6-29-2017

Hydrology, nutrient availability, and herbivory interacting to control ecosystem functions and services in created emergent freshwater wetlands

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Recommended Citation

Lodge, Kimberly A., "Hydrology, nutrient availability, and herbivory interacting to control ecosystem functions and services in created emergent freshwater wetlands" (2017). Thesis. Rochester Institute of Technology. Accessed from

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Hydrology, nutrient availability, and herbivory interacting to control ecosystem functions and services in created emergent freshwater wetlands

By:

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Environmental Science

Gosnell School of Life Sciences

College of Science

Environmental Science Program

Rochester Institute of Technology

Rochester, NY

June 29, 2017

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ACKNOWLEDGEMENTS

I would like to extend my deepest gratitude to my advisor, Dr. Christy Tyler, not only for her help and guidance throughout this project, but for taking me under her wing when I was a late transfer into the environmental science program. After years of not really knowing what I wanted to do with myself, she helped me to discover my desire to work in environmental restoration and conservation. I am so grateful she asked me to work with her over the summer at HANA and start what was the beginning of my thesis project. Her continued support throughout this process has allowed me to push myself academically, and to analyze and think critically about my work. I would also like to thank my committee members Dr. Carrie McCalley, Dr. Nathan Eddingsaas, and Dr. André Hudson; having such a diverse group of mentors from different scientific fields has helped me look at my project from many different angles. I also extend my thanks to the College of Science and Waste Management for funding and resources. This project would not have been possible if I didn’t have all of the students who helped out at HANA and with my project: Taylor Williams, Kaity Moranz, Lizzy Bruen, Rachel Allen, Brenna DeAngelis, Delanie Spangler, just to name a few. Nothing brings people closer than wading through the muck and listening to Disney songs while cutting cattails. I truly would not have been able to complete this project without the camaraderie, support, and long hours put in by everyone to get our work done. Lastly, I would like to thank my family and friends who unconditionally supported my decision to change majors and switch to the environmental science program, and who put up with my insanity as I raced towards the finish line. Thank you all so very much, it has meant the world to me.

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ABSTRACT

The loss of natural wetlands to anthropogenic development has warranted the creation of wetlands to mitigate the reduction of valuable ecosystem functions and services. However, the complex interactions between the main drivers of wetland community structure – hydrology, nutrient availability and herbivory – makes creation of fully functional wetland replacements challenging. In this study, we examined the interactions among these drivers, and their impacts on plant diversity and nitrogen removal in two created wetlands with different land use histories: A1N (previous gravel depository) and A3 (previous cattle pasture). We established paired plots protected from – and open to – large wetland grazers and compared vegetation abundance and diversity, soil characteristics, and soil respiration and potential denitrification in each wetland. At A1N, a permanently flooded emergent marsh with high observed grazer densities (predominantly waterfowl) and low nutrient availability, grazing significantly reduced plant growth and diversity. In contrast, at A3, a seasonally flooded wetland with lower grazer densities and high nutrient availability, grazing enhanced overall plant diversity and decreased invasive species cover. The effects of grazers varied seasonally and increased over time, eventually leading to a reduction in soil organic matter at both sites. In light of significant site differences, potential denitrification was limited by differential hydrology (very wet, anaerobic versus very dry, aerobic), nitrogen or carbon availability, and grazing (low or high levels) at both sites. These results suggest the possibility of long-term grazer-induced shifts in community composition and delivery of key ecosystem services in young, vulnerable created wetlands. To improve created wetland design and function, we suggest that the impact of prior land use on present nutrient availability must be considered, and sites must be heterogeneous in both physical and bathymetric structure, to provide conditions for diverse plant communities, both aerobic and anaerobic biogeochemical processes, and balanced habitat use by wetland grazers.

Chapter 1: Background

1.1 Wetland ecosystems

The unique hydrologic patterns of wetlands distinguish them from other terrestrial and aquatic ecosystems, and provide an important transitional zone between the two. Wetlands provide numerous ecosystem services including water filtration (Coveney et al. 2002), climate regulation (Kayranli et al. 2010), flood control (Mitsch & Gosselink 1993), nutrient cycling (Aerts et al. 1999; DeAngelis et al. 1989), and habitat diversity (Zedler & Kercher 2005) etc., making them some of the most economically and ecologically valuable on earth; estimated to be worth \$140,174 ha^{-1} yr^{-1} (Costanza et al. 2014) The main drivers of diversity and ecosystem function in wetlands are hydrology, nutrient availability, and herbivory, which exert strong individual influences on community dynamics. However, the complexity of the interactions among these factors, and their impact on community structure and function are not fully understood in natural wetlands. This gap in knowledge creates additional problems as we try to address these interactions in the planning, design and management of created wetlands.

Wetland creation has recently become more common in light of natural wetland destruction due to human development; by 1984, more than half of the wetlands in the United States were drained or filled for activities including agriculture and landfill expansion (USEPA 2008). Since the introduction of the Wetland No Net Loss Act of 1989, wetland creation is mandatory as compensatory mitigation for the loss of natural wetlands, per requirements of Section 404 of the Clean Water Act. The “no net loss” policy assumes that created wetlands will provide the same level of functionality as natural wetlands

(Robertson 2000; Bendor 2009). However, given the complexity of abiotic and biotic interactions, and the vast degree of small- (water levels, soil nutrients) and large-scale (landscape influences) heterogeneity within and between these ecosystems, predicting and meeting trajectories of development is difficult to achieve (Galatowitsch & van der Valk 1996; Campbell et al. 2002; Fennessy et al. 2008). The act of constructing a wetland, often in areas previously used for other purposes, can lead to altered structural and functional states resulting in reductions to overall biological and biogeochemical function when compared to reference sites (Moreno-Mateos et al. 2012). It is therefore important to understand how multiple factors influence wetland structure and function, in order to design and manage created wetlands with positive outcomes.

1.2 Driving factors of community structure and function

Wetland structure and function is primarily driven by hydrology, which varies both spatially and temporally, and depends on the surrounding geomorphology and climate. Wetlands are typically created by altering hydrology of land previously used for other purposes; design decisions made during planning and construction can impact flood storage, groundwater recharge and discharge, evaporation, and other factors, which in turn impact plant community composition (emergent vs submerged vs meadow) and distribution, nutrient availability, and overall water chemistry (Carter 1996; Newman et al. 1996). Wetlands that are permanently, intermittently, or seasonally flooded will allow for different degrees of oxygen penetration to the soil, influencing anaerobic and aerobic microbial processes which play a key role in regulating wetland ecosystem services (Hernandez & Mitsch 2007). There are also strong correlations between hydrologic conditions and the presence of specific aquatic herbivores, such as geese and ducks, which choose specific nesting and feeding sites based on water depths (Murkin et al. 1997; Clausen 2000; Lor & Malecki 2006).

Classically, it was viewed that nutrient availability was the other main factor driving community structure within wetland ecosystems from the bottom-up, specifically in salt marsh ecosystems (Teal 1962). In terrestrial and aquatic ecosystems, nutrient availability is strongly linked to the growth, productivity and survivorship of microbial and plant communities; most North American temperate wetlands are either phosphorus limited or co-limited by phosphorus and nitrogen (Bedford et al. 1999; Elser et al. 2000; Gusewell et al. 2003). Wetlands themselves may retain high quantities of nutrients from upstream sources and surrounding land uses, though the efficiency of nutrient uptake and transformation differs depending on the microbial or plant species present, and can lead to the proliferation of some species over others based on the level and form of nutrients available (Hobbie 1992). Plants and microorganisms are in constant competition with each other for these nutrients (Kaye & Hart 1997; Bardgett et al. 2003), and nutrient removal processes, performed by both, becomes an important wetland service for water filtration and clean drinking water for organisms downstream.

More modern views acknowledge the role of herbivores in exerting top-down influence on ecosystem structure and function. Aquatic herbivores have strong influences on community dynamics by limiting above- and/or belowground plant growth and survivorship of plants, especially during the early establishment and development of communities (Lauridsen et al. 1993) (Figure 1.1); this makes created wetlands especially vulnerable to the effects of grazing and highlights the need to understand how the impact of ecosystem drivers may be unique in these kinds of wetlands. Based on species-specific dietary requirements, herbivores often select plants based on nutrient content, palatability, and overall accessibility (Evers et al. 1998; Jefferies & Rockwell 2002; Goranson et al. 2004). This preferential selection can shift and alter species composition, by promoting the growth of unpalatable and/or invasive plants, because native plant species are often preferred by native grazers (Clay et al. 1993), facilitating the spread of species undamaged by herbivores (Grosholz et al. 2009). The scope of impacts is regulated seasonally, often based on migration patterns, though the greatest damage to plant growth does not always correspond to the time(s) of the year when grazer densities are highest (Perrow et al. 1997; Chaichana et al. 2011). This has important implications for long-term success of plant species and community development.

Herbivores may also impact nutrient availability and alter microbial processes within the soil. After being consumed plants must reallocate resources and uptake nutrients for recovery and new shoot growth; this may decrease nutrient pools within the soil (Gao et al. 2008). Herbivores may also deposit nutrient-rich feces while foraging in the wetland, or remove nutrients by consuming plant material before moving to another nearby system (Kitchell et al. 1999; Vanni 2002). Removing plant biomass can decrease the accumulation of labile litter at the end of the growing season (Van Wijnen et al. 1999) (Figure 1.1). These outcomes can go on to further impact soil processes like decomposition, nitrification, and denitrification, which heavily depend on the availability of labile carbon and nitrogen sources within the soil (Le Roux et al. 2003; Vaieretti et al. 2013).

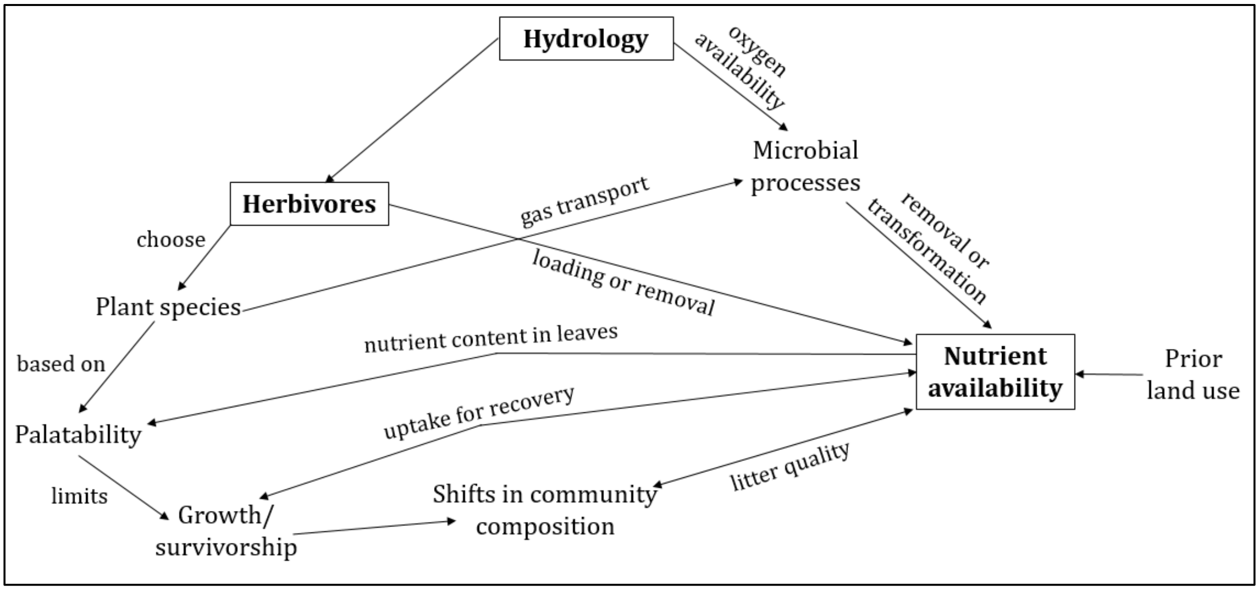

Figure 1.1: Conceptual diagram of hypothetical interactions between hydrology, nutrient availability, and herbivory, and their impacts on community dynamics in created wetlands.

1.3 Habitat diversity and nitrogen removal

In this study, we focused on two main wetland services: habitat diversity and nitrogen removal. Habitat diversity, provided by emergent vegetation, is a crucial service to guarantee the greatest number of resources for the greatest number of species (Murkin et al. 1997; Lor & Malecki 2006). Emergent plants provide the physical structure of wetland habitat and are an important source of food for many species, especially grazing water birds. They also provide temporary removal of nitrogen by the uptake of nutrients, which are required for both growth and reproduction (Brix 1994; Brix 1997), as well as providing a pathway for carbon sequestration or greenhouse gas emissions depending on the species (Kayranli et al. 2010). However, plants only account for a small fraction of overall removal (Brix 1997) and the nutrients will be released again when the plant dies and decomposes (McLatchey & Reddy 1998). Microbial processes account for the greatest and most permanent fraction of nitrogen removal, by converting soluble reactive to gaseous forms (Hanson et al. 1994; Coveney et al. 2002). These processes, which depend on oxygen availability, also rely on plant species which help to transport oxygen to anaerobic soils or those that release carbon through their roots and help fuel heterotrophic bacteria (Armstrong 1964; Zhai et al. 2013) (Figure 1.1).

The interactions between abiotic and biotic factors are complex and variable across ecosystems, making them difficult to understand and replicate to enhance biodiversity and/or nitrogen removal as part of created wetland management goals (Figure 1.1). For example, resource limitations or excess due to hydrology and/or prior land use can impact how plants and microorganisms respond to herbivory. It is crucial to test different combinations of these interactions in created wetlands in order to improve and aid decision-making during wetland design and management. Without understanding the context of these factors, created wetlands will likely continue to fail to meet optimum performance standards when compared to natural wetlands.

1.4 Overview of study

The overall objective of this study was to determine the impact of interacting factors – hydrology, nutrient availability, and herbivory – on key ecosystem services (nitrogen removal and plant diversity) in two created wetlands, with different past land use histories. The goals are to help improve the design and management of created wetlands before and after construction, ensure the provision of vital ecosystem services lost when wetlands are destroyed, and have implications for invasive species control and nutrient removal for water filtration. Our main hypotheses are: 1) past land uses will result in wetlands differing in nutrient availabilities, 2) hydrology will influence grazing pressure and therefore the overall impact of grazers, and will regulate microbial processes associated with nitrogen removal, and 3) grazers will limit plant growth and diversity, and nitrogen removal through consumption of preferential plant species and altering soil properties. We carried out our study at both sites by creating plots open to- and protected from-large grazers and evaluated environmental conditions (nutrients, water depth), vegetation data, and microbial processes (respiration and potential denitrification) in these plots. In Chapter 2, we focused on the impact of environmental conditions and grazers on plant growth and diversity. In Chapter 3, we focused on the impact of land use on environmental conditions and nitrogen removal, and the overall impact of grazers on nitrogen removal. We found that the impact of grazers was seasonally regulated depending on plant growth, water availability, and nutrient availability. Different interactions between hydrology, nutrient availability, and herbivory resulted in different overall impacts on plant diversity and nitrogen removal.

Chapter 2: Impact of abiotic and biotic factors on plant diversity and community structure

2.1 Introduction

Heterogeneity and the resulting biodiversity of wetland ecosystems provides numerous resources to the organisms that live there, including habitat and refugia, food, raw materials, disturbance regulation, and recreational/cultural services (Zedler & Kercher 2005). A key aspect of wetland diversity comes from the emergent plant community, which provide a great number of resources for a large variety of species (Murkin et al. 1997; Lor & Malecki 2006). The main drivers of plant diversity and wetland function – hydrology, nutrient availability, and herbivory – interact in complex and dynamic ways that are not fully understood in natural wetlands. This poses a difficult task as we try to address these interactions in the planning and design of created wetlands, which often fail to provide the same level of functionality as natural wetlands. Created wetlands often follow a different trajectory of plant succession and community development than natural wetlands because they are prone to early colonization by invasive species and tend to have lower species richness (Campbell et al. 2002; Edwards & Proffitt 2003; Zedler & Kercher 2005). It is therefore important to understand how multiple factors influence wetland structure and function, in order to more successfully design and manage created wetlands better in the future.

Wetland hydrology is characterized by seasonal, intermittent, or permanent flooding regimes, each of which results in unique wetland structure and function scenarios. Flooding regimes regulate soil and water chemistry by determining oxygen availability and soil redox status, which indirectly controls the availability of nutrients for competing plants and microorganisms (Reddy et al. 2000). Variations in surface water depth and duration of saturation also strongly influences the number, type, and distribution of individual plant species, affecting plant community diversity that in turn provides an important food source for wetland grazers (Fretwell et al. 1996). Similarly, hydrology plays an important role in determining wetland utilization by herbivores, such as waterfowl that choose habitat based on water depth (Clausen 2000). Shallow wetlands are typically used by wading species, whereas deeper wetlands provide better habitat for diving birds (Colwell & Taft 2000). As wetlands are created by altering hydrology of land previously used for other purposes, decisions made during planning and construction can result in wetlands that do not provide suitable habitat for all desirable waterfowl species, changing the overall impact of grazers. The consequences of these alterations in created wetlands are not well understood.

These aquatic herbivores carry out key top-down controls on community dynamics through the selection of plant species based on nutrient content and palatability (Goranson et al. 2004). At high densities, herbivores can significantly reduce above- and belowground biomass of preferred plants, driving plant competition as unpalatable species gain a competitive advantage (Oene et al. 1999) and ultimately leading to long-term reductions in plant community competition with a reduction in community diversity (Evers et al. 1998; Jefferies & Rockwell 2002). Created wetlands may be especially vulnerable to shifts in community composition, because their plant communities are young and less resistant. The result of grazing, therefore, may be more profound when compared to natural wetlands, and warrants study. Foraging behaviors also have strong implications for invasive species success in created wetlands, as native plant species are often preferred by native grazers, facilitating the spread of non-native invasive plants (Clay et al. 1993; Grosholz et al. 2009).

Herbivores may also alter soil dynamics through direct disruptions to the soil layers during root or rhizome removal, and indirect alterations to nutrient pools in the soil (Iacobelli & Jefferies 1990). The deposition of nutrient-rich feces while foraging can increase both nitrogen and phosphorus levels; however, removal of nutrient-rich plant material before moving on to another area can decrease local nutrient availability (Kitchell et al. 1999; Vanni 2002). Also, reallocation of resources by damaged plants, and increased nutrient uptake for recovery growth may lead to decreased nutrient pools and root exudation of labile carbon (Holland et al. 1995; Gao et al. 2008). Continued reductions in plant biomass may further decrease carbon availability due to reductions in litter input during end-of-season senescence (van Wijnen et al.1999). This could pose further problems in the development of communities in created wetland, which have already been found to contain significantly lower organic matter than comparable natural wetlands (Campbell et al. 2002; Fennessy et al. 2008).

The impact of wetland herbivores on plant communities can be significant, but interactions with nutrient availability and hydrology likely contribute to the community-level response and resilience to the disturbance. Resource limitation in nutrient-poor ecosystems may prevent plant re-growth after grazing; however grazing in nutrient-rich ecosystems may actually facilitate competition and diversity (Maschinski & Whitham 1989; Proulx & Muzumder 1998). Considering the constant competition for resources between plants and microorganisms, the level of nutrients within the system becomes important to combat detrimental impacts of grazers. Unique to created wetlands is the legacy of prior land use on present nutrient availability; this link is not clearly defined, but may have cascading impacts on the ability of plant communities to recover from grazing events. These impacts can be especially pronounced during migration periods or at over-wintering grounds when grazer populations are at their highest and may result in limiting the re-establishment of individuals in subsequent growing seasons (Perrow et al. 1997).

The overarching goal of our study was to evaluate the interactions between hydrology, nutrient availability, and herbivory in created wetlands in order to inform the design and management of similar systems before and after construction. These interactions were evaluated in two created, emergent freshwater wetlands with different prior land use histories: wet and low nutrients, dry and high nutrients. While these systems don't allow a full factorial analysis of driving forces, the contrasting nature of these systems allows a unique opportunity for comparison of the controls on wetland structure-function relationships in created wetlands. We hypothesized that: 1) grazing pressure will be higher in created wetlands that are permanently, as opposed to seasonally, flooded, and will shift seasonally in time with migration cycles, 2) the presence of grazers will decrease both plant growth and diversity, when compared to areas not grazed, and 3) the removal of plant matter by grazers will decrease soil nutrient pools and organic matter.

2.2 Methods

2.2.1 Site description

This study was conducted between June 2014 and October 2016 in two created wetlands at High Acres Nature Area (HANA) in Perinton, New York, USA that are owned and managed by Waste Management of New York and New England, LLC (Figure 2.1, top panel). Area 1 North (A1N) served as a gravel-mining depository until approximately the mid-1960s, before being abandoned and left fallow (Stantec 2009). Prior to mining, the area was used for agricultural purposes. Approximately 1.87 hectares of shallow marsh were created in 2009 with the goal of providing wildlife habitat, flood storage, and pollution/sediment removal. Immediately following construction, invasive cattail species (*Typha latifolia* and *Typha angustifolia*) colonized the majority of the site, leading to extensive invasive species control executed via manual cutting, pulling, and herbicide applications (glyphosate) starting in 2010. Following initiation of this study, no intentional invasive plant control was conducted in the vicinity of the treatment plots. A variety of native plants were also planted at the time of construction, and in subsequent years. Broadleaf arrowhead (*Sagittaria latifolia)*, water plantain (*Alisma plantago-aquatica*), and smartweed (*Polygonum* spp.) were the dominant species at the start of the study. The hydrology of A1N appears to be mainly driven by groundwater flow from an adjacent abandoned quarry pond and from precipitation. During construction, a culvert was installed in the southeast corner of the wetland, directing flow from A1N to another constructed wetland cell to the south and allowing for water level control in A1N and maintenance of standing water year-round (Figure 2.1, bottom left panel).

Area 3 (A3) was used as a cattle pasture prior to construction of approximately 1.63 hectares of wooded wetland and shallow marsh area in 2012. *Typha* spp. seed heads were cut each summer and plants were sprayed with herbicide (glyphosate) each fall since 2013, avoiding experimental plots. Native shrub and emergent wetland species were planted throughout this area such that *Typha* spp., *A. plantago-aquatica*, and *Polygonum* spp. dominated this area at the initiation of this study. The hydrology of A3 is driven by groundwater flow from an adjacent hillside and precipitation.

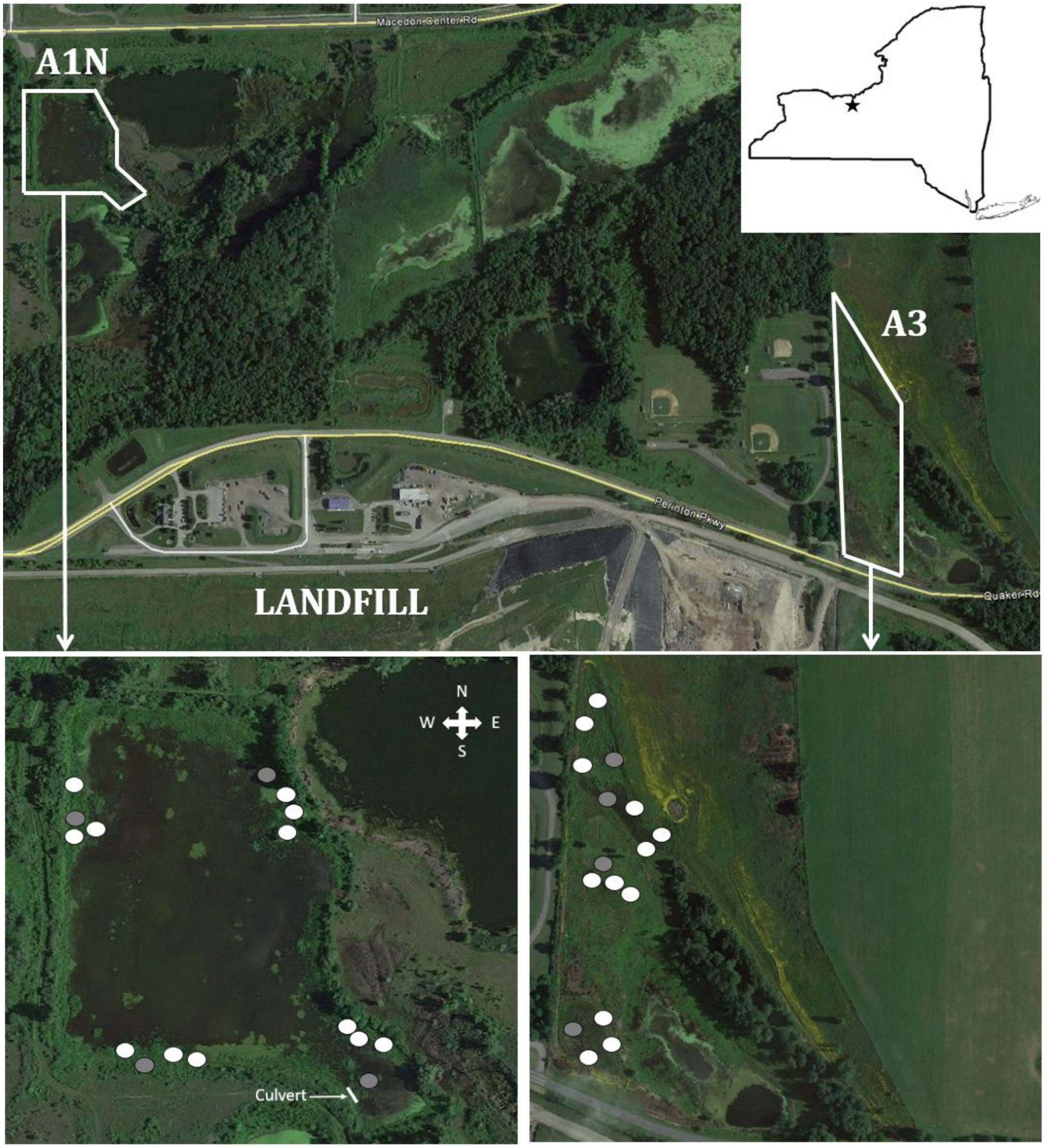

Figure 2.1: Map of HANA and wetlands of study (top panel), Area 1 North (A1N; bottom left panel) and Area 3 (A3; bottom right panel), located in Perinton, NY. (white= pair of caged and uncaged plots, gray= pair with additional cage control plots). Note the deeper Quarry Pond to the east of A1N separated by a narrow berm. The ponds in the southeast corner of A3 were pre-existing ponds previously used to water cattle.

2.2.2 Characterizing grazing pressure

Abundance of large wetland herbivores was quantified from September 2015 through September 2016 by the authors and trained volunteers. We recorded the number and species of grazers present (including tracks and houses/nests), their behavior (foraging, swimming, nesting, etc.), date, and time of day, during all visits to the two wetlands. The frequency of observation varied between the two wetlands, but is sufficient to demonstrate differences in grazer identity and density between sites. Results were converted to average density per species per unit area (calculated using ArcGIS mapping software) across seasons (spring, summer, fall, and winter; Marklund et al. 1992).

2.2.3 Experimental design: herbivore exclusion

In June 2014, 16 pairs of 1 x 1 m caged (herbivore exclusion) and uncaged control (open to herbivores) plots arranged in blocks of 4 pairs, were established randomly in A1N and across distinct zones of A3 (64 total plots). Paired caged and uncaged plots were 1 m apart and at least 3 m from another pair (Figure 1, bottom panels). Caged plots were set up by wrapping galvanized hardware cloth (1.27 cm mesh, 1.22 m tall) around four polyvinyl chloride pipes (PVC); uncaged plots were marked with PVC pipes only. In May 2015, four additional three-sided cages were established in each wetland, one with each block, as cage controls to evaluate any cage effects.

2.2.4 Characterizing hydrologic conditions and soil nutrient availability

Hydrologic conditions were evaluated by averaging surface water depths from 3 points in every plot in spring (May), early summer (June), mid-summer (July), and fall (September), starting in June 2014 and ending in September 2016. Three soil cores (2.5 cm diameter x 10 cm deep) were extracted from each plot with an auger in fall 2014, fall 2015, and spring and fall 2016 (spring= May, fall= September) and subdivided for organic matter and nutrient analysis. Soil organic matter content was determined using the loss on combustion method (Heiri et al. 2001). Inorganic nitrogen was extracted by shaking with 2M potassium chloride (Keeney and Nelson 1982). Ammonium was analyzed using the phenol-hypochlorite method and a Shimadzu 1800 spectrophotometer (Solorzano 1969). Nitrate+nitrite was measured with the cadmium reduction method and a Lachat Quikchem 8500 autoanalyzer (Lachat 2003). Total inorganic nitrogen (TIN) was calculated by summing extractable ammonium and nitrate+nitrite. Total phosphorus (TP; spring and fall 2016 only) was extracted from soil samples by adding magnesium nitrate to soil dried at 60°C, ashing in a 550°C oven for two hours, and dissolving in sulfuric acid before analysis using the ammonium molybdate method (Murphy & Riley 1962).

2.2.5 Plant growth and diversity

Vegetation measurements were collected at three to four time points during the growing season in all plots starting in June 2014 and ending in September 2016. Percent cover of each species was estimated by at least 2 observers per plot and averaged to eliminate observer bias. Plant diversity was evaluated using species richness (S) and the Shannon-Weiner Diversity Index (H'). Plant stem density and height were measured in May, June, July and September of 2016 for all species. Stem heights were taken for the three tallest stems of each species and averaged per plot.

Belowground biomass was measured in September 2016. One soil core (10 cm diameter x 25 cm depth) was collected from each plot using an auger, washed through a 1 mm mesh sieve to remove soil particles, dried (60^{O}C) and weighed (Evers et al. 1998).

2.2.6 Statistical analyses

All statistical analyses were completed using JMP 13 Pro statistical software. Prior to selection of statistical analysis method, each dataset was checked for normality and homogeneity of variance. Intra-site heterogeneity was evaluated by adding a block effect, to encompass different zones of each wetland, as a random factor into every analysis of variance (ANOVA). Results of the block effects are listed in Appendix D.

Grazer density was analyzed using a one-way Kruskal-Wallis test to compare total average individuals per hectare in A1N and A3, to determine differences between sites overall and within seasons (i.e. spring A1N vs spring A3, etc.).

We made between site comparisons of organic matter, total inorganic nitrogen, and total phosphorus using a one-way ANOVA, only including uncaged control plots. We also used a full-factorial three-way ANOVA to compare intra-site differences between these variables with treatment (caged/uncaged), season (spring and fall), and year (2014-2016), when applicable, as fixed factors.

For statistical analyses of stem height, density, and cover of individual species we only included species with a percent cover ≥5% in at least five plots across the growing season in order to focus on the most relevant species in each wetland and those that likely contribute the most to overall community structure; for analyses of total plant cover, *S*, and *H'* we included minor species as well. *Polygonum* spp. were grouped together, as were

Typha spp. for plant height analyses. We made between site comparisons of total plant cover, *S*, and *H'* using a one-way analysis of variance (ANOVA), only including uncaged control plots in this analysis. Using a full factorial three-way ANOVA we compared differences within sites for all other plant variables with treatment (caged/uncaged), season (spring, early summer, mid-summer, fall), and year (2014-2016), when applicable, as fixed factors. Invasive species data was not compared between A1N and A3, only within A3, because there was typically <1% cover in A1N in any one season. For all variables, when significant differences were found, a Tukey's HSD post hoc analysis was used to elucidate differences among treatments.

2.3 Results

2.3.1 Hydrology

A1N was permanently flooded throughout the year, whereas A3 was seasonally flooded and was typically fully dry by early July. The average water depth between May and September was consistently deeper in A1N (2014: 16.0 ± 5.4 cm, 2015: 16.1 ± 6.9 cm, and 2016: 13.5 ± 4.7 cm; mean ± SD) than A3 (2014: 7.5 ± 5.6, 2015: 7.1 ± 7.4, 2016: 2.9 ± 5.8 cm) (Figure 2.2). A drought in 2016 decreased average water depths in both wetlands, and resulted in A3 completely drying by mid-June.

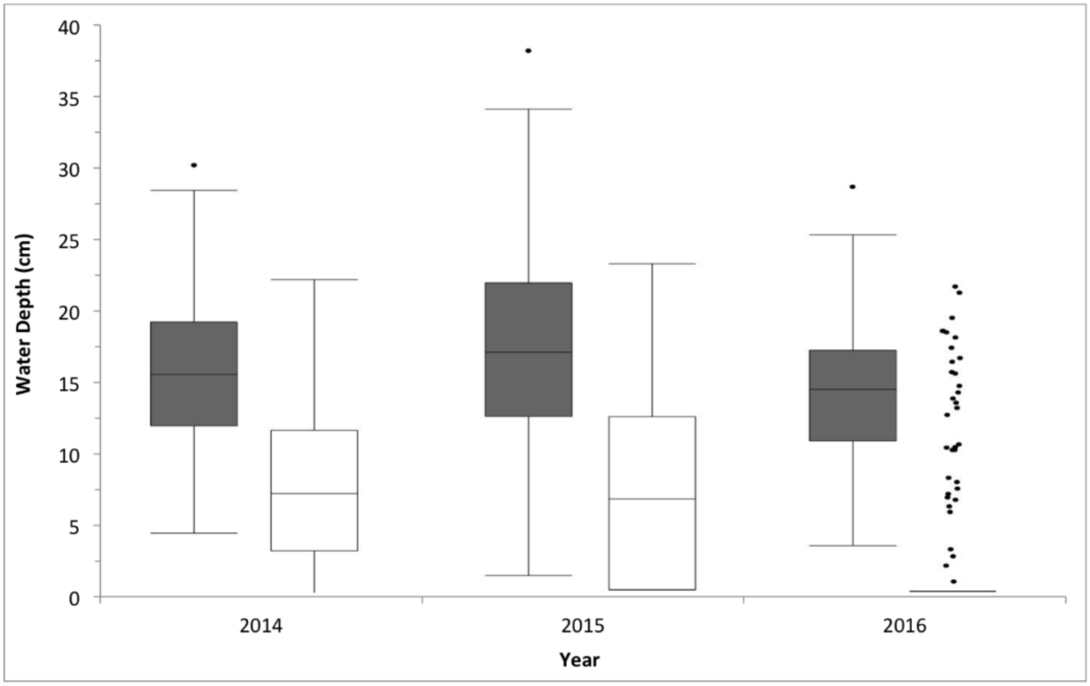

Figure 2.2: Boxplots showing water depths across the growing season (May-September) in A1N (gray) and A3 (white) from 2014-2016. New York State experienced an extreme drought beginning in June 2016.

2.3.2 Grazing Pressure

At both sites we observed large grazers, including Canada goose (*Branta 20anadensis*), ducks (*Anas* spp.), whitetail deer (*Odocoileus virginianus*), North American beaver (*Castor 20anadensis*), and the common muskrat (*Ondatra zibethicus*). Waterfowl comprised the majority of grazer abundance at both sites: 99-100% and 66-100% of grazers were waterfowl at A1N and A3, respectively. Overall grazer density in A1N was significantly greater than in A3 (41.2 ± 7.4 and 4.9 ± 1.5 individuals ha^{-1}, respectively; χ^2 =41.9, p <0.0001; Figure 2.3), but the relative difference varied across seasons. Grazer density was roughly 90 (summer) and 8 (fall, peak) times higher in A1N than A3 (χ^2 =18.2 and 26.4, respectively, p <0.0001), but were similar in spring and winter (χ^2 <1, p =0.95 and χ^2 =1.3, p =0.25, respectively).

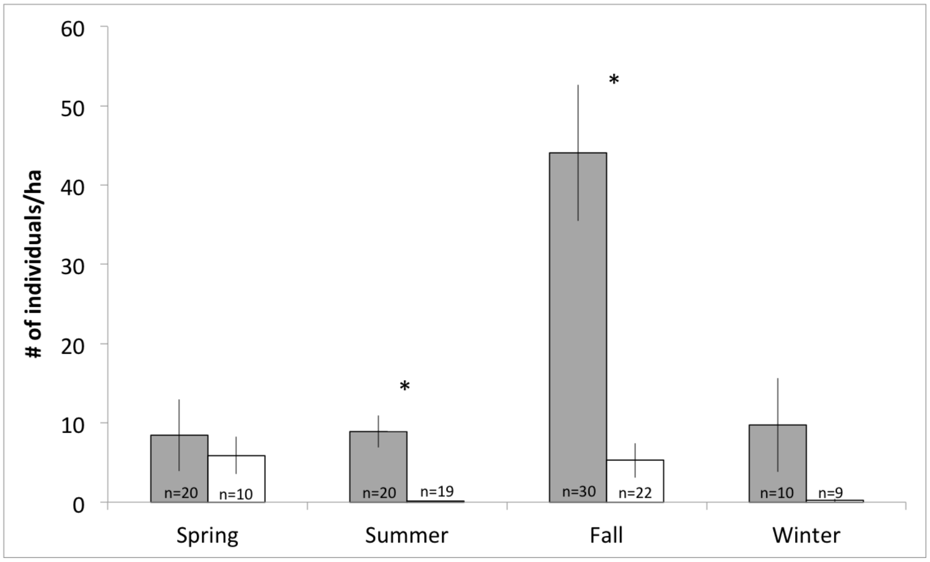

Figure 2.3: Large grazer density observed at A1N (gray) and A3 (white) wetlands between September 2015 and September 2016 (spring= March-May, summer= June-Aug, fall= Sept-Nov, winter= Dec-Feb; mean ± SE). Text values on bars are the number of individual observations per season. * indicates significant difference between sites within a season (p <0.0001)

2.3.3 Nutrient availability

Soil nutrients and organic matter were consistently higher in A3 relative to A1N (Table 2.1): organic matter content (OM) was 1.5 times greater (13.4 ± 0.5 versus 7.5 ± 0.4 %; p <0.0001); total inorganic nitrogen (TIN) was 3 times greater (17.1 ± 3.4 versus 6.2 ± 1.8 mg/kg; p <0.0001); total phosphorus (TP) was 1.5 times greater (1002.7 ± 53.2 versus 704.3 ± 28.0 mg/kg; p <0.0001), respectively. In A1N, grazing significantly reduced soil OM (caged= 8.9 ± 0.4 %, uncaged= 7.5 ± 0.4 %; p =0.046; Table 2.2, Appendix A). This trend was similar in A3, though not significant (caged =14.1 ± 1.1, uncaged =13.4 ± 1.1 %; p =0.54; Table 2.2, Appendix A). Spring flush of TIN resulted in significantly higher concentrations (up to 3 times higher) in spring 2016 than all fall concentrations in A1N (season x year p <0.0001; Table 2.2, Appendix A). This trend was similar in A3 in 2014 and 2015 only (season x year p =0.002; Table 2.2, Appendix A). There were no significant effects of grazing on TIN at either site, though in A1N uncaged plots were slightly higher than caged plots (6.2 ± 1.8 versus 4.8 ± 0.6 mg/kg, respectively; p =0.09). In A1N, there were no significant effects of either season or grazing on TP (Table 2.2, Appendix A); in A3, fall concentrations of TP were 1.2 times higher than spring (p <0.0001), and were negatively affected by grazing (p =0.02; Table 2.2, Appendix A). There were significant block effects found for OM at both sites, and TP at A3 only (Appendix D).

Table 2.1: Results of one-way ANOVAs comparing sites for both environmental and plant characteristics in the uncaged control plots. Significant p-values are bolded (*p <0.0001).

Factor	**Avg ± SE**		**Site**	
	A1N	A3	F	*p*
Organic matter (OM, %)	7.5 ± 0.4	13.4 ± 0.5	$F_{1,111}$= 87.8	**<0.001***
Total inorganic nitrogen (TIN, mg/kg)	6.2 ± 1.8	17.1 ± 3.4	$F_{1,111}$= 17.1	**<0.001***
Total phosphorus (TP, mg/kg)	704.3 ± 28.0	1002.7 ± 53.2	$F_{1,63}$= 42.6	**<0.001***
Total plant cover (TC, %)	48.4 ± 5.5	55.1 ± 6.4	$F_{1,351}$= 2.5	0.11
Species richness (*S*)	2.2 ± 0.3	3.9 ± 0.5	$F_{1,351}$= 73.3	**<0.001***
Shannon-Weiner (*H'*)	0.4 ± 0.1	0.9 ± 0.1	$F_{1,351}$= 74.4	**<0.001***
Belowground biomass (BGB, g/m²)	19.2 ± 1.9	21.8 ± 3.5	$F_{1,31}$= 0.4	0.51

Table 2.2: Results of one-, two-, and three-way ANOVAs examining the effect of year (2014-2016), season (spring, early summer, mid-summer, and fall), and grazing treatment (caged, uncaged) on environmental and plant characteristics at both wetland sites. Significant p-values are bolded (*p <0.0001). Yr= year, Seas= season, Tr= treatment

Factor	Year		Season		Treatment		Yr x Seas		Yr x Tr		Seas x Tr		Yr x Seas x Tr	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
A1N														
OM	$F_{2,126}$= 2.3	0.11	$F_{1,127}$= 1.8	0.19	$F_{1,127}$= 4.1	**0.046**	$F_{6,122}$= 1.6	0.21	$F_{2,126}$= 0.5	0.59	$F_{3,125}$= 1.0	0.32	$F_{6,122}$= 0.2	0.80
TIN	$F_{2,110}$= 4.0	**0.021**	$F_{1,111}$= 38.4	**<0.001***	$F_{1,111}$= 3.0	0.09	$F_{6,106}$= 14.3	**<0.001***	$F_{2,110}$= 0.6	0.55	$F_{3,109}$= 3.4	0.07	$F_{6,106}$= 1.2	0.30
TP	---	---	$F_{1,63}$ <0.1	0.90	$F_{1,63}$= 0.4	0.53	---	---	---	---	$F_{1,63}$= 3.1	0.08	---	---
TC	$F_{2,350}$= 6.1	**0.002**	$F_{3,349}$= 128.2	**<0.001***	$F_{1,351}$= 79.8	**<0.001***	$F_{6,356}$= 19.5	**<0.001***	$F_{2,350}$= 14.7	**<0.001***	$F_{3,349}$= 6.8	**<0.001**	$F_{6,356}$= 8.4	**<0.001***
S	$F_{2,350}$= 5.5	**0.004**	$F_{3,349}$= 28.2	**<0.001***	$F_{1,351}$= 14.8	**<0.001**	$F_{6,356}$= 7.7	**<0.001***	$F_{2,350}$= 3.2	**0.044**	$F_{3,349}$= 0.8	0.52	$F_{6,356}$= 1.1	0.40
H'	$F_{2,350}$= 5.3	**0.006**	$F_{3,349}$= 10.6	**<0.001***	$F_{1,351}$= 23.3	**<0.001***	$F_{6,356}$= 3.3	**0.004**	$F_{2,350}$= 5.9	**0.003**	$F_{3,349}$= 0.2	0.88	$F_{6,356}$= 0.6	0.74
BGB	---	---	---	---	$F_{1,31}$= 6.0	**0.021**	---	---	---	---	---	---	---	---
A3														
OM	$F_{2,126}$= 1.1	0.33	$F_{1,127}$= 0.8	0.39	$F_{1,127}$= 0.4	0.54	$F_{6,122}$ <0.1	0.97	$F_{2,126}$ <0.1	0.98	$F_{3,125}$= 0.5	0.49	$F_{6,122}$ <0.1	0.97
TIN	$F_{2,110}$= 5.2	**0.007**	$F_{1,111}$= 25.6	**<0.001***	$F_{1,111}$= 0.2	0.63	$F_{6,106}$= 11.4	**<0.001***	$F_{2,110}$= 0.4	0.64	$F_{3,109}$ <0.1	0.97	$F_{6,106}$= 0.7	0.51
TP	---	---	$F_{1,63}$= 33.1	**<0.001***	$F_{1,63}$= 5.8	**0.020**	---	---	---	---	$F_{1,63}$ <0.1	0.85	---	---
TC	$F_{2,350}$= 0.1	0.92	$F_{3,349}$= 102.7	**<0.001***	$F_{1,351}$= 3.8	0.05	$F_{6,356}$= 14.1	**<0.001***	$F_{2,350}$ <0.1	0.99	$F_{3,349}$= 0.6	0.61	$F_{6,356}$= 0.6	0.70
S	$F_{2,350}$= 20.7	**<0.001***	$F_{3,349}$= 18.3	**<0.001***	$F_{1,351}$= 9.6	**0.002**	$F_{6,356}$= 7.2	**<0.001***	$F_{2,350}$= 4.4	**0.013**	$F_{3,349}$= 0.9	0.42	$F_{6,356}$= 1.0	0.42
H'	$F_{2,350}$= 2.5	0.09	$F_{3,349}$= 6.4	**<0.001**	$F_{1,351}$= 17.3	**<0.001***	$F_{6,356}$= 2.0	0.07	$F_{2,350}$= 5.9	**0.003**	$F_{3,349}$= 1.1	0.37	$F_{6,356}$= 0.8	0.55
INV	$F_{2,350}$= 5.2	**0.006**	$F_{3,349}$= 10.4	**<0.001***	$F_{1,351}$= 3.3	0.07	$F_{6,356}$= 1.0	0.41	$F_{2,350}$= 0.1	0.91	$F_{3,349}$= 1.6	0.20	$F_{6,356}$= 1.1	0.39
BGB	---	---	---	---	$F_{1,31}$ <0.1	0.84	---	---	---	---	---	---	---	---

2.3.4 Plant growth and diversity

Total plant cover was similar between A1N and A3 in the control plots (p=0.11; Table 1). In A1N, grazing significantly reduced plant cover, but a significant three-way interaction suggests that the impact of grazers varied by season and across years (p <0.0001; Table 2.2, Figure 2.4A). The greatest grazing effect occurred in mid-summer (July), the height of the growing season (caged =112.7 ± 6.0, uncaged =81.8 ± 7.8 %). These effects appeared to increase interannually, with the difference in cover between caged and uncaged plots (C-U) increasing from approximately 5% in 2014 to 55% in 2016. In A3, a significant two-way interaction also showed similar trends of total plant cover varying by season and across different years (p <0.0001; Table 2.2, Figure 2.4B). Grazers slightly reduced plant cover in A3 (caged =61.4 ± 6.4, uncaged =55.1 ± 6.4 %; p =0.05). There were no differences between three-sided cage-control plots and uncaged control plots at either site (A1N: p= 0.56; A3: p =0.24).

Plant diversity was significantly lower in A1N than A3 (*S* = 2.2 ± 0.3 and 3.9 ± 0.5, respectively, p <0.0001; *H'* = 0.4 ± 0.1 and 0.9 ± 0.1, respectively, p <0.0001; Table 2.1). In A1N, the substantial reduction in diversity with grazing echoed total plant cover and increased over time for both *S* and *H'* such that caged plots had 1.3 and 1.7 times higher *S* and *H'*, respectively than uncaged plots in 2016 (p =0.044 and p =0.003, respectively; Table 2.2, Figure 2.4C & 4E). Seasonal variation (peak in mid-summer) in *S* and *H'* also increased over time (*S*: p <0.0001; *H'*: p =0.004). In contrast to A1N, grazing increased plant diversity in A3 and this effect, again, increased over time (*S*: p =0.013; *H'*: p =0.003; Table 2.2, Figure 2.4D & 4F); in 2016, *S* and *H'* were 1.3 and 1.5 times higher in uncaged as opposed to caged plots (*S:* =4.9 ± 0.6 and 3.8 ± 0.6, respectively; *H'*: 1.0 ± 0.1 and 0.7 ± 0.1, respectively). A3 also showed similar seasonal variations in diversity, which increased over time (*S*: p <0.0001; *H'*: p =0.07). Grazing reduced invasive cover in A3, but not significantly (caged =8.6 ± 3.3, uncaged =5.9 ± 2.3 %; p =0.07; Table 2.2, Figure 2.5). Invasive cover was consistently highest in the fall (p <0.0001) and significantly decreased over the course of the study such that cover was 2.5 times higher in 2014 than in 2016 (10.3 ± 3.4 and 4.1 ± 1.9 %, respectively; p =0.006).

Belowground biomass in control plots was similar between A1N and A3 (p =0.51; Table 2.1). In A1N only, grazing significantly reduced belowground biomass by 30% (caged =260.2 ± 28.4, uncaged =180.1 ± 17.7 g/m^2; p =0.021; Table 2.2, Figure 2.6). Significant block effects were found impacting plant cover and diversity in both wetlands (Appendix D).

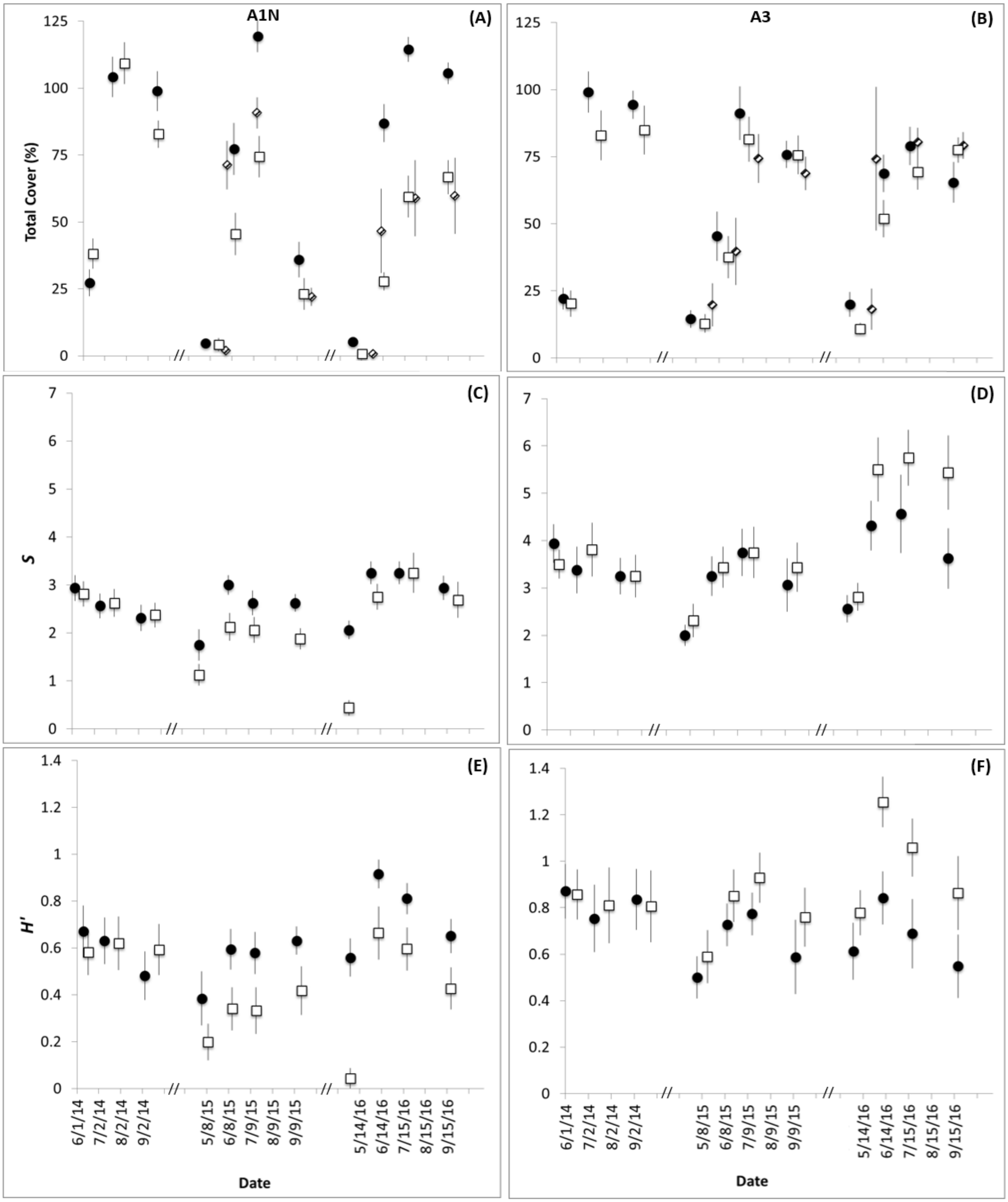

Figure 2.4: Mean ± SE plant characteristics measured in caged (black circle), uncaged (white square), and cage control (striped diamond) plots in study wetlands during the

growing seasons of 2014-2016. Panels: A= A1N, total cover; B= A3, total cover; C= A1N, species richness; D= A3, species richness; E= A1N, Shannon-Weiner diversity scores; F= A3, Shannon Weiner scores. Note that total cover may exceed 100% when plant canopies of individual species overlap.

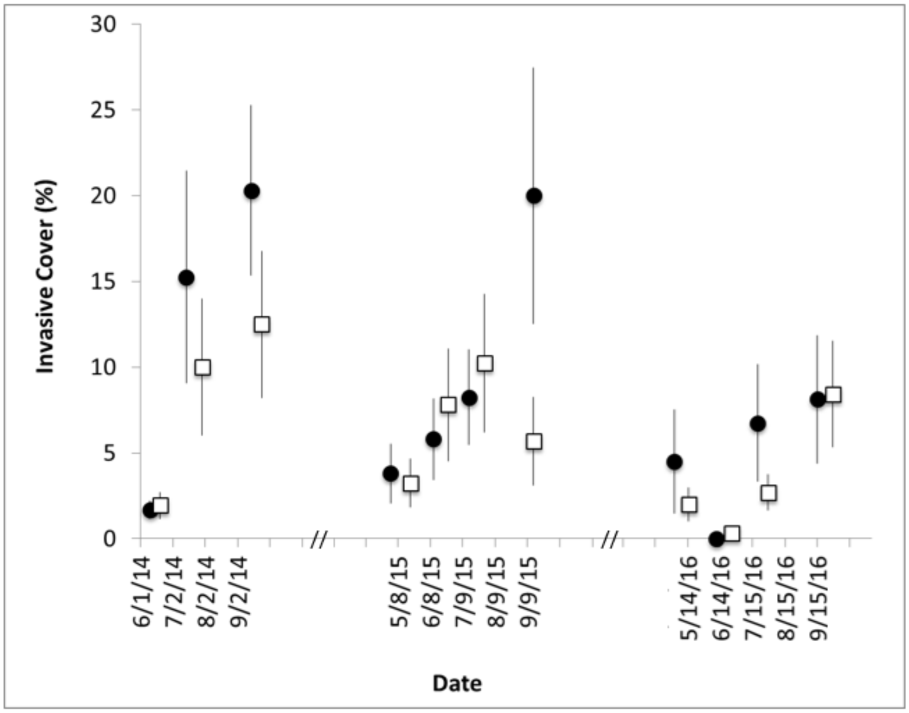

Figure 2.5: Mean ± SE invasive plant cover in caged (black circle) and uncaged (white square) plots in A3 wetland during the growing seasons of 2014-2016.

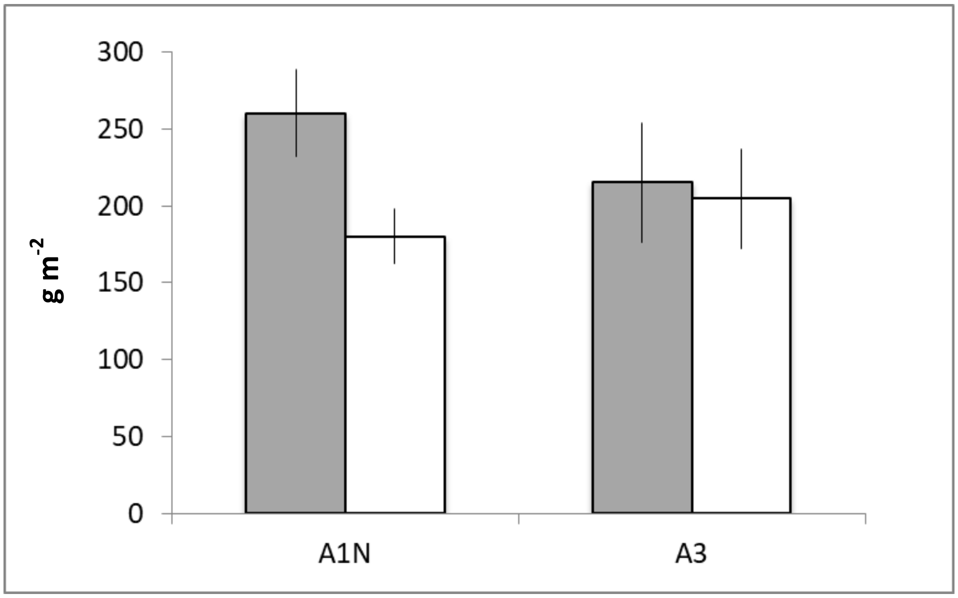

Figure 2.6: Mean ± SE belowground biomass (g m^{-2}) measured in caged (gray) and uncaged (white) plots in A1N and A3 wetlands in fall 2016. * indicates a significant difference between caged and uncaged plots ($p < 0.05$)

At both sites, stem height of the most common plant species was impacted more significantly by season and grazing treatment than stem density or individual species cover, but the trends were similar for all three variables (Figure 2.7; Appendices B & C). Differences in species composition led to differences in species-specific responses between sites. At A1N, where emergent wetland species dominated the community, the maximum effect of grazing coincided with the peak height and reduced stem height by 60-70% at peak growth (*A. plantago-aquatica*: caged =42.1 ± 6.8, uncaged =16.6 ± 4.1 cm; *Polygonum* spp.: caged =108.7 ± 6.7, uncaged =29.6 ± 8.1 cm; treatment x season $p < 0.001$ and $p < 0.0001$, respectively; Figure 2.7A, Appendix B). Grazing also significantly reduced the height of *S. latifolia*, another dominant emergent species, by approximately 18%, though this was not seasonally dependent (caged= 73.1 ± 6.1, uncaged= 60.1 ± 5.1 cm; $p = 0.041$).

In contrast, grazing significantly increased stem height for *Potamogeton amplifolius*, a submerged aquatic species, by approximately 35% (caged= 11.0 ± 0.3, uncaged= 17.1 ± 0.7 cm; $p < 0.0001$). For other emergent species, *Leersia oryzoides* and *Schoenoplectus tabernaemontani*, the reduction in stem height was not significant (Figure 2.7A, Appendix B).

The plant community of A3 was characterized by a mixture of wet meadow, grasses, and emergent species. *Eleocharis obtusa*, *L. oryzoides*, and *S. tabernaemontani*, three native species, were significantly taller in ungrazed plots ($p = 0.048$, $p = 0.02$, $p = 0.004$, respectively; Figure 2.7B, Appendix C). Stem height of *Erechtites hieracifolius*, another native species, was significantly greater in grazed plots, with a peak difference of 65% in fall (caged= 11.7 ± 5.8, uncaged= 33.5 ± 6.2 cm; treatment x season $p < 0.001$). Conversely, grazing reduced stem height of *Phalaris arundinacea* and *Typha* spp., invasive species, though not necessarily to the same degree ($F = 5.6$, $p = 0.02$ and $F = 0.9$, $p = 0.35$ for height; Figure 2.7B, Appendix C). There were significant block effects for stem height, density, and species cover for a number of species at both sites, underscoring the small-scale heterogeneity present at both sites (Appendices B & C).

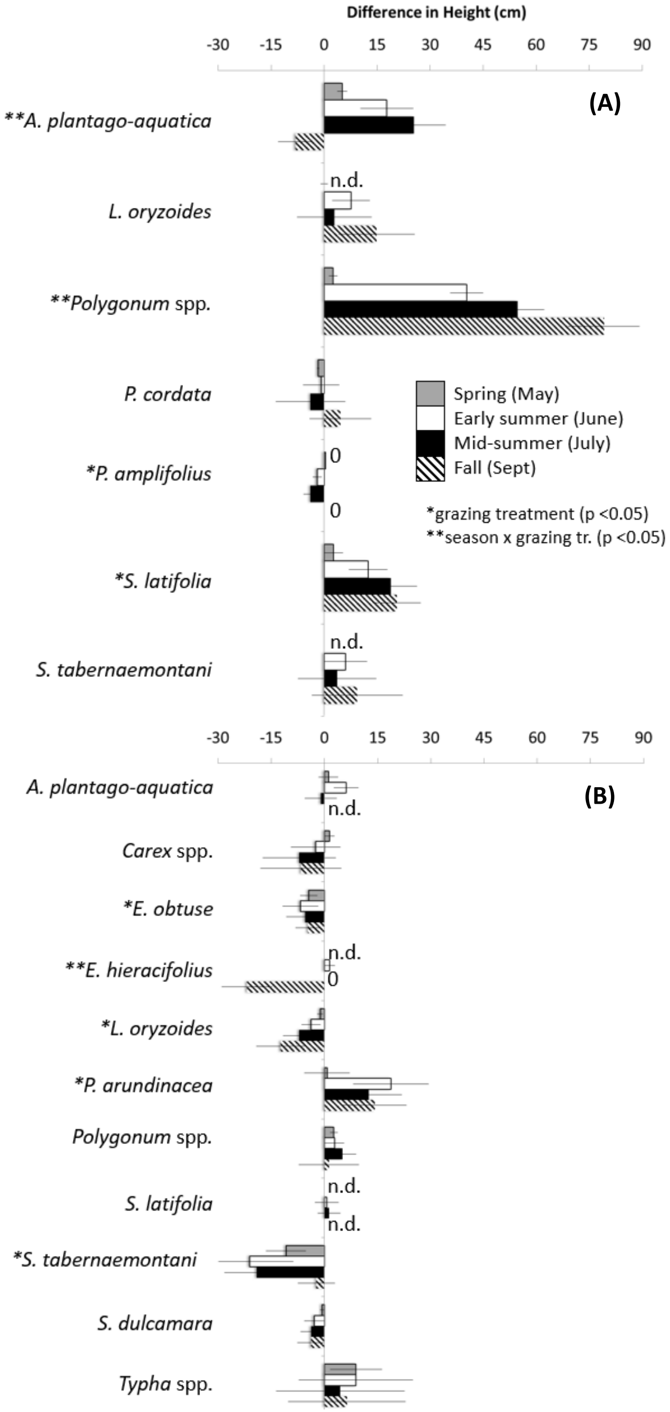

Figure 2.7: Mean ± SE difference in stem height (cm) between caged and uncaged (C-U) plots in A1N (A) and A3 (B) throughout the 2016 growing season. Positive values indicate caged > uncaged; negative values indicate uncaged > caged; values of 0 indicate caged = uncaged; n.d. (no data) indicates species not present.

2.4 Discussion

We found strong interactions between hydrology, nutrient availability, and herbivory at both created wetlands which drove the differences in response to grazer exclusion at each site. In A1N, high grazing pressure by waterfowl, permanent flooding, and low soil nutrients led to reductions in overall growth and diversity of emergent plant species, with implications for shifts in community structure as grazing opens up space and promotes submerged vegetation. In A3, low grazing pressure by waterfowl, seasonal flooding, and high soil nutrients led to enhanced plant growth and diversity, though trends did not suggest development of a desirable emergent plant community structure, despite reductions in undesirable invasive species. The impact of grazers was seasonal and increased over time, and highlighted increasing alteration of soil characteristics. This suggests long-term consequences for habitat provision and delivery of other ecosystem services that will ultimately influence the success of created wetlands in meeting economic and ecological goals.

Created wetlands typically require 15-20 years before plant communities are fully established and stable, because they frequently begin with bare, disturbed soil (Mitsch & Wilson 1996). The development of diverse communities may be hindered by the early colonization of aggressive invasive species (Zedler & Kercher 2005), necessitating management after initial construction. Created wetlands that are seeded and/or planted with native species demonstrate greater diversity over time, compared to those left to naturally colonize, which trend toward monocultures (Reinartz & Warne 1993; Balcombe et al. 2005). Other created and restored wetlands located in the United States, have similar species richness (2-6 species) as control plots in A1N and A3 across growing seasons (2.2 ± 0.3 and 3.9 ± 0.5 species, respectively), though these values were low when compared to natural reference wetlands (10-12 species) (Brown & Bedford 1997; Campbell et al. 2002; Matthews et al. 2009). Created wetlands may, in part, be failing to meet diversity expectations because young, less resistant plant communities are vulnerable to shifts in composition initiated by herbivores (Funk et al. 2008; Moreno-Mateos et al. 2012).

Temporal shifts in hydrologic conditions influenced spatial variability in grazer access and resulted in differences in waterfowl presence at both wetlands. Spring flooding in A3, led to similar grazer abundances when compared to A1N. However, the drying of A3 was uneven, so that some areas were more accessible to waterfowl for longer throughout the growing season. However, this does not take into account possible grazing by insects, or nocturnal or crepuscular mammals, which we did not observe during this study. In A1N, the greatest grazing impacts were observed in plots closest to a concealed goose thoroughfare between Quarry Pond and the created wetland. There were also abiotic variations in soil nutrients within each site: prior to creation of A3, cattle entered, grazed, and defecated more heavily on the east side of the site as is now reflected in higher legacy nutrient concentrations in the soil; sediment accumulation and goose nesting near the culvert in A1N explains higher nutrients in plots closest to this area. Despite this small-scale heterogeneity within both wetlands, further investigation by removing blocks and/or analyzing them separately did not affect the overall results of the analyses and impacts that grazers had on the plant communities.

The abundance and identity of grazers influenced the impact they would have on plant communities. High waterfowl grazing pressure, as seen in A1N, significantly limited both aboveground plant cover and belowground biomass; this trend was fairly consistent across the dominant species (Table 2.2, Figure 2.3A & 2.5). However, despite low waterfowl grazing pressure in A3, grazing did appear to influence plant diversity without reducing total plant cover, suggesting the importance of other grazer species that we may not have observed. The increasing grazer impacts over the duration of the study suggests a cumulative effect on plant communities, highlighting the importance of long term studies. Further, since hitting historic lows in the 1930s, waterfowl populations have continued to increase due to extensive conservation efforts, and factors like climate change widening their habitat ranges and increased agricultural land providing easily available food (Fox et al. 2005; Gauthier et al. 2005; Baldassarre et al. 2006). Increasing waterfowl abundance observed in other aquatic systems have been shown to have detrimental impacts on community structure as populations exceed the limitations normally set by cold winter temperatures, and can result in vegetation losses up to 98% (Jefferies & Rockwell 2002; Gauthier et al. 2005). This general trend may be counter-productive to restoration efforts in emergent wetlands, depending on other environmental conditions at a site. The selection of specific plant species for consumption also influences community structure and is important to consider when seeding and planting created wetlands after construction.

In A1N, some species' growth (*A. plantago-aquatica*, *Polygonum* spp., and *S. latifolia*) was more limited by grazing than others (Figure 2.7A, Appendix B); grazers generally eat plants that are palatable and labile, and avoid plants with higher concentrations of anti-herbivore phenolic compounds (Goranson et al. 2004; Harrison et al. 2017). The choice may also be a matter of convenience; these were the three most common emergent species at the site and could provide for the most optimal foraging (Charnov 1976). Species-specific selections can shift community composition and diversity by promoting unpalatable and recalcitrant species, leading to decreased species richness (Levin et al. 2006). This may explain the negative response in community species richness and Shannon-Weiner index scores to the presence of grazing (Figure 2.4C & 4E). Community shifts are also reflected in the increased height, density, and cover of *P. amplifolius*, a submerged species, possibly as a result of grazers opening space and having greater access to the available light (Mitchell 1989). Regardless, removing significant portions of photosynthetic surface area, likely made it more difficult for these plants to produce necessary energy for growth, necessitating the reallocation of stored belowground resources towards recovery, and limiting the expansion of belowground root networks and aboveground distribution (Hik & Jefferies 1990; Maron & Crone 2006; Gao et al. 2008). This is also consistent with the decrease in organic matter found in uncaged plots. We can infer that the decrease in carbon resulted from the removal of plant material, which limited the accumulation of labile detritus, enhancing the relative proportion of recalcitrant material at the end of the growing season (Van Wijnen et al. 1999; Vaieretti et al. 2013). Additional study, however, is needed to determine the quality of carbon in caged versus uncaged plots.

Differences in hydrology, nutrient availability, and grazing pressure in A3 resulted in contrasting impacts on the dominant species when compared to A1N, suggesting dissimilar trajectories of wetland development under variable grazing scenarios. Lower observed grazing intensity resulted in neutral or positive trends in growth for many common native species; this contrasts with the most common invasive species at the site

(*P. arundinacea* and *Typha* spp.), which were negatively impacted by grazer presence, and was consistent with observations of significant leaf damage by vertebrates (deer clipping) and invertebrates (snail radulations) on these species (Figure 2.7B, Appendix C). *P. arundinacea* and *Typha* spp., typically the tallest plants by early to mid-summer, may have aided in protecting some of the shorter native species from herbivore access (Mulder & Ruess 1998). Low to moderate levels of grazing may also lead to more balanced competition among species, promoting greater overall survivorship and diversity (Connell 1978). Without the mediating control of grazers in caged plots, invasive species cover increased and diversity decreased as native species were out-competed (Figure 2.4D, 2.4F & 2.6). This is in contrast with other studies that suggest that invasive species have a competitive advantage because native grazers prefer native plants, or because invasive species contain novel chemical defenses that native grazers are not adapted to deal with (Blossey & Notzold 1995; Callaway & Ridenour 2004); *P. arundinacea* and *T. angustifolia* are non-native invasive species, whereas *T. latifolia* is a native invasive. The waterfowl herbivores (*B. canadensis* and *Anas* spp.) observed at the two sites are generalist herbivores; their selection of plant species may have favored plant accessibility rather than palatability or nutritional quality.

The impact of grazer presence on overall height and dominance of plant species was greatest at the summer height of vegetation growth, though this did not correspond with peak grazer abundance, which occurred in the fall. Similar trends have been observed in natural aquatic systems, where high fall and overwintering waterfowl abundance has led to subsequent reductions in plant growth and distribution in the following summer season (Perrow et al. 1997; Chaichana et al. 2011). The interaction between grazer intensity and timing, along with individual physiologic responses, determine whether specific plant species can compensate for the herbivory.

In general, plants that are not limited by nutrients can compensate or respond positively to herbivory (Maschinski & Whitham 1989). It is challenging to directly link antecedent land use to present soil conditions and nutrient availability, however, gravel substrates are often difficult to re-vegetate due to low nutrient-holding capacities (Johnson 1987); the low soil nitrogen, phosphorus and organic matter, and low overall plant diversity at A1N, a former gravel depository, reflects these trends. In contrast, cattle deposit large quantities of nutrients into the soil through excretion of nutrient-rich feces (McGechen 2003), as reflected in the substantially higher nutrients and organic matter found at A3. The balance of nutrient availability is crucial; very high nutrient availability can lead to monocultures, because some plants are released from limitation (Bedford et al. 1999). Grazing in the early stages of the growing season (April and May) may have less of an impact because of spring nutrient flushes, allowing for neutral growth compensation by the plants, when compared to periods of higher grazing intensity and lower nutrient availability (June-September) (Maschinski & Whitham 1989). This nutrient-dependent response will have different outcomes in wetland plant communities subjected to different grazing intensities and timing of habitat usage.

Large- and small-scale heterogeneity, designed or not, lead to differences in hydrology, nutrient availability, and grazer intensity, affecting overall ecosystem diversity and function. Each of these factors needs to be addressed in a mindful manner before, during, and after wetland construction in order to ensure that they will meet long-term mitigation goals. The creation of physical and spatial heterogeneity within wetlands includes variations in bathymetry, deep and shallow areas, stream rivulets, and microtopography (Mitsch & Wilson 1996). Building distinct hydrological units and water control structures into wetland design will help to balance wetland use by grazer populations by providing separate areas for nesting and breeding, foraging, and open water that may be used seasonally. (Yallop et al. 2004). This will also encourage the growth and development of multiple vegetation communities that may differ based on water depth, which will provide different resources to herbivores based on varying diets, or habitat requirements and provide resilience in the face of extreme grazing or environmental fluctuations. Using small-scale protective enclosures to deter grazers, especially geese, initially after plantings will also help plant communities develop by protecting individuals during their most vulnerable growth period.

We found that the effects of large herbivores was not only species-specific – depending on hydrology and nutrient availability – but varied temporally depending on the grazing intensity, hydrology, and nutrient availability. Based on our findings, our experimental wetlands do not appear to be developing along the same trajectory as natural emergent wetlands; species richness is low in both wetlands and our data suggests possible shifts in species composition away from emergent communities in A1N (to submerged species) and A3 (to meadow species and grasses). However, these wetlands are still young, less than 10 years old, and like most created wetlands will require sustained long-term monitoring and management to promote equivalency with a natural state. If current positive trends in waterfowl populations continue, plant communities in created wetland may face short-term difficulties in community establishment and development, and long term patterns of degradation in regards to diversity. This may also result in changes in functional states and primary production, as opening space may lead to the proliferation of submerged aquatic vegetation instead of emergent vegetation, as we may have observed at A1N (Mitchell 1989). As grazers continue to manipulate nutrient pools within the soil, wetlands also become susceptible to reductions in carbon sequestration and increased emissions of greenhouse gases (Kayranli et al. 2010; Winton & Richardson 2016). Also, as wetlands face more summer drought conditions due to the consequences of climate change, created wetlands that lack water storage capacity or water level control structures may be in danger of shifting plant composition from wetland species to grassland species, as seen in A3, leading to different impacts of grazers over time. Overall, further study is required to understand whether grazers can shift community composition from one functional state to another, whether this will then impact future grazing, and the implications this may have on other ecosystem services, such as carbon storage and nutrient removal.

Chapter 3: Impact of abiotic and biotic factors on nitrogen removal

3.1 Introduction

As areas of depressed land, wetlands accumulate nutrients from other areas throughout the watershed. This makes nutrient removal processes that occur in wetlands invaluable for water filtration and preserving downstream water quality for use by other organisms, including humans. Denitrification, the conversion of nitrate (NO_3^-) to nitrogen gas (N_2) or nitrous oxide (N_2O) by anaerobic heterotrophic bacteria, is one of the main processes for nitrogen removal in aquatic ecosystems (Vymazal 2005; Bulc 2006). Three main drivers of wetland ecosystem function – hydrology, nutrient availability, and herbivory – interact in complex ways to regulate services such as denitrification (Teal 1962; Anderson & Low 1976; Carter 1996). Understanding these interactions is especially pertinent when designing, constructing, and managing wetlands that are created to mitigate for the loss of natural wetlands destroyed for human development (US EPA 2008). Due to prior land use legacies, artificially made hydrologic regimes, and altered soil properties, created wetlands often face a unique combination of these ecosystem drivers and fail to provide the same level of function as their natural wetland counterparts. Created wetlands often have lower decomposition rates and soil organic matter (Campbell et al. 2002; Fennessy et al. 2008), which may limit heterotrophic bacterial activity, and limit their ability to efficiently remove nitrogen. It is therefore important to study the interactions of hydrology, nutrients, and grazing specifically in the context of created wetlands to optimize the ecosystem service outcomes we desire.

Hydrology dictates whether a wetland will be permanently, seasonally, or intermittently flooded and in turn drives the availability of oxygen in the soil. Since denitrification is a process carried out by anaerobic bacteria, oxygen availability is an important limiting factor (Paul & Beauchamp et al. 1989). Other processes such as nitrification, the conversion of ammonia or ammonium (NH_3/NH_4^+) to nitrite (NO^{2-}) then nitrate (NO_3^-), that are aerobic and rely on oxygen, are necessary for converting unusable nitrogen species into forms than can be used by denitrifying bacteria (Hernandez & Mitsch 2007). Therefore, the availability of nutrients like nitrate and labile organic matter are also important limiting factors for heterotrophic denitrifiers (Ballantine et al. 2014). The strong correlations between hydrologic conditions and the presence of specific aquatic herbivores, such as geese and ducks (Murkin et al. 1997; Clausen 2000; Lor & Malecki 2006), are also relevant, as grazers can manipulate nutrient and carbon pools based on their foraging behavior (Figure 3.1).

Plants provide a carbon substrate, in the form of detritus at the end of the growing season and root exudates during active growth (Whiting and Chanton 1993; Ding et al. 2005; Laanbroek 2010). Removing biomass of palatable species, which may be more nutrient-rich and easily broken down by microbes, can also influence litter quality and the accumulation of carbon-rich detritus (Van Wijnen et al. 1999). Grazing may also increase the uptake of nutrients by those plants for recovery growth; this may increase temporary nutrient immobilization and decrease nutrient pools within the soil (Gao et al. 2008). However, aquatic herbivores, like waterfowl, may enhance decomposition rates and soil nutrient concentrations through the deposition of nutrient-rich feces (Kitchell et al. 1999)

(Figure 3.1). Small- and large-scale variability in nutrient and carbon content may have cascading impacts on overall ecosystem function.

Soil properties and availability of nutrients in created wetlands may also be influenced by the legacy of the prior land use, though the link between past use and present conditions is not clearly defined. Wetlands constructed on former agricultural lands may result in soils depleted of nutrients, however soil amendments like liming and fertilization may result in increased nutrient levels (Compton et al. 1998; Knops & Tilman 2000; Richter & Roelcke 2000; Foster et al. 2003). Nutrient availability within the soil provides the basis for fueling growth and survivorship of microbial and plant species, and the balance of competition between them has implications for nitrogen removal (Figure 3.1).

Wetland plants also play an important role in permanently flooded wetlands by transporting oxygen through diffusion from roots to soil; this – oxidized rhizosphere – brings oxygen to normally anoxic soils (Armstrong 1964). However, the presence of aquatic herbivores can change the dynamics that nutrient availability and plant species play in nitrogen removal. Herbivore clipping may limit or shut down gas transport, preventing formation of the oxidized rhizosphere, which is important for nitrogen cycling in low oxygen wetland soils (Winton & Richardson 2016) (Figure 3.1). Waterfowl populations have increased dramatically since historic lows in the 1930s, due to strong conservation efforts (Baldassarre et al. 2006). At the same time, by 1984, more than half of the historic wetland area in the United States was lost (US EPA 2008); this has led to higher numbers of waterfowl concentrating in a smaller area of remaining wetland habitat. The interactions between hydrology, nutrient availability, and herbivory on nitrogen removal in natural wetlands are complex; taking into the account the added variability of prior land use, altered hydrology and soil properties makes predicting outcomes and meeting management goals in created wetlands even more difficult.

The objective of this study was to determine the impact of interacting factors (hydrology, nutrient availability, and herbivory) on nitrogen removal in two created emergent freshwater wetlands with different prior land use histories: wet and low nutrients, dry and high nutrients. We hypothesized that: 1) the created wetland with higher nutrient availability will result in higher rates of potential nitrogen removal, though not necessarily similar to natural wetlands, and 2) the removal of plant matter by grazers will decrease nutrient pools and organic matter within the soil, and this will result in changes to rates of potential nitrogen removal.

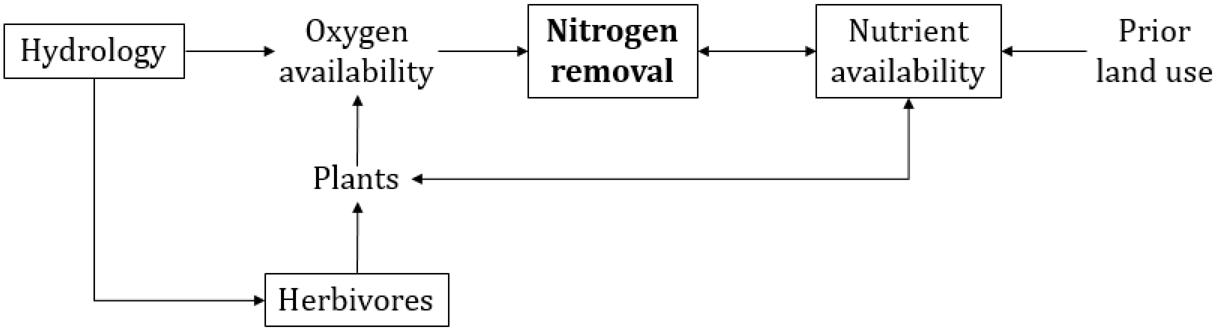

Figure 3.1: Schematic of interactions between hydrology, nutrient availability, and herbivory on their impacts on nitrogen removal.

3.2 Methods

3.2.1 Site description

This study was conducted between May 2016 and October 2016 in two created wetlands at High Acres Nature Area (HANA) in Perinton, New York, USA that are owned and managed by Waste Management of New York and New England, LLC (Chapter 2.2 Figure 2.1). Area 1 North (A1N) served as a gravel-mining depository until approximately the mid-1960s, before being abandoned and left fallow (Stantec 2009). Prior to mining, the area was used for agricultural purposes. A variety of native plants were planted at the time of construction and in subsequent years, and *Typha latifolia* (invasive) was cut during the growing season of 2011-2014 to enhance habitat diversity. Broadleaf arrowhead (*Sagittaria latifolia)*, water plantain (*Alisma plantago-aquatica*), and smartweed species (*Polygonum* spp.) were the dominant species at the start of the study. Area 3 (A3) was a cattle pasture prior to construction in 2012. Native shrub and emergent wetland species were planted throughout; *Typha* spp., *A. plantago-aquatica*, and *Polygonum* spp. dominated at the initiation of this study.

The observed abundance of wetland grazers, reported previously, was significantly higher in A1N than A3, though this difference varies depending on the season (Chapter 2.3, Figure 2.2). The greatest differences were seen in summer and fall (A1N: summer =8.9 ± 2.0, fall =44.1 ± 8.6 individuals ha^{-1}; A3: summer =0.1 ± 0.1, fall =5.3 ± 2.2 individuals ha^{-1}); grazing densities were not different in spring and winter. Waterfowl comprised 99-100% and 66-100% of the large grazers in A1N and A3, respectively.

3.2.2 Experimental design: herbivore exclusion

We conducted our analyses in 8 pairs of 1 x 1 m caged (herbivore exclusion) and uncaged control (open to herbivores) plots in blocks of 4 pairs that were established randomly, in A1N and across distinct zones of A3, (32 total plots, 2 blocks per site) (Chapter 2.2, Figure 2.1). Cages were maintained throughout the subsequent years and during the measurements described below, had been in place for 24 months. As described elsewhere, three-sided cage control plots were also established and demonstrated no difference in vegetation properties relative to uncaged plots over the three year experiment (Chapter 2).

3.2.3 Characterizing hydrologic conditions and soil and porewater nutrient chemistry

Hydroperiod was evaluated by averaging standing surface water depth from 3 points in every plot in spring (May), early summer (June), mid-summer (July), and fall (September) 2016. Three soil cores (2.5 cm diameter x 10 cm deep) were extracted from each plot with an auger in May and September 2016 and subdivided for organic matter, nutrients, and pH analysis. Soil organic matter content was determined by loss on combustion (Heiri et al. 2001). Extractable inorganic nitrogen was measured by extraction with 2M potassium chloride (Keeney and Nelson 1982). Ammonium in the supernatant was analyzed using the phenol-hypochlorite method on a Shimadzu 1800 spectrophotometer (Solorzano 1969), and nitrate+nitrite was measured with the cadmium reduction method using a Lachat Quikchem 8500 Autoanalyzer (Lachat 2003). Total phosphorus was extracted from soil samples by adding magnesium nitrate to soil dried at 60 ^{o}C, combusting at 550 ^{o}C for two hr, and dissolving in hydrochloric acid before analysis using the ammonium molybdate method (Murphy & Riley 1962). Soil pH was measured by creating a 2:1 (v/v) slurry of dionized water to soil, stirring vigorously until a uniform suspension was achieved (Gelderman and Mallarino 2012) and measuring with a Hach pH probe calibrated with pH =4, 7, and 10 buffers.

Porewater nutrient chemistry was evaluated in 2016, in suction lysimeters inserted to 15 cm depth in A1N plots (Chambers & Odum 1990). Lysimeters were not installed in A3, because of the lack of groundwater due to drought conditions. Water samples were collected under an anaerobic headspace in spring and fall 2016 and immediately filtered (0.45 μm PES filter) prior to freezing and later analyzed for ammonium and nitrate as described above. Porewater redox potential and temperature were measured with a Hach Intellical™ ORP Electrode (HACH 2014) in the spring and fall by drawing water from the lysimeters into a sampling chamber that had been flushed with nitrogen gas.

3.2.4 Soil respiration and potential denitrification

Soil respiration was evaluated by measuring the build-up of CO_2 gas in 200 mL septa jars, containing the soil (30-40 grams wet weight), using a LI-COR LI-820 CO_2 Gas Analyzer (LI-COR 2002). Soil was collected from each plot in the fall (September) using a soil auger (2.5 cm diameter x 10 cm depth). The CO_2 concentration was measured in the dark over a roughly 30 minute period under aerobic and anaerobic (accomplished by flushing with N_2 gas) conditions. After the analysis, each soil sample was dried in a 60^oC oven, and weighed to calculate soil moisture and dry weights. All rates are expressed per g dry weight.

Samples for potential denitrification measurements were taken using a soil auger (2.5 cm diameter x 10 cm depth) from each plot in the spring (late May to early June) and fall (September) of 2016. Samples were split in two: one for denitrification and one to be dried in a 60°C oven to calculate dry weight. This process was measured using the acetylene block method, adapted from Groffman et al. (1999). Acetylene (C_2H_2) blocks the final step in microbial denitrification – the conversion of N_2O to N_2 gas – and the subsequent buildup of N_2O can be used to estimate potential denitrification rates. This method does not distinguish between nitrate converted to nitrogen gas or converted to nitrous oxide, and can lead to underestimations of nitrogen removed in aquatic systems because this is not the only mechanism of removal in wetlands (Brix 1994; Groffman et al. 1999).

Briefly, soil samples (approx. 30-40 grams wet weight) were placed in 250 mL septa jars, flushed with N_2 gas to create an anoxic environment (2 cycles, 3 minutes), acetylene was added (25 mL), and incubated under four separate conditions by adding a media solution: plus carbon (dextrose), plus nitrogen (potassium nitrate), plus carbon and nitrogen, and controls with nothing added (media: 100 mg/kg N, 40 mg/kg C, and 10 mg/kg chloramphenicol). This methodology does not replicate exact field conditions for denitrification, only optimum potential rates. Gas samples (5 mL) were collected from incubation vials at approximately 30, 60, 120, and 180 minutes, and injected immediately into a Shimadzu Greenhouse Gas Analyzer Gas Chromatograph for analysis. N_2O data, in ppm, was converted to moles/gram dry soil, and rates were derived by regression over time.

3.2.6 Statistical analyses

All statistical analyses were completed using JMP 13 Pro statistical software. Prior to selection of statistical analysis method, each dataset was checked for normality and homogeneity of variance. Intra-site heterogeneity was evaluated by adding a block effect, to encompass different zones of each wetland, as a random factor into every analysis of variance (ANOVA). Results of the significance of the block effects are listed in Appendix D.

Using a full-factorial three-way ANOVA we compared organic matter content, soil nutrients (nitrate, ammonium, total phosphorus), and soil pH with site (A1N, A3), treatment (caged, uncaged), and season (spring, fall) as fixed factors. Using a full factorial two-way ANOVA we compared porewater nutrients (nitrate, ammonium, phosphate), redox potential, and temperature in A1N with season (spring, fall) and grazing treatment (caged, uncaged) as fixed factors.

Soil respiration and potential denitrification rates were normalized by taking the inverse square root of the rates prior to analysis using a full-factorial three-way ANOVA for comparison of potential denitrification rates with site (A1N, A3), season (spring, fall), and grazing treatment (caged, uncaged) as fixed factors. Using a one-way ANOVA , we compared potential denitrification rates with nutrient treatment (carbon+nitrogen, +carbon, +nitrogen, control) as a fixed factor for both wetlands, separately. We used a full factorial two-way ANOVA for comparison of aerobic and anaerobic respiration rates with site (A1N, A3) and grazing treatment (caged/uncaged) as fixed factors. For all ANOVAs, when significant difference were found a Tukey's HSD post hoc analysis was used to elucidate differences among treatments.

3.3 Results

3.3.1 Hydrology

A1N and A3 had distinct hydrologic characteristics throughout the growing season of 2016: A1N was permanently flooded, A3 was flooded during early spring, but was fully dry by mid-June (Chapter 2.3, Figure 2.2). During summer 2016, New York State experienced an extreme drought, affecting both wetlands differently. In June, the culvert in A1N was closed preventing further water from flowing out, and continued to be fed through subsurface recharge from the adjacent deeper abandoned quarry pond such that water was held in the wetland (May= 8.3 ± 3.0, June= 13.5 ± 3.8, July= 17.1 ± 3.1, September= 15.1 ± 3.6 cm). A3 lacks a water control structure (May= 11.2 ± 6.4, June= 0.3 ± 1.2, July= 0.0 ± 0.0, September= 0.0 ± 0.0 cm; mean ± SD).

3.3.2 Soil and porewater nutrients

Soil properties were distinct between sites and followed a predictable pattern based on prior land use. Organic matter content was 1.5 times higher in A3 than A1N (14.6 ± 1.1 and 8.5 ± 0.7 %, respectively; $p < 0.0001$; Tables 3.1 & 3.2). Despite this difference, grazing resulted in a significant overall reduction in organic matter content ($p = 0.031$). Site differences in extractable nitrate, ammonium, and total phosphorus were seasonally dependent, but in general, significantly higher at A3 than A1N (site x season $p < 0.0001$, $p = 0.005$, $p < 0.001$, respectively; Tables 3.1 & 3.2). In both sites during spring and fall, extractable soil ammonium was greater than extractable soil nitrate (15 and 35 times higher in A1N, respectively; 100 and 5 times higher in A3, respectively; Table 3.1). Soil pH significantly varied by season: in fall was more acidic than spring (7.4 ± 0.1 and 7.6 ± 0.1, respectively; $p < 0.001$; Tables 3.1 & 3.2). There were no significant effects of grazing on soil nitrate, ammonium, total phosphorus, or soil pH (Table 3.2).

In A1N, porewater chemistry was not significantly affected by the grazing treatment, however there were some significant seasonal variability (Table 3.2). Porewater ammonium followed a similar trend as soil ammonium, with 32 times higher values in spring than fall (28.4 ± 6.2 mg/L and 0.9 ± 0.2 mg/L, respectively; $p<0.0001$; Tables 3.1 & 3.2). Phosphate was not significantly affected by season. Porewater temperature was also significantly higher in the spring than in the fall (25.8 ± 0.2 ^{o}C and 20.3 ± 0.3 ^{o}C, respectively; $p < 0.0001$). Finally, the oxidation-reduction potential was negative and reducing, more so in spring than fall (-133.2 ± 3.8 mV and -84.4 ± 5.5 mV, respectively; $p < 0.0001$). All soil characteristics, along with porewater phosphate and oxidation-reduction potential demonstrated significant block effects (Appendix D).

Table 3.1: Mean ± SE soil chemistry measured in A1N and A3, and porewater chemistry measured in A1N in 2016. C= caged, U= uncaged

Factor	**Spring**				**Fall**			
	A1N		A3		A1N		A3	
	Avg ± SE				Avg ± SE			
	C	U	C	U	C	U	C	U
Soil								
Organic matter (OM, %)	8.8 ± 0.5	7.2 ± 0.4	15.8 ± 1.2	14.6 ± 1.2	9.3 ± 1.0	8.6 ± 1.0	14.1 ± 1.0	13.8 ± 1.0
Nitrate (NO_3^-, mg/kg)	0.7 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0.3 ± 0.2	0.1 ± <0.1	0.1 ± <0.1	2.3 ± 0.6	2.0 ± 0.5
Ammonium (NH_4^+, mg/kg)	10.6 ± 2.6	9.9 ± 2.5	25.4 ± 6.4	27.9 ± 7.0	2.4 ± 0.6	5.0 ± 1.3	10.6 ± 2.7	10.0 ± 2.5
Total phosphorus (TP, mg/kg)	744.6 ± 23.5	683.7 ± 29.7	990.0 ± 53.2	903.0 ± 48.7	696.8 ± 22.3	724.9 ± 26.2	1176.2 ± 61.7	1102.3 ± 57.7
pH	7.6 ± 0.03	7.7 ± 0.02	7.6 ± 0.1	7.6 ± 0.1	7.5 ± 0.04	7.5 ± 0.1	7.3 ± 0.2	7.3 ± 0.2
Porewater								
NO_3^- (mg/L)	0.4 ± 0.3	0.1 ± 0.0	---	---	<0.1	<0.1	---	---
NH_4^+ (mg/L)	25.7 ± 6.7	31.1 ± 5.6	---	---	1.0 ± 0.2	0.7 ± 0.2	---	---
Phosphate (PO_4^{3-}, mg/L)	<0.1	<0.1	---	---	<0.1	<0.1	---	---
Oxidation-reduction potential (ORP, mV)	-139.5 ± 4.3	-126.9 ± 5.6	---	---	-86.8 ± 7.7	-81.9 ± 8.1	---	---
Temperature (°C)	26.0 ± 0.2	25.6 ± 0.2	---	---	20.3 ± 0.3	20.2 ± 0.4	---	---

Table 3.2: Results of two- and three-way ANOVAs examining the effect of site (A1N, A3), season (spring, fall), and grazing treatment (caged, uncaged) on soil and porewater chemistry. Significant p-values are bolded (*p <0.0001). Seas= season, Tr= treatment

Factor	**Site**		**Season**		**Treatment**		**Site x Seas**		**Site x Tr**		**Seas x Tr**		**Site x Seas x Tr**	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Soil														
OM	$F_{1,127}$= 82.2	**<0.001***	$F_{1,127}$= 0.03	0.87	$F_{1,127}$= 4.8	**0.031**	$F_{1,127}$= 1.8	0.19	$F_{1,127}$= 0.8	0.39	$F_{1,127}$ <0.1	0.87	$F_{1,127}$= 0.3	0.59
NO_3^-	$F_{1,127}$= 19.7	**<0.001***	$F_{1,127}$= 13.9	**<0.001**	$F_{1,127}$= 0.2	0.70	$F_{1,127}$= 45.3	**<0.001***	$F_{1,127}$= <0.1	0.92	$F_{1,127}$= 0.3	0.56	$F_{1,127}$= 0.7	0.40
NH_4^+	$F_{1,127}$= 46.1	**<0.001***	$F_{1,127}$= 45.5	**<0.001***	$F_{1,127}$= 0.3	0.57	$F_{1,127}$= 8.4	**0.005**	$F_{1,127}$= <0.1	0.98	$F_{1,127}$= <0.1	0.97	$F_{1,127}$= 0.9	0.35
TP	$F_{1,127}$= 169.9	**<0.001***	$F_{1,127}$= 14.0	**<0.001***	$F_{1,127}$= 3.7	0.06	$F_{1,127}$= 15.0	**<0.001**	$F_{1,127}$= 1.6	0.21	$F_{1,127}$= 1.0	0.32	$F_{1,127}$= 0.6	0.46
pH	$F_{1,127}$= 3.3	0.07	$F_{1,127}$= 12.9	**<0.001**	$F_{1,127}$= 0.2	0.66	$F_{1,127}$= 1.7	0.19	$F_{1,127}$ <0.1	0.93	$F_{1,127}$ <0.1	0.94	$F_{1,127}$= 0.1	0.73
Porewater														
NO_3^-	---	---	$F_{1,31}$= 3.6	0.07	$F_{1,31}$= 1.0	0.32	---	---	---	---	$F_{1,31}$= 1.0	0.33	---	---
NH_4^+	---	---	$F_{1,31}$= 38.4	**<0.001***	$F_{1,31}$= 0.3	0.58	---	---	---	---	$F_{1,31}$= 0.4	0.53	---	---
PO_4^{3-}	---	---	$F_{1,31}$= 0.2	0.68	$F_{1,31}$= 0.1	0.75	---	---	---	---	$F_{1,31}$ <0.1	0.90	---	---
ORP	---	---	$F_{1,31}$= 69.7	**<0.001***	$F_{1,31}$= 2.3	0.15	---	---	---	---	$F_{1,31}$= 0.4	0.51	---	---
Temp	---	---	$F_{1,31}$= 337.6	**<0.001***	$F_{1,31}$= 0.4	0.51	---	---	---	---	$F_{1,31}$= 0.3	0.62	---	---

3.3.3 Soil respiration and potential denitrification

There were no significant effects of grazing on soil respiration at either site (Table 3.3, Figure 3.2). However, both aerobic and anaerobic respiration rates were significantly different between sites ($p < 0.001$ and $p < 0.0001$, respectively; Table 3.3): aerobic respiration in A1N was 7.5 times faster than in A3 (1.53 ± 0.57 and 0.20 ± 0.09 moles C g^{-1} day^{-1}, respectively; Figure 3.2); similarly, anaerobic respiration in A1N was 4 times faster than in A3 (0.91 ± 0.21 and 0.23 ± 0.05 moles C g^{-1} day^{-1}, respectively; Figure 3.2). This also corresponds with high soil moisture in A1N, which was more than two times higher than A3 (53.9 ± 3.5 and 23.0 ± 1.4%, respectively).

Potential denitrification rates were 7 times faster in A3 than A1N (0.14 ± 0.03 and 0.02 ± 0.0 moles N g^{-1} day^{-1}, respectively; $p < 0.0001$; Table 3.3, Figure 3.3A), and greater in spring than fall (0.10 ± 0.02 and 0.06 ± 0.02 moles N g^{-1} day^{-1}, respectively; $p < 0.0001$). Overall, grazing reduced potential denitrification by approximately 40% across all measurements (caged: 0.10 ± 0.02 moles N g^{-1} day^{-1}; uncaged: 0.06 ± 0.01 moles N g^{-1} day^{-1}; $p = 0.024$; Table 3.3, Figure 3.3A). Potential denitrification rates in A1N significantly increased with addition of nitrogen but not carbon ($p < 0.0001$; Table 3.3, Figure 3.3B); contrastingly, A3 significantly increased with addition of carbon but not nitrogen ($p = 0.014$; Table 3.3, Figure 3.3B). For both the grazing and limiting factors experiment, there were significant block effects ($p < 0.0001$, respectively; Appendix D).

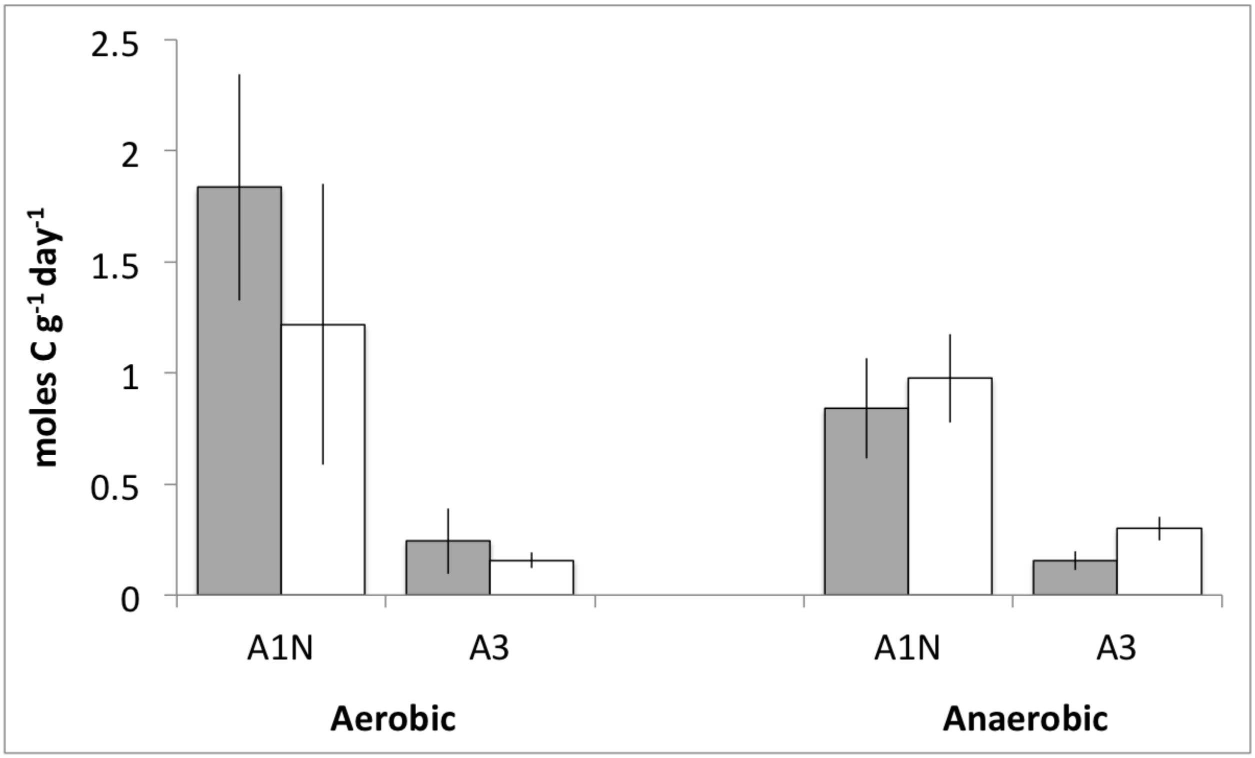

Figure 3.2: Mean ± SE rates of carbon released via soil respiration observed in caged (gray) and uncaged (white) plots at both A1N and A3 wetlands under aerobic and anaerobic conditions in fall 2016.

Table 3.3: Results of one-, two- and three-way ANOVAs examining the effects of site (A1N, A3), grazing treatment (caged, uncaged), and nutrient treatment (CN, N, C, control) on potential denitrification rates, and on soil respiration rates. Significant p-values are bolded (*p <0.0001). Resp= respiration, DNF= potential denitrification, Seas= season, Graz= grazing treatment

Factor	**Site**		**Season**		**Grazing Treatment**		**Nutrient Treatment**		**Site x Seas**		**Site x Graz**		**Seas x Graz**		**Site x Seas x Graz**	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Resp (moles C g^{-1} day^{-1})																
Aerobic	$F_{1,31}$= 18.3	**<0.001**	---	---	$F_{1,31}$= 1.2	0.28	---	---	---	---	$F_{1,31}$= 0.9	0.35	---	---	---	---
Anaerobic	$F_{1,31}$= 24.7	**<0.001***	---	---	$F_{1,31}$= 2.1	0.16	---	---	---	---	$F_{1,31}$= 0.1	0.75	---	---	---	---
DNF (moles N g^{-1} day^{-1})																
Grazing	$F_{1,159}$= 106.8	**<0.001***	$F_{1,159}$= 19.6	**<0.001***	$F_{1,159}$= 5.2	**0.024**	---	---	$F_{1,159}$= 1.8	0.18	$F_{1,159}$= 0.5	0.12	$F_{1,159}$= 0.7	0.40	$F_{1,159}$= 0.5	0.48
Limiting factors																
A1N	---	---	---	---	---	---	$F_{1,79}$= 26.7	**<0.001***	---	---	---	---	---	---	---	---
A3	---	---	---	---	---	---	$F_{1,79}$= 3.8	**0.014**	---	---	---	---	---	---	---	---

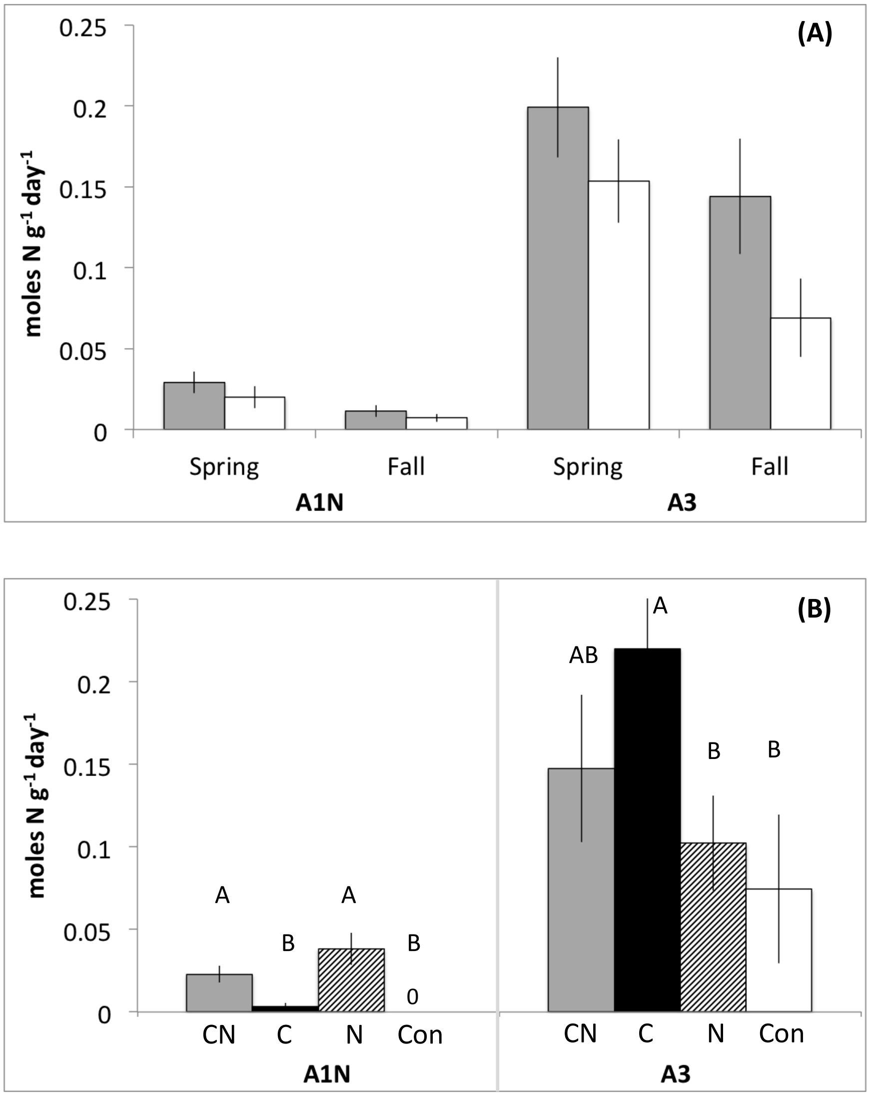

Figure 3.3: Mean ± SE rates of potential denitrification – (A) caged (gray) vs. uncaged (white) plots in spring and fall, (B) one-way analyses under different nutrient conditions (CN= carbon+nitrogen, C= +carbon, N= +nitrogen, Con= control), observed at both A1N and A3 wetlands in 2016.

3.4 Discussion

The distinct differences in hydrology, nutrient availability, and herbivory we found between the two sites demonstrated the importance of considering prior land use and water level control as part of wetland construction and management to ensure the ecosystem service outcomes we desire. Created wetlands are often constructed on land previously used for other purposes (i.e. agriculture, pastureland), and past activities on these lands, like crop tilling, can lead to alterations in soil properties, soil compaction, and/or homogenization unlike what would be found in a natural wetland (Galatowitsch and Van der Valk 1996; Campbell et al. 2002). Though it is difficult to make direct links between antecedent land use and present nutrient availability, significant differences in nutrient availability between the two sites may be a result of their contrasting prior land use histories. Wetlands constructed on gravel substrates such as A1N, the former gravel depository, are associated with low nutrient holding capacity, and may explain the relatively low soil organic matter, nitrogen, and phosphorus found there (Johnson 1987). In comparison, A3, a former pastureland, had significantly higher soil organic matter, nitrogen, and phosphorus, consistent with legacy nutrient deposition by cattle (McGechen 2003).

Drought conditions during the study affected the wetlands differently as well, reflecting two different wetland designs. A1N was constructed at a lower elevation, with a deep basin, and the groundwater connection to an adjacent deep pond and the installation of a depth-controlling culvert allows it to remain flooded for the entire year. A3 was constructed at a higher elevation with a very shallow basin; it is not connected to a large adjacent water source, and no water control structures were installed during construction to maintain wetland water storage. During the spring, both sites were flooded, and redox potentials in A1N indicated anaerobic conditions, therefore nitrification, an aerobic process producing nitrate, may be limited. During the spring and fall, soil ammonium was substantially higher than soil nitrate in A1N and A3 (Table 3.1), suggesting the need for tighter coupling of nitrification and denitrification. Low soil moisture likely led to low soil respiration observed in A3, and suggests that soils may be too aerobic for denitrification in spite of nitrogen availability (Linn and Doran 1984). The balance of oxygen availability is crucial to providing appropriate conditions for both processes to occur.

These large-scale variations, along with low and high levels of observed waterfowl grazing, led to potential denitrification that was limited by different factors in both created wetlands. When compared to other studies using the acetylene block method to measure potential denitrification in restored and natural freshwater wetlands (approximately 2.3-19.2 mg kg^{-1} d^{-1}), A1N and A3 (approximately 0.42 and 2.1 mg kg^{-1} d^{-1}, respectively) fall below the range of daily rates, despite the addition of both carbon and nitrogen sources (Hunter & Faulkner 2001; Clément et al. 2002; Dodla et al. 2008). This has important implications for management goals of overall nitrogen removal within these created wetlands.

Soil organic matter in control plots at A1N (7.9 ± 0.7 %) and A3 (14.2 ± 1.1 %) were comparable or higher, than other created and restored wetlands located in the eastern United States (3.1-11.8 % in created and restored wetlands, less than ten years old in Ohio, Pennsylvania and North Carolina), although these values may fall short when compared to natural wetlands at the same sites (11.5-28.9 %) (Edwards & Proffitt 2000; Campbell et al. 2002; Bruland et al. 2006; Fennessy et al. 2008). However, carbon soil amendments (top soil, biochar, straw) can increase organic matter in created wetlands (Ballentine et al. 2014). Extractable soil nitrate and ammonium concentrations in A1N (0.4 ± 0.1 and 7.0 ± 1.8 mg/kg, respectively) and A3 (1.2 ± 0.4 and 18.5 ± 4.7 mg/kg, respectively) demonstrated possible links between prior land use and present nutrient availability, and the disproportion of nitrate and ammonium availability when compared to restored wetlands located in New York State (approximately 1.7 and 2.0 mg/kg extractable nitrate and ammonium, respectively) (Ballentine et al. 2014). Knowledge of prior land was and preliminary soil studies to assess ambient nutrient availability will allow wetland managers to make decisions about possible soil amendments during construction, aiding in wetland development. Adding a substantial organic top soil, to land that is lacking in soil nutrients, will help to provide fuel for microbial activity and promote processes like nitrification and denitrification (Ballantine et al. 2014).

There is constant competition between plants and microbes for soil nutrients within the system, regulating nitrogen removal. Although, plants provide temporary removal of nutrients, immobilization by microbes provides more permanent pathways of nitrogen removal in created wetlands. Potential denitrification measurements may only provide information about a fraction of the nitrogen that is possibly removed from the system, but it is an important metric in assessment of whether a created wetland is adequately and efficiently removing nitrogen. Although, potential denitrification rates in uncaged plots in A3 were 8 times higher than in A1N, neither wetland is meeting expectations when compared to natural wetlands. When nitrogen – in the form of nitrate – was added to incubations from A1N, rates increased significantly; this did not occur when carbon was added, suggesting that denitrification was at least partially nitrogen limited (Table 3.3, Figure 3.3B). In A3, potential denitrification significantly increased when carbon was added, suggesting a different source of limitation for this process. However, higher nitrogen availability in the spring, possibly from spring fertilizer use and run off from surrounding agriculture and residential areas (Lambert et al. 1985; Quinn and Stroud 2002), or from nutrient build up over the winter released during spring thaw (Smith et al. 2010), resulted in higher denitrification in spring than in fall at both sites.

Abiotic conditions at the two sites do not appear to fully support the processes of nitrification and denitrification; limitations were likely further compounded by the presence of large grazers. We found that grazers reduced overall soil organic matter, despite differences in grazer abundance between the two wetlands. This was likely a result of the grazers removing plant biomass and decreasing the accumulation of detritus in the soil at the end of the growing season as observed at these sites and elsewhere (Chapter 2.3, Figures 2.4A & 4B; van Wijnen et al. 1999). Further, when grazed plants must reallocate resources for recovery growth, leading to decreased belowground growth (Chapter 2.3, Figure 2.6) and root exudates of labile carbon (Gao et al. 2008). This is significant, because carbon is necessary to fuel heterotrophic microbes responsible for nitrification and denitrification. Emergent macrophytes have extensive root systems and can transport gases, like oxygen, to low oxygen soils (Armstrong 1964). This function is crucial in wetlands, like A1N, that are deep and remain flooded throughout the season, and without it, nitrification may be severely reduced, if not completely eliminated. Grazing may result in the temporary reduction or shutdown of gas transport by emergent plants, due to the reallocation of resources, or due to the removal or destruction of roots and rhizomes as they forage (Kelker & Chanton 1997; Winton & Richardson 2017).

Denitrification varies significantly in created wetlands depending on the vegetation communities: emergent macrophytes >> forested edge and open water communities (Hernandez & Mitsch 2007). However, there is generally a lack of knowledge about which species are most efficient oxygen transporters, suggesting the need for further research. This may also be significant in terms of grazer impacts, as consumption of emergent plant species can open space and allow for the spread of submerged aquatic vegetation (Chapter 2; Mitchell 1989). Furthermore, promoting plant community diversity is necessary for wetland managers in order to balance damage by generalist grazers, like ducks and geese, and prevent limitations on plant-mediated oxygen transport that promotes coupled nitrification-denitrification in the rhizosphere and reductions in carbon exudates that fuel nitrogen removal processes.

Installing water control structures as part of wetland creation can allow mangers to create periods of intermittent flooding by lowering water levels during spring nutrient flushes, when soil ammonium may be high, to promote the conversion of ammonium to nitrate by nitrifying bacteria. The strategic decreasing or increasing of water levels to promote both nitrification and denitrification, also requires knowledge of surrounding land use, which may be responsible for spring nutrient run off. Water control structures are also relevant for wetlands that appear to be susceptible to drought; culverts may be necessary to improve water storage during desired periods, so that anaerobic conditions may be maintained in some areas to promote denitrifying activity. In wetlands that have already been constructed or ones where water control structures are not feasible, oxygen penetration facilitated by planting diverse communities of macrophytes, may help in providing conditions suitable for both processes. Further study is needed to investigate the role of herbivores in limiting species-specific emergent plant gas transport and root exudates that are vital for microbial processes associated with denitrification, and whether some plant species are more efficient at oxygen transport despite pressure from grazing.

We found that prior land use history may be used as an indicator of nutrient availability in created wetlands, and may be a useful way to assess the need for soil amendments in order to promote services such as denitrification. Since plants and microbes are continuously competing for resources in order to carry out their own processes, constructing wetlands that promote all steps of nitrification-denitrification is essential to provide the resources necessary for all groups of species. Our study also clearly demonstrated that grazing at both low and high intensities, and hydrologic extremes (deep and flooded vs shallow and dry) interact to limit denitrification by providing conditions that are only suitable for part of the process, preventing created wetlands from meeting goals of development and function. We can surmise that grazing may limit denitrification by removing biomass and preventing the accumulation of carbon based litter at the end of the growing season and during decomposition or by reducing the ability of plants to properly transport oxygen to the soil. As waterfowl populations increase 9from effective species' conservation and widening habitat ranges due to climate change (Fox et al. 2005; Baldassarre et al. 2006), wetlands will likely be exposed to higher and more sustained pressure from grazers, generating the need to create wetlands that promote heterogeneity to balance these effects.

Chapter 4: Conclusions

Our study demonstrated that the impact of land use legacies and hydrology have cascading and interwoven impacts on plant diversity and nitrogen removal within created wetlands. These impacts are further influenced by the role of herbivores in manipulating these services. Our results also show that a reduction in one ecosystem service can lead to a reduction in another, and in order to create a fully functional wetland, the parameters of success need to be clear so that a wetland can be designed to accomplish as many of the ecosystem service goals as possible (Figure 4.1).

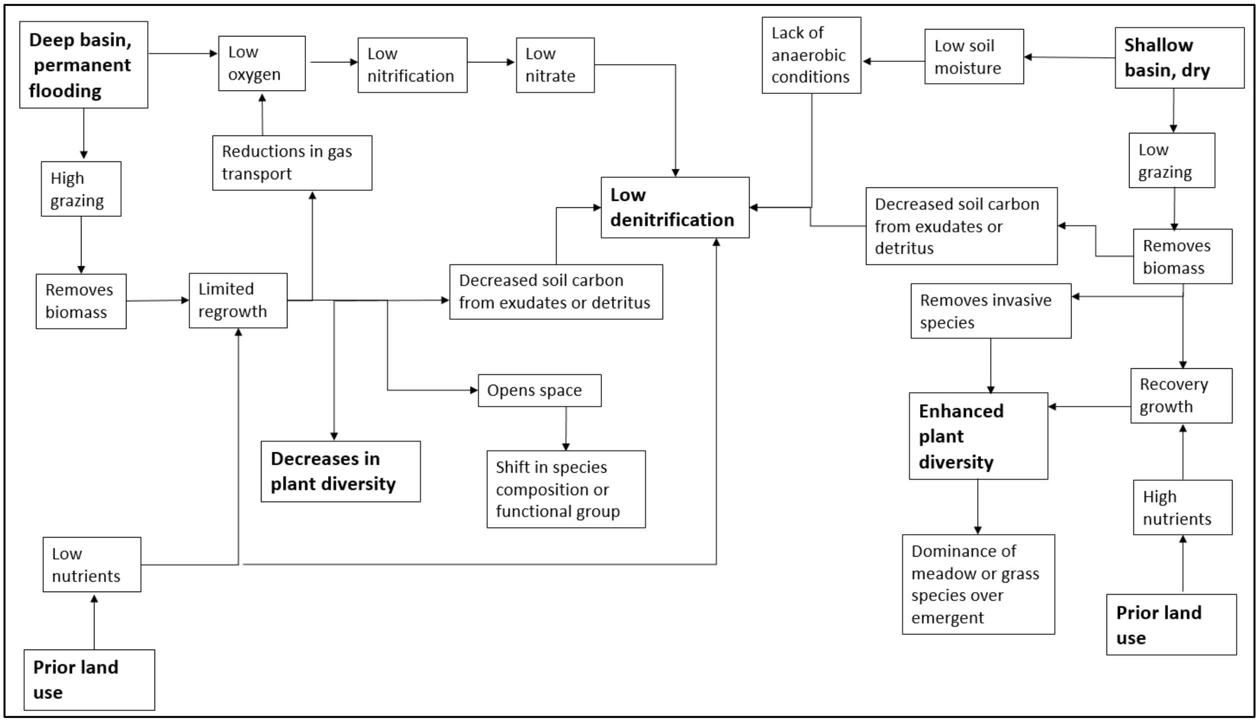

Figure 4.1: Interactions of abiotic and biotic factors resulting in reduced ecosystem services within our wetlands of study.

We observed two extreme hydrologic regimes: deep and flooded, shallow and dry. Both resulted in created wetlands that do not meet the same expectations of plant diversity and potential denitrification as natural wetlands. In A1N, permanent flooding guaranteed maximum access by aquatic herbivores, which led to high grazing events. Plant communities in young wetlands are especially vulnerable to intense grazing, because communities are not fully resilient and are thus susceptible to persistent disturbances. This may help to explain the significant reductions in plant cover and individual species' stem height and density (Chapter 2.3, Figure 2.4 & 2.7). Limited regrowth, possibly due to low nutrients resulting from this wetlands prior land use, also led to reductions in belowground growth and overall plant diversity (Figure 4.1). These results are also intertwined with low rates of potential denitrification rates measured at this site. Sustained low oxygen conditions, from continuous flooding, likely severely limited aerobic nitrification, resulting in limited nitrate available for denitrifying bacteria. This was further exacerbated by the removal of plant biomass due to grazing, limiting the accumulation of carbon-based detritus and exudates that heterotrophic bacteria need for food, but also limiting emergent plant species from introducing oxygen through their root systems (Armstrong 1964; Gao et al. 2008; Winton & Richardson 2017) (Figure 4.1).

Though the hydrology and land use history was very different in A3 than in A1N, the development of an equitable emergent plant community and potential denitrification rates were similarly limited. The rapid drainage of water from A3 quickly limited grazer access to the wetland, resulting in low grazing pressure. On the surface, high nutrients and low-level removal of biomass by grazers, resulting in increased plant diversity, may appear beneficial for this wetland to meet its goals for overall biodiversity (Chapter 2.3, Figure 2.4 & 2.5). However, due to lack of flooding for extended periods of time, community composition reflects the dominance of meadow and grass species over emergent plants

(Figure 4.1). This was further exacerbated by the 2016 drought. Like A1N, these results are linked to measurements of potential denitrification in A3. Little to no soil moisture likely resulted in decreased soil respiration and microbial activity. Also, the absence of water, decreased anaerobic conditions necessary for denitrification. Even at low grazing levels, the removal of biomass appeared to decrease carbon pools, similar to A1N and possibly limit denitrification as well (Figure 4.1). Our study clearly demonstrates the importance of considering multiple abiotic and biotic factors when designing a wetland, but also highlights the need to evaluate more than one ecosystem service in order to determine whether or not a created wetland is actually meeting expectations of a natural wetland replacement.

These results identify key areas that wetland designers and managers should focus on to improve wetland construction and prevent future cost-prohibitive challenges to wetland development. Though it is difficult to make direct links between antecedent land use and present soil conditions or nutrient availability, gathering information about past land use histories of possible wetland sites can help managers make better decisions about choosing what sites to use. If choice of site is not feasible, doing preliminary soil analysis will determine whether soil amendments should be added at the beginning. If a wetland is being constructed on land that is lacking in nutrient capacity, organic and nutrient-rich top-soil should be added; without it, establishing plant communities will be more vulnerable to intense grazing events, and processes like denitrification may be limited. Installing water level control structures is useful to be able to increase or decrease depths based on seasonal nutrient availability or grazer populations, so that oxygen can penetrate soils when needed or specific areas of a wetland can remain inaccessible to grazers for a period of time. In addition, or particularly if water control is not feasible, identifying macrophytes that are efficient gas transporters are crucial for promoting denitrification, and protecting seedlings from herbivore access via enclosures may be necessary for the populations to develop and establish properly.

Finally, site heterogeneity may be the best option for wetland design in order to optimize the site for multiple services. This includes physical/structural heterogeneity (multiple hydrologic units connected to each other, variations in depth/bathymetry throughout the wetland), and heterogeneity in flooding regimes. Physical variations in a created wetland will allow for separate units to be used at different times or for different reasons depending on whether grazers are using the wetland for nesting and breeding, or a migration stop-over site. Variations in depth will also allow variations in plant species to establish and grow into resilient communities. Intermittent flooding, or strategic raising and lowering of water levels using control devices like culverts will help provide a balance between aerobic and anaerobic conditions, and promote multiple microbial processes associated with denitrification.

Wetland creation can be improved by taking into account multiple variables that contribute to the functioning of natural wetland ecosystems. It is necessary to identify the factors that regulate a desired service, and consider other services that may also be impacted by these interactions.

Literature Cited

Aerts, R., Verhoeven, J. T. a, & Whigham, D. F. (1999). Plant-mediated controls on nutrient cycling in temperate fens and bogs. *Ecology*, *80*(7), 2170–2181. doi:10.1890/0012-9658(1999)080[2170:PMCONC]2.0.CO;2

Anderson, M. G., Low, J. B. (1976). Use of Sago pondweed by waterfowl on the Delta Marsh. *Journal of Wildlife Management*, 20, 233-242.

Armstrong, J., W. Armstrong, P. M. Beckett, J. E. Haider, S. Lythe, R. Holt, and A. Sinclair. 1996. Pathways of aeration and the mechanisms and beneficial effects of humidity-and Venturi-induced convections in Phragmites australis (Cav) Trin ex Steud. Aquatic Botany 54:177-197.

Balcombe, C. K., Anderson, J. T., Fortney, R. H., Rentch, J. S., Grafton, W. N., & Kordek, W. S. (2005). A comparison of plant communities in mitigation and reference wetlands in the mid-Appalachians. *Wetlands*, *25*(1), 130-142.

Baldassarre, G. A. Bolen, E. G., and Saunders, A. (2006). Influence of migrant tundra swans (*Cygnus columbianus*) and Canada geese (*Branta canadensis*) on aquatic vegetation at Long Point, Lake Erie, Ontario. *Hydrobiologia*. 567:195-211.

Ballantine, K. a., Groffman, P. M., Lehmann, J., & Schneider, R. L. (2014). Stimulating nitrate removal processes of restored wetlands. *Environmental Science and Technology*, *48*, 7365–7373. doi:10.1021/es500799v

Bardgett, R. D., Streeter, T. C., & Bol, R. (2003). Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology*, *84*(5), 1277-1287.

Bedford, B. L., Walbridge, M. R., & Aldous, A. (1999). Patterns in nutrient availability and plant diversity of temperate North American wetlands. *Ecology*, *80*(7), 2151–2169. doi:10.1890/0012-9658(1999)080[2151:PINAAP]2.0.CO;2

Bendor, T. (2009). A dynamic analysis of the wetland mitigation process and its effects on no net loss policy. *Landscape and Urban Planning*, *89*(1), 17-27.

Brix, H. (1994). Functions of macrophytes in constructed wetlands. *Water Science and Technology*, *29*(4), 71–78.

Brix, H. (1997). Do macrophytes play a role in constructed treatment wetlands? *Water Science and Technology*, *35*(5), 11–17. doi:10.1016/S0273-1223(97)00047-4

Brown, S. C., & Bedford, B. L. (1997). Restoration of wetland vegetation with transplanted wetland soil: an experimental study. *Wetlands*, *17*(3), 424-437.

Bruland, G. L., Richardson, C. J., & Whalen, S. C. (2006). Spatial variability of denitrification potential and related soil properties in created, restored, and paired natural wetlands. *Wetlands*, *26*(4), 1042-1056..

Bulc, T. G. (2006). Long term performance of a constructed wetland for landfill leachate treatment. *Ecological engineering*, *26*(4), 365-374.

Campbell, D. A., Cole, A., & Brooks, R. P. (2002). A comparison of created and natural wetlands in Pennsylvania, USA Deborah. *Ecology and Management*, *10*, 41–49. doi:10.1023/A

Carter, V. (1996). Wetland hydrology, water quality, and associated functions. *National Water Summary on Wetland Resources*, 35-48.

Chaichana, R., Leah, R., & Moss, B. (2011). Seasonal impact of waterfowl on communities of macrophytes in a shallow lake. *Aquatic Botany*, *95*(1), 39–44.

Chambers, R. M., & Odum, W. E. (1990). Porewater oxidation, dissolved phosphate and the iron curtain. *Biogeochemistry*, *10*(Hearn 1984), 37–52.

Charnov, E. L. (1976). Optimal foraging, the marginal value theorem. *Theoretical population biology*, *9*(2), 129-136.

Clausen, P. (2000). Modelling water level influence on habitat choice and food availability for Zostera feeding brent geese Branta bernicla in non-tidal areas. *Wildlife Biology*, *6*(November 2015), 75–87.

Clay, K., Marks, S., Cheplick, G. P., & Sep, N. (1993). Effects of Insect Herbivory and Fungal Endophyte Infection on Competitive Interactions among Grasses, *74*(6), 1767–1777.

Clément, J. C., Pinay, G., & Marmonier, P. (2002). Seasonal dynamics of denitrification along topohydrosequences in three different riparian wetlands. *Journal of Environmental Quality*, *31*(3), 1025-1037.

Colwell, M. A., & Taft, O. W. (2000). Waterbird communities in managed wetlands of varying water depth. *Waterbirds*, 45-55.

Compton, J. E., Boone, R. D., Motzkin, G., & Foster, D. R. (1998). Soil carbon and nitrogen in a pine-oak sand plain in central Massachusetts: role of vegetation and land-use history. *Oecologia*, *116*(4), 536-542.

Connell, J. H. (1978). Diversity in tropical rain forests and coral reefs. *Science*, *199*(4335), 1302-1310.

Conover, M. R., & Kania, G. S. (1994). Impact of interspecific aggression and herbivory by mute swans on native waterfowl and aquatic vegetation in New England. *The Auk*, *111*(3), 744-748.

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., … Turner, R. K. (2014). Changes in the global value of ecosystem services. *Global Environmental Change*, *26*, 152–158.

Coveney, M. ., Stites, D. ., Lowe, E. ., Battoe, L. ., & Conrow, R. (2002). Nutrient removal from eutrophic lake water by wetland filtration. *Ecological Engineering*, *19*(2), 141–159. doi:10.1016/S0925-8574(02)00037-X

DeAngelis, D. L., Mulholland, P. J., Palumbo, A. V, Steinman, A. D., Huston, M. A., & Elwood, J. W. (1989). Nutrient dynamics and food-wed stability. *Annual Review of Ecology and Systematics*, *20*(7), 71–95.

Ding, W., Cai, Z., & Tsuruta, H. (2005). Plant species effects on methane emissions from freshwater marshes. *Atmospheric Environment*, *39*(18), 3199-3207.

Dodla, S. K., Wang, J. J., DeLaune, R. D., & Cook, R. L. (2008). Denitrification potential and its relation to organic carbon quality in three coastal wetland soils. *Science of the Total Environment*, *407*(1), 471-480.

Edwards, K. R., & Proffitt, C. E. (2003). Comparison of wetland structural characteristics between created and natural salt marshes in southwest Louisiana, USA. *Wetlands*, *23*(2), 344-356.

Elser, J. J., Fagan, W. F., Denno, R. F., Dobberfuhl, D. R., Folarin, A., Huberty, A., ... & Sterner, R. W. (2000). Nutritional constraints in terrestrial and freshwater food webs. *Nature*, *408*(6812), 578-580.

Environmental Protection Agency. Section 404(b)(1) Guidelines for Specification of Disposal Sites for Dredged or Fill Material, 230 Title 40 Code of Federal Regulations. 253–306 (2008). Washington, D.C.

Evers, D. A., Sasser, C. E., Gosselink, J. G., Fuller, D. A., & Visser, J. M. (1998). The Impact of Vertebrate Herbivores on Wetland Vegetation in Atchafalaya Bay , Louisiana. *Estuaries*, *21*(1), 1–13.

Fennessy, M. S., Rokosch, A., & Mack, J. J. (2008). Patterns of plant decomposition and nutrient cycling in natural and created wetlands. *Wetlands*, *28*(2), 300–310. doi:10.1672/06-97.1

Foster, D., Swanson, F., Aber, J., Burke, I., Brokaw, N., Tilman, D., & Knapp, A. (2003). The importance of land-use legacies to ecology and conservation. *BioScience*, *53*(1), 77-88.

Fox, A. D., Madsen, J., Boyd, H., Kuijken, E., Norriss, D. W., Tombre, I. M., & Stroud, D. A. (2005). Effects of agricultural change on abundance, fitness components and distribution of two arctic-nesting goose populations. *Global Change Biology*, *11*(6), 881-893.

Fretwell, J. D., Williams, J. S., & Redman, P. J. (1996). *National Water Summary on Wetland Resources*(United States of America, Department of the Interior, US Geologic Survey).

Funk, J. L., Cleland, E. E., Suding, K. N., & Zavaleta, E. S. (2008). Restoration through reassembly: plant traits and invasion resistance. *Trends in ecology & evolution*, *23*(12), 695-703.

Galatowitsch, S. M., Valk, A. G. Van Der, Applications, S. E., Feb, N., Galatowitsch, S. M., & Valk, A. G. V. a N. D. E. R. (2010). The Vegetation of Restored and Natural Prairie Wetlands. *Ecological Applications*, *6*(1), 102–112.

Gao, Y. Z., Giese, M., Lin, S., Sattelmacher, B., Zhao, Y., & Brueck, H. (2008). Belowground net primary productivity and biomass allocation of a grassland in Inner Mongolia is affected by grazing intensity. *Plant and Soil*, *307*, 41–50.

Gauthier, G., Giroux, J. F., Reed, A., Bechet, A., & Belanger, L. (2005). Interactions between land use, habitat use, and population increase in greater snow geese: what are the consequences for natural wetlands?.*Global change biology*, *11*(6), 856-868.

Gelderman, R.H., and A.P. Mallarino. "Recommended Chemical Soil Test Procedures for the North Central Region." *North Central Regional Research Publication* 221 (2012).

Groffman P.M., Holland E., Myrold D.D., Robertson G.P. & Zou X. (1999) Denitrification. In: Robertson G.P., Bledsoe C.S., Coleman D.C. & Sollins P. (Eds) Standard Soil Methods for Long Term Ecological Research (pp 272–290). Oxford University Press, New York.

Goranson, C. E., Ho, C. K., & Pennings, S. C. (2004). Environmental gradients and herbivore feeding preferences in coastal salt marshes. *Oecologia*, *140*(4), 591-600.

Grosholz, E. D., Levin, L. A., Tyler, A. C., & Neira, C. (2009). Changes in Community Structure and Ecosystem Function Following *Spartina alterniflora* Invasion of Pacific Estuaries. In: Silliman, B. R., Grosholtz, E. D. and Bertness, M. D., eds. *Human Impacts on Salt Marshes: A Global Perspective* (pp. 23-40). Berkley, CA: University of California Press.

Güsewell, S., Koerselman, W., & Verhoeven, J. T. a. (2003). Biomass N : P Ratios as Indicators of Nutrient Limitation for Plant Populations in Wetlands BIOMASS. *Ecological Applications*, *13*(2), 372–384.

HACH Company (2014). *User Manuel: ORP/Redox Probe*. Loveland, CO.

Hanson, G. C., Groffman, P. M., & Gold, A. J. (1994). Denitrification in Riparian Wetlands Receiving High and Low Groundwater Nitrate Inputs. *Journal of Environmental Quality*, *23*, 917–922.

Heiri, O., Lotter, A. F., & Lemcke, G. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: reproducibility and comparability of results. *Journal of paleolimnology*, *25*(1), 101-110.

Hernandez, M. E., & Mitsch, W. J. (2007). Denitrification in created riverine wetlands: Influence of hydrology and season. *Ecological Engineering*, *30*, 78–88. doi:10.1016/j.ecoleng.2007.01.015

Hernandez, M. E., & Mitsch, W. J. (2007). Denitrification potential and organic matter as affected by vegetation community, wetland age, and plant introduction in created wetlands. *Journal of Environmental Quality*, *36*(1), 333-342.

Hobbie, S. E. (1992). Effects of plant species on nutrient cycling. *Trends in Ecology & Evolution (Personal Edition)*, *7*(10), 336–339. doi:10.1016/0169-5347(92)90126-V

Holland, J. N., Cheng, W., & Crossley, D. A. (1996). Herbivore-induced changes in plant carbon allocation: assessment of below-ground C fluxes using carbon-14. *Oecologia*, *107*(1), 87-94.

Hunter, R. G., & Faulkner, S. P. (2001). Denitrification potentials in restored and natural bottomland hardwood wetlands. *Soil Science Society of America Journal*, *65*(6), 1865-1872.

Iacobelli, A., & Jefferies, R. L. (1991). Inverse Salinity Gradients in Coastal Marshes and the Death of Stands of Salix : The Effects of Grubbing by Geese. *Journal of Ecology*, *79*(1), 61–73.

Jefferies, R. L., & Rockwell, R. F. (2002). Foraging geese , vegetation loss and soil degradation in an Arctic salt marsh. *Applied Vegetation Science*, *5*(1), 7–16. doi:10.1658/1402-2001(2002)005[0007:FGVLAS]2.0.CO;2

Johnson, L. A. (1987). Management of northern gravel sites for successful reclamation: a review. *Arctic and Alpine Research*, 530-536.

Kaye, J. P., & Hart, S. C. (1997). Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution*, *12*(4), 139-143.

Kayranli, B., Scholz, M., Mustafa, A., & Hedmark, A. (2010). Carbon Storage and Fluxes within Freshwater Wetlands: a Critical Review. *Wetlands*, *30*, 111–124. doi=10.1.1.469.9380&rep=rep1&type=pdf

Keeny, D.R. and Nelson, D.W. (1982). Nitrogen in organic forms. Pages 643-698 in Methods of soil analysis. Part 2 Agronomy No.9, *American Society of Agronomy*, Madison, WI.

Kelker, D., & Chanton, J. (1997). The effect of clipping on methane emissions from Carex. *Biogeochemistry*, *39*(1), 37-44.

Kitchell, J. F., Schindler, D. E., Herwig, B. R., Post, D. M., Olson, M. H., & Oldham, M. (1999). Nutrient cycling at the landscape scale: The role of diel foraging migrations by geese at the Bosque del Apache National Wildlife Refuge, New Mexico. *Limnology and Oceanography*, *44*(Wobeser 1981), 828–836. doi:10.4319/lo.1999.44.3_part_2.0828

Knops, J. M., & Tilman, D. (2000). Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology*, *81*(1), 88-98.

Kusler, J. A., & Kentula, M. E. (1990). *Wetland creation and restoration: the status of the science*. Washington, D.C.: U.S. Environmental Protection Agency, Environmental Research Laboratory.

Laanbroek, H. J. 2010. Methane emission from natural wetlands: interplay between emergent macrophytes and soil microbial processes. A mini-review. Annals of Botany 105:141-153.

Lachat Instruments (2003). Determination of Ammonium, Nitrate, Ortho-phosphate and Total Phosphorus. Loveland, CO.

Lambert, M. G., Devantler, B. P., Nes, P., & Penny, P. E. (1985). Losses of nitrogen, phosphorus, and sediment in runoff from hill country under different fertiliser and grazing management regimes. *New Zealand journal of agricultural research*, *28*(3), 371-379.

Lauridsen, T. L., Jeppesen, E., & Andersen, F. Ø. (1993). Colonization of submerged macrophytes in shallow fish manipulated Lake Væng: impact of sediment composition and waterfowl grazing. *Aquatic Botany*, *46*(1), 1-15.

Le Roux, X., Bardy, M., Loiseau, P., & Louault, F. (2003). Stimulation of soil nitrification and denitrification by grazing in grasslands: do changes in plant species composition matter?. *Oecologia*, *137*(3), 417-425.

Levin, L. A., Neira, C., & Grosholz, E. D. (2006). Invasive cordgrass modifies wetland trophic function. *Ecology*, *87*(2), 419-432.

Linn, D. M., & Doran, J. W. (1984). Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. *Soil Science Society of America Journal*, *48*(6), 1267-1272.

Lor, S., & Malecki, R. A. (2006). Breeding ecology and nesting habitat associations of five marsh bird species in Western New York. *Waterbirds*, *29*(4), 427–436.

Maron, J. L., & Crone, E. (2006). Herbivory: effects on plant abundance, distribution and population growth. *Proceedings of the Royal Society of London B: Biological Sciences*, *273*(1601), 2575-2584.

Maschinski, J., & Whitham, T. G. (1989). The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *The American Naturalist*, *134*(1), 1-19.

Matthews, J. W., Spyreas, G., & Endress, A. G. (2009). Trajectories of vegetation-based indicators used to assess wetland restoration progress. *Ecological Applications*, *19*(8), 2093-2107.

McGechan, M. B. (2003). Modelling phosphorus leaching to watercourses from extended autumn grazing by cattle. *Grass and Forage Science*, *58*(2), 151-159.

McLatchey, G. P., & Reddy, K. R. (1998). Regulation of Organic Matter Decomposition and Nutrient Release in a Wetland Soil. *Journal of Environmental Quality*, *27*(5), 1268–1274.

Mitchell, S. F. (1989). Primary production in a shallow eutrophic lake dominated alternately by phytoplankton and by submerged macrophytes. *Aquatic Botany*, *33*(1-2), 101-110.

Mitsch, W. J., & Gosselink, J. G. (1993). Wetlands Van Nostrand Reinhold. *New York*, 722.

Mitsch, W. J., & Wilson, R. F. (1996). Improving the success of wetland creation and restoration with know-how, time, and self-design. *Ecological applications*, *6*(1), 77-83.

MO Bio Laboratories, Inc. (2014). *PowerSoill® DNA Isolation Kit*. Carlsbad, CA. Retrieved from https://mobio.com/media/wysiwyg/pdfs/protocols/12888.pdf

Moreno-Mateos, D., Power, M. E., Comín, F. a., & Yockteng, R. (2012). Structural and Functional Loss in Restored Wetland Ecosystems. *PLoS Biology*, *10*(1), e1001247. doi:10.1371/journal.pbio.1001247

Mulder, C. P., & Ruess, R. W. (1998). Effects of herbivory on arrowgrass: interactions between geese, neighboring plants, and abiotic factors. *Ecological Monographs*, *68*(2), 275-293.

Murkin, H. R., Murkin, E. J., & Ball, J. P. (1997). Avian habitat selection and prairie wetland dynamics: A 10-year experiment. *Ecological Applications*, *7*(4), 1144–1159. doi:10.1890/1051-0761(1997)007[1144:AHSAPW]2.0.CO;2

Murphy, J. A. M. E. S., & Riley, J. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica chimica acta*, *27*, 31-36.

Newman Grace, J.B., Koebel, J.W., S. (1996). The Effects of Nutrients and Hydroperiod on Mixtures of Typha Domingensis, Cladium Jamaicense, and Eleocharis Interstincta: Implications for Everglades Restoration, *6*(3), 774–783.

Paul, J. W., & Beauchamp, E. G. (1989). Effect of carbon constituents in manure on denitrification in soil. *Canadian Journal of Soil Science*, *69*(1), 49-61.

Perrow, M. R., Schutten, J. H., Howes, J. R., Holzer, T., Madgwick, F. J., & Jowitt, A. J. D. (1997). Interactions between coot (Fulica atra) and submerged macrophytes: the role of birds in the restoration process. *Hydrobiologia*, *342-343*, 241–255. doi:10.1023/A:1017007911190

Proulx, M., & Mazumder, A. (1998). Reversal of Grazing Impact on Plant Species Richness in Nutrient-Poor vs. Nutrient-Rich Ecosystems. *Ecology*, *79*(8), 2581–2592.

Quinn, J. M., & Stroud, M. J. (2002). Water quality and sediment and nutrient export from New Zealand hill-land catchments of contrasting land use. *New Zealand journal of marine and freshwater research*, *36*(2), 409-429.

Reddy, K., D'Angelo, E., & Harris, W. (2000). *Biogeochemistry of Wetlands. In'Handbook of Soil Science'.(Ed M Sumner) pp*. G-89-G-119.(CRC Press: Boca Raton, FL).

Reinartz, J. A., & Warne, E. L. (1993). Development of vegetation in small created wetlands in southeastern Wisconsin. *Wetlands*, *13*(3), 153-164.

Richter, J., & Roelcke, M. (2000). The N-cycle as determined by intensive agriculture–examples from central Europe and China. *Nutrient Cycling in Agroecosystems*, *57*(1), 33-46.

Robertson, M. M. (2000). No net loss: wetland restoration and the incomplete capitalization of nature. *Antipode*, *32*(4), 463-493.

Smith, J., Wagner-Riddle, C., & Dunfield, K. (2010). Season and management related changes in the diversity of nitrifying and denitrifying bacteria over winter and spring. *Applied Soil Ecology*, *44*(2), 138-146.

Solorzano, L. (1969). Determination of ammonia in natural waters by the phenolhypochlorite method 11.This research was fully supported by US Atomic Energy Commission Contract No. ATS (11-1) GEN 10, PA 20. *Limnology and oceanography*, *14*(5), 799-801.

Stantec Consulting (2009). High Acres Landfill – Wetland Mitigation Report Towns of Macedon and Perinton Wayne and Monroe Counties, New York, pg 10-26.

Vaieretti, M. V., Cingolani, A. M., Harguindeguy, N. P., & Cabido, M. (2013). Effects of differential grazing on decomposition rate and nitrogen availability in a productive mountain grassland. *Plant and soil*, *371*(1-2), 675-691.

van Oene, H., Van Deursen, E. M., & Berendse, F. (1999). Plant-herbivore interaction and its consequences for succession in wetland ecosystems: A modeling approach. *Ecosystems*, *2*(2), 122-138.

van Wijnen, H. J., van der Wal, R., & Bakker, J. P. (1999). The impact of herbivores on nitrogen mineralization rate: consequences for salt-marsh succession. *Oecologia*, *118*(2), 225-231.

Vanni, M. J. (2002). Nutrient Cycling By Animals in Freshwater Ecosystems. *Annual Review of Ecology and Systematics*, *33*(1), 341–370.

Verhoeven, J. T. A., Koerselman, W., & Meuleman, A. F. M. (1996). Nitrogen-or phosphorus-limited growth in herbaceous, wet vegetation: relations with atmospheric inputs and management regimes. *Trends in Ecology & Evolution*, *11*(12), 494-497.

Vymazal, J. (2005). Horizontal sub-surface flow and hybrid constructed wetlands systems for wastewater treatment. *Ecological engineering*, *25*(5), 478-490.

Whiting, G. J., & Chanton, J. P. (1993). Primary production control of methane emission from wetlands.

Winton, R. S., & Richardson, C. J. (2016). Top-down control of methane emission and nitrogen cycling by waterfowl. *Ecology*.

Yallop, M. L., O'Connell, M. J., & Bullock, R. (2004). Waterbird herbivory on a newly created wetland complex: potential implications for site management and habitat creation. *Wetlands Ecology and Management*, *12*(5), 395-408.

Zedler, J. B., & Kercher, S. (2005). WETLAND RESOURCES: Status, Trends, Ecosystem Services, and Restorability. *Annual Review of Environment and Resources*, *30*(1), 39–74.

Zhai, X., Piwpuan, N., Arias, C. A., Headley, T., & Brix, H. (2013). Can root exudates from emergent wetland plants fuel denitrification in subsurface flow constructed wetland systems?. *Ecological engineering*, *61*, 555-563.

APPENDICES

Appendix A: Avg ± SE soil characteristics measured in A1N and A3, throughout the study period.

Factor	Fall 2014		Fall 2015		Spring 2016		Fall 2016	
	C	U	C	U	C	U	C	U
A1N								
Organic matter (%)	8.3 ± 0.9	6.9 ± 0.7	9.0 ± 1.0	7.2 ± 0.7	8.8 ± 0.5	7.2 ± 0.4	9.3 ± 1.0	8.6 ± 1.0
Total inorganic nitrogen (mg/kg)	4.0 ± 0.6	7.1 ± 3.5	1.5 ± 0.3	2.0 ± 0.5	11.3 ± 1.1	10.5 ± 1.1	2.4 ± 0.3	5.1 ± 1.9
Total phosphorus (mg/kg)	---	---	---	---	744.6 ± 23.5	683.7 ± 29.7	696.8 ± 22.3	724.9 ± 26.2
A3								
Organic matter (%)	13.0 ± 0.9	12.5 ± 1.0	13.4 ± 1.1	12.5 ± 1.0	15.8 ± 1.2	14.6 ± 1.2	14.1 ± 1.0	13.8 ± 1.0
Total inorganic nitrogen (mg/kg)	15.4 ± 5.1	15.1 ± 3.3	2.6 ± 0.4	7.9 ± 3.7	25.6 ± 3.0	28.2 ± 5.4	12.9 ± 1.7	12.0 ± 1.4
Total phosphorus (mg/kg)	---	---	---	---	990.0 ± 53.2	903.0 ± 48.7	1176.2 ± 61.7	1102.3 ± 57.7

Appendix B: Results of two-way ANOVAs examining the effect of season (spring, early summer, mid-summer, fall) and grazing treatment (caged/uncaged) on stem height (cm), density, and cover (%) for major plant species in A1N. Minor species not analyzed: *Asclepias incarnata*, *Carex* spp., *Epilobium* spp., *Juncus effuses*, *Lythrum salicaria***, *Nymphaea odorata*, *Sparganium americanum*, *Typha* spp.** Significant p-values are bolded (*p <0.0001; **invasive species).

Factor	**Avg ± SE**		**Season**		**Treatment**		**Seas x Tr**		**Block**	
	C	U	$F_{3,125}$	*p*	$F_{1,127}$	*p*	$F_{3,125}$	*p*	$F_{3,125}$	*p*
A. plantago-aquatica										
Height	19.6 ± 3.1	9.6 ± 1.7	15.98	**<0.001***	11.82	**<0.001**	6.32	**<0.001**	0.35	0.789
Density	5.0 ± 1.0	2.6 ± 0.6	3.30	**0.023**	4.58	**0.034**	1.04	0.376	1.85	0.142
Cover	4.6 ± 1.1	1.2 ± 0.2	4.75	**0.004**	10.22	**0.002**	2.57	0.058	2.90	**0.038**
L. oryzoides										
Height	14.2 ± 4.1	7.8 ± 3.2	4.75	**0.004**	1.54	0.216	0.40	0.752	0.06	0.979
Density	4.4 ± 1.6	0.3 ± 0.1	1.15	0.332	6.58	**0.012**	0.87	0.459	1.88	0.136
Cover	3.5 ± 1.6	0.2 ± 0.1	1.18	0.322	4.53	**0.036**	0.82	0.485	1.75	0.161
Polygonum spp.										
Height	62.3 ± 5.4	18.1 ± 3.0	84.25	**<0.001***	185.62	**<0.001***	24.39	**<0.001***	5.34	**0.002**
Density	21.0 ± 2.2	3.5 ± 0.8	6.44	**<0.001**	68.53	**<0.001***	2.10	0.104	4.11	**0.008**
Cover	21.9 ± 2.6	2.6 ± 0.6	18.84	**<0.001***	89.19	**<0.001***	13.60	**<0.001***	2.73	**0.047**
P. cordata										
Height	7.8 ± 3.2	8.3 ± 2.8	1.85	0.142	0.01	0.915	0.18	0.908	1.59	0.194
Density	1.0 ± 0.4	1.7 ± 0.6	0.93	0.429	0.98	0.324	0.07	0.979	1.33	0.269
Cover	1.1 ± 0.5	2.9 ± 1.5	0.88	0.456	1.28	0.260	0.38	0.770	1.68	0.175
P. amplifolius										
Height	0.5 ± 0.3	1.9 ± 0.7	4.37	**0.006**	4.28	**0.041**	2.22	0.089	9.57	**<0.001***
Density	1.3 ± 0.8	2.0 ± 0.8	2.90	**0.038**	0.47	0.493	0.34	0.797	10.37	**<0.001***
Cover	1.7 ± 1.0	1.4 ± 0.5	1.33	0.269	0.08	0.780	0.02	0.996	8.16	**<0.001***
S. latifolia										
Height	73.1 ± 6.1	60.1 ± 5.1	256.13	**<0.001***	18.84	**<0.001***	2.44	0.068	2.16	0.097
Density	17.0 ± 1.8	12.8 ± 1.2	56.62	**<0.001***	9.20	**0.003**	1.36	0.258	7.49	**<0.001**
Cover	44.1 ± 4.6	29.9 ± 3.6	74.68	**<0.001***	16.27	**<0.001***	2.95	**0.036**	1.58	0.197
S. tabernaemontani										
Height	8.4 ±3.8	2.9 ± 2.1	1.01	0.389	1.62	0.206	0.12	0.948	3.40	**0.020**
Density	1.1 ± 0.6	0.2 ± 0.1	0.27	0.847	2.15	0.145	0.29	0.835	3.04	**0.032**
Cover	0.8 ± 0.5	0.1 ± 0.0	0.81	0.809	2.72	0.102	0.45	0.720	3.05	**0.031**

Appendix C: Results of two-way ANOVAs examining the effect of season (spring, early summer, mid-summer, fall) and grazing treatment (caged/uncaged) on stem height (cm), density, and cover (%) for major plant species in A3. Minor species not analyzed: *Acer saccharum*, *Andropogon gerardii*, *Artemisia vulgaris***, *Asclepias incarnate*, *Aster* spp., *Cornus sericea*, *Daucus carota*, *Echinochloa crus-galli*, *Epilobium* spp., *Juncus effuses, Juncus inflexus, Lactuca serriola, Lythrum salicaria***, *Mimulus ringen, Ranunculus scelergtus, Rosa multiflora***, *Rumex crispus, Solidago arguta, Sparganium americanum*, *Thinopyrum intermedium*, *Verbena hastate*. Significant p-values are bolded (*p <0.0001; **invasive species).

Factor	**Avg ± SE**		**Season**		**Treatment**		**Seas x Tr**		**Block**	
	C	U	$F_{3,125}$	*p*	$F_{1,127}$	*p*	$F_{3,125}$	*p*	$F_{3,125}$	*p*
A. plantago-aquatica										
Height	14.0 ± 2.2	12.4 ± 1.8	23.21	**<0.001***	0.61	0.437	0.62	0.601	20.35	**<0.001***
Density	4.7 ± 1.1	6.9 ± 1.5	13.88	**<0.001***	2.09	0.151	0.73	0.534	7.58	**<0.001**
Cover	4.7 ± 1.2	3.7 ± 0.9	8.91	**<0.001***	0.58	0.447	1.04	0.378	5.50	**0.001**
Carex spp.										
Height	7.8 ± 2.6	11.5 ± 3.7	3.28	**0.023**	0.74	0.391	0.22	0.886	3.15	**0.028**
Density	0.8 ± 0.3	1.7 ± 0.8	0.81	0.490	0.95	0.331	0.49	0.687	1.33	0.266
Cover	0.6 ± 0.2	0.6 ± 0.2	2.80	**0.043**	0.00	1.00	0.16	0.923	3.59	**0.016**
E. obtusa										
Height	7.6 ± 2.1	12.9 ± 2.3	3.38	**0.021**	3.97	**0.048**	0.04	0.989	16.29	**<0.001***
Density	2.5 ± 0.9	17.9 ± 5.3	3.00	**0.033**	9.78	**0.002**	1.92	0.130	5.94	**<0.001**
Cover	0.4 ± 0.1	4.0 ± 1.4	2.07	0.109	7.52	**0.007**	1.27	0.288	5.86	**<0.001**
E. hieracifolius										
Height	3.3 ± 1.6	8.4 ± 2.4	28.97	**<0.001***	5.99	**0.016**	7.23	**<0.001**	4.39	**0.006**
Density	0.5 ± 0.4	1.6 ± 0.7	8.67	**<0.001***	2.28	0.134	2.37	0.074	3.56	**0.016**
Cover	0.5 ± 0.3	1.6 ± 0.6	13.86	**<0.001***	4.06	0.046	4.38	**0.006**	4.25	**0.007**
L. oryzoides										
Height	3.0 ± 1.5	9.1 ± 2.4	4.25	**0.007**	5.00	**0.027**	0.78	0.507	2.18	0.094
Density	0.3 ± 0.2	4.1 ± 1.6	0.87	0.457	5.63	**0.019**	0.55	0.652	2.61	0.055
Cover	0.1 ± 0.0	3.3 ± 1.7	0.84	0.472	3.81	0.053	0.73	0.535	2.31	0.080
P. arundinacea**										
Height	27.4 ± 6.1	15.8 ± 3.6	3.86	**0.011**	5.60	**0.020**	0.61	0.607	45.34	**<0.001***
Density	8.5 ± 2.3	3.7 ± 0.9	0.79	0.504	6.22	**0.014**	0.38	0.765	28.43	**<0.001***
Cover	10.6 ± 3.3	2.5 ± 0.7	0.18	0.908	7.65	**0.007**	0.09	0.964	15.85	**<0.001***
Polygonum spp.										
Height	27.0 ± 3.7	24.0 ± 3.3	57.09	**<0.001***	0.94	0.333	0.07	0.977	12.20	**<0.001***
Density	27.4 ± 4.5	28.1 ± 4.7	15.95	**<0.001***	0.02	0.890	0.33	0.806	21.42	**<0.001***
Cover	27.2 ± 4.3	25.2 ± 3.9	14.61	**<0.001***	0.24	0.626	2.58	0.057	33.26	**<0.001***
S. latifolia										
Height	3.5 ± 1.4	3.0 ± 1.1	5.90	**<0.001**	0.11	0.746	0.04	0.988	12.05	**<0.001***
Density	2.4 ± 1.3	1.3 ± 0.6	2.86	**0.040**	0.75	0.387	0.26	0.858	7.24	**<0.001**
Cover	2.1 ± 1.4	0.9 ± 0.4	1.85	0.142	0.77	0.383	0.29	0.834	3.98	**0.010**
S. tabernaemontani										
Height	5.1 ± 2.6	18.4 ± 4.3	0.45	0.718	8.43	**0.004**	0.88	0.455	10.89	**<0.001***
Density	0.4 ± 0.2	3.9 ± 1.1	0.79	0.501	12.51	**<0.001**	1.05	0.373	11.21	**<0.001***
Cover	0.2 ± 0.1	1.9 ± 0.7	0.86	0.465	7.54	**0.007**	0.74	0.528	7.17	**<0.001**
S. dulcamara**										
Height	0.8 ± 0.7	3.6 ± 1.6	0.94	0.424	2.61	0.109	0.19	0.903	5.09	**0.002**
Density	0.1 ± 0.1	0.3 ± 0.2	9.43	**<0.001***	2.48	0.118	2.57	0.057	4.47	**0.005**
Cover	0.1 ± 0.1	0.5 ± 0.2	0.43	0.734	3.19	0.077	0.05	0.987	2.87	**0.040**
Typha spp.**										
Height	39.6 ± 7.6	32.5 ± 6.4	5.98	**<0.001**	0.89	0.348	0.02	0.996	26.18	**<0.001***
Density	1.3 ± 0.3	1.2 ± 0.3	3.27	**0.024**	0.13	0.717	0.01	0.999	28.59	**<0.001***
Cover	2.0 ± 0.4	2.6 ± 0.7	2.47	0.065	0.85	0.357	0.39	0.757	18.73	**<0.001***

Appendix D: Results of Chapter 2 (top) and 3 (bottom) ANOVAs examining random block effects. Significant p-values are bolded (*p <0.0001)

Factor	**Block effect**	
	F	*p*
A1N		
Organic matter	$F_{3,125}=2.8$	**0.041**
Total inorganic nitrogen	$F_{3,109}=1.9$	0.14
Total phosphorus	$F_{3,61}=1.3$	0.28
Total plant cover	$F_{3,349}=3.4$	**0.017**
Species richness	$F_{3,349}=4.7$	**0.003**
Shannon-Weiner	$F_{3,349}=4.3$	**0.006**
Belowground biomass	$F_{3,29}=1.5$	0.24
A3		
Organic matter	$F_{3,125}=20.1$	**<0.001***
Total inorganic nitrogen	$F_{3,109}=6.0$	**0.001**
Total phosphorus	$F_{3,61}=35.1$	**<0.001***
Total plant cover	$F_{3,349}=5.3$	**0.001**
Species richness	$F_{3,349}=53.4$	**<0.001***
Shannon-Weiner	$F_{3,349}=49.8$	**<0.001***
Invasive cover	$F_{3,349}=14.4$	**<0.001***
Belowground biomass	$F_{3,29}=1.5$	0.32

Factor	**Block effect**	
	F	*p*
Soil		
Organic matter	$F_{3,125}=4.3$	**0.006**
Nitrate	$F_{3,125}=3.3$	**0.022**
Ammonium	$F_{3,125}=3.5$	**0.017**
Total phosphorus	$F_{3,125}=19.2$	**<0.001***
pH	$F_{3,125}=20.1$	**<0.001***
Aerobic respiration	$F_{1,31}=1.2$	0.71
Anaerobic respiration	$F_{1,31}=1.5$	0.79
Pot. denitrification	$F_{1,159}=19.8$	**<0.001***
Porewater		
Nitrate	$F_{1,31}=0.6$	0.47
Ammonium	$F_{1,31}<0.1$	0.90
Phosphate	$F_{1,31}=6.6$	**0.016**
Redox	$F_{1,31}=8.9$	**0.006**
Temperature	$F_{1,31}=2.9$	0.11