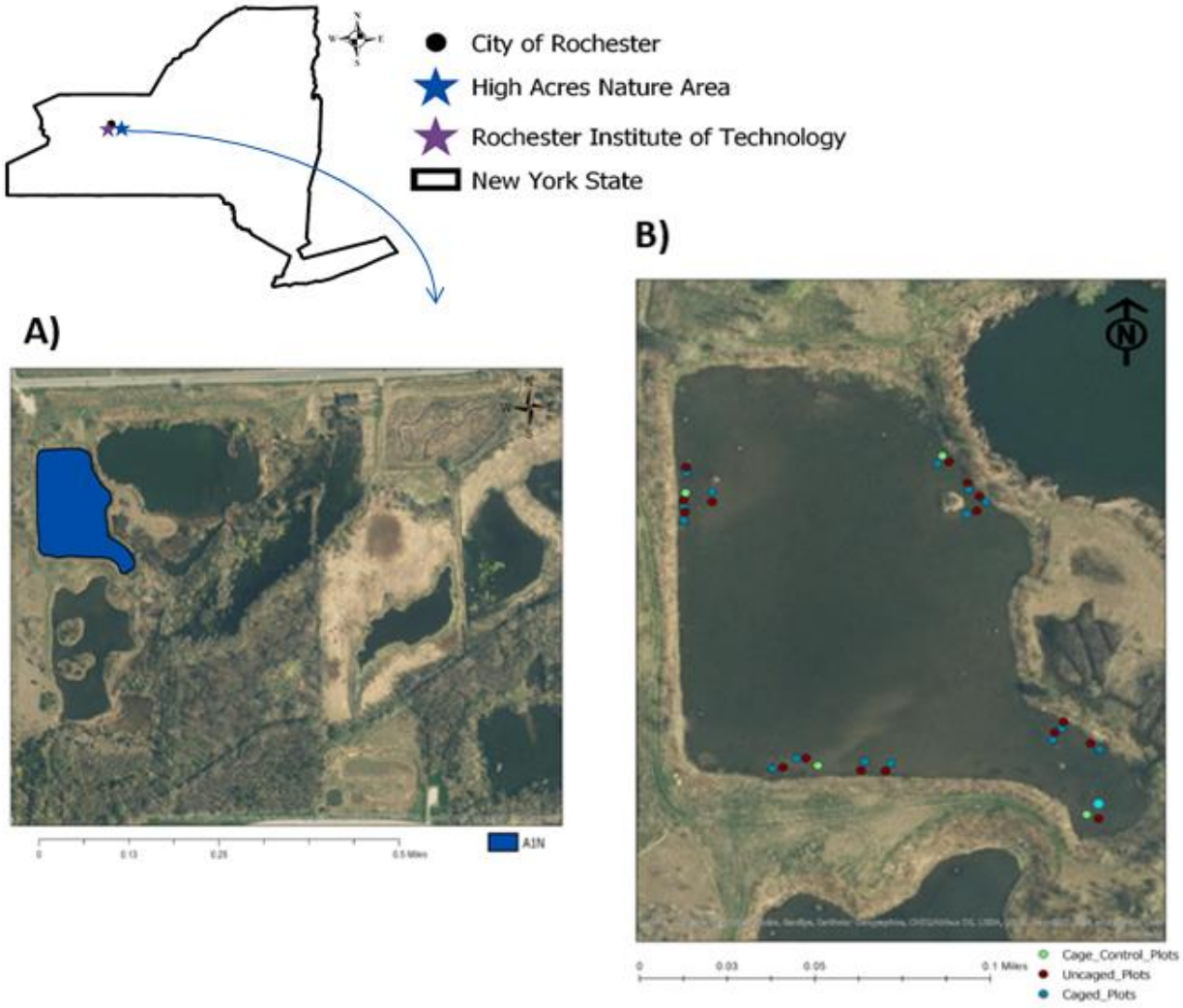
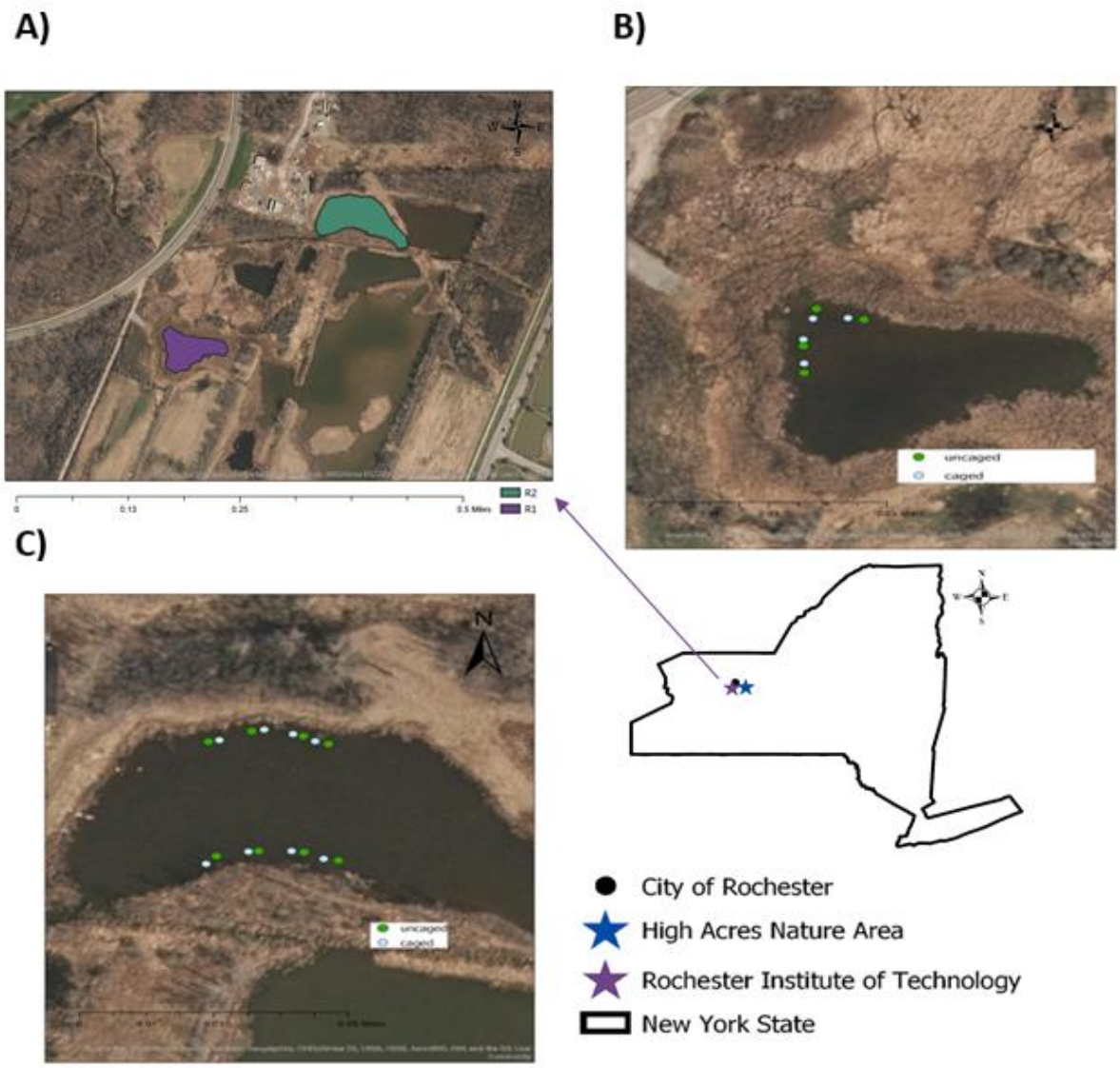
APPENDICES

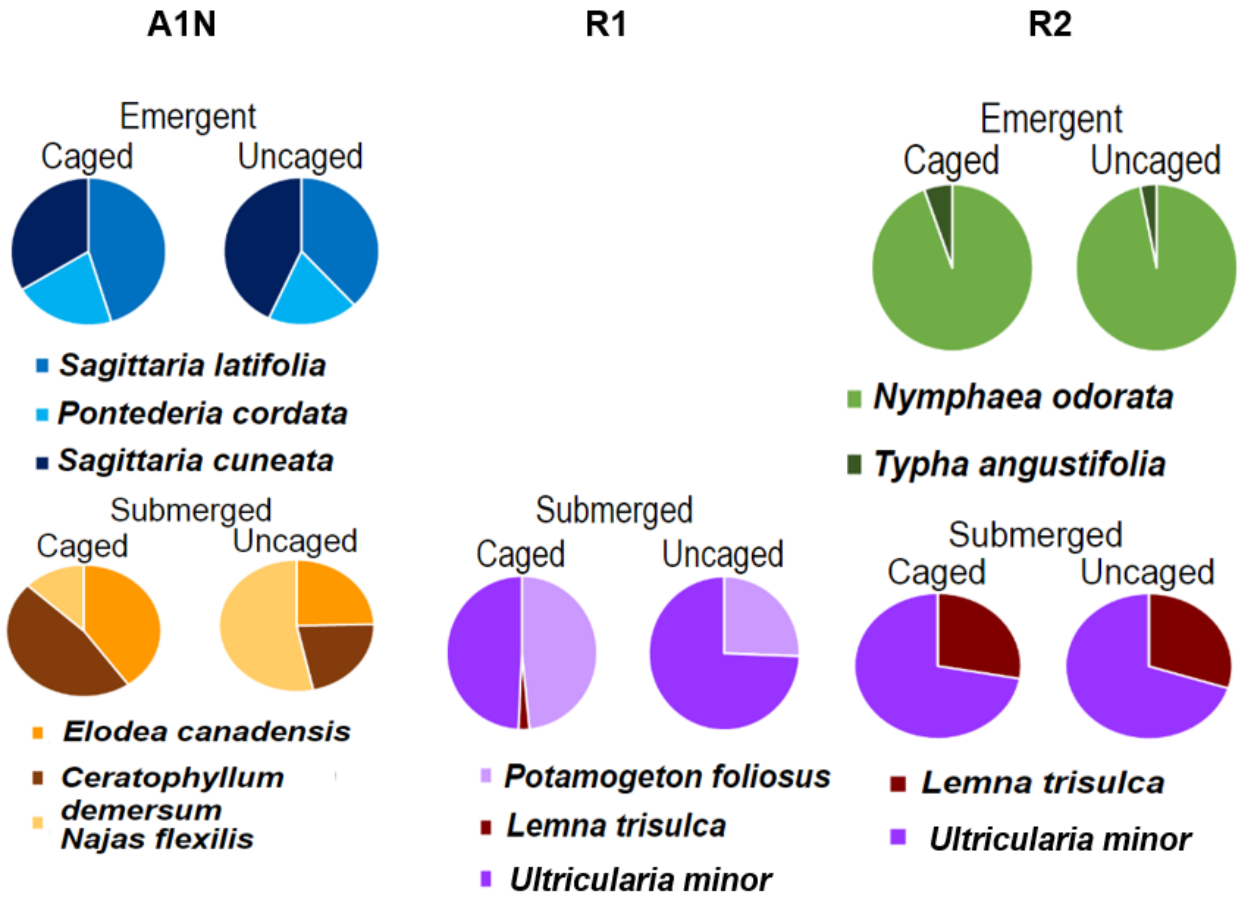


Appendix A: Map of HANA (High Acres Nature Area) located in Fairport, NY. A). Study site located in the blue polygon referred to as A1N. B). Map of vegetation plots located in HANA. The blue circles represent the caged plots, the red circles represent the uncaged plots, and the green circles represent the cage-control plots.



Appendix B: Map of Rochester Institute of Technology's Campus in Rochester, New York. A). R1 and R2 are two created wetlands used as study sites. B.) Map of R1 located in the southeast of Rochester Institute of Technology's campus in Rochester, NY. The light blue circles represent the caged plots and the green circles represent the uncaged plots. C.) Map of R2 located in the southeast of Rochester Institute of Technology's campus in Rochester, NY. The light blue circles represent the caged plots and the green circles represent the uncaged plots.

Appendix C: Pie graphs displaying the relative proportion of the dominant submerged and emergent macrophytes in A1N, R1, and R2. Note that R1 rarely had emergency macrophytes.



Appendix D: List of macrophytes collected in caged and uncaged plots in A1N, R1, and R2 during the fall of 2019 for C:N ratio analysis.

Area	Caged	Uncaged
Fall		
A1N		
	Sagittaria latifolia	*Sagittaria latifolia*
	Sagittaria latifolia	*Sagittaria latifolia*
	Sagittaria latifolia	*Sagittaria latifolia*
	Leersia oryzoides	*Sagittaria latifolia*
	Leersia oryzoides	*Sagittaria latifolia*
	Pontederia cordata	*Pontederia cordata*
	Lythrum salicaria	*Pontederia cordata*
	Typha latifolia	*Typha latifolia*
R1		
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
R2	*Nymphaea odorata*	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*
	Nymphaea odorata	*Nymphaea odorata*

Appendix E: Results of the mean ± C:N ratios of different macrophyte species collected in caged and uncaged plots (C= caged, U= uncaged) in A1N, R1, and R2.

Macrophyte C:N	**A1N**		**R1**		**R2**	
	Avg ± SE					
	C	U	C	U	C	U
Species						
Leersia oryzoides	28.1 ± 3.0	---	---	---	---	---
Lythrum salicaria	32.0 ± 0.7	---	---	---	---	---
Nymphaea odorata	---	---	33.5 ± 1.8	30.4 ± 2.4	33.1 ± 2.5	30.5 ± 2.2
Pontederia cordata	36.1 ± 0.2	25.0 ± 3.5	---	---	---	---
Sagittaria latifolia	22.8 ± 5.7	18.8 ± 1.9	---	---	---	---
Typha latifolia	30.9 ± 0.4	35.7 ± 0.7	---	---	---	---

Appendix F: List of macrophyte species observed in wetlands A1N, R1, and R2 throughout the growing seasons of 2018 and 2019. Obl= Obligate, E= Emergent, S= Submerged

Scientific Name	Common Name	Wetland Indicator Status	Submerged or Emergent
A1N			
Alisma subcordatum	Water plantain	Obl	E
Ceratophyllum demersum	Coontail	Obl	S
Elodea canadensis	Waterweeds	Obl	S
Leersia oryzoides	Rice cutgrass	Obl	E
Lythrum salicaria	Purple loosestrife	Obl	E
Nymphaea odorata	White water lily	Obl	E
Pontederia cordata	Pickeral weed	Obl	E
Potamogeton foliosus	Leafy pondweed	Obl	S
Potamogeton nodosus	American pondweed	Obl	E
Sagittaria latifolia	Broadleaf arrowhead	Obl	E
Typha angustifolia	Narrowleaf cattail	Obl	E
Typha latifolia	Broadleaf cattail	Obl	E
R1			
Caltha palustris	Marsh marigold	Obl	E
Potamogeton foliosus	Leafy pondweed	Obl	S
Schoenoplectus tabernaemontani	Soft stem bulrush	Obl	E
Typha latifolia	Broadleaf cattail	Obl	E
Utricularia minor	Bladderwort	Obl	S
R2			
Lemna trisulca	Forked duckweed	Obl	S
Nymphaea odorata	White water lily	Obl	E
Potamogeton foliosus	Leafy pondweed	Obl	S
Typha angustifolia	Narrowleaf cattail	Obl	E
Typha latifolia	Broadleaf cattail	Obl	E
Utricularia minor	Bladderwort	Obl	S

Appendix G: List of macrophyte species used for nitrogen fixation incubation experiments conducted in the spring, summer, and fall of 2019.

Area		Macrophyte species collected
Spring		
A1N		
	Caged	*Elodea canadensis*
	Uncaged	*Elodea canadensis*
R1		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*
R2		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*
Summer		
A1N		
	Caged	*Ceratophyllum demersum*
	Uncaged	*Ceratophyllum demersum*
R1		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*
R2		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*
Fall		
A1N		
	Caged	*Ceratophyllum demersum*
	Uncaged	*Ceratophyllum demersum*
R1		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*
R2		
	Caged	*Utricularia minor*
	Uncaged	*Utricularia minor*

Appendix H: Results of one-way ANOVA examining the relationship between average light measurements and grazing treatment (C= caged, U= uncaged) in A1N, R1, and R2. Significant p-values are bolded, p<0.001 are starred (*).

Irradiance Measurements	**A1N**		**R1**		**R2**	
	Avg ± SE					
	C	U	C	U	C	U
Spring						
Surface (µMol)	1836.4 ± 23.1	1835.4 ± 27.3	1869.2 ± 20.3	1858.9 ± 28.4	1854.3 ± 38.1	1830.1 ± 21.6
	$F_{1,16}$=0.0, p=0.98		$F_{1,8}$=0.09, p=0.78		$F_{1,8}$=0.31, p=0.60	
Benthic (µMol)	382.6 ± 152.1	122.4 ± 49.5	12.0 ± 2.0	19.3 ± 7.5	23.6 ± 2.5	24.9 ± 1.1
	$F_{1,16}$=2.6, p=0.13		$F_{1,8}$=0.88, p=0.38		$F_{1,8}$=0.22, p=0.65	
Late summer						
Surface (µMol)	1852.1 ± 17.3	1827.1± 22.1	1841.2 ± 34.5	1839.0 ± 27.3	1881.2 ± 16.5	1840.2 ± 29.7
	$F_{1,16}$=0.79, p=0.39		$F_{1,8}$=0.0, p=0.95		$F_{1,8}$=1.5, p=0.27	
Benthic (µMol)	91.7 ± 38.0	67.3 ± 9.3	12.8 ± 1.0	23.8 ± 4.8	14.2 ± 3.2	19.6 ± 7.0
	$F_{1,16}$=0.39, p=0.54		$F_{1,8}$=5.1, p=0.06		$F_{1,8}$=0.48, p=0.52	