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The impact of herbivory on methane cycling in a created wetland

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ABSTRACT

Wetlands have been identified as one of the largest sources of atmospheric methane (CH_4), an important greenhouse gas with 28 times the global warming potential of CO_2. Due to the complexity of wetland ecosystems, they have also been identified as one of the largest sources of uncertainties in the global CH_4 budget. Net CH_4 emissions are controlled by microbial production, consumption by methanotrophs, and transport to the atmosphere through diffusion, ebullition, and plant-mediated transport. Herbivory has the potential to alter these processes, with recent studies showing both positive and negative effects of herbivory on emissions and uncertainty in which components of the CH_4 cycle are most impacted by grazing. To examine the effects of herbivory on CH_4 emissions in wetlands, we completed an *in situ* study and a simulated greenhouse herbivory study. The *in situ* study, completed at High Acres Nature Area (Perinton, NY), included pairs of plots protected from- and open to grazing, where we quantified CH_4 flux, primary production, vegetation cover, porewater CH_4 concentrations, and potential rates of CH_4 production and oxidation. The simulated herbivory experiment included clipping the stems of *Typha latifolia* and *Sagittaria latifolia* at multiple levels of damage above- and below the water level to examine the impact of plant damage and biomass removal on emissions. The results of our study showed significant effects of herbivory, *in situ* emissions were 1-4 times higher in ungrazed plots compared to grazed plots. Changes in vegetation cover, emergent cover was 1.7-2.9 times higher in caged plots than uncaged plots, likely played an important role in the observed differences in CH_4 emissions. Higher vegetation cover facilitates CH_4 movement through plants, increasing net emissions. In the greenhouse, we observed increased emissions when plants were clipped above the water level and decreased emissions when plants were clipped below the water level, consistent with the key role plant transport plays in CH_4 emissions. We also observed a significant effect of species on CH_4 emissions, emissions from *Sagittaria latifolia* were 2-6 times that of *Typha latifolia*. We conclude that herbivory has a significant effect on CH_4 emissions, where plant damage caused by grazing can yield an immediate increase in emissions, as observed in our clipping experiment, however, long-term herbivory reduces plant cover, resulting in lower substrate for CH_4 production and fewer opportunities for transport through plants, leading to a net decrease in emissions, as captured in our *in situ* measurements.

OVERVIEW

Wetlands provide many important ecosystem services that in turn make them one of the most ecologically and economically important ecosystems in the world (Costanza et al. 2014). However, due to the substantial loss of natural wetlands, created wetlands are increasingly common (EPA 2008). Unfortunately, it has been found that created wetlands often fail to replicate the functions of their natural counterparts, where they have been shown to have lower organic matter, lower species richness, lower total vegetation cover, and higher invasive species cover (Campbell et al. 2002; Moreno-Mateos et al. 2012; Zelder & Callaway 1999). To compensate for the observed differences in created wetlands, management techniques are often used to help improve ecosystem services and restore lost function. One management technique, herbivore exclusion, is implemented in created wetlands to help restore vegetation, where it has been found that herbivore exclusion can increase vegetation cover, improve species richness and improve vegetation diversity (Lodge 2017; Spangler 2019). Impacts on vegetation, therefore, lead to increased productivity, soil organic matter, and soil carbon, improving soil health and the wetland's ability to sequester carbon (Spangler 2019). For these reasons, herbivore exclusion is a beneficial management technique in created wetlands.

The role of herbivores is wetlands is important as it has been found that the population of one important and common wetland grazer, the Canada Goose (*Branta candensis*) has been exponentially rising since the 1960s (N. American Breeding Bird Survey). In addition, the conditions of created wetlands, such as the permanent standing water and young vegetation, often create the perfect habitat for waterfowl, attracting large populations, which in turn leads to a larger impact than that observed in a natural wetland counterpart. For these reasons, any impact of waterfowl in a created wetland may, therefore, be exacerbated and waterfowl may have significant control over many ecosystem services in created wetlands.

Unfortunately, it has been shown that one additional impact of herbivores is the ability to significantly impact methane (CH_4) emissions in wetlands. CH_4 is an important greenhouse gas (GHG) that has been shown to have 28 times the global warming potential (GWP) as CO_2. For this reason, CH_4 is an incredibly potent GHG and has a significant positive effect on climate change. Although, in the literature, it is unclear if herbivory causes an increase or decrease in CH_4 emissions (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richardson 2017; Spangler 2019), and the component of the CH_4 cycle that they impact most is disputed.

This study took place in a created wetland and aimed to examine herbivores net impact on wetland CH_4 emissions. It quantifies net emissions in the presence and absence of grazers, as well as examining the impact of herbivores on the vegetation community and important processes such as soil CH_4 production and oxidation. Furthermore, we examine the impact of grazing on plant-mediated transport by simulating grazing in a greenhouse experiment. Overall, this study attempts to examine all important aspects of the CH_4 cycle in wetlands that could be impacted by grazing.

Chapter one explores the impact of herbivores in a created wetland using herbivore exclusion as a research technique to quantify CH_4 emissions in plots open to- and protected from grazing. This study utilized herbivore exclusion plots established in 2014, allowing our study to examine the long term effects of grazing on the CH_4 cycle in this created wetland. This allowed us to examine the long term impact of grazing on the vegetation community, as well as soil microbial processes including CH_4 production and oxidation. This study considerably expands on our understanding of the impact of herbivores on CH_4 emissions by attempting to examine all three of the main processes that influence overall CH_4 emissions, including production, consumption, and transport processes. Finally, our results aim to inform land managers about the benefits and potential substantial tradeoffs of implementing herbivore exclusion plots in created wetlands.

Chapter two explores one process of the CH_4 cycle in wetlands; plant-mediated CH_4 transport and the immediate impact of grazers on plant related CH_4 emissions. As herbivory has been found to significantly alter vegetation, it has been suggested that this will lead to substantial impacts on plant-mediated transport. To examine this process, we completed a simulated herbivory mesocosm in the greenhouse, in which we clipped plant stems to quantify the immediate impact of grazing that we were unable to quantify in the *in situ* study (Chapter 1). This experiment examined two wetland species broadleaf cattail (*T. latifolia*), an abundant and commonly studied wetland species, and broadleaf arrowhead (*S. latifolia*), a common wetland species that to our knowledge has yet to be studied. The results of this experiment will expand our understanding of the initial impacts of grazing on vegetation that may not be captured in many herbivore exclusion studies. In addition, it will provide information regarding the emissions of the two species to broaden the research examining if different species of vegetation can transport different amounts of CH_4.

Finally, chapter three joins the findings from the *in situ* experiment (chapter 1) and the simulated herbivory mesocosm (chapter 2) to provide overall conclusions about the impact of herbivory on wetland CH_4 emissions. We provide overall suggestions for land managers and how to best utilize herbivore exclusion and herbivory in created wetlands.

Chapter 1: The impact of herbivores on methane cycling in created wetlands

1.1 Introduction

Freshwater wetlands provide valuable ecosystem services such as carbon sequestration (Kayranli et al. 2009; Villa & Bernal 2018), flood control (Mitsch & Gisselink 1993), and water purification (Coveney et al. 2002), making them one of the most ecologically and economically valuable ecosystems in the world, valued at over \$140,000 ha^{-1} $year^{-1}$ (Costanza et al. 2014). Wetlands are defined by their distinctive hydrology, hydric soils, and vegetation communities (USEPA 2002), and for this reason, have been thought to be primarily controlled by bottom-up factors. Although it has been shown that herbivory has a significant top-down effect on wetlands, altering vegetation community composition and reducing vegetation growth and survivorship (Hirota et al. 2005; Lauidsen et al. 1993; Silliman & Zieman 2001; Sondergaard et al. 1996). These effects are exacerbated in created wetlands, which often have controlled, permanent standing water and young vegetation, which inadvertently creates the perfect habitat for waterfowl (Isola et al. 2000), particularly Canada geese (*Branta candensis*) and ducks (*Anas* spp.) (Murkin et al. 1997). This is of particular concern as the Canada Goose population has been exponentially rising since the 1960s throughout the US (N. American Breeding Bird Survey).

Herbivores have been shown to alter plant communities in wetlands, significantly decreasing aboveground biomass (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richardson 2017) and below-ground biomass (Bodelier et al. 2006; Winton & Richardson 2017). Reductions in aboveground biomass when grazers are present have been observed to approach 90% (Hiorta et al. 2005). Herbivores also preferentially graze native vegetation, creating room for invasive species to colonize and inhibiting the establishment of native species (Clay et al. 1993). Furthermore, consumption of vegetation by herbivores can lead to lower organic matter inputs and increased nutrient losses, altering microbial processes and impacting greenhouse gas emissions from wetlands (Lodge 2017; Wijen et al. 1999; van den Wyngaert et al. 2002). In particular, studies have shown that herbivores have a significant impact on CH_4 emissions, with multiple studies showing higher CH_4 emissions in grazed plots, compared to ungrazed plots (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richardson 2017). Dingemans et al. (2011) observed CH_4 emissions that were 5 times higher in plots grazed by Greylag Geese (*Anser anser*) compared to ungrazed plots in a *Phragmites australis* dominated lake. Hirota et al. (2005)

observed similar trends, where CH_4 emissions were 4 times higher in plots grazed by livestock compared to ungrazed plots in an emergent wetland and Winton & Richards (2017) observed a 230% increase in CH_4 emissions in marsh plots grazed by Tundra Swan (*Cygnus columbianus*) The opposite trend has also been observed, where CH_4 emissions significantly decreased when grazers were present in the system (Bodelier et al. 2006: Spangler 2019). Bodelier et al. (2006) concluded that biomass removal and foraging activity by Bewick's swans (*Cygnus columbianus bewickii*) resulted in a net decrease in CH_4 emissions in a freshwater lake and Spangler (2019) observed lower CH_4 emissions in plots grazed by primary Canada geese (*Branta candensis*) compared to ungrazed plots in a freshwater wetland.

Methane emissions from wetlands are of concern since wetlands are one of the largest sources of atmospheric CH_4 (Whalen 2005) and have been identified as one of the largest sources of uncertainties in the global CH_4 budget (Kirschke et al. 2013). Methane emissions in wetlands are a product of production, oxidation, and transport processes (Bodelier et al. 2006, Dingemans et al. 2011; Whalen 2005), all of which have the potential to be impacted by herbivory, making it unclear which is the key driver of observed changes in emissions with and without grazers (Bodelier et al. 2006; Dingemans et al. 2011; Hirota et al. 2005; Spangler 2019; Winton & Richards 2017).

Methane production, or methanogenesis, is completed by methanogenic *Archaea* under anerobic conditions, during the final steps of decay (Hanson & Hanson 1996; Segers 1998; Whalen 2005). Acetotrophic methanogens utilize acetate (CH_3COOH) and break it down into CO_2 and CH_4 and hydrogenotrophic methanogens utilize H_2 to reduce CO_2 into CH_4 and water (Hanson & Hanson 1996). Herbivory removes biomass therefore decreasing substrate for this process to occur, as well as decreasing CO_2 uptake via primary production, resulting in lower C provisions overall (Bodelier et al. 2006; Falk et al. 2013; Hirota et al. 2005; Kelsey et al. 2016). Also, it has been found that there is a positive correlation between CH_4 production and vegetation density (Bodelier et al. 2006) and it has been shown that herbivory has a significant negative impact on CH_4 production rates (Bodelier et al. 2006; Falk et al. 2014). Other studies found no differences in CH_4 production between grazed and ungrazed plots (Dingemans et al. 2011; Winton & Richardson 2017), but some still hypothesized significant herbivory effects that were not captured in *in-vitro* assays that may not reflect *in situ* activity (Dingemans et al. 2011). Other studies did not isolate CH_4 production, but hypothesized significant herbivory effects due

observed effects of herbivory on biomass, decrease C provisions for the process to occur (Hirota et al. 2005; Spangler 2019).

After undergoing methanogenesis, the product, CH_4, still holds potential energy that can be captured through CH_4 oxidation (Megonigal et al. 2004). Methanotrophs consume CH_4 in aerobic environments and release CO_2 (Segarra 2015). This process typically occurs at the air-water interface where there is sufficient O_2, or at the plant roots which leak O_2 as they photosynthesize (Whiting & Chanton 1993). Areas with higher vegetation densities have been shown to have higher rates of CH_4 oxidation and it's been suggested that CH_4 oxidation can consume nearly half of total wetland CH_4 emissions (Shultz et al. 2011; Segarra et al. 2015). Herbivory can significantly impact the concentration of O_2 in the soil and water column through reduced plant O_2 transport to the soil, which suggests impacts on CH_4 oxidation rates (Bodelier et al. 2006; Dingemans et al. 2011; Winton & Richardson 2017). Bioturbation of sediment caused by Bewick swans (*Cygnus columbianus beickii*) leads to significant increases in the rate of CH_4 oxidation in a freshwater lake, which was hypothesized to be one of the main drivers leading to significant negative effects of herbivory on CH_4 emissions (Bodelier et al. 2006). Other studies did not isolate CH_4 oxidation rates, but hypothesized significant herbivory effects due to significant effects of herbivory on vegetation cover, decreasing sediment O_2 concentrations (Dingemans et al. 2011) or significantly higher CH_4 porewater concentration in grazed plots versus ungrazed plots (Winton & Richardson 2017).

Methane produced in wetlands is transported from the wetland through diffusion, ebullition, and plant-mediated transport (Segers 1998; Shultz et al. 1991; Whalen 2005). Diffusion is the slow movement of CH_4 that builds up within the sediment, through the water column, and to the atmosphere. This pathway provides multiple opportunities for CH_4 to become oxidized, due to slow movement through aerobic environments within the water column and surface sediment (Whalen 2005; Hanson & Hanson 1996; Happel & Chanton 1995). Ebullition is the transport of CH_4 in bubbles that builds up within the soil and then moves rapidly through the water column and to the atmosphere, resulting in a low possibility of CH_4 oxidation (Chanton & Martens 1988; Whalen 2005). Ebullitive fluxes have high spatial and temporal distribution and therefore are difficult to quantify, but are often the dominant transport pathway when vegetation cover is low (Grünfeld &Brix 1999; van der Nat & Middleburg 1998). When vegetation cover is high, CH_4 emissions are typically dominated by plant-mediated transport (Grünfeld & Brix 1999;

Whalen 2005). It has been shown that vegetated areas emit 10 times the amount of CH_4 as adjacent non-vegetated areas, due to plant-mediated transport (Whiting et al. 1991; Whiting & Chanton 1992).

Plant-mediated transport is the transport of gases from the soil, through the plant tissues, to the atmosphere, through internal spaces within the plant culm referred to as lacunae or aerenchyma in emergent aquatic macrophytes (Dacey 1981; Whalen 2005). Many plants transport gases through passive diffusion, although it has been shown that some plant species can more efficiently transport gases through the active process of convective flow (Armstrong 1979; Brix et al. 1996). Convective flow occurs when gases travel along pressure gradients caused by temperature or humidity differences between the plant internals and the outside air (Armstrong & Armstrong 1991; Askaer et al. 2010; Brix et al. 1992; Dacey et al. 1981; Whalen 2005). Plant-mediated transport is a significant pathway for CH_4 transport in emergent wetlands (Sebacher et al. 1985), often accounting for more than half of total CH_4 flux emissions (Chanton et al. 1989) or as high as 97% of all CH_4 emissions (Kelker & Chanton 1997). Plant species that transport CH_4 through diffusion show minimal diurnal variation in fluxes (Whiting & Chanton 1996), where convective CH_4 transport shows a distinctive daily flux pattern that tracks light intensity, causing variation in flux rate thought the day (Chanton et al. 1993; Whiting & Chanton 1996). Convective plant-mediated transport has been shown to occur in some wetland species including *Phragmites australis* (Armstrong & Armstrong 1991; Grünfeld & Brix 1999; Kaki et al. 2001*), Typha spp*. (Bendix et al. 1994; Kaki et al. 2001; Tornbjerg et al. 1994; Whiting & Chanton 1996), and *Nymphaea odorata* (Dacey 1981).

Studies have suggested that herbivory could have a large impact on CH_4 emissions by altering rates of CH_4 transport through plants (Ding et al. 2005; Dingemans et al. 2011; Falk et al. 2013; Hirota et al. 2005; Kelker & Chanton 1997; Kelsey et al. 2016; Petruzzella et al. 2015; Rietl et al. 2017). Research suggests that when herbivores graze, they damage the topmost part of the stem, or remove leaves, reducing the distance CH_4 has to travel from the sediment to reach the atmosphere (Dingemans et al. 2011; Hirota et al. 2005; Kelsey et al. 2016), in turn reducing resistance, allowing the CH_4 to move more efficiently through the plant (Dingemans et al. 2011; Hirota et al. 2005; Kelker & Chanton 1997; Kelsey et al. 2016). Or similarly, removal of the top of the stem leads to a more efficient pathway for CH_4 to exit the stem, versus exiting through the base of the stem where CH_4 is typically released in un-damaged plants (Whiting & Chanton

1996; van der Nat et al. 1998). This idea has been supported by simulated herbivory studies such as Kelker & Chanton (1997), where sealing clipped *Carex* spp. stems with petroleum jelly caused the CH_4 flux to decrease to rates similar to un-clipped *Carex*. Other studies suggest that when plants are damaged, primary production is reduced, which is positively correlated with CH_4 emissions in some species, specifically species shown to complete convective plant-mediated transport, leading to decreased emissions when grazers are present (Falk et al. 2014). Furthermore, when herbivores remove vegetation entirely, CH_4 emissions can decrease by nearly 85% when compared to control vegetated plots (Falk et al. 2013). Similar results are observed when vegetation is grazed below the water level, inhibiting CH_4 transport via plant-mediated transport, such as clipping *Carex* spp. below the water level or removing it entirely, which led to significant decreases in CH_4 emissions (Kelker & Chanton 1997; Noyce et al. 2014).

Previous research has shown that herbivory can significantly impact CH_4 emissions, with most studies observing significant increases in emissions in the presence of herbivores. What is still unknown is what process is most affected by herbivores that is leading to this result; CH_4 production, oxidation, or transport processes. Previous research finds conflicting results, in which studies identify varying mechanisms as the main driver of the change in net emissions. In this study, we examined all three mechanisms to determine how herbivory impacts overall CH_4 emissions from a freshwater wetland. We complete an *in situ* study using caged (herbivores prevented from grazing) and uncaged (herbivores able to graze) and quantify CH_4 flux emissions, as well as herbivores impact on vegetation. In addition, we analyze porewater CH_4, primary production, and herbivore presence. Finally, using soil incubations we assess CH_4 production and oxidation. We hypothesized that herbivore exclusion would lead to a net increase in CH_4 emissions. This would occur through increased plant-mediated transport due to increased stem densities by grazers, and increased CH_4 production due to increased root biomass supplying more C substrate, which will outweigh increased rates of CH_4 oxidation associated with higher belowground O_2 transport by roots.

1.2 Methods

1.2.1 Site description

This study took place at High Acres Natura Area (HANA), which is owned and managed by Waste Management of NY, LLC, in Perinton, NY, USA. HANA includes natural and created wetlands that are part of several long-term research projects. This project took place in a created wetland, Area 1 North (A1N), which was a gravel mine repository until approximately the mid-1960s when it was abandoned (Figure 1.1). In 2009, it was converted into a wetland as part of a mitigation project. After its creation, the wetland was dominated by invasive cattail species (*Typha latifolia* and *Typha angustifolia*), which were removed by manual cutting and pulling, as well as the application of herbicide (glyphosate). After research began at the site in 2014, no further invasive plant removal was conducted within the treatment plots. Currently, arrowhead (*Sagittaria* spp.), water plantain (*Alisma plantago-aquatica*) and water lily (*Nymphaea odorata*) are the dominant vegetation species. Water depth in A1N is controlled by an adjustable culvert and the wetland is fed via groundwater flow from an adjacent quarry pond as well as precipitation and has standing water year-round.

Previous research at this site found that A1N has low soil organic matter (OM, 7.5$\pm$0.4%) and that herbivory significantly decreased OM (Lodge 2017). A1N soil OM content is significantly lower than other sites at HANA (Lodge 2017), and lower than that seen in similar herbivore exclusion study (Dingemans et al. 2011: 8-13% OM). Similarly, Spangler (2019) showed that the soil carbon content ranged from 5-7% and significantly decreased due to herbivory. Additionally, A1N has been characterized as a low nutrient site by Lodge (2017) who found total inorganic nitrogen (TIN) content was 6.2+1.8 mg/kg and total phosphorus (TP) was 704.3+28.0 mg/kg.

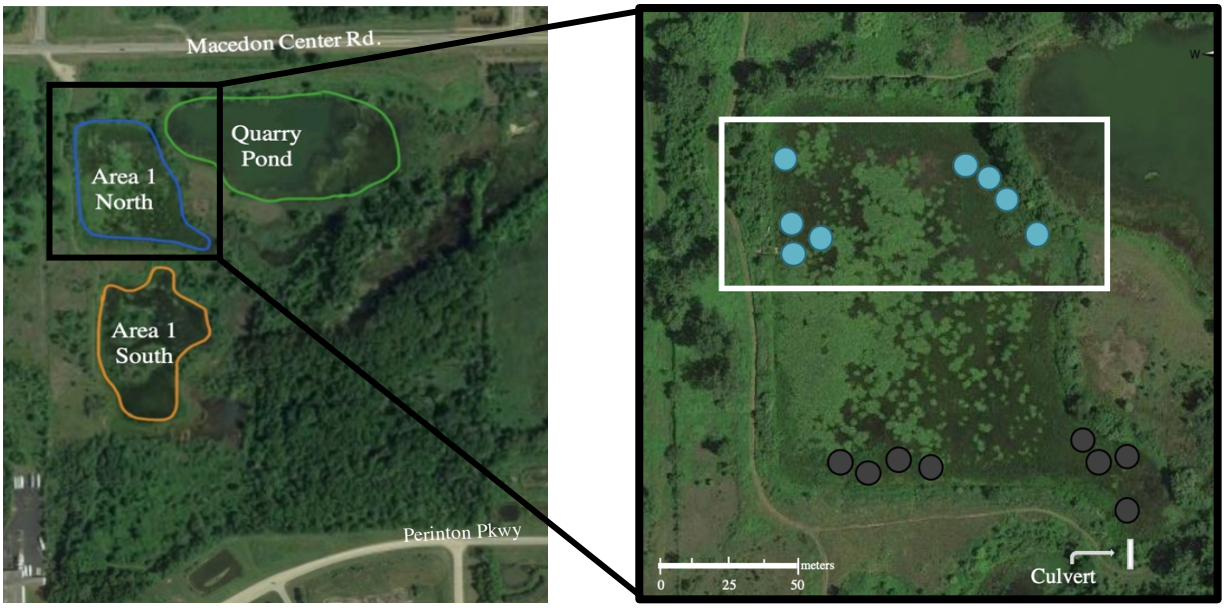


Figure 1.1: Map of High Acres Nature Area (HANA, left), and Area 1 North (A1N) shown in blue and enlarged (right), each circle represents one pair of caged and uncaged plots and the box indicates the east and west blocks used in the current study.

1.2.2 Experimental Design: Herbivore exclusion

In June of 2014, 16 pairs of 1x1 m caged (large herbivores excluded) and uncaged (opened to grazing) plots were established (Lodge 2017). The plots were arranged into 4 blocks, where each block contained 4 pairs of caged and uncaged plots (Figure 1.1, left). The paired cages were placed within 1 m of each other and the pairs were at least 3 m apart. Cages were created by marking the plot with 4 PVC poles and wrapping the caged plots in galvanized hardwire mesh (1.27 cm mesh, 1.22 m tall). Uncaged plots were marked only with PVC poles. The field component of the study took place in a subset of the plots established, where measurements were completed in the two northern blocks, called 'east' and 'west', outlined in Figure 1.1 (right), for a total of 8 plots. One cage control plot, consisting of 3 sides of galvanized hardwire mesh, was established in each block. It was determined that the cage itself had no effect on herbivory or plant cover (Lodge 2017), and therefore these plots were not used in this study. Removable boardwalks were used to reduce sediment disturbance during measurements. Semi-permanent wood boardwalk supports were installed on opposite sides of each plot using 'H' design (USGS SET design). During measurements, an aluminum plank was suspended between the supports. Within the 8 pairs of plots examined the following were analyzed; vegetation cover,

in situ gas fluxes, soil incubations rates, and porewater CH_4 concentrations, and are described below.

1.2.3 Herbivore presence

Previous research showed that the dominant large grazer at HANA is the Canada Goose (*Branta canadensis*) (Spangler 2019; Lodge 2017), whose population has been exponentially rising in the region since the 1960's (N. American Breeding Bird Survey). Additional grazers observed at HANA are deer, muskrat, and ducks.

The presence and composition of wetland herbivores have been quantified by previous researchers and volunteers, starting in September of 2015 (Lodge 2017; Spangler 2019). In this study, we continued to monitor grazers abundances, following the method outlined by Lodge (2017). Herbivore density and species identity was recorded, as well as the date, time, weather, and the herbivores' general behavior. On average a total of 36 observations were recorded each year from 2015 to 2019, typically during the hours of 7am to 4pm, with more frequent observations in the spring, summer, and fall, than in the winter. In this study we analyzed the presence of waterfowl in A1N to find the average number of individuals per unit area across seasons (spring: March-May, summer: June-August, and fall: September-November).

1.2.4 Vegetation surveys and grazer damage

Vegetation surveys were conducted coincident with *in situ* gas flux measurements in spring, summer, and fall. All species present and their percent cover in each plot were recorded. Percent cover in each plot may exceed 100% when plant canopies of vegetation species overlap. The number of stems of each species, as well as the heights of the three tallest plants of each species, were recorded. Herbivore damage was determined by estimating the amount of total leaf and stem tissue area per vegetation species that had visible signs of large herbivore grazing. For our analysis, vegetation species were grouped into three categories; emergent, submergent, and floating. For the purpose of our study, emergent refers to vegetation that is rooted in the sediment, but the majority of the plant is above the water level, floating are leaved plants on the surface of the water that are rooted or unrooted, and submerged are wholly underwater and can be rooted or unrooted.

1.2.5 In situ Gas flux Measurements

Carbon gas flux measurements were quantified using the static chamber method (Caroll & Crill 1997). The chamber was 1x1 m and was created to fit over the PVC pipes that define the caged and uncaged plots. The top and side were transparent greenhouse film which was rolled down into the water during measurements. The chamber was equipped with two internal fans, to circulate air within the chamber, and a cooling system which circulated cold water through a heat exchanger situated over the fans to regulate chamber temperature. Temperature and solar radiation (inside and outside of chamber) were recorded using a Li-COR 2π light sensor for every sampling period (Appendix Table ii).

Chamber fluxes were measured in ambient light and with the chamber darkened by an opaque tarp. The concentration of CO_2 concentration was measured continuously using a Li-COR infrared gas analyzer (IRGA) model Li-830 and samples for CH_4 analysis were collected at 0, 5, 10, 15, 20 and 30 min after chamber closure. Methane samples were analyzed on a gas chromatograph (GC) fitted with an FID detector (Shimadzu GC-2104). We determined concentration of CH_4 from simultaneously run standards in replicates of 10 at the beginning, middle and end of each 120 sample run. Gas fluxes were calculated using a linear regression of concentration versus time and measurements with an $r^2 \leq 0.80$ were omitted. Measurements in 2018 were made in summer (July) and fall (September), and in 2019 in spring (end May- early June), summer (July), and fall (October). All flux measurements were made between the hours of 09:00 and 16:00.

The net carbon balance of A1N was roughly estimated using primary production and CH_4 emission results from our study. GPP and ER were adjusted to determine the amount of CO_2 taken in (GPP) and released (ER) from the wetland per season (spring, summer, and fall). To complete this, first daily estimates were made assuming approximately 10 hours of sunlight per day throughout the growing season, and assuming consistent ER every day throughout the growing season. For this estimate, we omitted CO_2 and CH_4 release due to winter soil respiration, and therefore, winter GPP and ER were assumed to be 0. Spring GPP and ER (not analyzed) were assumed to be the same as summer for this estimate. Estimates were made per season by determining the approximate number of days per season and extrapolating daily fluxes (g CO_2 m^{-2} yr^{-1} per season). CH_4 emissions were converted to CO_2 equivalents with the assumption that CH_4 has 28 times the global warming potential (GWP) as CO_2 and extrapolated to determine the

amount of CH_4 released per season in caged and uncaged plots (CH_4 in terms of g CO_2 m^{-2} yr^{-1} per season). For each season, the amount of CO_2 taken in was added to the amount of CH_4 released, in terms of CO_2 equivalent, to determine the net carbon balance in caged and uncaged plots.

1.2.6 Soil incubations

One soil sample was collected from each plot in the summer and fall of 2018 and the spring, summer, and fall of 2019. Soil collection followed the completion of chamber flux measurements. Samples were collected to a depth of 10 cm using a 5 cm diameter corer, stored on ice in the field, and then stored at 4° C in the lab for no longer than 24 hours before being processed. Samples were then homogenized and sieved through a 2.38 mm sieve to remove rocks and large roots. A subset of soil was separated, massed, and dried at 60° C to determine soil moisture content. The change in mass was then used to determine moisture content and calculate the dry mass of soil used in incubations.

Rates of potential CH_4 and CO_2 were measured using anaerobic, water-saturated incubations in the dark. Two replicates were conducted per plot. For each incubation, 50 g of wet soil was placed in a wide-mouth, 16 oz mason jar with equal parts sparged water. The jar was flushed with N_2 to create an anaerobic environment and then jars were incubated for 2 weeks at 22°C to allow the microbial communities to acclimate. The jars were then re-flushed with N_2 and sampling began 24 hours later. Gas samples were collected every 24-48 hours over 10-14 days and analyzed on a GC with FID detector and a methanizer to determine CH_4 and CO_2 concentrations. Headspace volume removed from jars when sampling was replaced with N_2 gas.

Rates of potential CH_4 oxidation were measured using aerobic incubations (Larmola et al. 2010). Two replicates were conducted per plot. For each replicate, 100 g of wet soil was added to a wide-mouth, 16oz mason jar. Jars were then spiked with 10% CH_4 to reach a headspace concentration of 1% CH_4. Gas samples were collected at 0, 2, 12, 24, 36, 48, and 72 hours after spiking with CH_4, and samples were analyzed for CH_4 concentration using a GC-FID. Headspace volume removed from the jars when sampling was replaced with room air.

For all incubations, sample concentrations of CH_4 and CO_2 were determined from simultaneously run standards in replicates of 10 at the beginning, middle, and end of each 120 sample run. CH_4 and CO_2 production rates were estimated using a linear regression of sample

concentration as a function of time elapsed since the final N_2 purge, where data with an $r^2 \leq 0.80$ was omitted. For CH_4 oxidation, rates were estimated using a linear regression of sample concentration as a function of time elapsed since the spike of CH_4, where data with an $r^2 \leq 0.80$ was omitted.

1.2.7 Porewater

Porewater samples were collected using lysimeters installed in the caged and uncaged plots in A1N at HANA. Lysimeters were installed in the plots such that the bottom of the lysimeter was approximately 15 cm below the soil surface (Chambers & Odum 1990). Upon collection, the lysimeter was flushed of all water using a syringe, then allowed to refill with water. A water sample was taken, mixed with equal parts air, and then shaken for 2 min. A subset of the headspace was then transferred to an evacuated vial and analyzed for CH_4 concentration on a GC-FID. Concentrations of porewater CH_4 were determined from simultaneously run standards in replicates of 10 at the beginning, middle and end of each 120 sample run, where final concentration was determined in mmol CH_4. Porewater samples were collected in the summer and fall of 2019.

1.2.8 Statistical Analyses

All statistical analyses were completed using JMP Pro 15 Statistical Software. All data was tested for normality using The Anderson-Darling goodness of fit test and tested for homogeneity of variances using Levene's test. Full-factorial three-way analysis of variance (ANOVA), with plot as a random variable, were used to test for significant effects of year (2018, 2019), season (spring, summer, fall), and treatment (caged, uncaged) on vegetation cover measurements. Full-factorial two-way ANOVA, with plot as a random variable, was used to test for significant effects of date (year and season), and treatment (caged, uncaged) on *in situ* gas fluxes, primary production measurements, and soil incubations. Effects of date were analyzed as some measurements were not completed in all 3 seasons in both years. For all ANOVAs, when significant effects were found, a Tukey's HSD post hoc test was used to identify significant differences. For data that did not meet the requirements of normality and homogeneity of variances, including waterfowl density, a Kruskal Wallis test was used to determine the effects of year, season, or treatment.

1.3 Results

1.3.1 Herbivore presence

The presence of the dominant group of herbivores, waterfowl, was found to significantly differ by season (p=0.01), but not year (p=0.9, Figure 1.2). Waterfowl were observed in the highest density during the fall seasons (2018 fall waterfowl density: 12±3 ind. ha^{-1} day^{-1}, and 2019 fall waterfowl density: 8±3 ind. ha^{-1} day^{-1}, Figure 1.2). Similar densities were seen in 2018 and 2019 spring and summer seasons with densities ranging between 2-5 ind. ha^{-1} day^{-1}. Species of waterfowl observed included the Canada goose (*Branta canadensis*), mallard duck (*Anas platyrhynchos*), green-winged teal duck (*Anas carolinensis*), wood duck (*Aix sponsa*), Northern Shoveler (*Spatula clypeata*), and common gallinule (*Gallinula galeata*). Other herbivores observed included deer (*Cervidae* family) and muskrat (*Ondatra* spp.), but the number of individuals observed of these two herbivores accounted for only 0.1% of total grazer observations across 2018 and 2019.

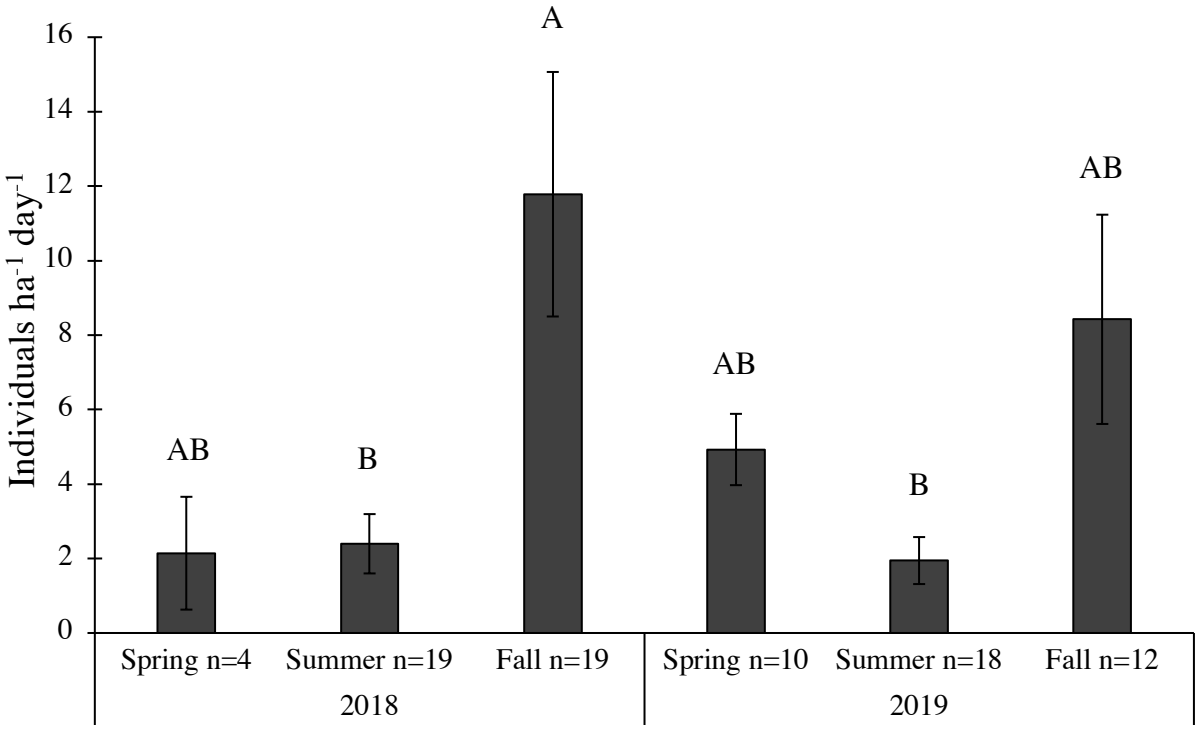


Figure 1.2: Waterfowl densities observed in A1N in the 2018 and 2019 season (spring: March-May, summer: June-Aug., fall: Sept.-Nov.; avg ± SE, number of observation days specified on graph).

1.3.2 Vegetation surveys and grazer damage

There was significant variation in total vegetation cover across years ($p=0.004$) and seasons ($p<0.0001$), with the highest vegetation cover in summer 2019 and the lowest in spring 2018 (Figure 1.3, Table 1.1). There was also a trend towards higher total vegetation cover in caged plots, however this was not significant ($p=0.1$). Additionally, no interactions between year, season, and treatment were significant. Fall measurements were made after all plants had senesced, therefore this time period was not included in the analysis. Large herbivore damage was found to be highest in the summer of 2019 in caged plots (2019 summer caged: 13 ± 4 %, uncaged: 27 ± 9 %), where a significant interaction was found between year, season and treatment ($p=0.03$, Table 1.1)

Emergent vegetation cover was found to differ by year, season, and treatment (year: $p=0.002$, season: $p<0.0001$, treatment: $p=0.01$, Figure 1.3, Table 1.1). Emergent plant cover was highest in the summer of 2018 and lowest in the spring of 2019. Across all dates, caged plots had higher emergent cover ($p=0.01$), with values close to double that observed in uncaged plots. Emergent cover ranged from 33-85% in caged plots and 16-48% in uncaged plots. No interactions between year, season, and treatment were significant, but the interaction between year and season trended towards being a significant effect ($p=0.1$). Floating vegetation cover was not affected by treatment ($p=0.2$), but significantly differed by year ($p<0.0001$) and season ($p=0.005$), with a significant interaction between year and season ($p=0.01$, Figure 1.3, Table 1.1). Summer 2019 had higher floating cover than any other time period (summer 2019 caged: 61+9, uncaged: 27+11). Spring 2018, summer 2018 and spring 2019 dates were similar with floating cover ranging from 4-20%. No significant year, season, treatment, or interaction effects was seen in submerged cover, where cover ranged from 4-32% across all measurement dates (Figure 1.3, Table 1.1).

In both 2018 and 2019 the most prominent species were *Sagittaria* spp. in both caged and uncaged plots (Appendix, table i). White waterlily (*Nymphaea odorata*), another dominant plant, has been increasing in percent cover since 2017, with the highest cover recorded in 2019 ($p<0.001$, Appendix, Figure i) making up a majority of the floating cover recorded in 2019 (Figure 1.3). Waterlily percent cover also different by season ($p=0.008$), with higher cover in the summer than spring, and by treatment ($p=0.03$), with higher cover in uncaged plots compared to

caged plots, with a significant interaction between year and treatment ($p<0.001$) and season and treatment ($p=0.01$, Table 1.1).

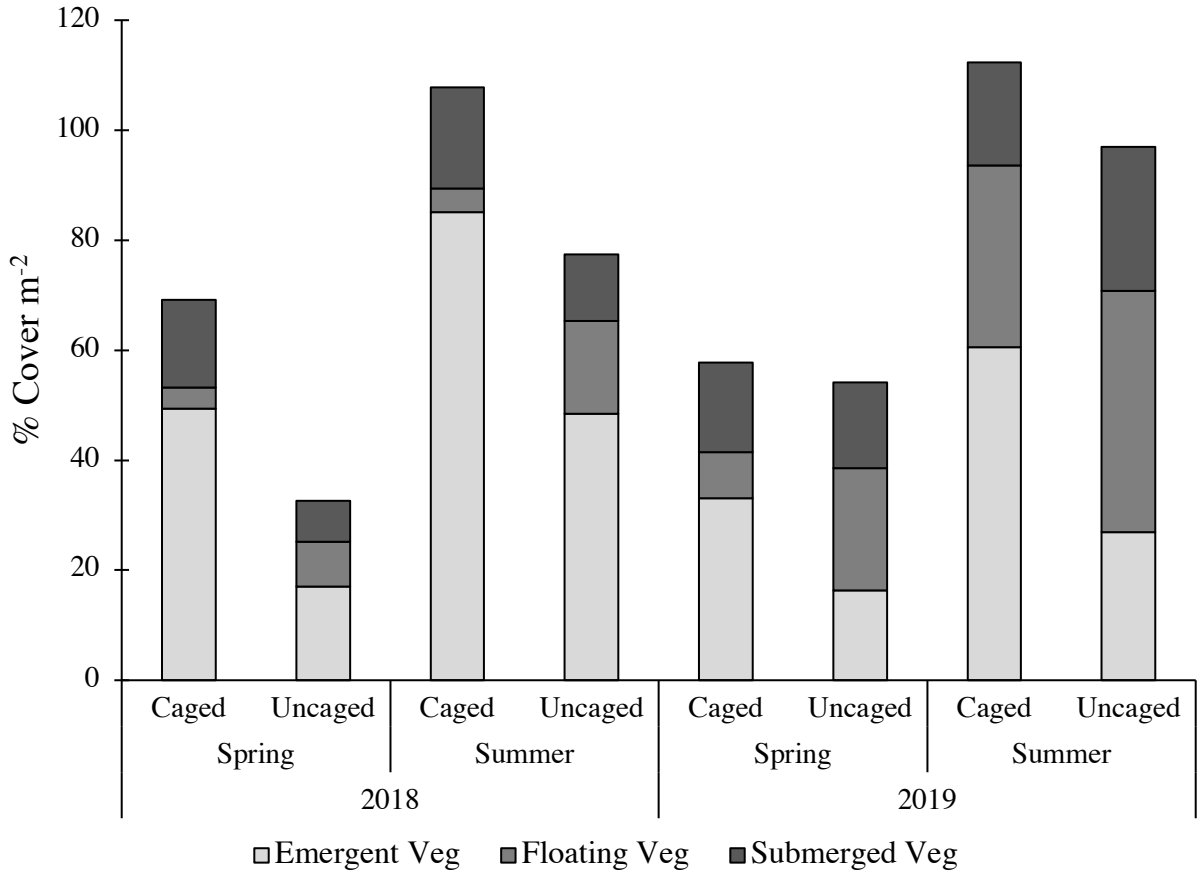


Figure 1.3: Average percent cover of emergent, floating, and submergent vegetation species in caged and uncaged plots in A1N in 2018 and 2019, avg ± SE (n=8).

1.3.3 In situ Gas Flux Measurements

Under ambient light conditions, CH_4 fluxes varied by date ($p=0.0007$) and by treatment ($p=0.006$), but the interaction was not significant ($p=0.5$, Figure 1.4, Table 1.2). Strong treatment effects were seen in 2018 summer and 2019 spring, where caged emissions were higher than uncaged emissions (summer 2018 caged: 12.3+4.51, uncaged: 4.56+2.81 mg C m^{-2} hr^{-1}, spring 2019 caged:15.1±3.03, uncaged: 7.27+2.17 mg C m^{-2} hr^{-1}). Fall 2018 CH_4 emissions were lower than all other dates (2018 fall caged: 0.65±0.18, uncaged: 0.77±0.11 mg C m^{-2} hr^{-1}) and fall of 2019 were low as well (2019 fall caged: 6.80±1.17, uncaged: 1.71±3.39 mg C m^{-2} hr^{-1}). Methane fluxes under dark conditions did not vary by date or by treatment (Appendix, Figure ii). There were also no differences between CH_4 emissions under ambient light and in the dark ($p=0.8$).

Gross primary production (GPP) and ecosystem respiration (ER) were significantly affected by date (GPP: $p<0.0001$, ER: $p<0.0001$), treatment (GPP: $p=0.04$, ER: $p=0.003$), and the interaction (GPP: $p=0.04$, ER: $p=0.01$, Table 1.2). GPP and ER were greater in caged plots and greater in the summer compared to the fall (Figure 1.5, A & B). GPP ranged from -0.3 to -0.9 g CO_2 m^{-2} hr^{-1} in the summer of 2018 and 2019, where caged plots were 60% greater on average than uncaged plots. ER ranged from 0.1 to 0.23 g CO_2 m^{-2} hr^{-1} in the summer of 2018 and 2019, where caged plots were 40% higher than uncaged plots. NEE did not differ between caged and uncaged plots ($p=0.2$), but it did vary across measurement dates ($p<0.0001$, Figure 1.5, C, Table 1.2). Additionally, the interaction between date and treatment trended towards being an effect on NEE, but was not significant ($p=0.1$). NEE in the summer of 2018 was significantly more negative than other time periods (summer 2018 caged: -0.67+0.27, uncaged: -0.42+0.26 g CO_2 m^{-2} hr^{-1}), and significantly higher during the fall of 2019 than the other time periods (fall 2019 caged: 0.08+0.01, uncaged: 0.05+0.02 g CO_2 m^{-2} hr^{-1}, Figure 1.5, C). A regression analysis on summer measurements, when vegetation cover was highest, concluded that there was no significant relationship between CH_4 flux emissions and GPP ($p=0.73$, $r^2=0.01$) and no significant relationship between CH_4 flux emissions and NEE ($p=0.91$, $r^2=0.001$). Additionally, A1N was found to be a carbon source, where the yearly net carbon budget for A1N was found to be 1060 g CO_2 m^{-2} yr^{-1} in caged plots and 336.5 g CO_2 m^{-2} yr^{-1} in uncaged plots.

Additionally, a regression analysis found a significant relationship between total vegetation cover and CH_4 emissions in the spring and summer measurement periods ($p=0.05$, $r^2=0.13$), where increased CH_4 fluxes were observed when vegetation cover was higher. There was a trend towards a similar relationship between CH_4 flux and emergent vegetation cover, but this was not significant ($p=0.10$, $r^2=0.09$). Furthermore, no significant relationships were found between CH_4 emissions and dominant vegetation species including *Sagittaria* spp. ($p=0.48$, $r^2=0.02$), *Nymphaea odorata* ($p=0.54$, $r^2=0.01$), or *Pontederia cordata* ($p=0.83$, $r^2=0.002$).

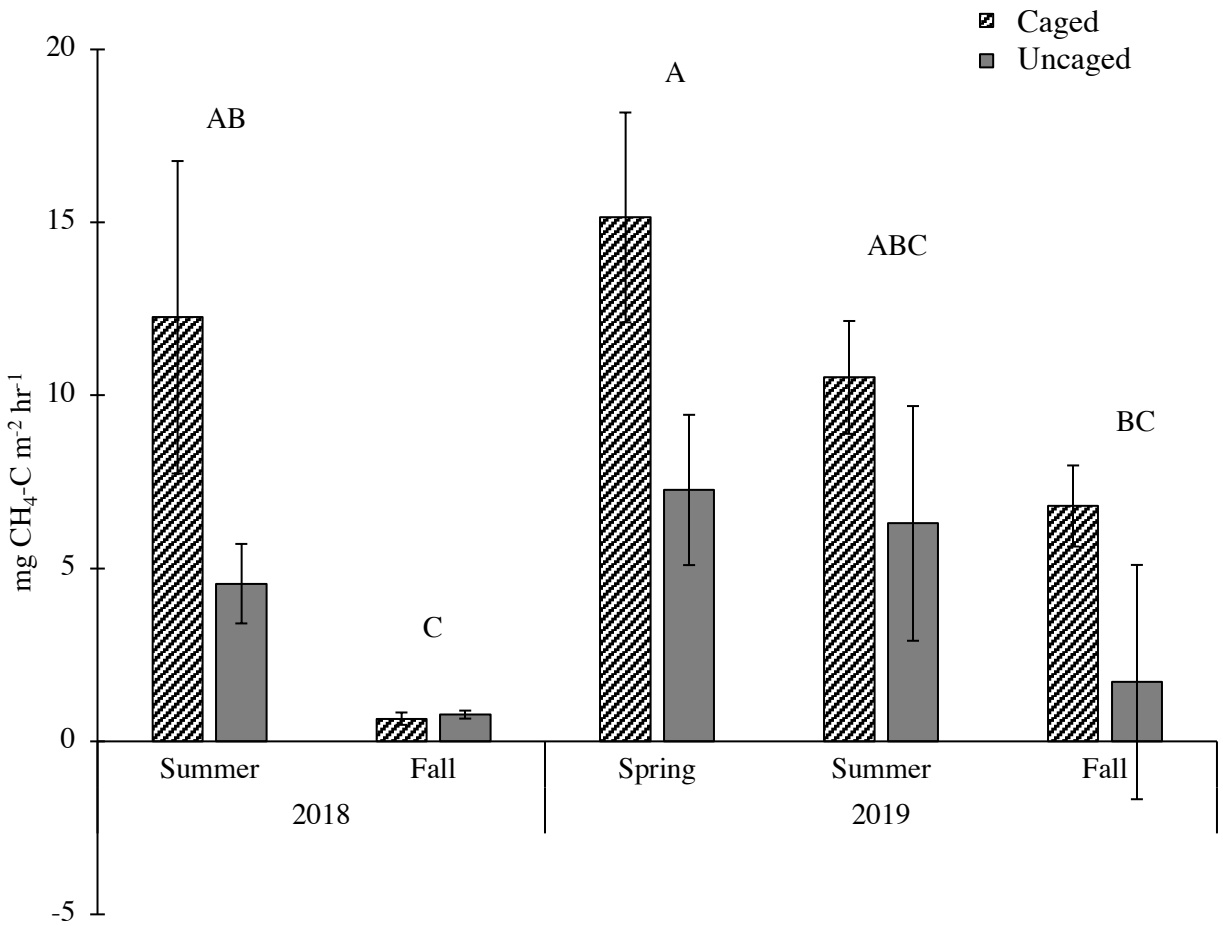
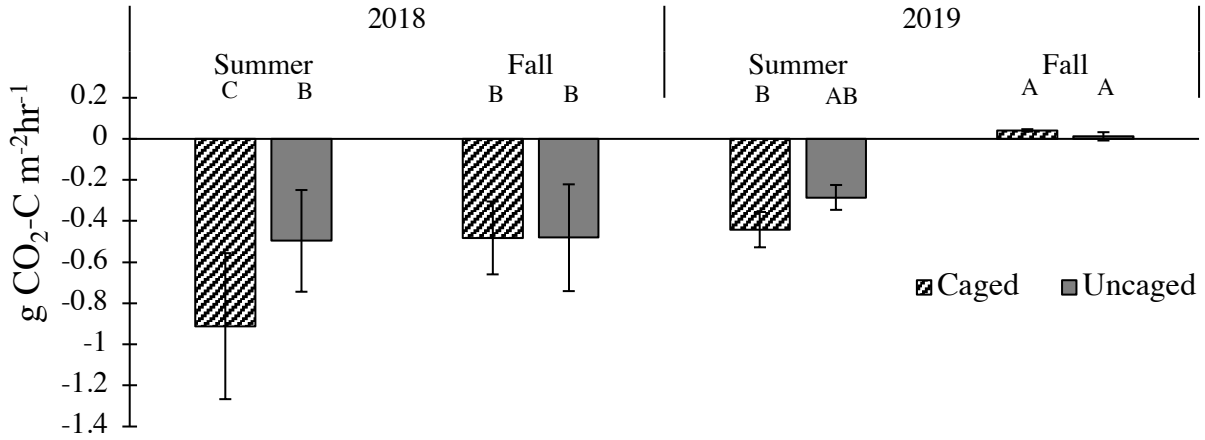
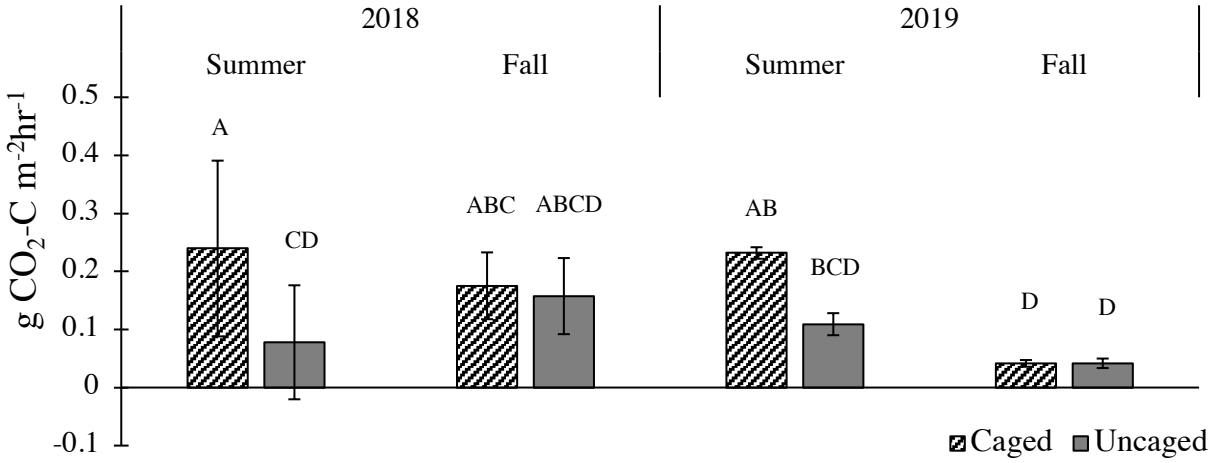


Figure 1.4: *In situ* CH_4 emissions under ambient light in caged and uncaged plots from 2018 to 2019 (n=3-7), avg ± SE, where letters indicate significant differences between dates.

A) Gross Primary Production



B) Ecosystem Respiration



C) Net Ecosystem Exchange

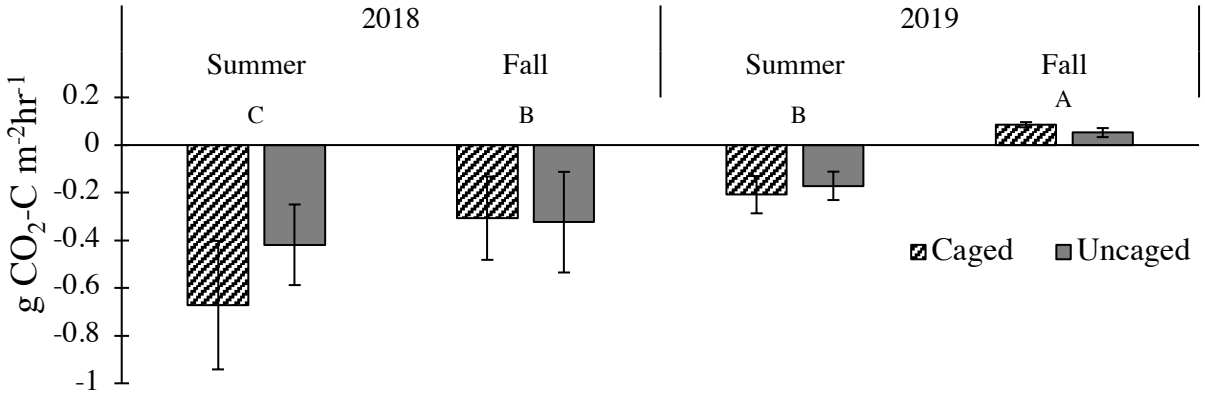


Figure 1.5**:** Results in caged and uncaged plots, avg ± SE (n=8) for **A)** Gross primary productivity (GPP), where letters represent significant differences, **B)** ecosystem respiration (ER), where letters represent significant differences, and **C)** net ecosystem exchange (NEE), where letters indicate significant differences between dates. Measurement dates were as follows, 2018 summer: June-July, 2018 fall: Aug.-Sept., 2019 Summer: July, 2019 fall: Oct.

1.3.4 Soil incubations

Potential rates of CH_4 and CO_2 production varied significantly across measurement dates (CH_4: $p<0.0001$, CO_2: $p<0.0001$) and there was a significant interaction between date and treatment (CH_4: $p=0.004$, CO_2: $p=0.04$, Figure 1.6, Table 1.2). There was no significant treatment effect for CH_4 and CO_2 (CH_4: $p=0.9$, CO_2: $p=0.2$). Summer and fall of 2019 had similar CH_4 production rates, with fluxes ranging from 0.008 to 0.01 mg C day^{-1} g dry $soil^{-1}$, whereas spring 2019 and summer 2018 had lower CH_4 production rates with values ranging from 0.001 to 0.003 mg C day^{-1} g dry $soil^{-1}$. Fall 2018 had intermediate production rates, with caged rates nearly half that of uncaged plots (caged: 0.0039 ± 0.0007, uncaged: 0.0072 ± 0.0007 mg C day^{-1} g dry $soil^{-1}$). Production rates of CO_2 were lower in the summer and fall of 2018 compared to summer and fall of 2019, with rates of 0.002 to 0.003 mg C day^{-1} g dry $soil^{-1}$ in 2018 and 0.01 mg C day^{-1} g dry $soil^{-1}$ in 2019. CO_2 production rates were substantially lower in spring 2019 with rates of 0.0004 ± 0.00004 mg C day^{-1} g dry $soil^{-1}$ in caged plots and 0.0003 ± 0.00005 mg C day^{-1} g dry $soil^{-1}$in uncaged plots. For both CO_2 and CH_4 production, significant differences between caged an uncaged plots were only observed in fall 2018, with higher rates of both CO_2 and CH_4 production in sediments from uncaged plots.

Potential rates of CH_4 oxidation were significantly different across the measurement dates ($p<0.001$), but did not differ by treatment ($p=0.5$, Figure 1.7, Table 1.2). Oxidation rates were lowest in summer 2018 (caged: 0.009 ± 0.0009, uncaged: 0.0103 ± 0.001 mg CH_4-C day^{-1} g dry $soil^{-1}$) and generally high throughout the entire 2019 season ranging from 0.008 to 0.086 mg CH_4-C day^{-1} g dry $soil^{-1}$ (Figure 1.7). In the spring and summer of 2019 caged oxidation rates trended towards being higher than uncaged rates. Additionally, no significant interaction between date and treatment was observed ($p=0.2$).

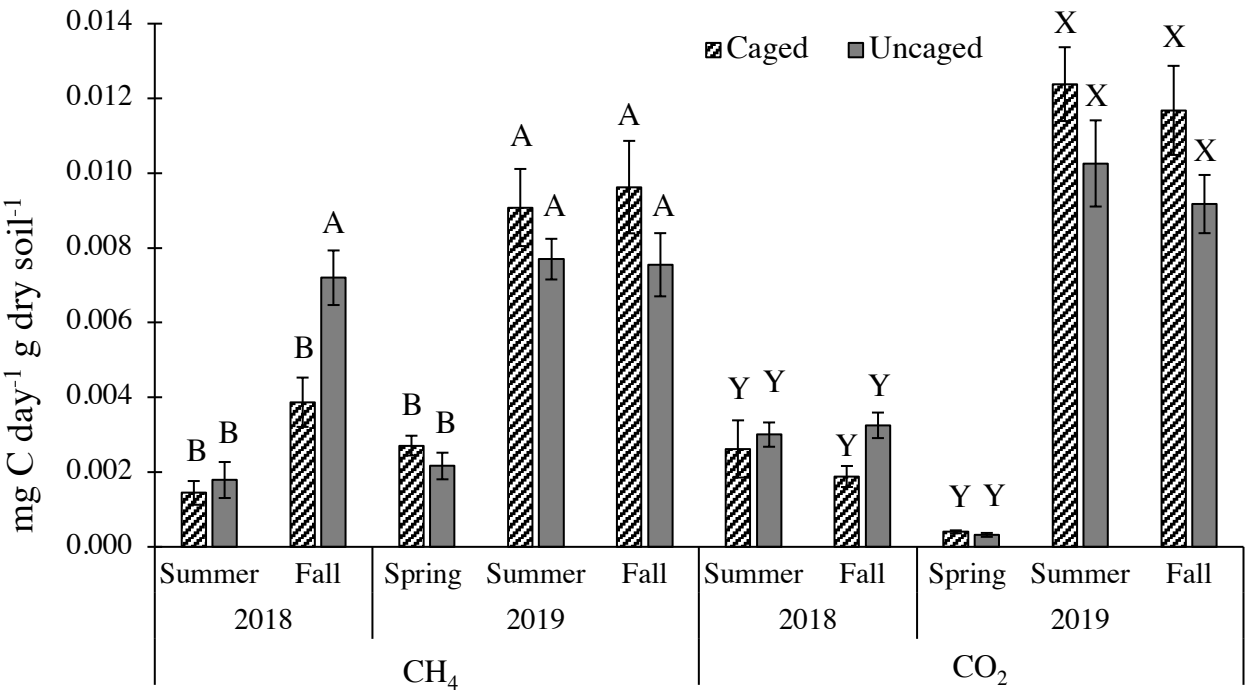


Figure 1.6: Potential CH_4 and CO_2 production in caged and uncaged plots from 2018 and 2019 season, average ± SE, n=8, where letters represent significant differences.

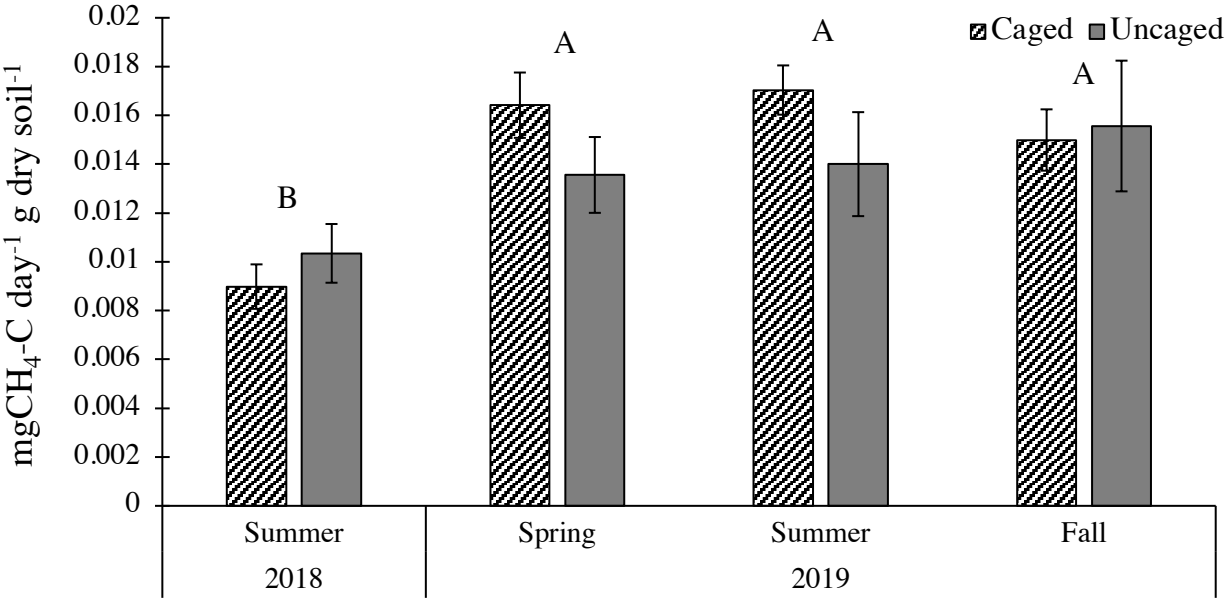


Figure 1.7: Potential CH_4 oxidation rates in caged and uncaged plots from summer 2018 throughout the 2019 season, average ± SE, n=8, where letters represent significant differences between dates.

1.3.5 Porewater

Porewater CH_4 concentrations were found to not significantly differ by season (p=0.5), treatment (p=0.1), or the interaction (p=0.7), although caged plots trended towards being higher compared to uncaged plots in both seasons (Figure 1.8, Table 1.2). Porewater CH_4 concentrations ranged from 0.001 to 1.4 mM CH_4.

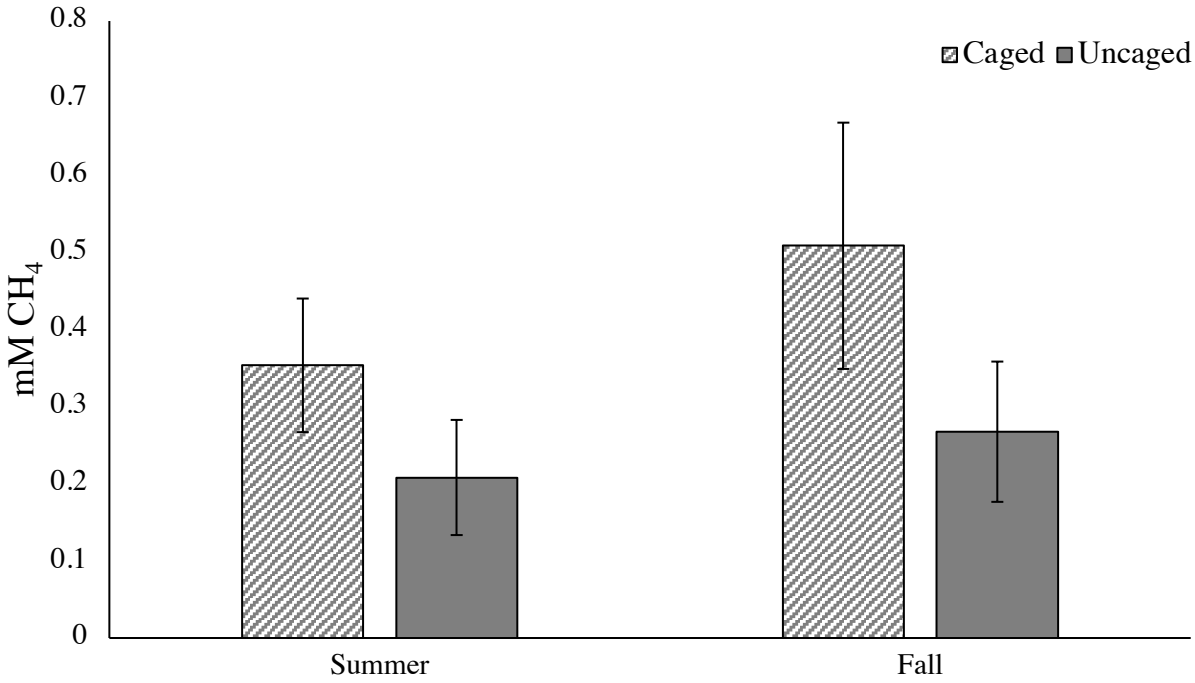


Figure 1.8: Average porewater CH_4 concentration at a depth of 15 cm below the soil in caged and uncaged plots in summer and fall of 2019, avg $\pm$ SE, n=8.

Table 1.1: Results of two- and three-way ANOVA examining the effects of year, season, treatment (cage, uncaged), and the interactions, with plot as a random variable. Significant p-values are bolded (*p<0.0001).

Factor	Year		Season		Treatment		Year x season		Year x treatment		Season x treatment		Year x season x treatment	
	F	p	F	p	F	p	F	p	F	p	F	p	F	p
Vegetation % Cover														
Total	$F_{1,64}$=9.26	**0.0040**	$F_{1,64}$=36.0	**<0.0001***	$F_{1,64}$=3.06	0.1022	$F_{1,64}$=0.53	0.4702	$F_{1,64}$=1.31	0.2596	$F_{1,64}$=0.07	0.7938	$F_{1,64}$=0.30	0.5868
Emergent	$F_{1,64}$=10.8	**0.0021**	$F_{1,64}$=29.8	**<0.0001***	$F_{1,64}$=8.15	**0.0127**	$F_{1,64}$=2.33	0.1347	$F_{1,64}$=0.94	0.3391	$F_{1,64}$=1.20	0.2790	$F_{1,64}$=0.52	0.5157
Floating	$F_{1,64}$=25.8	**<0.0001***	$F_{1,64}$=14.4	**0.0005**	$F_{1,64}$=1.68	0.2161	$F_{1,64}$=6.51	**0.0145**	$F_{1,64}$=0.27	0.6054	$F_{1,64}$=0.13	0.7155	$F_{1,64}$=0.60	0.4418
Submerged	$F_{1,64}$=1.52	0.2246	$F_{1,64}$=0.70	0.4061	$F_{1,64}$=0.07	0.8014	$F_{1,64}$=0.03	0.8675	$F_{1,64}$=1.22	0.2763	$F_{1,64}$=0.18	0.6769	$F_{1,64}$=0.05	0.8209
Waterlily	$F_{2,144}$=24.6	**<0.0001***	$F_{1,144}$=7.22	**0.0083**	$F_{1,144}$=1.06	**0.0389**	$F_{2,144}$=5.14	0.3494	$F_{2,144}$=11.6	**<0.0001***	$F_{1,144}$=6.56	**0.0117**	$F_{2,144}$=0.93	0.3962
Herbivore Damage	$F_{1,64}$=21.2	**<0.0001***	$F_{1,64}$=4.27	**0.0451**	$F_{1,64}$=2.99	0.1055	$F_{1,64}$=0.16	0.6887	$F_{1,64}$=0.42	0.5221	$F_{1,64}$=0.04	0.8348	$F_{1,64}$=5.10	**0.0291**
Porewater														
CH_4 concentration	---	---	$F_{1,30}$=0.20	0.6644	$F_{1,30}$=0.36	0.3602	---	---	---	**---**	$F_{1,30}$=0.299	0.1249	---	---

Table 1.2: Results of two-way ANOVA examining the effects of treatment (cage, uncaged), date (season and year) and the interaction, with plot as a random variable. Significant p-values are bolded (*p<0.0001).

Factor	Date		Treatment		Treatment x Date	
	F	p	F	p	F	p
In-Situ CH_4 Fluxes						
Light	$F_{3,52}$=8.03	**0.0007**	$F_{1,52}$=5.73	**0.0057**	$F_{3,52}$=1.25	0.5147
Dark	$F_{3,42}$=1.66	0.1985	$F_{1,42}$=1.10	0.4452	$F_{1,42}$=0.62	0.6052
Primary Production						
NEE	$F_{3,58}$=24.8	**<0.0001***	$F_{1,58}$=1.63	0.2315	$F_{3,58}$=1.96	0.1388
GPP	$F_{3,59}$=24.2	**<0.0001***	$F_{1,59}$=5.10	**0.0433**	$F_{3,59}$=3.17	**0.0353**
ER	$F_{3,61}$=9.88	**<0.0001***	$F_{1,61}$=13.7	**0.0026**	$F_{3,61}$=4.10	**0.0126**
Soil Incubations						
CH_4 Production	$F_{3,80}$=42.3	**<0.0001***	$F_{1,80}$=0.02	0.8911	$F_{3,80}$=4.44	**0.0035**
CO_2 Production	$F_{3,80}$=99.9	**<0.0001***	$F_{1,80}$=99.9	0.2486	$F_{3,80}$=2.75	**0.0367**
CH_4 Oxidation	$F_{3,64}$=9.07	**<0.0001***	$F_{1,64}$=0.38	0.5484	$F_{3,64}$=0.23	0.2282

1.4 Discussion

Herbivores had a strong effect on CH_4 emissions throughout our study (Figure 1.4; Table 1.1). Fluxes were observed to be 1-4 times higher in ungrazed plots compared to grazed plots, with generally higher emissions in the spring and summer measurement periods, compared to fall measurements. Overall, flux emissions were observed in the range of 1-16 mg CH_4-C m^{-2} hr^{-1}, similar to those reported in natural wetlands (1-27 mg CH_4 m^{-2} hr^{-1}, Bridgham et al. 2006; Dingemans et al. 2011; Winton & Richardson 2017). Similar effects of herbivory have been observed in other studies (Bodelier et al. 2006; Falk et al. 2013; Noyce et al. 2014). Bodelier et al. (2006) concluded that CH_4 emissions decreased when Bewick's Swans (*Cygnus columbianus bewickii*) were caged in an enclosure attributed to decreased CH_4 production rates and increased CH_4 oxidation rates during their one season of study. Falk et al. (2013) who simulated herbivory in a Greenland Arctic mire found a 26% decrease in yearly CH_4 emissions after vegetation was clipped for 1 year. This treatment effect is lower than what was observed throughout our study, but is likely due to the difference in study location and climate. Lastly, Noyce et al. (2014) observed significantly lower CH_4 emissions when *Carex* stems were clipped throughout their 4-year study in a New Hampshire fen, compared to unclipped plots. Emissions were approximately 1.5 times lower in clipped plots compared to unclipped plots (Noyce et al. 2014), falling in the range of our observations in this study. Furthermore, these studies found similar seasonal trends to our study where emissions were highest in the summer and lower in the fall, but differed from our study as they observed lower emissions in the spring than in the summer (Dingemans et al. 2011; Falk et al. 2014; Noyce et al. 2014).

We attribute higher flux emissions in caged plots to significant effects of herbivory on vegetation (Figure 1.3; Table 1.1). Throughout our study, we observed emergent vegetation cover that was 1.7-2.9 times higher in caged plots than uncaged plots (Figure 1.3; Appendix Table i). We also observed significantly higher vegetation cover in 2019 and in the summers versus the spring. High emergent vegetation cover coupled with warm air temperatures in the summer could lead to the high *in situ* CH_4 emissions observed in the summer flux measurements. Although, spring 2019 CH_4 emissions were similar to summer emissions, which we believe may have been influenced by the average stem count in spring 2019 being similar to that in the summer of 2019, despite significantly less total vegetation cover. This idea is supported by Greenup et al. (2000) who observed CH_4 emissions were correlated with the number of plant

stems, although they concluded that total above- and belowground biomass had the strongest correlation with CH_4 emissions. Overall, we see a stronger correlation of *in situ* CH_4 emissions to emergent vegetation cover, where higher CH_4 emissions are seen in the summer of 2018, where emergent cover is also higher, compared to the summer 2019. A regression analysis showed a significant relationship between total vegetation cover and CH_4 flux emissions, where we observed that as total vegetation cover increased, emissions also increased. This trend suggests a significant impact of plant-mediated transport on total CH_4 emissions in our wetland. We also examined the relationship between the dominant vegetation species and CH_4 emissions and found that the dominant species in our wetland do not have major controls on emissions, indicating that one species is likely not responsible for emissions at our site. Although we believe more research should be completed as it has been shown some vegetation species contribute more CH_4 than others (Bhullar et al. 2014; Ding et al. 2005; Hirota et al. 2005; Rietl et al. 2017). Furthermore, fall CH_4 emissions patterns may be influenced by the presence of standing dead vegetation, which was observed to be 28% and 18% in caged and uncaged plots, respectively, in the fall of 2019 when fall fluxes were higher, where no standing vegetation was observed in 2018 fall when fall fluxes were lower than any other season. This idea is also supported by Greenup et al. (2000) who observed CH_4 emissions were weakly correlated with the number of dead plant culms.

The vegetation observations in this study are similar to those observed by previous researchers in A1N, where Lodge (2017) saw significantly higher total plant cover in caged plots throughout the 2016 season, and Spangler (2019) observed aboveground biomass was significantly higher in caged plots, compared to uncaged plots, in the summer of 2018. Furthermore, due to similar aboveground vegetation trends, it is likely that belowground biomass was higher in caged plots compared to uncaged plots, as seen by Lodge (2017) and Spangler (2019) in A1N throughout our study. Additionally, herbivores were observed throughout the course study in similar densities to those observed by previous researchers in A1N (Figure 1.2, Lodge 2017; Spangler 2019), where we observed significant large grazer damage in uncaged plots throughout our study. Lastly, our vegetation observations align with those seen in other herbivore exclosure studies, where Hirota et al. (2005) observed that aboveground biomass decreased by nearly 90% in the presence of herbivores and Dingemans et al. (2011) observed lower vegetation cover and more damaged stems in grazed plots compared to ungrazed plots.

Higher vegetation cover in ungrazed plots has the potential to provide more opportunities for CH_4 to move rapidly to the atmosphere through plants, generating higher CH_4 fluxes. Previous research has shown that vegetation has a strong influence on CH_4 emissions. In clipping studies when vegetation is clipped below the water level or clipped stems are sealed, emissions drop by 35-97% (Ding et al. 2005; Kelker & Chanton 1997; Rietl et al. 2017) and Kelker & Chanton (1997) estimated plant-mediated transport accounted for 97% of the total CH_4 flux in their study. Although, herbivory has been shown to increase emissions through reduced resistance and distance CH_4 must travel to reach the atmosphere (Dingemans et al. 2011; Hirota et al. 2005; Kelker & Chanton 1997; Kelsey et al. 2016). However, it has been suggested that the grazing of stems may impact CH_4 emissions only short term (Hirota et al. 2005). Herbivores in our study system were observed to graze vegetation by removing the topmost part of the plant, most notably in *S. latifolia*, which could allow CH_4 to easily escape from the damaged stem. However, this increase in emission may only be observed immediately after the stem is damaged and therefore not reflected in our *in situ* fluxes. A simulated herbivory study showed increased emissions when stems were clipped, but after an immediate increase in CH_4 flux after clipping, fluxes slowly decreased over time (Kelker & Chanton 1997). High concentrations of CH_4 within plant stems may be released from the stem immediately after grazing, and then CH_4 fluxes would stabilize to a lower rate in uncaged plots. In caged plots, vegetation would still be transporting CH_4 through plant-mediated transport using a less efficient pathway in the intact stem (Whiting & Chanton 1996; van der Nat et al. 1998), but leading to overall higher emissions from caged plots compared to uncaged plots. This idea has some support by the weak trend observed in porewater CH_4 concentrations between caged and uncaged plots. We observed slightly lower porewater CH_4 concentrations in uncaged plots and could therefore support our idea that damaged stems quickly, and efficiently transport CH_4, depleting the belowground supply of CH_4. Porewater CH_4 was observed to range from 0.002-1.37 mMol CH_4, where uncaged plots were slightly lower, but no significant treatment effect was found. We suggest that uncaged plots may have slightly lower CH_4 within the sediment due to the damaged plant culms transporting not only the CH_4 built-up within the plant, but from the soil as well.

Herbivory also had a significant effect on GPP and ER, but we observed no substantial effect of herbivory on CH_4 production or oxidation (Figure 1.6; Figure 1.7). Increased rates of GPP in caged plots could lead to increased C substrate within the soil, as supported by previous

research in my study site, where belowground biomass, organic matter, and soil carbon was observed to be higher in caged plots, compared to uncaged plots (Lodge 2017; Spangler 2019), suggesting higher supply of methanogenic substrate. Increased substrate could yield higher rates of CH_4 production and contribute to the observed higher rates of emission (Bodelier et al. 2006; Hirota et al. 2005). We did not see higher rates of CH_4 production in caged-plot soils in our incubations, however, the conditions of our incubations didn't directly simulate field conditions. On average our observed potential rate of CH_4 production ranged from 0.002-0.02 mg CH_4-C day^{-1} g dry $soil^{-1}$ and CH_4 oxidation ranged from 0.004-0.086 mg CH_4-C day^{-1} g dry $soil^{-1}$, but this excluded influence from plants such as a continued supply of O_2 or C substrate. Primary production results indicate that plants are likely depositing organic material and O_2 continuously throughout the growing season which could increase CH_4 production and oxidation from what was shown in the incubation results. Due to higher vegetation cover and GPP in caged plots, as well as higher belowground biomass, we therefore hypothesize CH_4 production and oxidation would be higher in caged plots compared to uncaged plots, but is not reflected in our incubation results due to ideal laboratory conditions. This idea is also supported by the slightly higher porewater CH_4 concentrations we observed in caged plots, compared to uncaged plots, suggesting higher rates of CH_4 production. Furthermore, we believe plant senescence and increased labile C in the fall drive higher production rates observed in our incubations in the fall measurements compared to those in summer and spring. We also hypothesize that the most labile C substrate may have been depleted by microbes during our two week acclimation period, leaving substrate that is less labile and therefore leading to lower CH_4 production rates than what occurs in the field where labile carbon is continuously added.

Our incubation results are similar to previous studies that observed no effect of herbivory on CH_4 production (Dingemans et al. 2011; Winton & Richards 2017) as well as increased rates of production in the fall compared to the summer (Dingemans et al. 2011). Our results are also similar to previous studies that observed no effect of herbivory on CH_4 oxidation (Dingemans et al. 2011), although it is hypothesized that removal of vegetation has the ability to significantly reduce oxidation by reducing sediment O_2 concentrations (Dingemans et al. 2011; Winton & Richards 2017) or increase oxidation due to grazing bioturbating the sediment increasing sediment O_2 concentrations (Bodelier et al. 2006; Dingemans et al. 2011). Lastly, our incubation results showed generally higher potential CO_2 production rates in 2019, compared to 2018,

which may have been driven by increased total vegetation cover over the years, but no treatment effect was observed in 4 out of 5 incubation periods. Similar to the other incubations, due to the conditions of the measurements, such as no added C substrate, microbes may have been less active than what would have been observed *in situ*.

Our analysis found no significant relationship between primary production and CH_4 emissions in summer measurements, when vegetation cover was highest. On average we see that in the summer of 2018, GPP, NEE and CH_4 emissions are greater when compared to the summer of 2019, but regression analysis showed that there was no significant correlation between primary production measurements and CH_4 flux emissions (Figure 1.4; Figure 1.5). Although, each year we see GPP and CH_4 fluxes decrease from summer to fall, suggesting an effect of active vegetation. We also observed higher GPP and CH_4 emissions in caged plots, compared to uncaged plots, further suggesting vegetation does have a strong influence on CH_4 emissions. Strong positive relationships have been found between primary productivity and CH_4 emissions (Falk et al. 2014; Whiting & Chanton 1993), where it is suggested that photosynthetically active vegetation will deposit more methanogenic substrate, generating higher CH_4 emissions (Joabsson et al. 1999), but we do not see a significant relationship in our study.

Our net carbon balance estimate suggested the A1N has a significant effect on climate change, and that CH_4 emissions offset the positive impact of C uptake through photosynthesis. We found that the carbon balance of A1N is 1060 g CO_2 m^{-2} yr^{-1} in caged plots and 336.5 g CO_2 m^{-2} yr^{-1} in uncaged plots. These results suggest that a substantial amount of carbon is being released by the wetland on a yearly basis, regardless of the strong productivity observed by vegetation, although we note that this is only an estimate. These results suggest that A1N is a source of carbon in both caged and uncaged plots, but caged plots significantly increase the carbon release, mostly due to the GWP of CH_4 emissions. This suggests that herbivore exclusion plots have a significant negative tradeoff and a significant impact on climate change. For these reasons, we suggest that herbivore exclusion should be used only when necessary to prevent unnecessary CH_4 emissions which leads to more GHG released.

Overall, we conclude that herbivory significant decreases CH_4 emissions, although this idea contradicts previous herbivore exclosure studies (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richards 2017) and clipping studies (Kelker & Chanton 1997; Petruzzella et al. 2015; Rietl et al. 2017) that observed increased CH_4 in the presence of herbivores. These results

indicate that vegetation species and wetland conditions may play a significant role in herbivory impacts on CH_4 emissions. Many studies suggest that there are significant differences in plant species ability to transport CH_4 (Bergstrom et al. 2006; Bhullar et al. 2014; Chanton 2005; Davidson et al. 2016; Ding et al. 2005; Greenup et al. 2000; Joabsson et al. 2001; Kao-Kniffin et al. 2010; Rietl et al. 2017; van der Nat et al. 1998) and therefore demonstrates the need for additional research in various wetland systems. Additionally, our wetland has been shown to have low OM and nutrients, which may have led to reduced CH_4 emissions overall, prompting the need for additional research. Furthermore, as stated previously, we believe a significant reason why our results may contradict those in the literature may be due to the timing of our flux measurements, that did not capture the immediate release of plant-mediated CH_4, but instead shows the long term effect of herbivory over the course of the 6-year exclosure study.

1.5 Conclusions

Wetlands are one of the largest sources of atmospheric CH_4, yet they are also one of the largest sources of uncertainties in the global CH_4 budget. This research suggests that one major impact on CH_4 emissions from wetlands may be herbivores, where they have been shown to have a significant top-down effect on vegetation (Hirota et al. 2005; Lauidsen et al. 1993; Silliman & Zieman 2001; Sondergaard et al. 1996), leading to potential changes in CH_4 emissions. Additionally, these effects may be exacerbated in created wetlands, where permanent standing water and young vegetation create the perfect habitat for waterfowl (Isola et al. 2000). In our study, we saw significant effects of herbivory on CH_4 emissions, where ungrazed plots were observed to have 1-4 times higher emissions compared to grazed plots. We propose that substantial effects of herbivory on vegetation, where emergent vegetation cover was found to be 1.7-2.9 times higher in caged plots than uncaged plots, is the key driver of the observed CH_4 emissions. This is supported by our results that show as total vegetation cover increases, CH_4 emissions increased as well. Significantly higher vegetation cover in caged plots allows for more opportunities for CH_4 to move through plants to the atmosphere increasing net emissions. Furthermore, higher biomass likely supplies more methanogenic substrate and increases CH_4 production. The results of these interactions lead to net increases in CH_4 emissions when herbivores are excluded. Furthermore, our net carbon balance shows that this wetland is a significant source of carbon, especially when herbivores are excluded. Although herbivore

exclusion has been proven to be very beneficial by increasing vegetation cover, improving biodiversity, improving soil quality, and improving carbon sequestration (Lodge 2017; Spangler 2019), there are significant negative tradeoffs when it comes to CH_4 emissions. Increased emissions in caged plots, make these areas significant sources of CH_4 and carbon. For this reason, we believe that land managers should only use herbivore exclusion when necessary, such as in newly constructed wetlands to support the establishment of native vegetation, and for short periods of time. Although, we advise managers of wetlands with healthy vegetation communities or little impact by grazers not to exclude grazers in order to avoid unnecessary CH_4 emission increases.

Chapter 2: The impact of herbivory on methane fluxes in a greenhouse clipping experiment to simulate herbivory

2.1 Introduction

Wetlands are one of the largest sources of atmospheric methane (Whalen 2005), a greenhouse gas with 28 times the global warming potential of CO_2 (Myhre et al. 2013). Wetlands are also one of the largest sources of uncertainties in the global CH_4 budget (Kirschke et al. 2013). Methane emissions from wetlands are a product of the following processes: CH_4 production, CH_4 oxidation, and CH_4 transport (Bodelier et al. 2006, Dingemans et al. 2011; Whalen 2005). CH_4 production is when organic matter is consumed by methanogenic *Archaea* under anaerobic conditions in the final steps of decay within the soil (Hanson & Hanson 1996; Segers 1998; Whalen 2005). CH_4 oxidation is when CH_4 is consumed by methanotrophs in aerobic environments, which typically occurs at the air-water interface or around plant roots which leak O_2, where the bi-product, CO_2, is released (Megonigal et al. 2004; Segarra 2015; Whiting & Chanton 1993). Oxidation has considerable control on CH_4 emissions from wetlands, in which Segarra et al. (2015) estimated oxidation can nearly half total CH_4 emissions from wetlands. Lastly, CH_4 is transported to the atmosphere via diffusion, ebullition, and plant-mediated transport (Segers 1998; Shultz et al. 1991; Whalen 2005). Diffusion is the slow transport of CH_4 from the sediment, through the water column, to the atmosphere (Hanson & Hanson 1996; Whalen 2005), in which a significant amount of CH_4 can be oxidized due to oxic conditions within the water. Ebullition is the rapid release of CH_4 bubbles from the soil, resulting in high spatial and temporal variability, and a low possibility of oxidation (Chanton & Martens 1988; Whalen 2005). Ebullition is often the dominant pathway in unvegetated areas (Grünfeld & Brix 1999), but when vegetation is high, plant-mediated transport dominates (Grünfeld & Brix 1999; Whalen 2005). Plant-mediated transport is the process of CH_4 moving within the internal spaces of a plant, referred to as lacunae or aerenchyma, leading to the efficient transport of CH_4 from the soil to the atmosphere (Dacey 1981; Whalen 2005).

Plant-mediated transport has been shown to have significant control over total emissions, in which previous studies have shown it accounted for 22-97% of total emissions from the wetland systems (Carmichael et al. 2014; Grünfeld et al. 1999; Kelker & Chanton 1997). Greenup et al. (2000) also observed that CH_4 emissions were highly correlated with the number

of *Eriophorum vaginatum* stems in their study plots. What is still unclear is how herbivory, or grazing of stems, impacts these emissions, as simulated herbivory studies have seen increases (Kelker & Chanton 1997; Petruzzella et al. 2015; Rielt et al. 2017) and decreases (Falk et al. 2013; Noyce et al. 2014) in emissions when plants were clipped.

Herbivory overall has been shown to significantly impact vegetation, reducing aboveground biomass (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richardson 2017), below-ground biomass (Bodelier et al. 2006; Winton & Richardson 2017) or removing vegetation entirely. For example, Hirota et al. (2005) observed a nearly 90% reduction in aboveground biomass in the presence of herbivores and Dingemans et al. (2011) observed that every stem in their study plot had been grazed (58 stems m^{-2}). Due to the significant damage brought upon vegetation by grazers, it has been suggested that herbivory has the potential ability to alter plant-mediated transport of CH_4 (Ding et al. 2005; Dingemans et al. 2011; Falk et al. 2013; Hirota et al. 2005; Kelker & Chanton 1997; Petruzzella et al. 2015; Rietl et al. 2017). Many *in situ* studies have shown that herbivory leads to an increase in CH_4 emissions, in which herbivore exclusion studies observed emissions that were 2-5 times higher in grazed plots versus ungrazed plots (Dingemans et al. 2011; Hirota et al. 2005; Winton & Richardson 2017). Other herbivory studies have observed the opposite result, in which grazing leads to reduced emissions (Bodelier et al. 2006; Spangler 2019). In simulated herbivory experiments, where stems were clipped above the water level, CH_4 emissions were observed to increase significantly by 26-350% (Ding et al. 2005; Kelker & Chanton 1997; Petruzzella et al. 2015; Rietl et al. 2017). These results are significant as it has been shown that herbivores often graze stems above the water level, leaving stems emerging out of the water (Dingemans et al. 2011; Kelsey et al. 2016; personal observation). Although, other clipping studies found the opposite results, where emissions decreased when stems were clipped above the water level (Falk et al. 2014; Noyce et al. 2014). Furthermore, in clipping experiments when stems were clipped below the water level CH_4 emissions were observed to decrease by 73-97% (Ding et al. 2005; Greenup et al. 2000; Kelker & Chanton 1997), or when vegetation was removed entirely emissions were observed to decrease by 29-85% (Falk et al. 2014; Whalen 2005).

It has also been shown that different plant species mediate different amounts of CH_4 emissions prompting the need for additional research on other dominant wetland species (Bergstrom et al. 2006; Bhullar et al. 2014; Chanton 2005; Davidson et al. 2016; Ding et al.

2005; Greenup et al. 2000; Joabsson et al. 2001; Kao-Kniffin et al. 2010; Rietl et al. 2017; van der Nat et al. 1998). This is attributed to the two main mechanisms in which plants transport CH_4: diffusive and convective through flow. Many plants have been suggested to transport CH_4 via passive diffusion, where *Carex* spp., *Oryza sativa,* and *Peltandra virginica*, have been shown to undergo this mechanism (Chanton & Dacey 1991; Ding et al. 2004; Kelker & Chanton 1997; van Bodegom et al. 2001). Other vegetation species undergo convective transport where gases travel along pressure gradients caused by temperature or humidity differences between the plant internals and the outside air (Armstrong & Armstrong 1991; Askaer et al. 2010; Brix et al. 1992; Dacey et al. 1981; Whalen 2005). Species shown to under-go convective transport include *Phragmites australis* (Armstrong & Armstrong 1991; Grünfeld & Brix 1999; Kaki et al. 2001*), Typha spp*. (Bendix et al. 1994; Kaki et al. 2001; Tornbjerg et al. 1994; Whiting & Chanton 1996), *Nymphaea odorata* (Dacey 1981), and *Eleocharis sphacelata* (Sorrell et al. 1997). For species utilizing diffusive flux, only small variations are observed throughout the day and are attributed to small variations in temperature (Whiting and Chanton 1996; van der Nat et al. 1998). Species utilizing convective flow have been shown to have significant correlations between CH_4 emissions and the time of day or PAR (Chanton et al. 1993; Whiting & Chanton 1996).

Overall, herbivory has been shown to significantly impact vegetation and can alter CH_4 emissions, and our study examines the direct impact of herbivory on vegetation related emissions. Our study is unique as it studies a species of vegetation that to our knowledge has yet to be studied and we include multiple levels of damage to increase our understanding of the effects of herbivory on CH_4 emissions. We examine the effects of herbivory at two levels of damage above the water level, as well as damaging the vegetation below the water level. In addition, we study two vegetation species, one that is well studied; *Typha latifolia*, and another that to our knowledge has yet to be examined; *Sagittaria latifolia*. We hypothesize that herbivory will significantly increase emissions when plants are damaged above the water level, and significantly decrease emissions when plants are damaged below the water level. We also hypothesize significant differences in emissions between the two species examined, as significant research indicates species vary in their ability to transport CH_4.

2.2 Methods

2.2.1 Experimental Design

This experiment was conducted in wetland mesocosms (40 total), constructed from 5-gallon plastic buckets (height: 36.2 cm, diameter: 31.8 cm), in the greenhouse on the Rochester Institute of Technology (RIT) campus, Rochester, NY. Each mesocosm was filled with a homogenized mixture of equal parts soil (collected from an emergent wetland on the south side of the RIT campus, dominated by *Typha* and *Phragmites*) and purchased sand (Quikrete, fine, screened sand) to a height of 20 cm. The mesocosms were then filled with tap water to height of 10 cm above the soil level (water level was kept consistent throughout the entire study). Two emergent, wetland species were analyzed, *Typha latifolia* (broadleaf cattail), collected from a newly constructed wetland near the Millseat landfill, Bergen, NY and *Sagittaria latifolia* (broadleaf arrowhead), purchased from Southern Tier Consulting, Inc., West Clarksville, NY. *Typha latifolia* was chosen due to the fact that it is a well-studied, common wetland species. *Sagittaria latifolia* was chosen as to our knowledge it has yet to be studies and it is commonly found in wetlands in the Rochester, NY area and dominates a research wetland at High Acres Nature Area where RIT research is completed (HANA, A1N). Each mesocosm was planted with 6 individuals of a single species (20 mesocosms per species) in the June of 2019.

One week prior to gas flux measurements, the number of stems and plant heights were recorded. In addition, *S. latifolia* leaf dimensions (length and width at the broadest point) were recorded as a secondary measurement to understand growth. The experimental design consisted of 4 levels of treatment, 1) 30% of the total stems were clipped to 10 cm above the water level, 2) 60% of the total stems were clipped to 10 cm above the water level, 3) 100% of the total stems were clipped below the water level, and 4) control group, where no clipping occurred (Table 2.1). The 20 mesocosm of each of the two species were divided into the 4 levels of treatment by grouping mesocosms with similar numbers of stems, as new stems had grown during the growth period, with the goal of reducing variability within the treatment group. *T. latifolia* heights ranged from 90-135cm and *S. latifolia* heights ranged from 35-75cm, with leaf dimensions of 20x10cm on average. A flux chamber was designed using 3 opaque, 5-gallon buckets, where the bottoms of two were removed and attached to each other using acrylic sealant and heavy-duty tape. A septa hole was drilled at the top of the chamber and a sampling port was attached.

Table 2.1: Experimental design of 4 groups (treatment 1, 2, 3 and control) and the damage level (% of total stems clipped), as well as if the stems were clipped 10 cm above the water level or below the water level (clipping method), the collection time and number of replicates.

Treatment	Damage Level	Clipping method	Flux collection time	Replicates per species
Baseline	NONE	---	Prior to clipping	20
1	30%	Above water level	Immediately after clipping	5
2	60%	Above water level	Immediately after clipping	5
3	100%	Below water level	Immediately after clipping	5
Control	NONE	---	---	5
Recovery	NONE	N/A	One month after clipping	20

2.2.2 Clipping and gas flux measurement

Methane emissions were measured prior to clipping ("baseline flux"), as well as immediately following the clipping of the stems ("clipping flux"). Sampling took place in two events; one in August of 2019 where clipping occurred, and a second in September of 2019 where no additional clipping was completed to understand plant recovery. For each mesocosm, no clipping was completed, the chamber was attached, and a "baseline flux" was collected over 25 mins with a headspace sample collected at 5, 10, 15, 20 and 25 mins after chamber attachment. The chamber was removed, the plants were given time to rest, then the stems were clipped according to the treatment level (Table 2.1, control was not clipped), and the chamber was immediately reattached and a "clipping flux" was collected over 25 mins where headspace samples were collected at 5, 10, 15, 20 and 25 mins after chamber attachment. All clipped biomass was collected and dried to determine clipped biomass. This process was repeated for all 40 mesocosms. After one month, a "recovery flux" was collected, where the chamber was attached, no clipping occurred, and samples were collected in the same manor. At the time of each flux measurement, the greenhouse temperature was recorded.

All samples were run on a gas chromatograph (GC) fitted with an FID detector (Shimadzu GC-2104). We determined the concentration of CH_4 from simultaneously run standards in replicates of 10 at the beginning, middle, and end of each 120-sample run. The estimated CH_4 flux was estimated using a linear regression of sample concentration as a function of time elapsed, where data with an r^2 of 0.80 was omitted. The difference between pre clipping ("baseline flux", no stems damaged) and post clipping ("clipping flux") CH_4 flux rates were analyzed to determine how herbivory impacts CH_4 emissions (%Δ in CH_4 emissions) from

vegetation. In addition, the baseline flux emissions and recovery flux emissions were analyzed to determine the plants abilities to transport CH_4 regardless of herbivory, to examine differences in emissions between the two species, and to understand the recovery ability of the plants.

2.2.3 *Porewater*

Porewater was collected using a piezometer installed in each mesocosm prior to flux measurements. Piezometers were created using 25 cm long PVC tubes (dimeter: 1.9 cm) with mesh bottoms to collect pore water from a depth of 10 cm below the soil surface, and deployed several days before measurements began, to quantify porewater CH_4. A water sample was collected from each mesocosm 24 hours after the flux measurements were collected. The piezometer was flushed of all water, allowed to refill with porewater and then a water sample was taken. The water sample was then shaken with equal parts air for 2 minutes, then a subset of the headspace was transferred to an evacuated vial. The samples were then analyzed for CH_4 concentration on a CG-FID with simultaneously run standards in replicates of 10 at the beginning, middle and end, where final concentration was determined in μmol CH_4.

2.2.4 Vegetation biomass

All clipped biomass was collected, dried, and weighted. After the experiment had concluded in September all aboveground and below ground biomass was collected, dried and weighed as well. Total aboveground biomass was collected by clipping at the soil water interface. Total belowground biomass was collected by washing all roots of soil using a garden hose and a sieve.

2.2.5 Statistical analyses

All statistical analyses were completed using JMP Pro 14 Statistical Software. All data were tested for normality using The Anderson-Darling goodness of fit test and tested for homogeneity of variances using Levene's test. Full-factorial two-way analysis of variance (ANVOA), with mesocosm replicate as a random factor, were used to test for significant effects of treatment (30%, 60% and 100% damage, as well as control if applicable) and species (*T. latifolia* and *S. latifolia*) on percent change in CH_4 flux, unclipped CH_4 fluxes, porewater CH_4

concentrations, and biomass measurements. For all ANOVAs, when significant effects were found, a Tukey's HSD post hoc test was used to identify significant differences.

2.3 Results

2.3.1 Effects of clipping on CH_4 flux

When analyzing the change in emissions from before and after clipping (%Δ in CH_4 flux) in all mesocosms (n=40), there was a significant treatment effect (p=0.001), where the greatest positive changes in emissions were seen in the 60% damage treatment, and significant negative changes in emissions were seen in 100% damage (Figure 2.1, Table 2.3). There was a trend towards a species effect, but it was not significant (p=0.1) and the interaction was not significant (p=0.7).

For *T. latifolia* the highest change in emission was observed in the 60% damage treatment, where emissions were 241% greater on average after clipping when compared to the baseline flux (Figure 2.1, Table 2.2). Additionally, when *T. latifolia* was clipped below the water level (100% damage treatment), the emissions were reduced by 115% on average compared to the baseline flux (Figure 2.1, Table 2.2). At the 30% treatment level, *T. latifolia* emissions increased by 93% on average compared to the control, but the 30% damage treatment and the 60% damage treatment were statistically similar.

For *S. latifolia* the highest emission was also observed in the 60% damage treatment, where emissions were 70% greater on average after clipping compared to the baseline flux (Figure 2.1, Table 2.2). For *S. latifolia*, reduced emissions were also observed for the 100% damage treatment, where emissions were 177% lower when stems were clipped below the water than the baseline flux (Figure 2.1, Table 2.2). Lastly, the 30% damage treatment and 60% damage treatment were statistically similar, where emissions increased by 38% after clipping compared to the control.

Furthermore, for all mesocosm, when stems were clipped above the water, we observed a linear increase throughout the whole 25-minute flux period. Similarly, for all mesocosm clipped below the water, we observed a linear decrease through the whole 25-minute flux period.

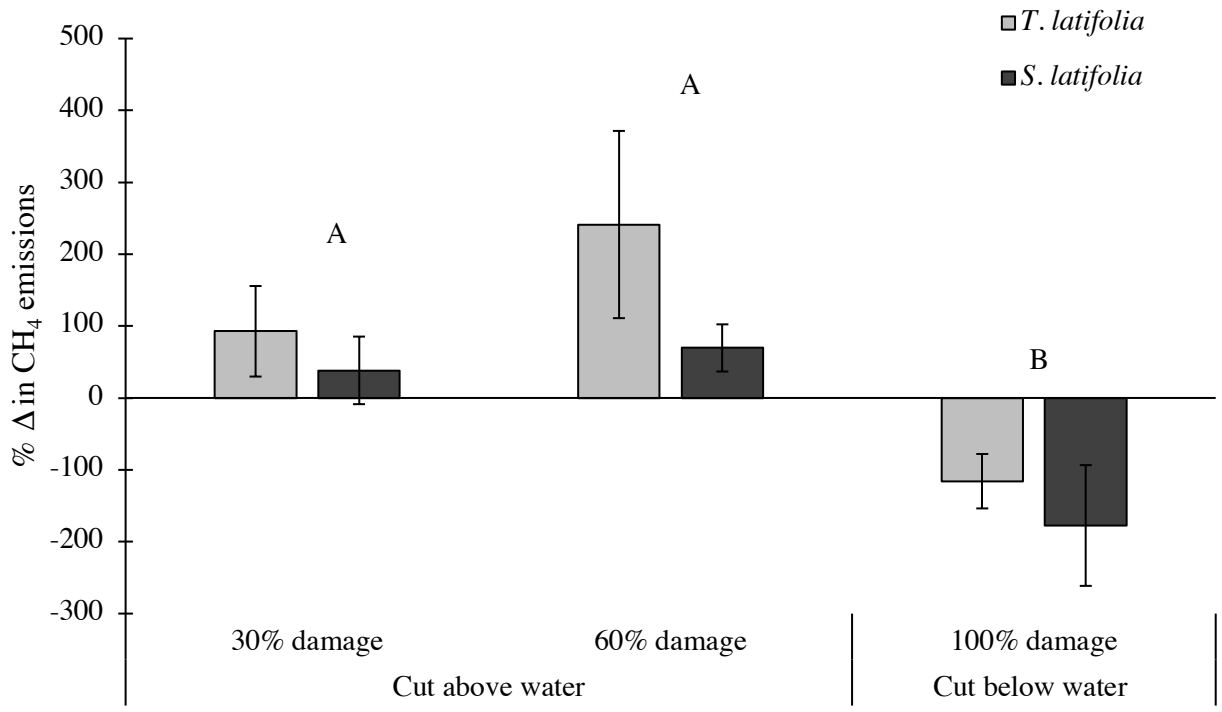


Figure 2.1: %Δ in CH_4 emissions (mg CH_4-C m^{-2} hr^{-1}) from baseline flux (no clipping) to clipping flux at three treatment levels (30% and 60% clipped 10cm above the water level, and 100% clipped below the water level) for broadleaf cattail (*T. latifolia*) and broadleaf arrowhead (*S. latifolia*), avg ± SE, n=5, where letters represent significant differences between treatments.

2.3.2 Baseline and recovery CH_4 flux

When observing CH_4 emissions before clipping (baseline CH_4 flux), significant differences were seen between species, where *S. latifolia* had significantly higher emissions than *T. latifolia* ($p<0.0001$, Figure 2.2, Table 2.3). Baseline flux emissions for *S. latifolia* were observed to be more than 6 times higher than *T. latifolia* (*S. latifolia*: 0.248±0.035 μg CH_4-C m^{-2} hr^{-1}, *T. latifolia*: 0.050+0.016 μg CH_4-C m^{-2} hr^{-1}, Figure 2.2, Table 2.2). No significant treatment effect nor interaction effect was observed on baseline flux comparisons (treatment: $p=0.9$, interaction: $p=0.15$). Per stem CH_4 emissions were 0.008±0.003 and 0.037±0.016 μg CH_4-C m^{-2} hr^{-1} for *T. latifolia* and *S. latifolia*, respectively.

There was also a significant species effect on recovery CH_4 fluxes ($p=0.02$), where *S. latifolia* had significantly higher emissions compared to *T. latifolia* (Figure 2.2). The recovery *S. latifolia* CH_4 flux emissions were observed to be about 2 times higher than *T. latifolia (S. latifolia*: 0.211±0.015 μg CH_4-C m^{-2} hr^{-1}, *T. latifolia*: 0.095+0.046 μg CH_4-C m^{-2} hr^{-1}, Figure 2.2,

Table 2.2). No significant treatment effect nor interaction effect was observed on recovery fluxes (treatment: p=0.45, interaction: p=0.53.14).

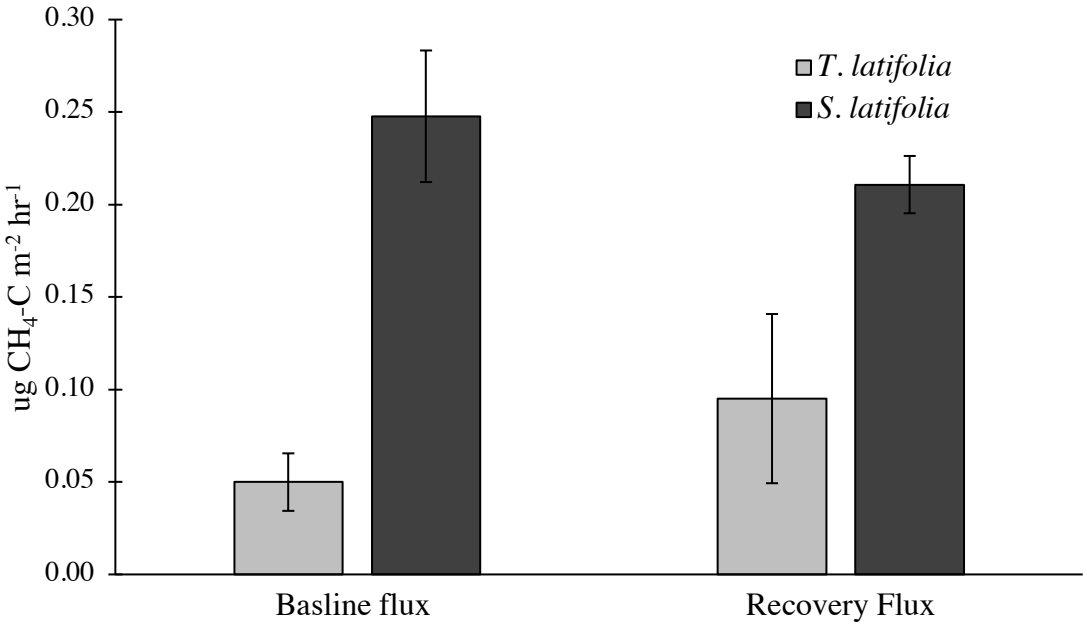


Figure 2.2: Baseline and recovery CH_4 flux emissions for broadleaf cattail (*T. latifolia*) and broadleaf arrowhead (*S. latifolia*), avg $\pm$ SE, n=20, two stars represent p<0.0001 between species, one star indicates p<0.5.

2.3.2 Porewater

Porewater CH_4 was found to significantly differ by species (p=0.008), where on average *S. latifolia* had significantly higher porewater CH_4 concentrations when compared to *T. latifolia* (Figure 2.3). No treatment effect was found (p=0.7), although there was a trend towards an interaction effect between species and treatment (p=0.1, Table 2.3). Porewater CH_4 concentrations ranged from 0.009-0.209 mM CH_4 for *T. latifolia* and from 0.038-0.308 mM CH_4 for *S. latifolia*. When analyzing the species separately, treatment was not a significant effect for *S. latifolia* (p=0.57), but was a significant effect for *T. latifolia*, where the porewater concentration was significantly higher when no clipping occurred (p=0.06, Table 2.3).

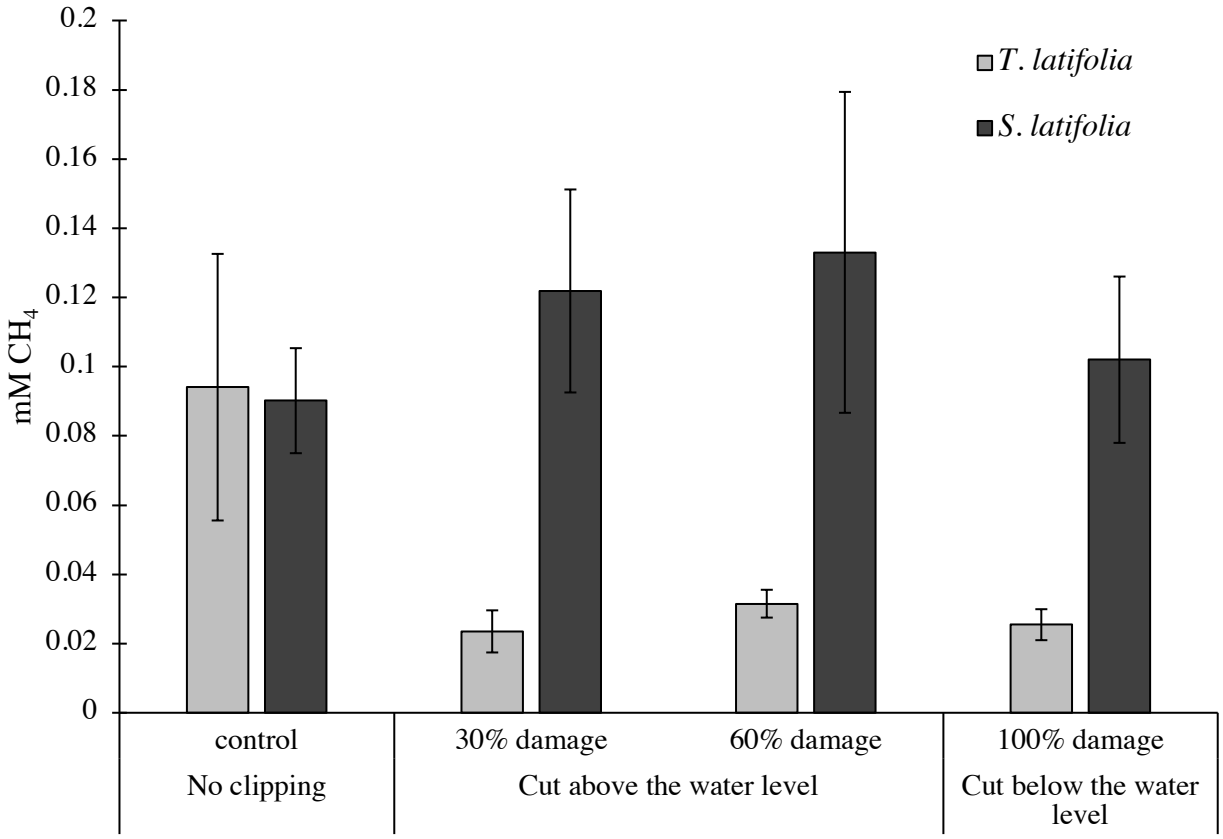


Figure 2.3: Porewater CH_4 at 10cm below the soil in control mesocosms, as well as after clipping in the treatment mesocosms (30% and 60% damage above the water level, and the 100% damage below the water level) for broadleaf cattail (*T. latifolia*) and broadleaf arrowhead (*S. latifolia*), avg $\pm$ SE, n=5. Treatment was not a significant effect (p=0.7), species was significantly different (p=0.008), and the interaction trended towards being a significant effect (p=0.16).

2.3.3. Biomass and stem count

As expected, clipped biomass was affected by treatment, where the weight of dried biomass was significantly greater in the 100% damage treatment, and lowest in 30% damage treatment ($p<0.0001$, Table 2.3, Appendix Table iii). The average dry weight of biomass clipped for *T. latifolia* was 5.28 g, 9.35 g, and 11.82 g for treatment 1, 2, and 3 respectively, and for *S. latifolia* was 1.82 g, 4.45 g, and 6.83 g, for treatment 1, 2, and 3, respectively. Clipped biomass also varied by species, where more biomass by dry weight was removed from *T. latifolia* mesocosms, but was not affected by the interaction (species: $p<0.0001$, interaction: $p=0.6435$, Table 2.3).

Aboveground biomass (collected one month after clipping) was affected by treatment, species and the interaction, where the highest aboveground biomass by dry weight was observed

in *T. latifolia* control mesocosms and the lowest in the *T. latifolia* 100% damage mesocosms (treatment, species and interaction: $p<0.0001$, Table 2.3). Overall, the control mesocosms had the highest aboveground biomass and the 100% damage had the least. No significant effects were observed for belowground biomass (Table 2.3).

Additionally, we found a correlation between the absolute percent change in CH_4 emissions and clipped biomass by dry weight, where with increased biomass removed, the absolute change in flux increased as well (*T. latifolia*: $p=0.007$, $r^2=0.36$, *S. latifolia*: $p=0.035$, $r^2=0.23$). *T. latifolia* recovery CH_4 emissions were correlated with aboveground biomass ($p=0.02$, $r^2=0.26$) and belowground ($p=0.02$, $r^2=0.27$), but not stem count ($p=0.86$, $r^2=0.001$). *S. latifolia* recovery CH_4 emissions were correlated with belowground biomass ($p=0.002$, $r^2=0.43$), trended towards being correlated with aboveground biomass ($p=0.07$, $r^2=0.17$), but not stem count ($p=0.72$, $r^2=0.007$). Furthermore, above- and belowground biomass were strongly correlated with stem count at the time of biomass collection for *T. latifolia* (aboveground: $p<0.0001$, $r^2=0.8$, belowground: $p<0.001$, $r^2=0.6$,), but not *S. latifolia*: (aboveground: $p=0.37$, $r^2=0.2$, belowground: $p=0.12$, $r^2=0.1$). For this reason, we did not estimate biomass at the time of the clipping experiment, but do note that *T. latifolia* had higher biomass than *S. latifolia*.

Table 2.2: CH_4 flux ($\mu g\ CH_4\ m^{-2}\ hr^{-1}$) prior to clipping (baseline) and after clipping in the three levels of treatment (30%, 60% and 100% damage) for each species, as well as the % change from baseline flux to treatment flux for each species (%Δ in CH_4), average ± SE.

CH_4 flux ($\mu g\ CH_4\ m^{-2}\ hr^{-1}$)					
	Control	Treatment			Final
	No clipping	Cut above water level		Cut below water level	No clipping
	Baseline	30%	60%	100%	Recovery
T. latifolia	0.050 ± 0.015	0.027 ± 0.012	0.130 ± 0.036	0.006 ± 0.030	0.095 ± 0.046
S. latifolia	0.320 ± 0.080	0.399 ± 0.089	0.405 ± 0.134	- 0.081 ± 0.146	0.211 ± 0.015
%Δ in CH_4 flux from baseline to treatment					
		Cut above water level		Cut below water level	
		30%	60%	100%	
T. latifolia		92.75 ± 63.0	241.3 ± 130	- 115.8 ± 37.9	
S. latifolia		38.37 ± 47.0	69.60 ± 32.8	- 177.4 ± 84.0	

Table 2.3: Results of one- and two-way ANOVA examining the effects of treatment (30% damage, 60% damage, 100% damage), species (*T. Latifolia* and *S. latifolia*) and the interaction, with replicate as a random variable. Significant p-values are bolded (*p<0.0001).

Factor	Treatment		Species		Treatment x Species	
	F	p	F	p	F	p
%Δ in CH_4 flux						
	$F_{1,30}$=8.61	**0.0016**	$F_{1,30}$=2.55	0.1241	$F_{1,30}$=0.41	0.6690
Baseline CH_4 flux						
	$F_{1,40}$=0.20	0.8933	$F_{1,40}$=24.8	**<0.0001***	$F_{1,40}$=1.91	0.1489
Recovery emissions						
	$F_{1,40}$=0.90	0.4537	$F_{1,40}$=5.58	**0.0244**	$F_{1,40}$=0.75	0.5314
Porewater CH_4						
All mesocosms	$F_{1,40}$=0.45	0.7219	$F_{1,40}$=13.8	**0.008**	$F_{1,40}$= 1.81	0.1655
T. latifolia only	$F_{1,20}$=2.94	**0.0652**	---	---	---	---
S. latifolia only	$F_{1,20}$=0.58	0.5684	---	---	---	---
Biomass						
Clipped biomass	$F_{2,29}$=21.0	**<0.0001***	$F_{1,29}$=38.0	**<0.0001***	$F_{2,29}$=0.45	0.6435
Aboveground biomass	$F_{3,40}$=29.4	**<0.0001***	$F_{1,40}$=35.7	**<0.0001***	$F_{3,40}$=10.2	**<0.0001***
Belowground biomass	$F_{3,40}$=1.50	0.2342	$F_{1,40}$=0.11	0.7485	$F_{3,40}$=1.22	0.3182

2.4 Discussion

The results of our study supported our hypothesis that herbivory leads to significant CH_4 emissions when stems are clipped above the water, as well as decreased emissions when stems are clipped below the water. We observed that when stems were clipped above the water level, emissions increased by as much as 241% or 70% on average for *T. latifolia* and *S. latifolia,* respectively. We also observed that when stems were clipped below the water level emissions decreased by 115% and 177% for *T. latifolia* and *S. latifolia,* respectively.

Our results suggest that herbivory could have significant positive effects on CH_4 emissions if stems were cut above the water level, where herbivores are often observed grazing stems above the water level. Many studies have found similar results where after stems are clipped above the water level CH_4 emissions increase by 5-350% (Ding et al. 2005; Kelker et al. 1997; Petruzzella et al. 2015; Rietl et al. 2017). Each study included simulating herbivory by clipping stems above the water level and each found significant increases in emissions, where emissions increased by 36% from *Carex lasicarpa*, *Carex meyeriana* and *Deyeuxia augustifoli* by only approximately 5% (Ding et al. 2005), *Carex aquatilis* and *Carex rostrate* by 26% (Kelker & Chanton 1997), and *Eleocharis equisetoides* by 350% (Petruzzella et al. 2015).

Furthermore, we see support for the slight correlation we observed in our study between the level of damage (30% and 60% damage treatments) and the change in CH_4 emissions after clipping, where Petruzzella et al. (2015) observed that as stem damage increases, emissions increase as well. Our results contradict two studies that found that CH_4 fluxes were significantly lower in clipped plots compared to unclipped plots (Falk et al. 2014; Noyce et al. 2014) although, we suggest our results are not comparable to these studies as they took place over multiple years. Instead, the combination of these results may suggest that clipping causes an immediate increase in emissions (captured by our experiment as well as Ding et al. 2005; Kelker et al. 1997; Petruzzella et al. 2015; Rietl et al. 2017), and after stems are damaged emissions will decrease over time. This idea is supported by Kelker & Chanton (1997) who observed that emissions returned to a level similar to that observed before clipping after only 24 hours after clipping had occurred. Additionally, Greenup et al. (2000) found significant correlations between CH_4 emissions and the number of green stems present, as well as a weak correlation between emissions and the number of dead stems present. This idea could suggest that living vegetation plays the largest role in CH_4 flux, and that dead vegetation may still contribute, but to a lesser extent. Although, Dingemans et al. (2011) suggested that plant related emissions from dead vegetation are reduced as senesced stems often fill with water, preventing most CH_4 plant-mediated transport.

Our experiment suggests that when stems are clipped below the water level, CH_4 emissions are significantly reduced by as much as 180%. This result suggests that in our simulated mesocosm, the CH_4 emissions are dominated by plant-mediated transport, and therefore when the plant no longer has access to the atmosphere emissions significantly decrease. Similar trends were observed in previous studies that clipped plants below the water level, where emissions were observed to decrease by 56-86% (Ding et al. 2005; Greenup et al. 2000; Kelker & Chanton 1997). Ding et al. (2005) observed a 73-86% decrease after *C. lasiocarpa and C. meyeriana* were clipped below the water level and Greenup et al. (2000) observed a decrease in emissions of 56% when *Eriophorum vaginatum* was clipped below the water. Additionally, Kelker & Chanton (1997) found emissions were 3% or less of their control value (in-tact vegetation) after *C. aquatilis* and *C. rostrate* were clipped below the water level. Additionally, studies that removed vegetation all together found similar trends, where CH_4 emissions decreased by 84-300% after vegetation was removed (Falk et al. 2014; Shultz et al. 2018;

Whalen 2005). This result further indicates the significant influence of plant-mediated transport on wetland systems.

Our study also found significant differences in emissions between the two species examined; *S. latifolia* and *T. latifolia*. Specifically, significant differences were observed prior to clipping, where *S. latifolia* had emissions that were 6 times that of *T. latifolia*. Additionally, we observed the recovery emissions from *S.* latifolia were 2 times higher than *T. latifolia* despite *S. latifolia* having significantly lower aboveground biomass at the time of the recovery flux collection. This is particularly interesting as no study to our knowledge has estimated the CH_4 flux of *S. latifolia*. Many studies have shown significant differences between various plant species (Bergstrom et al. 2006; Bhullar et al. 2013; Bhullar et al. 2014; Chanton 2005; Davidson et al. 2016; Ding et al. 2005; Greenup et al. 2000; Joabsson et al. 2001; Kao-Kniffin et al. 2010; Rietl et al. 2017; van der Nat et al. 1998). For example, Ding et al. (2005) found that per stem emissions from *Carex* spp. were nearly double those from *D. angustifolia,* and Rietl et al. (2017) found that emissions from *Panicum hemitomon* were 56% higher than all other species analyzed (S*agittaria lancifolia*, *Echinochloa walteri*, and *Eleocharis macrostachya*). In addition to species level studies, some studies suggested that differences can be attributed to the vegetation functional group, where tussocks have been shown to mediate more CH_4 compared to graminoids, which mediated more than forbs (Bhullar et al. 2013; Kao-Kniffin et al. 2010). This is likely due to the way in which the plant transports CH_4 and how open or restricted their aerenchyma are to the movement of gases. Due to limited research on *Sagittaria* spp. we suggest more research is completed to help understand why we observed a large difference in emissions from our two species.

Previous studies have indicated that CH_4 emissions from vegetation can be driven by GPP and/or PAR, driven by the outflow of O_2 from plant roots that creates an inflow of CH_4 into the plant culm (Brix et al. 1996; Dacey et al. 1981; Garnet et al. 2005; Grünfeld & Brix 1999; Sebacher et al. 1985). Plants observed to have CH_4 emissions correlated with GPP or PAR include Phragmites (Brix et al. 1996; Grünfeld & Brix 1999), *Carex stans*, *Dupontia psilosantha* and *Eriophorum scheuchzeri* (Falk et al. 2014), *Peltandra virginica*, *Orontium aquaticum, Juncus effusus*, and *Taxodium distichum* (Garnet et al. 2005). Although previous research has shown that emissions from *Typha* spp. (Chanton et al. 1993; Whiting & Chanton 1996) and *Sagittaria* spp. (Sebacher et al. 1985; Harden & Chanton 1994) are independent of PAR. Rather

Typha has shown to be correlated with time of day, where emissions were observed to peak in the afternoon, instead of being correlated with PAR (Chanton et al. 1993; Whiting & Chanton 1996). Additionally, *Sagittaria lancifolia* was observed to release CH_4 from the plant stem, versus the leaves (Harden & Chanton 1993), supporting the fact that emissions are not driven by GPP or PAR which is typically associated with CH_4 leaving through open stomata (Garnet et al. 2005; Greenup et al. 2000). This suggests that if herbivores are removing leaves as they graze, significant increases in CH_4 emissions could occur, as openings are reviled in the plant stem. Moreover, we believe this information supports our use of opaque chambers, instead of transparent chambers allowing in light. Although our use of opaque chambers means that light intensity was not a variable in our study, and we did not manipulate sampling time and therefore cannot make conclusions about the impact of time of day on CH_4 emissions.

Our experiment also found significant differences in porewater concentrations between the two species, despite similar belowground biomass. Kelker & Chanton (1997) suggested that belowground conditions regulate plant-mediated transport by altering the amount of CH_4 in the porewater than can be emitted. Higher belowground biomass would likely lead to more CH_4 oxidation, although we see no difference in belowground biomass, yet a significant difference in porewater, where porewater CH_4 was significantly higher in *S. latifolia* mesocosms on average, compared to *T. latifolia* mesocosms. Additionally, Greenup et al. (2000) suggest that more belowground biomass leads to more root surface area, which will increase the amount of CH_4 moving in the plant stem, although our results do not support this idea. We also see *T. latifolia* has significantly more aboveground biomass, on average, and therefore aboveground biomass is likely not driving this observation. Therefore, we suggest that potentially, *S. latifolia* may positively impact CH_4 production to a greater extent, leading to higher porewater concentrations and therefore higher plant-mediated transport (Greenup et al. 2000).

Our findings also show that herbivory has a significant impact on vegetation health, as aboveground biomass significantly differed by the clipping treatment. Aboveground biomass differed by species, but for both species, the control mesocosm (that received no clipping one month prior) had the highest aboveground biomass, on average. The mesocosms receiving 100% damage treatment (where all stems were clipped below the water level one month prior), had the lowest aboveground biomass after the recovery period, on average, over 9 times less than the control mesocosms for *S. latifolia* and half of the control average for *T. latifolia*. Our results

suggest, that herbivory may have a significant impact on vegetation cover, and depending on the level of damage inflicted through grazing, may reduce plant-mediated CH_4 emissions in the long term if vegetation cover is reduced over time.

Lastly, the range of emissions observed in this study when stems were clipped above water was 0.000006 to 0.0009 mg CH_4 m^{-2} hr^{-1}. Our observations were substantially lower than many studies (3.5 to 43 mg CH_4 m^{-2} hr^{-1}; Ding et al. 2005; Falk et al. 2014), which we believe is due to our low stem count (*S. latifolia*: n=5-24, *T. latifolia*: n=6) and the low organic matter mixture of sand and soil used in the mesocosms. For example, Ding et al. (2005), had stem densities of approximately 1000-2000 and observed emissions significantly higher than ours when stems were clipped above the water level. Similarly, our average below water clipping emissions (-0.00004 mg CH_4 m^{-2} hr^{-1}) and average baseline fluxes (0.0001 mg CH_4 m^{-2} hr^{-1}) were significantly lower than other values seen in the literature. Limited soil quantity (14300 L soil per mesocosm) and no added nutrients throughout the experiment (such as soil amendments) likely resulted in lower substrate availability for methanogens as its been shown the nutrient concentration of soil impact soil CH_4 production and emissions (Rietl et al. 2017). Our results also do not isolate plant-mediated emissions, and therefore may also reflect changes in diffusive and ebullitive flux. More research should be completed, specifically with more replicates per treatment to reduce variability, as well as in light and dark conditions and with manipulated measurement times, to further examine the effect of PAR and time of day on these species.

Chapter 3: Conclusions

Our study showed that herbivory has a significant impact on CH_4 emissions in wetland ecosystems by altering vegetation, which has a strong ability to transport CH_4 (Dacey 1981; Whalen 2005). From *in situ* flux measurements, we observed that ungrazed plots had 1-4 times the CH_4 emissions compared to grazed plots. This appears to be driven by the differences in vegetation cover between the plots, where ungrazed plots had nearly double the emergent vegetation cover and a significant relationship was found between total vegetation cover and emissions. Our clipping experiment demonstrated that plant-mediated transport is the dominant transport pathway for vegetated mesocosms, where we observed a >100% decrease in emissions when stems were clipped below the water level, this suggests that plant-mediated transport may dominate *in situ* emissions as well. This result is consistent with other studies that found plant-mediated transport is the dominant pathway of CH_4 emissions in wetlands (Chanton et al. 1989; Kelker & Chanton 1997; Sebacher et al. 1985). Furthermore, mesocosms with both species emitted CH_4, even given the extremely low stem count and *in vitro* conditions. Additionally, significant differences in species, where *S. latifolia* was observed to emit 2-6 times as much CH_4 as *T. latifolia* when no clipping occurred, could explain variation between *in situ* plots. Vegetation at HANA is not controlled and therefore species composition varies across the wetland and between plots, although significant the cover of *S. latifolia* is observed in caged and uncaged plots in 2019. Additional research should be completed to determine which species at HANA may contribute the most to CH_4 emissions.

Furthermore, we observed significant increases in CH_4 emissions after clipping above the water level, where emissions increased by 70-240% over the course of our 25 minute flux period. We suggest that *in situ* emissions were captured after a majority of the CH_4 had been released from the plant and potentially the porewater supply as well. This is supported by the trend towards lower porewater CH_4 concentrations in grazed plots and decreased porewater CH_4 concentrations after clipping observed for *T.* latifolia. For example, when a stem is grazed, the plant emits CH_4 efficiently, immediately, but after some time all the built-up CH_4 within the culm of the plant and the porewater surrounding the plant roots are depleted and therefore the plant will return to a similar rate of emissions as before being grazed, or the emission rate will decrease due to a reduced supply of CH_4 available to transport. Furthermore, the *in situ* herbivore exclusion plots were established in 2014 (Lodge 2017), suggesting our results show long term

effects of herbivory over the course of the 5-year study, where our clipping experiment shows the immediate impact of a single grazing event.

We also conclude that CH_4 production and CH_4 oxidation may have a significant impact on emissions, but not to the extent of plant-mediated transport. No notable differences were observed in production or oxidation rates in incubations, suggesting that the activity of microbial communities captured under laboratory conditions are not impacted by herbivory, however, dynamic feedbacks between plants and microbes in the field could result in changes in production or oxidation not captured in incubations. Both vegetation and herbivory may alter O_2 transport to the sediment, positively impacting oxidation. Vegetation can also impact substrate availability for CH_4 production, which may have contributed to significant species effects on CH_4 emissions from the clipping experiment as well as differences in CH_4 fluxes in caged vs uncaged plots.

Overall, our study shows that herbivory has significant effects on CH_4 emissions, where they have the ability to immediately increase emissions, but over the course of multiple years leads to significant decreases in CH_4 emission. The clipping experiment revealed emissions can increase by as much as 240% after stems are clipped above the water level, but *in situ* results show emissions are 1-4 times lower in grazed plots 4 and 5 years into the herbivore exclusion study. Additionally, we found that our wetland was a significant source of carbon, and that herbivore exclusion plots elevated carbon emissions. Although there are significant benefits of herbivore exclusion such as increasing vegetation cover, improving biodiversity, and improving soil quality (Lodge 2017; Spangler 2019), there are significant negative tradeoffs when it comes to CH_4 emissions. For this reason, we suggest that land managers implement herbivore exclusion in created wetlands in short periods of time when needed to restore ecosystem function, but advise land managers of healthy wetlands not to implement these plots to avoid unnecessary CH_4 emission increases.

APPENDIX: **Supplementary Results**

Table i: Average total cover per species present in caged (top) and uncaged (bottom) plots in spring and summer of 2018 and 2019. Data from 2018 courtesy of Spangler 2019 and Squier 2020.

		2018		2019	
Caged Species	**Veg. Group**	**Spring**	**Summer**	**Spring**	**Summer**
Sagittaria sagittifolia	emergent	21.25 ± 6.6	43.1 + 8.2	10.3 ± 2.9	1.90 ± 1.3
Sagittaria latifolia	emergent	0	2.50 + 0.9	8.50 ± 3.4	43.8 ± 8.3
Pontederia cordata	emergent	5.25 ± 2.5	4.38 + 4.4	2.00 ± 1.9	3.13 ± 3.1
Typha latifolia	emergent	1.25 ± 1.3	3.13 ± 3.1	0.75 + 0.8	1.88 + 1.9
Leersia oryzoides	emergent	17.5 ± 12.1	21.8 ± 9.4	3.88 ± 3.7	3.13 ± 3.1
Eleocharis obtusa	emergent	0	0	4.50 ± 2.3	1.25 ± 0.8
Polygonum spp.	emergent	0	0	1.63 ± 1.1	3.38 ± 2.1
Lythrum salicaria	emergent	0.63 ± 0.6	0.88 ± 0.9	0.25 ± 0.3	0.63 ± 0.6
Alisma plantago-aquatica	emergent	3.25 ± 1.6	1.88 ± 1.3	1.38 ± 1.2	1.50 ± 1.2
Lemna minor	floating	3.50 ± 1.5	2.13 ± 1.0	3.25 ± 2.1	28.1 ± 8.7
Nymphaea odorata	floating	0.38 ± 0.3	2.13 ± 1.2	5.13 ± 2.5	5.00 ± 2.1
Potamogeton pusillus	submerged	2.13 ± 0.9	1.50 ± 1.2	3.75 ± 2.5	5.63 ± 2.7
Ceratophyllum demersum	submerged	11.9 ± 6.0	10.0 ± 3.7	0	2.50 ± 1.9
Elodea canadensis	submerged	1.87 ± 1.9	6.88 ± 2.8	12.5 ± 12	10.6 ± 8.7

		2018		2019	
Uncaged Species	**Veg. Group**	**Spring**	**Summer**	**Spring**	**Summer**
Sagittaria sagittifolia	emergent	10.9 ± 1.8	40.0 ± 7.7	2.25 ± 0.8	0.63 ± 0.6
Sagittaria latifolia	emergent	0	0	3.25 ± 3.1	12.5 ± 3.1
Pontederia cordata	emergent	5.25 ± 5.0	6.90 ± 6.2	10.9 ± 11	13.8 ± 11
Lemma minor	floating	0.75 ± 0.5	1.25 ± 0.7	1.00 ± 0.7	6.75 ± 2.1
Nymphaea odorata	floating	7.38 ± 2.5	15.6 ± 8.7	21.1 ± 11	37.1 ± 10
Potamogeton pusillus	submerged	3.13 ± 1.9	2.00 ± 0.9	3.13 ± 2.1	11.9 ± 5.7
Ceratophyllum demersum	submerged	4.38 ± 2.6	9.38 ± 2.7	0	10.0 ± 5.9
Elodea canadensis	submerged	0	0.63 ± 0.6	12.5 ± 9.3	4.38 ± 3.1

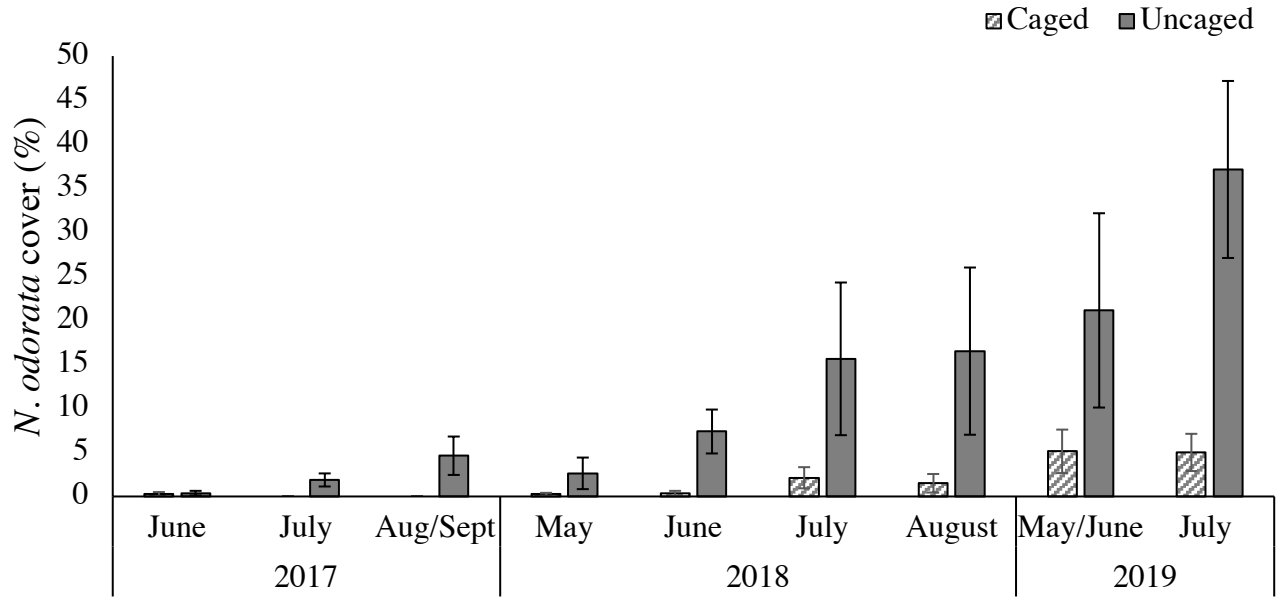


Figure i: Average White water lily (*Nymphaea odorata*) percent cover in caged and uncaged plots in the growing season from June 2017 to July 2019, avg ± SE, n=8. Data from 2017-2018 from Spangler 2019.

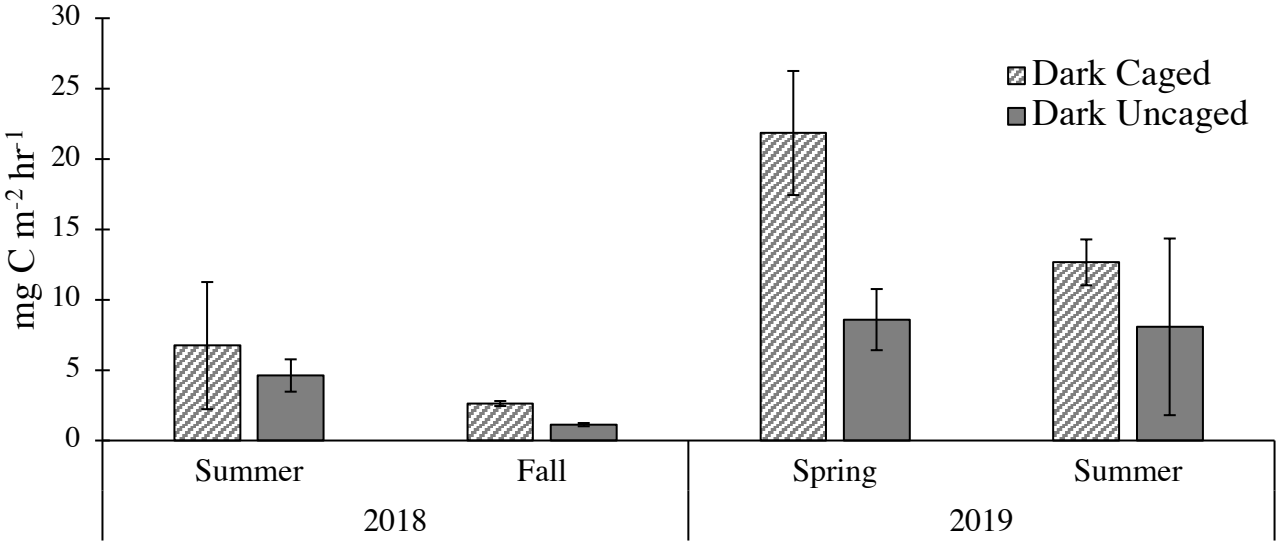


Figure ii: *In situ* CH_4 emissions in dark conditions in caged and uncaged plots from 2018 to 2019, avg ± SE (n=3-8). There was no significant effects of date or treatment. Fall 2019 samples in dark conditions were not able to be run due to COVID-19, but were samples.

Table ii: Average temperature (°C) and light response (μmol) during spring, summer, and fall chamber flux measurements in 2018 and 2019 outside of the chamber and within the chamber, avg± SE.

Factor	Average Temperature (°C)		Average Light Response (μmol)	
	In chamber	Out chamber	In chamber	Out chamber
2018				
Summer	30.9±0.6	NA	1690±50	696±42
Fall	25.6±1.2	NA	1525±52	545±35
2019				
Spring	29.6±1.2	30.1±0.9	906±136	1473±63
Summer	31.8±0.8	33.2±0.7	1370±108	1456±78
Fall	19.9±1.2	20.0±1.9	2827±151	4211±473

Table iii: Biomass (g dry weight) of clipped biomass removed prior to clipping flux, and above- and belowground biomass collected after the recovery flux, average ± SE

	Clipped	Aboveground	Belowground
T. latifolia			
Control	---	25.9 ± 2.57	57.2 ± 5.23
30% damage	5.28 ± 0.76	26.0 ± 1.25	48.5 ± 7.00
60% damage	9.35 ± 0.46	23.2 ± 1.38	40.7 ± 4.07
100% damage	11.8 ± 0.93	2.77 ± 1.48	8.45 ± 2.53
S. latifolia			
Control	---	14.1 ± 2.66	30.4 ± 4.23
30% damage	1.82 ± 0.17	11.6 ± 1.05	21.8 ± 3.42
60% damage	4.45 ± 0.94	12.6 ± 2.07	17.3 ± 1.96
100% damage	6.83 ± 1.32	7.57 ± 0.72	27.6 ± 12.0

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