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Greenhouse Gas Fluxes from Created Wetlands: How Management Techniques Impact Emissions and Implications for Climate Change

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Greenhouse Gas Fluxes from Created Wetlands: How Management Techniques Impact Emissions and Implications for Climate Change

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TABLES AND FIGURES

ABSTRACT

Wetlands are one of the most valuable ecosystems, providing services such as carbon sequestration and nitrogen removal. Studies suggest that created wetlands may not function the same as natural wetlands and management techniques, such as organic matter addition (OM), have been proposed to promote natural functions. The objective of this study was to understand the effects of OM additions on greenhouse gas (GHG) emissions in created wetlands with different vegetation, hydrology and soil characteristics. This study was conducted from 2016 to 2017 at two created wetlands (A2S and A3A) at High Acres Nature Area in Fairport, New York. There was high seasonal and inter-annual variability in weather conditions during the study period and rainfall and temperature were the dominant factors controlling GHG fluxes within both wetlands. Drought condition during 2016 limited soil respiration and C uptake by plants. In 2017, when moisture conditions were more typical, OM addition increased soil respiration rates at A2S in the fall. There was a trend towards higher ecosystem respiration at this time; however, OM addition also increased gross primary production, resulting in no net change in CO_2 exchange. Due to dry conditions, methane (CH_4) emissions were low during much of the study. When emissions were high, fluxes were significantly higher in the light than the dark at A2S, but not A3A, suggesting that vegetation differences between the site impact CH_4 transport pathways. While OM addition did not change anaerobic CH_4 or CO_2 production potential, there were significant differences between the sites, with higher production rates in A2S, where hydrologic conditions in the field may have selected for microbial communities adapted to anaerobic environments. These findings highlight the importance of precipitation and hydrology in controlling C cycling in created wetlands and suggest that wetland characteristics will influence their responses to management techniques.

1. Introduction

Wetlands are a transitional habitat between terrestrial and aquatic ecosystems that provide important ecological services. Wetlands help preserve biodiversity by providing habitat for many species (Ghermandi et al., 2010) and enhance water quality by removing nutrients (Ghermandi et al., 2010; Vymazal, 2007; Brix et al., 2000; Reddy et al., 1999; White et al., 1999). They also play a significant role in the global carbon (C) cycle and although wetlands compromise only 5-8% of the terrestrial land surface they store over one third of the earth's carbon. In addition to sequestering C, wetlands are also the largest natural source of the greenhouse gas (GHG) methane (CH_4), producing 20-40% of global emissions (Solomon, 2007). The global warming potential of CH_4 is 25 times greater than carbon dioxide (CO_2) over a 100-year time horizon (Lowe & Zealand, 2007), therefore CH_4 production in wetlands often makes them a GHG source, despite considerable C fixation and storage in soils and plants.

Wetlands can have a net positive or negative global warming potential, depending on interactions between biotic and abiotic components, especially soil, vegetation, and hydrology. Photosynthesis and respiration are key processes determining the C balance of wetlands (Kayranli et al., 2010; Whalen, 2005; Boon & Lee, 1997). Net ecosystem exchange (NEE) reflects the net amount of C that is fixed and stored and is the difference between gross primary production (GPP), the total uptake of CO_2 by photosynthesis, and ecosystem respiration (ER), the CO_2 that is respired back to the atmosphere by both heterotrophic and autotrophic organisms. Under anaerobic conditions, C fixed by plants can be converted to CH_4 by methanogens, microorganism in the *Archaea* domain (García et al., 2000).

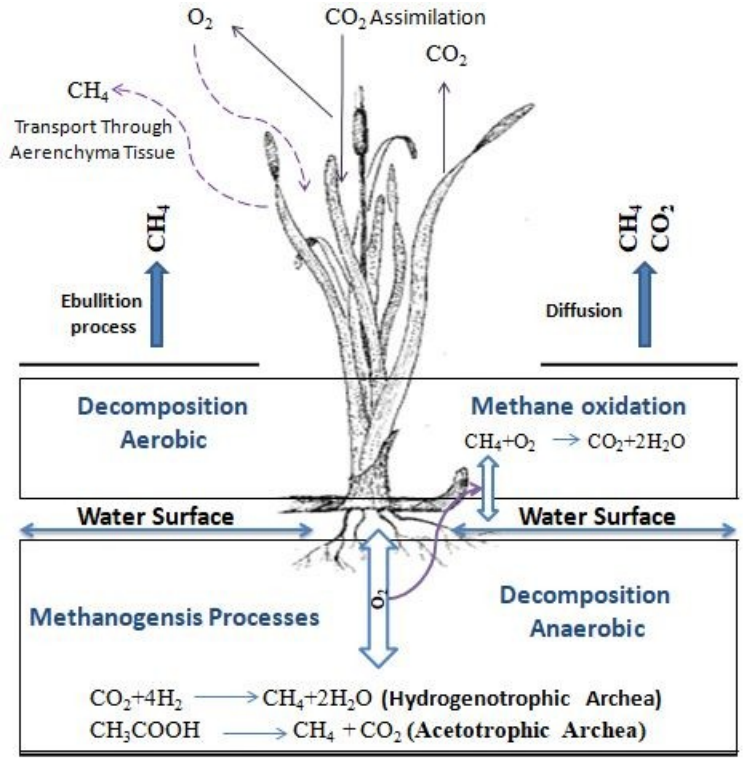

Figure 1. Wetland C cycle and GHG production, consumption and emission.

Anaerobic conditions slow decomposition and enhance the ability of wetlands to store C in their soils, however, they also enable CH_4 production and more than 15% of the C fixed in wetlands through photosynthesis may be released to the atmosphere as CH_4 (Brix et al., 2001). The balance between CH_4 and CO_2 emission will determine whether a given wetland has a positive or negative global warming potential (Figure 1).

GHG fluxes in wetland ecosystems are sensitive to environmental conditions including hydrology, temperature, and soil chemistry. Hydrology exerts overarching control over wetland C cycling, with inundated conditions generally enhancing CH_4 production (Olefeldt et al., 2017; Bansal et al., 2016; Whalen, 2005; Bubier & Crill, 2003; Griffis et al., 2001), while a drop in the standing water can result in an increase in CH_4 oxidation and shift in decomposition towards aerobic processes that yield CO_2 (Olefeldt et al., 2017; Hou et al., 2013; Sulman et al., 2010; Ise et al., 2008; Whalen, 2005). Temperature positively affects both microbial activities (e.g. methanogenesis) and the rate of C fixation by plants (Klein & Werf, 2014; Liikanen et al., 2006; Søvik et al., 2006; Whalen, 2005; Whiting & Chanton, 1993). This means that considering seasonal temperature patterns is important when studying GHG emissions in wetlands.

Nutrient availability affects wetland GHG fluxes by influencing plant and microbial communities. Primary production in wetlands is often limited by N availability (LeBauer & Treseder, 2008; Reddy & DeLaune, 2008; Güsewell & Koerselman, 2002; Vitousek & Howarth, 1991), however, phosphorus (P) limitation (Zhang et al., 2012) or N and P co-limitation (Elser et al., 2007) have also been observed. Nutrient availability can affect wetland CH_4 emissions indirectly by altering vegetation (Liu & Greaver, 2009), which provide C substrate and CH_4 transport, and directly by affecting the activity of methanogenic and methanotrophic microbial communities (Kim et al., 2015; Bodelier, 2011; Nesbit & Breitenbeck 1992). Because N and P availability have been found to both negatively (Kim et al., 2015; Bodelier, 2011) and positively (Liu & Greaver, 2009) impact CH_4 production and consumption it can be difficult to predict how nutrient availability will impact net CH_4 emissions in a particular wetland.

Globally, wetlands are being filled and destroyed by anthropogenic activities and to counteract these losses there are ongoing efforts to restore and construct wetlands to preserve their valuable ecosystem services (Vymazal, 2007). For example, in the United States the Wetland No Net Loss Act of 1989 requires wetland creation to mitigate human impacts on natural systems. However, ecosystem properties between these created wetlands and natural

systems may not be equivalent and this may result in differences in GHG emission. Multiple studies have shown that created wetlands do not duplicate many of the biotic and abiotic characteristics of natural wetland systems, especially vegetation and soil features. Created wetlands are often characterized by low species richness and dominance by invasive plant species such as *Typha* spp. and *Phragmites* spp. (Svitok et al. 2011; Hartzell et al., 2007; Vymazal & Kropfelová 2005; Balcombe et al. 2005). Soils in created wetlands also tend to have lower soil organic matter (SOM) content than natural wetlands (Hossler & Bouchard, 2010; Bantilan-smith et al., 2009; Bruland & Richardson, 2005; Campbell et al. 2002; Stolt et al., 2000) and research suggests that it could take up to 300 years for created wetlands to replicate the ability of natural systems to sequester soil organic carbon (SOC) (Hossler & Bouchard, 2010).

While many studies have found that plant and soil properties in created wetlands differ from natural systems, it is less clear how these differences translate into changes in GHG emissions. For example, Maltais-Landry et al. (2009) found that created wetlands produce 2 to 10 times more CH_4 than natural systems. However, Nahlik & Mitsch (2010) found that CH_4 emissions from created wetlands were lower than emissions from natural systems. This raises a question about the balance between GHG production and C sequestration in created wetlands. In their review of C storage and fluxes in freshwater wetlands, Kayranli et al. (2010) argued that constructed wetlands could become a source of GHG's and concluded that further research is required. Additional measurements of GHG fluxes in created wetlands are an important next step, especially because patterns of GHG emission from created wetlands are likely to be highly variable and responsive to multiple ecological and landscape factors.

Management techniques such as organic matter (OM) addition have been proposed as a strategy to help restore ecosystem function in created wetlands; however, we have limited understanding of how this impacts C cycling in these systems. Organic matter addition has been shown to enhance soil C content, resulting in soils that more closely match natural wetlands (Ballantine et al., 2012). However, OM addition also provides C substrate that feeds microbial activity (Balch et al., 1979), therefore, it can positively impact both CH_4 and CO_2 emissions. OM addition in restored freshwater wetlands in western New York resulted in a 20% increase in potential net of CH_4 production (Ballantine et al., 2015). In contrast, a study in a mitigation wetland in Virginia found that heavy OM loading increased soil CO_2 fluxes, but did not affect

CH_4 emissions (Winton & Richardson, 2015). It is likely that the impact of OM addition on wetland GHG emissions will be influenced by environmental factors such as hydrology, nutrient availability and plant community composition, thus it is important to study these impacts in created wetlands that differ in biotic and abiotic conditions.

The objective of this study was to quantify the effect of organic matter (OM) addition on CH_4 and CO_2 fluxes from created wetlands that differ in vegetation, land-use history, and hydrologic regime. I hypothesized that CO_2 and CH_4 fluxes will differ across the study area due to hydrology, vegetation structure, and nutrient availability differences: (1) Water availability would be the dominant driver of GHG emissions, with the highest CH_4 fluxes occurring when water was above the soil surface, while GPP, NPP, and ER would be highest when soil moisture was high, but standing water was not present. (2) When soil moisture was high, adding OM would enhance both CH_4 emissions and soil respiration due to an increase in C availability for microbial activities. (3) Under low moisture conditions, OM addition would enhance ER due to an increase in soil C content boosting heterotrophic respiration, while CH_4 fluxes would exhibit small changes or no changes, depending upon the size of anaerobic zone.

2. Methods

2.1. Site description

This study took place at two wetland sites, Area 3A (A3A) and Area 2 South (A2S), at High Acres Nature Area (HANA) in Fairport, NY (Figure 2). The distance between the sites is approximately one kilometer. These wetlands are managed by Waste Management of New England and New York, LLC and were created to comply with the Clean Water Act "No Net Loss" policy to mitigate the loss of natural wetlands. A2S was created in 2009, and A3A was created in 2012. The land-use history of these sites differ, A2S was used for row crop agriculture where as A3A was previously used as a livestock pasture. They also have different vegetation and hydrology. A2S is dominated by *Typha* spp. (*Typha latifolia* and *Typha angustifolia*, broad and narrow leaf cattail) and *Phalaris arundinacea* (reed canary grass. A3A is more diverse, with upland species including *Polygonum persicaria* (smart weed), *Solidago canadensis* (common goldenrod), *Epilobium* (willow herb), *Schoenoplectus tabernaemontani* (softsteam bulrush), *Cladium mariscoides* (sawgrass), *Daucus carota* (Queen Anne's lace), *Phragmites australis*

(common reed), and with some presence of *Typha* spp. The elevation of A2S is lower than A3A. Standing water in A2S is more common than in A3A, which is only flooded during spring thaw.

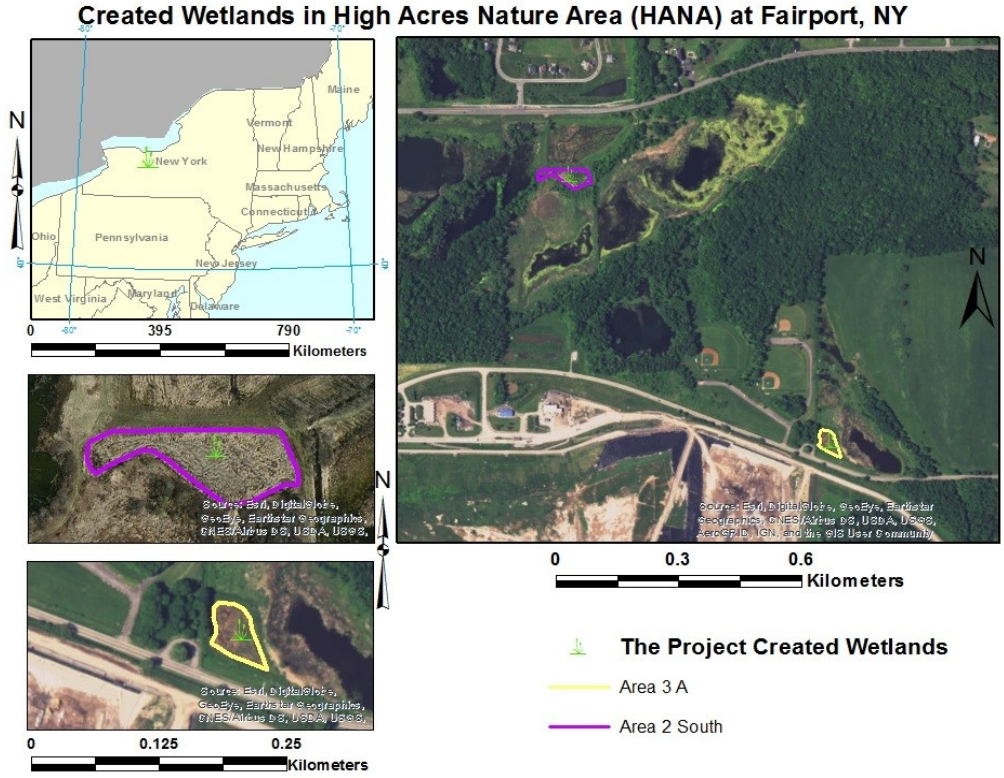

Figure 2. Map of study sites at High Areas Nature Area. A2S has a purple boundary while A3A has a yellow boundary.

2.2. Experimental Design

Ten transects (30m x 2m) were established in A2S in 2014 and in A3A in 2015. Eight of these transects (1, 2, 3, 4, 5, 6, 9, and 10) were used for the measurements in this project and two, $1m^2$, plots were randomly established in each of these transects. Organic matter, 7 cm of leaf litter compost with an approximate concentration of 250 g C/m^2 (Williams, unpublished data), was applied to half of the transects while the rest of transects were used as control plots (Figure 3). Transects were paired to account for a known hydrologic gradient at the site. Organic matter was applied annually at both sites. At A2S it was applied July 2014, May 2015, June 2016 and June 2017. At A3A it was applied in May of 2015, 2016, and 2017.

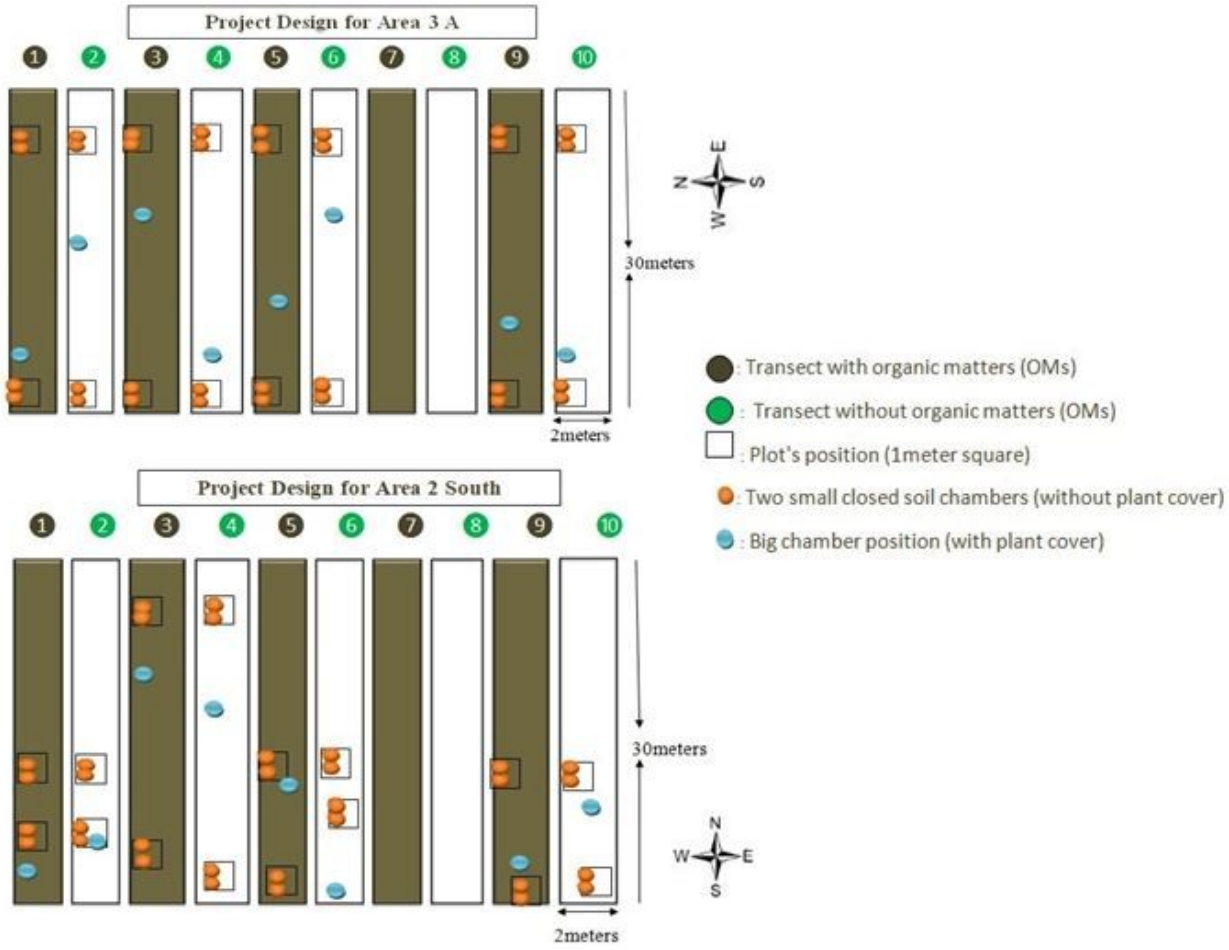

Figure 3. Experimental design at A2S and A3A wetlands.

2.3. Gas Flux Measurements

Soil fluxes of CH_4 and CO_2 were measured from all plots in late June/early July, August, and November of 2016 and in May, July and September of 2017 using small flux chambers. Whole ecosystem CO_2 and CH_4 fluxes were measured using big flux chambers in August and October of 2016 and May, July, and September of 2017.

Soil fluxes were measured using cylindrical polycarbonate soil chambers. Two soil chamber bases were installed in all plots in June 2016 (Figure 4). The soil chambers were constructed based on a design from Ryden et al. (1987) and are 9.5 cm^2 in diameter and 30 cm tall (Figure 5). All new plants were removed from the small soil chambers. For ecosystem flux measurements, a single big chamber base (60 cm by 60 cm) was installed adjacent to one of the plots in each transect in June 2016. The big chamber was constructed based on the design of Carroll & Crill (1997) and is 186cm tall, large enough to encompass wetland vegetation at the

site. The chamber has a cooling system and temperature inside the chamber was monitored and maintained within 2 °C of the outside temperature.

For each flux measurement four (20mL) gas samples were collected from each chamber. Gas samples were taken every 10 min and 15 min for small and big chambers respectively. For small chamber measurements a 10-minute pause between placing the lid and collecting the first gas sample was used to help avoid disturbances caused by chamber installation. For the big chamber, CO_2 measurements were also made in the field using an infra-red gas analyzer (LI-820) and the first 5 min following chamber closure were used to calculate fluxes. Big chamber measurements were taken under both light and dark conditions (Figure 6). For the dark condition, an opaque cover was used to block light penetration into the chamber (Figure 6). Gas samples were analyzed within 24hrs or put in evacuated vials and stored for later analysis. Methane and CO_2 concentration was analyzed using a Shimadzu GC-2014 gas chromatograph with a flame ionization detector (FID) and methanizer.

Fluxes were calculated using the slope of the linear relationship between gas concentration and time, using the following equation: flux = slope * (1/(air temp + 273.15)) *101326 Pa * (1/8.314 m^3 Pa mol^{-1} K^{-1}) * (1/chamber height). Soil CO_2 fluxes were only used if there was a linear change in CO_2 concentration over time ($R^2 \geq 0.8$) or the change in CO_2 concentration was very low ($R^2 \leq 0.2$ and concentration range <100 ppm or any R^2 value for a concentration range < 25 ppm). Methane data from small chambers was not used because time zero concentrations were 2-3 orders of magnitude higher than atmospheric concentrations or fluxes were so low that it was impossible to interpret the data. Big chamber CH_4 fluxes were excluded if time zero CH_4 concentrations were higher than atmospheric concentration. Big chamber CO_2 measurements made in the dark were excluded if they had a negative slope, which occasionally occurred in 2016, either due to chamber leakage or incomplete shrouding.

Figure 4. Big chamber and small chamber bases in the field.

Figure 5. Field measurement of soil respiration using small chambers.

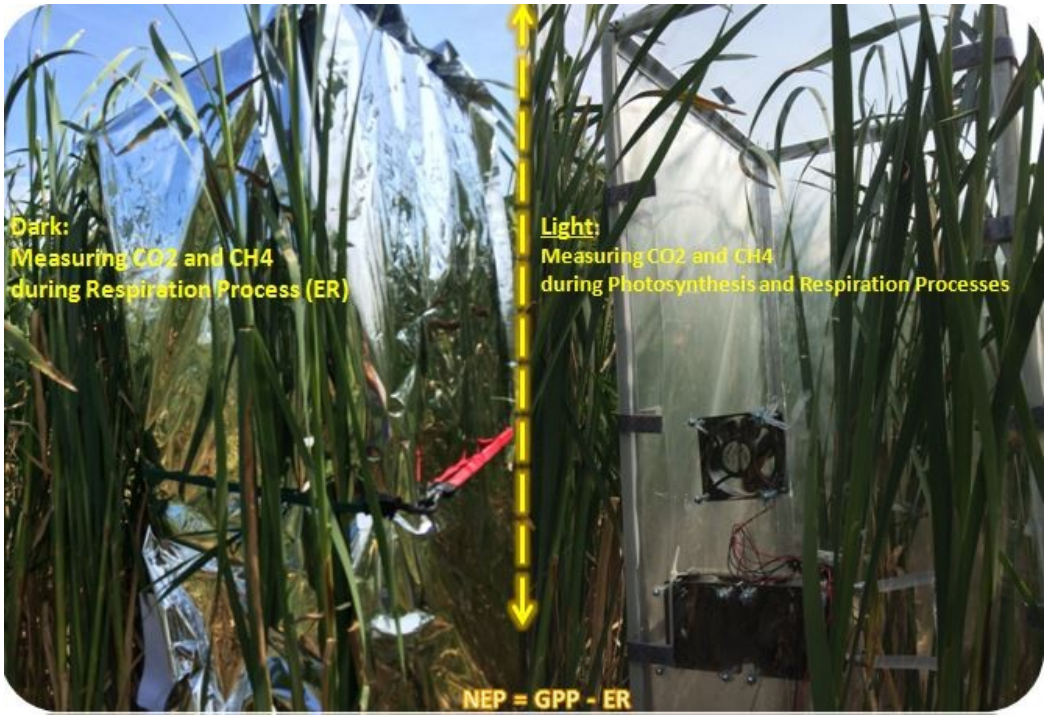

Figure 6. Field measurements of NPP, GPP and ER using a big chamber.

2.4. Soil incubations

Soils samples were collected on the 15th and 22nd of September (2017) for A2S and A3A respectively, using a tulip bulb corer (0.10 m depth and 0.06 m diameter) from each plot in both sites (32 samples total). Soil samples were stored on ice while being transported to the lab and incubations begin within 48 hrs of collection. Soil samples were homogenized, large roots and stones removed, and then 10g of soil and an equal amount of nanopure water was placed in a 250 ml clear glass jar and sealed with a screw cap and septa. The jars were immediately purged with N_2 gas three times (1 minute each) and shaken for 30 seconds between each flush. A 20 ml gas sample was taken on day 1, 2, 4, 6, 9, and 12. Concentrations of CH_4 and CO_2 were measured using a Shimadzu GC-2014 gas chromatograph with a flame ionization detector (FID) and methanizer. Methane and CO_2 production rates were calculated during the time period with the highest, linear production rate.

2.5. Soil chemistry

Soil samples were collected in early and late fall of 2016 using an Auger (0.02 m ID). Soil samples were collected to a depth of approximately 15 cm, stored in Whirl-pak bags and

stored in the lab at -20 °C. Soil samples were divided into 4 subsamples of 5 g each, 2 subsamples were used for soil moisture analysis and 2 were used for extractable nutrients.

Extractable including nitrate (NO_3) and ammonium (NH_4) were measured in all soil samples. Two subsamples of soil were shaken with 2M KCL on a rotoshaker for 45 min and then decanted, filtered (0.45 µm) and frozen at -20 °C in whirl-pak bags. Nitrate was measured on a Lachat QuikChem 8500 Autoanalyzer using the cadmium reduction method (Lachat, 2003). Ammonium was measured using the phenol-hypochlorite method with sodium nitroprusside as a catalyst (Solorzano, 1969). A spectrophotometer (Shimadzu UV 1800 Spectrophotometer) was used to measure the absorption at 630 nm and a standard curve was used to determine the NH_4 concentration in each sample.

Total soil phosphorus (P) was measured by adding 50% w/v magnesium nitrate ($Mg(NO_3)_2$) to 0.1 g of oven-dried soil. The mixed solution was ashed in a muffle furnace (2 h at 550 °C) and then once cooled, 10 mL of 1 M HCl was added, and samples were shaken for 16 h and then left to settle overnight. Finally, samples were diluted (10x) and measured at 880 nm using a Shimadzu UV 1800 Spectrophotometer (Murphy & Riley, 1962).

Soil properties including soil moisture content (MC%), bulk density, and organic matter content (OM%) and pH were measured on all soil samples. Moisture content was measured using the gravimetric method by drying soils for 48hrs at 60°C. Bulk density was calculated using the dry soil weight (24 hrs under 60°C) divided by the volume of the soil core used to collect samples. Organic matter content was measured using the loss on combustion method (Brimhall et al, 2002). Soil pH was measured with a Hach probe by creating a 2:1 (v/v) slurry of dionized water to soil, stirring vigorously to create a uniform suspension (Gelderman and Mallarino 2012).

2.6. Vegetation composition

Vegetation survey's in the field were used to quantify the percent cover of individual species from the big chamber bases. Vegetation percent cover was quantified at both study sites in July and September 2017.

2.7. Statistical Analysis

All statistical analyses were completed using JMP 13 Pro statistical software, except for stepwise regression, which was conducted in R. For all field measurements (soil respiration, NPP, GPP, ER, and ecosystem CH_4 fluxes), a 2-way ANOVA was used to test for differences between sites and measurement dates as well as the interaction between these factors in control plots. A paired t-test was used to test for the effect of organic matter addition on gas fluxes on each measurement date within each site. A paired t-test was also used to test for significant differences between CH_4 fluxes in the light and the dark at each site. For this analysis, only pairs with a positive CH_4 emission in the light were used. Within each site the effect of treatment and temporal variability on gas fluxes was examined using two methods, a 2-way ANOVA (treatment, date, treatment*date) and a linear mixed model with treatment, soil or air temperature, and rainfall as predictor variables and block as a random factor. Three rainfall variables were tested, days since rain, rainfall amount in the past 7 days and rainfall amount in the past 30 days. Only a single rainfall variable was used in each test and the best rainfall predictor was selected for each response variable.

A step-wise regression approach using Akaike's Information Criterion (AIC) as the model selection criterion was used to identify a sub-set of environmental predictor variables that best explained soil respiration rates during the subset of dates where detailed soil chemistry data was available (early and late fall 2016). Model selection was done using the stepAIC package in R and the relative importance of the predictor variables in the selected model was then calculated using the relaimpo R package. Variables used in the model selection process included continuous variables (Total P, Inorganic N, OM%, soil temperature, days since rain, rain during past 7 days, rain during past 30 days, moisture content, and pH) and categorical variables (site and treatment).

For the incubation experiment, a 2-way ANOVA with a Tukey post-hoc test was used to test for significant differences between sites and treatments.

3. Results

3.1 Soil properties and vegetation

There were differences in soil physiochemical properties between the wetland sites and between control and compost plots (Table 1, Appendix A, Huang unpublished data). Averaged across the growing season, soils at A2S had higher OM%, MC%, and inorganic N concentrations (NH_4^+ and NO_3^-) than A3A, whereas soils at A3A had higher total phosphorus content. Soil pH was close to the neutral at both sites, however, it was higher in A3A compared to A2S. There were no differences in bulk density or porosity between the sites. The OM content of soils at both sites were 2-3% higher in compost amended plots. Compost addition also increased inorganic N concentrations at both sites.

TABLE 1. Summary of soil physiochemical features in compost and control plots at each site. Data are seasonal averages from 2016 (average ± SE, n=4).

Factor	Control		Compost	
	A3A	A2S	A3A	A2S
μgNH_4-N/g dried soil	18.7±2.7	36.8±4.7	21.4±4.2	39.7±4.6
μgNO_3-N/g soil	41.1±5.3	50.5±10.1	49.2±3.5	70.9±2.6
mg P/kg dried soil	105.6±4.3	91.2±3.7	109.2±1.9	107.2±4.8
Organic matter (OM)%	12.6±0.9	15.9±0.9	15.3±1.1	17.9±0.7
Moisture Content (MC)%	10.8±1.3	18.4±1.3	13.2±0.5	17.5±0.9
pH	7.6±0.05	7.1±0.06	7.7±0.05	7.2±0.05
Bulk Density	0.9±0.06	0.8±0.02	0.8±0.06	0.7±0.03
Soil Porosity	66.9±2.3	70.1±1.3	70.1±2.1	73.6±1.2

Total percent plant cover at the two wetlands was similar, however, the species composition was very different (Table 2, Appendix B). During 2017, the dominant plant species at A2S was *Phalaris arundinacea*, which averaged 41.9±11.2 and 19.7±9.1% in control and compost plots respectively. Compost amended plots in A2S also had substantial cover of *Typha*

spp., with 7.7 ± 3.6% *Typha latifolia* and 2.5±0.4% *Typha angustifolia*. No one plant species was dominant at A3A, with several species, including *Eupatorium perfoliation*, *Persicaria punctata*, *Polygonum spp.*, *Juncus effusus*, and *Solidago* spp. representing 3-7 % cover.

TABLE 2. Summary of the percent cover of major plant species (>1% cover) and total percent vegetation cover in control and compost plots at both sites. Data are seasonal averages from big chamber bases in 2017 (average ± SE, n=4).

Species	**Control**		**Compost**	
	A3A	A2S	A3A	A2S
Phalaris arundinacea	0	41.9±11.2	2.4±1	19.4±9.1
Lythrum salicaria	0.3±0.3	0	0.1±0.1	1.4±1.2
Typha angustifolia	0.1±0.1	0.1±0.1	1.1±0.7	2.5±0.4
Typha latifolia	0	0.9±0.4	0	7.7±3.6
Eupatorium perfoliatum	4.9±2.3	0	4.6±2.1	0
***Polygonum* spp.**	2.8±2.4	0.4±0.1	0	0
Persicaria punctata	3.8±2.1	0	6.9±3.4	0
***Alisma* spp.**	0	0.6±0.2	0	2±1.3
Juncus effusus	6.3±6.3	0	0.4±0.3	0
***Solidago* spp.**	1.3±1.3	0	0.5±0.5	0
Total Percent Cover	40.6±3.6	54±4.5	21.5±3.6	32.5±8.5

3.2 Environmental conditions (Rainfall, hydrology, and temperature)

Neither site had standing water during the measurement period in 2016 (Table 3). In 2017, A3A also had no standing water, however, in A2S the water level was above the soil surface in the spring and summer, averaging 9.3 and 2.8 cm during May and July respectively and then dropped below the soil surface in September (Table 3). Precipitation data from a weather station (Station: USC00304952) in the nearby town of Macedon, NY showed that in 2016 precipitation was low in the beginning of the growing season and then increased in the fall, whereas in 2017, precipitation levels were consistent across study period (Figure 7).

Soil temperature was measured during each chamber measurement. In 2016 the highest temperatures were in early and late summer (June/July, August) and averaged 18 and 21 °C, respectively. Temperature then decreased during early and late fall (September/October, November), with averages of 14 and 8 °C, respectively. In 2017, soil temperature was similar in May and September, averaging 10-13 °C at both sites and the highest temperature, 16 °C, was in July (Figure 7).

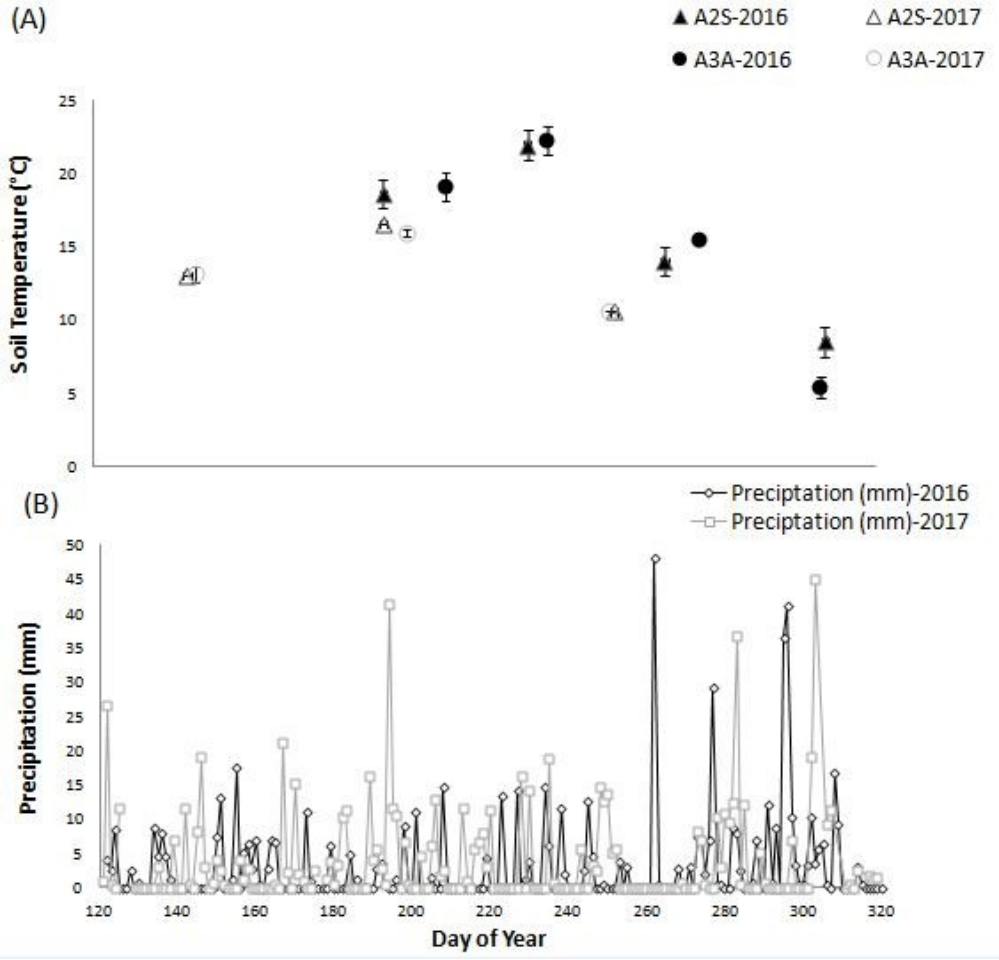

Figure 7. (A) Soil temperature measured in the field and (B) daily precipitation totals recorded close the study site during the study period in 2016 and 2017.

TABLE 3. Standing water depth measured at both sites during flux measurements in 2016 and 2017 (average ± SE, n=8).

Site	Date	Year	Standing Water Depth (cm)
A3A	June	2016	0
	August	2016	0
	October	2016	0
	November	2016	0
	May	2017	0
	July	2017	0
	September	2017	0
A2S	July	2016	0
	August	2016	0
	September	2016	0
	November	2016	0
	May	2017	9.3±0.8
	July	2017	2.8±1.6
	September	2017	0

3.3 Soil Respiration

Soil respiration rates were not significantly different in control plots in the two wetland sites, with fluxes ranging from 0.052 to 5.66 g CO_2 -C m^{-2} d^{-1} in A3A and 0.027 to 4.35 g CO_2 - C m^{-2} d^{-1} in A2S (Figure 8). In August 2016, rainfall occurred between measurements in the two sites, resulting in 5-fold higher fluxes in A3A, which was measured after the rain event. There was significant variability in soil respiration rates across the two-year measurement period ($F_{11,35}$=19.5, p<0.001) as well as significant date by site interactions ($F_{11,35}$=9.4, p<0.0001, Table 4, Figure 8). In 2016, soil respiration was lowest early in the summer (late June/early July) and then fluxes increased following late summer rainfall. In 2017, soil respiration was lowest in May, increased during the summer and peaked in July at both sites. In A3A, soil respiration then declined in the fall, whereas in A2S, rates remained high into early September.

Across all sites and treatments, soil respiration was significantly influenced by the amount of rainfall in the proceeding 7 days ($F_{1,96}$ = 5.6, p = 0.01, Table 4). When the analysis was run within an individual site, the effect of rainfall was only significant within A3A ($F_{1,42}$=22.2, p<0.0001, Table 4), however, soil temperature was a significant predictor of respiration rates in both wetlands ($F_{1,48}$=4.4, p = 0.04, $F_{1,45}$=21.1, p<0.0001) respectively (A2S, A3A, Table 4). During most of the study period there was no difference between soil respiration

rates in control and compost plots, however, at A2S in September 2017 compost plots had more than double the soil respiration rate of control plots ($t(3)=3.58$, $p = 0.04$, paired t-test, Figure 8).

During early and late fall of 2016 soil chemistry was analyzed in conjuncture with soil respiration measurements (Huang, unpublished data) enabling quantification of the main factors contributing to variability across the sites. Step-wise regression using Akaike's Information Criterion (AIC) showed that 79% of the variability in soil respiration during this time period could be explained by site, soil temperature, rainfall amount during the proceeding 30 days, total inorganic N ($NH_4 + NO_3$) and OM%. Rainfall during the proceeding 30 days was the most important predictor of soil respiration rates, accounting for 40% of the total variability. This was followed by site (22%), soil temperature (15%), total inorganic N (13%) and finally OM% (8%).

TABLE 4. Results of two-way ANOVA and linear mixed-models examining the response of soil respiration to site and date for control plots and site, treatment, soil temperature and rainfall amount for all plots (2016-2017). Significant p-values are bolded (*p<0.0001).

	Site		**Date**		**Site*Date**			
	F	p	F	p	F	p	F	p
Both Sites	$F_{11,35}$=0.8	0.39	$F_{11,35}$=19.5	**<.0001***	$F_{11,35}$=9.4	**<.0001***		
	Site		**Treatment**		**Temperature**		**Rainfall (mm) in the past 7 days**	
	$F_{1,4}$=0.20	0.67	$F_{1,91}$=0.001	0.96	$F_{1,97}$=1.3	0.24	$F_{1,96}$=5.6	**0.01**
	Treatment		**Temperature**		**Rainfall (mm) in the past 7 days**			
A2S	$F_{1,47}$=0.6	0.44	$F_{1,48}$=4.4	**0.04**	$F_{1,43}$=0.1	0.77		
A3A	$F_{1,42}$=1.3	0.27	$F_{1,45}$=21.1	**<.0001***	$F_{1,42}$=22.2	**<.0001***		

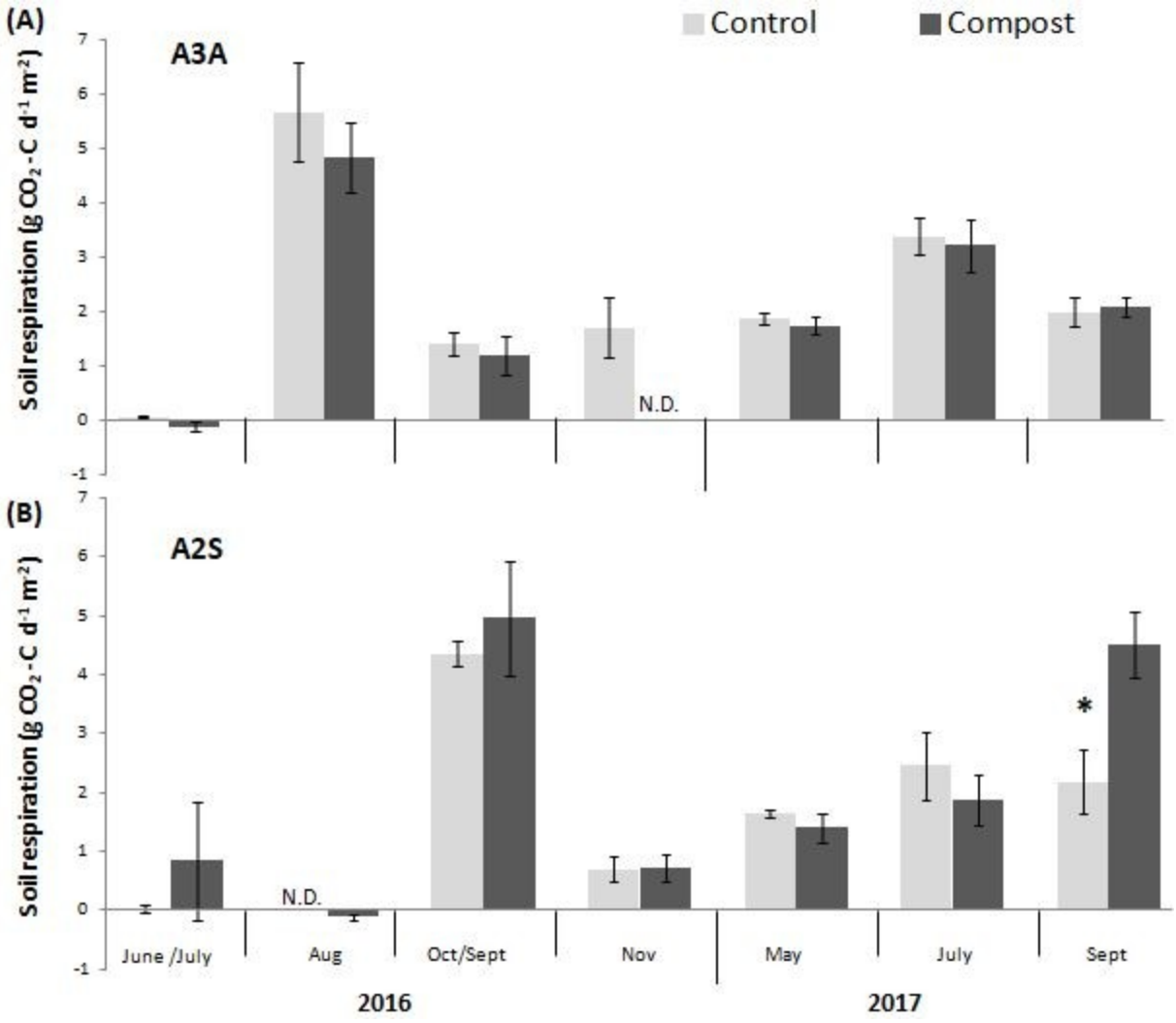

Figure 8: Soil respiration rates measured in control and compost plots in (A) A3A (measurements started 6/28/16, 8/23/16, 10/1/16, 11/1/16, 5/26/17, 7/18/17, and 9/9/17) and (B) A2S (measurements started 7/12/16, 8/18/16, 9/22/16, 11/2/16, 5/24/17, 7/13/17, and 9/10/17). N.D. refers to no data. Values are mean ± SE and * indicates a significant difference between control and compost transects (paired t-test, $p<0.05$).

3.4 Ecosystem C Flux (NPP, ER, and GPP)

There were no significant differences in NPP, GPP or ER between the two wetland sites, however, all fluxes showed a significant temporal pattern (NPP: $F_{4,25}=7$, $p=0.0006$; ER: $F_{3,19}=3.91$, $p=0.02$; GPP: $F_{3,21}=7.4$, $p=0.001$, Table 5, Figure 9) and there was a significant interaction between site and date for NPP and GPP (NPP: $F_{4,25}=5.8$, $p=0.001$; GPP: $F_{3,21}=6.1$, $p = 0.004$, Table 5, Figure 9). Also, there was a marginally significant interaction between site and date for ER ($p=0.09$, Table 5). There was net C uptake in the control plots across all

measurement dates, with NPP ranging from close to zero to -16.4 ± 4.9 g C m^{-2} d^{-1} in A3A and from -1.8 ± 0.4 to -20.1± 1.9 g C m^{-2} d^{-1} in A2S (Figure 9). At both sites, CO_2 exchange was low in 2016 and there was no difference between summer and fall measurements. In 2017, the seasonal pattern in ecosystem CO_2 fluxes in control plots at the two sites differed. In A3A, NPP, GPP and ER all peaked in July, whereas in A2S, ER increased across the growing season while GPP decreased across the same time period, resulting in maximum CO_2 uptake in the spring (Figure 9, Appendix C). Multiple regression analysis with a linear mixed model showed a significant effect of temperature (NPP: $F_{1,63}$=5.4, p<0.02; GPP: $F_{1,53}$=10.4, p<0.002; ER: $F_{1,16}$=4.7, p=0.04) and rainfall (ER: $F_{1,51}$=13.9, p<0.0005; GPP: $F_{1,49}$=11.4, p<0.001; NPP: $F_{1,59}$=11.7, p<0.001) on NPP, GPP and ER as well as a significant effect of treatment on ER ($F_{1,44}$=7.9, p<0.007, Table 6). During most of the study period paired t-tests did not show a significant difference between NPP, GPP and ER in control and compost plots, however, GPP (p < 0.008) and ER (p=0.06) in A2S compost plots was double that of control plots in September 2017 and NPP was an order of magnitude lower in A3A compost plots compared to control plots in August 2016 (p = 0.004).

TABLE 5. Results of two-way ANOVAs examining the effect of site (A3A, A2S) and measurement date on NPP, ER, and GPP (2016-2017). Significant p-values are bolded.

	Site		**Date**		**Site*Date**	
	F	p	F	p	F	p
NPP	$F_{1,25}$=1.4	0.23	$F_{4,25}$=7.1	**0.0006**	$F_{4,25}$=5.8	**0.001**
ER	$F_{1,19}$=0.6	0.44	$F_{3,19}$=3.9	**0.02**	$F_{3,19}$=2.4	0.09
GPP	-	-	$F_{3,21}$=8.1	**0.001**	$F_{3,21}$=6.1	**0.004**

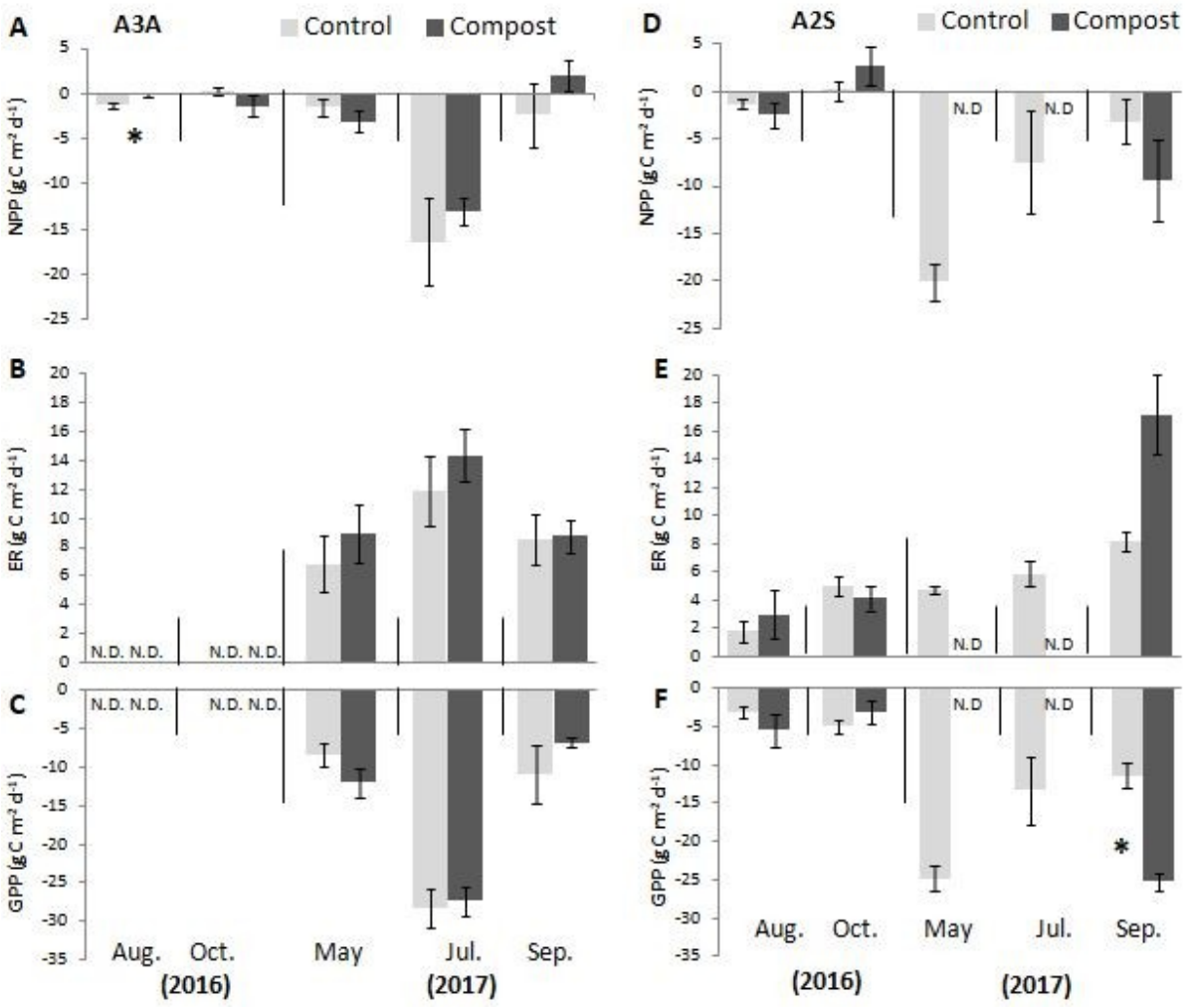

Figure 9. NPP (A, D), ER (B, E), and GPP (C, F) during growing and fall seasons of 2016-2017 in A3A (A-C) and A2S (D-F). Values are mean ± SE. * indicates a significant difference in a paired t-test ($p<0.05$). N.D. refers to no data.

TABLE 6. Results of linear mixed-models examining the effect of site, treatment, temperature, and rainfall on NPP, ER, and GPP (2016-2017) at both sites. Significant p-values are bolded.

	Site		**Treatment**		**Temperature**		**Rainfall**		**Type**
	F	p	F	p	F	p	F	p	
NPP	$F_{1,7}$=4.4	0.07	$F_{1,58}$=0.09	0.76	$F_{1,63}$=5.4	**0.02**	$F_{1,59}$=11.7	**0.001**	Days since rain
ER	$F_{1,2}$=0.06	0.82	$F_{1,44}$=7.9	**0.007**	$F_{1,16}$=4.7	**0.04**	$F_{1,51}$=13.9	**0.0005**	Rainfall (mm) in the past 7 days
GPP	$F_{1,7}$=2.8	0.13	$F_{1,49}$=2.1	0.14	$F_{1,53}$=10.4	**0.002**	$F_{1,49}$=11.4	**0.001**	Rainfall (mm) in the past 30 days

3.5 Methane Flux

Methane emissions from both wetlands were variable and the majority of measurements made during the summer and fall of 2017 had to be omitted due to soil disturbance causing ebullition events during chamber deployment. The highest CH_4 emissions occurred at A2S in the spring of 2017, with fluxes of 74.9 ± 18.9 mg CH_4-C m^{-2} d^{-1} (Figure 10). At A3A, CH_4 emissions were highest, but also extremely variable, in the fall of 2016 (55.5 ± 43.0 mg CH_4-C m^{-2} d^{-1}) and summer of 2017 (58.8 ± 65.0 mg CH_4-C m^{-2} d^{-1}, Figure 10). At both sites, CH_4 emissions were near zero or there was a net uptake of CH_4 in the summer of 2016 (Figure 10). There were no significant differences in CH_4 emissions from control plots at the two sites, however, measurement date had a significant impact on fluxes ($F_{2,12}$ = 3.8, p = 0.05, Table 7, Figure 10) and there was a marginally significant site by date interaction (p = 0.07). Further investigation into environmental drivers of CH_4 emission showed that while site and temperature did not have a significant effect on CH_4 fluxes, days since it last rained was a significant predictor of CH_4 emissions ($F_{1,11}$=4.8, p = 0.05). Within each site, neither days since rain or temperature were a significant predictor of CH_4 emissions, however, there was a trend towards rainfall having an effect in A2S (p = 0.07). Organic matter addition did not have a significant effect on CH_4 emissions at either site. In chambers that were emitting CH_4, emissions in the light were approximately double those in the dark, however, this difference was only significant in A2S (p = 0.05, Figure11).

TABLE 7. Results of two-way ANOVA (site and measurement date) and linear mixed-model (site, temperature, and day since rain) examining predictors of CH_4 fluxes from control plots at both sites. Significant p-values are bolded.

Factor	**Site**		**date**		**Site*date**	
	F	p	F	p	F	p
CH_4 flux	$F_{1,12}$=1.5	0.25	$F_{2,12}$=3.8	**0.05**	$F_{2,12}$=3.5	0.07
	Site		**Temperature**		**Days Since Rain**	
	$F_{1,12}$=2.7	0.13	$F_{1,11}$=0.3	0.59	$F_{1,11}$=4.8	**0.05**

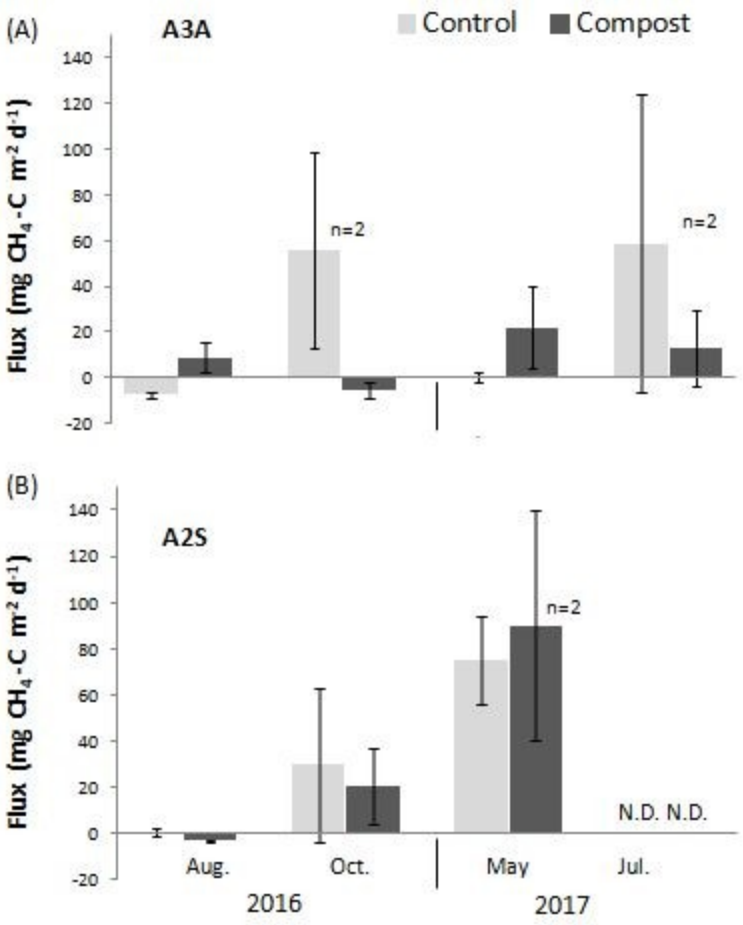

Figure 10. Methane fluxes from control and compost plots during 2016 and 2017 in (A) A3A and (B) A2S. N.D. refers to no data. Data with n=2 refers to sites/dates where the sample size was only 2, these data points were not used for statistical analysis.

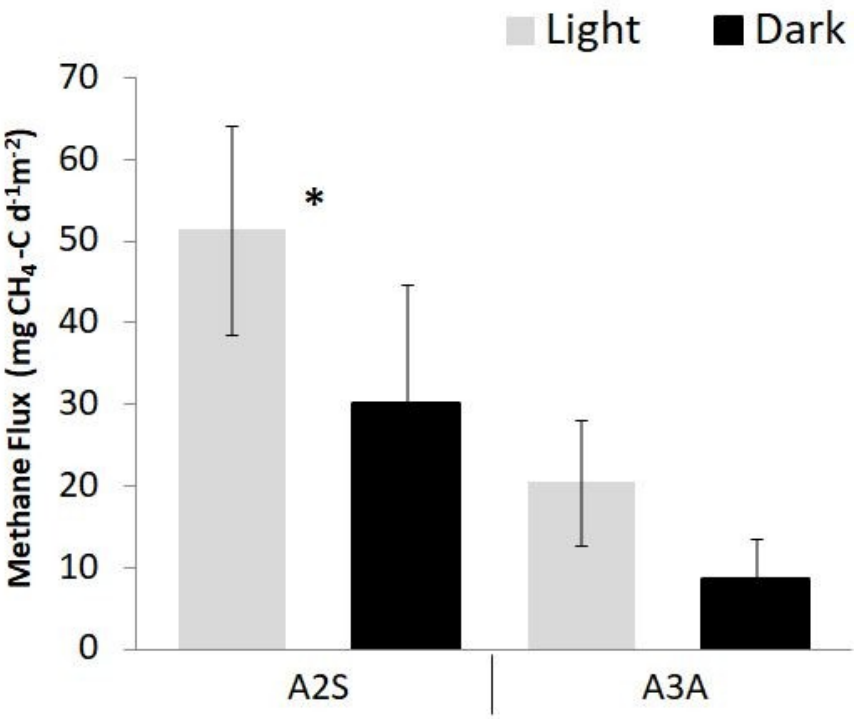

Figure 11: Methane fluxes under light and dark conditions in each wetland. Values are mean ± SE. * indicates a significant difference in CH_4 flux between the light in dark condition (paired t-test, $p<0.05$).

3.6 Soil Incubations

Potential rates of anaerobic CH_4 and CO_2 production were significantly different between the two sites, as was the CH_4:CO_2 production ratio ($F_{3,12}=4.1$, $p<0.001$, Figure 12, Table 8). Production rates of CO_2 and CH_4 and the CH_4:CO_2 production ratio were significantly higher in soils from A2S ($p < 0.01$). In A3A CO_2 production was the dominant form of anaerobic respiration (4.7 ± 0.4 and 7.1±0.8 μg d^{-1} g dry $soil^{-1}$ in control and compost respectively) and there was minimal CH_4 production (0.03 ± 0.01 and 0.17 ± 0.06 μg d^{-1} g dry $soil^{-1}$ in control and compost respectively), resulting in CH_4:CO_2 production ratios near zero. In contrast, CH_4 and CO_2 production rates were a similar magnitude in A2S resulting in CH_4:CO_2 production ratios that approached one (0.4± 0.2 and 0.9 ± 0.2 in control and compost respectively). Although soils amended with organic matter were not significantly different from control soils, there was a trend towards higher CO_2 and CH_4 production rates in compost soils from both sites (Figure 12, Table 8).

TABLE 8. Results of two-way ANOVAs examining the effect of site and treatment on CH_4 and CO_2 production rates and their ratio in anaerobic incubations. Significant p-values are bolded.

Factor	Site		Treatment		Site*Treatment	
	F	p	F	p	F	p
CH_4 production rate ratio	$F_{3,12}$=3.2	**0.008**	$F_{3,12}$=1.3	0.23	$F_{3,12}$=1.2	0.25
CO_2 production rate ratio	$F_{3,12}$=3.7	**0.003**	$F_{3,12}$=1.5	0.15	$F_{3,12}$=0.7	0.52
CH_4/CO_2 production rate ratio	$F_{3,12}$=4.1	**0.001**	$F_{3,12}$=1.6	0.14	$F_{3,12}$=1.5	0.16

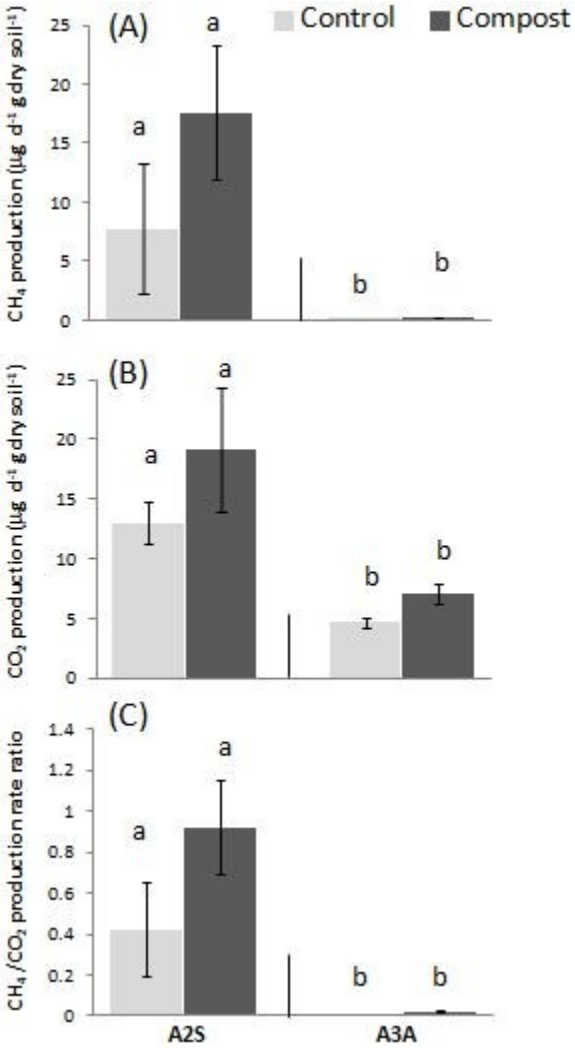

Figure 12. (A) CH_4 production rates, (B) CO_2 production rates and (C) CH_4/CO_2 production ratios from anaerobic incubations of soils collected from each site. Values are mean ± SE and letters indicate statistical differences between averages.

4. Discussion

4.1 Overview

This project focused on understanding the impact of OM addition on GHG fluxes including soil respiration, CH_4 emission and ecosystem CO_2 exchange (NPP, ER, and GPP) in two wetlands with differing hydrology, vegetation, and soil chemistry. We found that seasonal and inter-annual weather conditions, especially rainfall and temperature, were the dominant factor controlling GHG emissions within both wetlands. Drought conditions during 2016 limited both soil respiration and plant activity, negatively impacting soil and ecosystem CO_2 exchange. This reveals the sensitivity of wetland ecosystems to fluctuations in weather, particularly the timing and amount of precipitation. In 2017, moisture conditions were more typical and OM addition significantly increased soil respiration in A2S when soils were wet, but not inundated. During this same time window, GPP was also significantly higher in the organic matter amended plots, however, increases in respiration meant that there was no net change in CO_2 exchange. Unlike A2S, A3A was mostly dry across both years and while OM did not have a significant effect on soil respiration, NPP was slightly lower in August 2016, possibly due to lower respiration rates. Methane emissions were highly variable and were low during much of the study due to dry conditions, however, when emissions were high, fluxes were significantly higher in the light than the dark at A2S, suggesting that active transport is important in this wetland. Further, incubations showed that there was higher CO_2 and CH_4 production potential under anaerobic conditions in A2S that in A3A and that there is minimal potential for CH_4 production in A3A. This could be due to the microbial community not being adapted to anaerobic conditions in A3A. This supports the hypothesis that climate and hydrological regime are important controllers of CO_2 uptake and GHG emissions in created wetlands.

4.2 Soil Respiration

Temperature and precipitation were key drivers of soil respiration at both wetland sites. This is consistent with other studies that have found temperature and moisture to be important factors controlling soil biogeochemical processes. A 10 °C increase in temperature has been found to double decomposition rates, increasing wetland soil respiration under aerobic conditions (Rasmussen et al., 1998; Sylvia et al., 1998; Christensen, 1993). Drought conditions, such as

what we observed at HANA in 2016, have also been shown to inhibit soil respiration. Low precipitation coupled with high temperatures leads to dry soil conditions, which can suppress soil biogeochemical processes (Cook & Orchard, 2008; Ågren & Wetterstedt, 2007). For example, an experiment conducted at the Harvard forest found that drought resulted in a decrease in soil respiration (Borken et al., 2006). This is due to soil water content being a crucial factor for microbial activity (Wilson & Griffin, 1975) and extreme conditions of continuous drought inhibit respiration, because water is required for hydrolytic reactions during respiration (Malone, Starr, Staudhammer, & Ryan, 2013). This also explains the observed increase in soil respiration following precipitation in late summer and fall of 2016.

Because soil respiration includes root respiration, plant stress during drought can also negatively affect soil respiration. This could be another factor explaining the low rates of soil respiration observed in early summer 2016. Studies have shown that photosynthesis in wetlands is highest when the water table is high (Malone et al., 2013; Adkinson et al., 2011), and that lowering of the water table has a negative effect on photosynthesis (Lafleur et al., 1997). Because studies have found a positive relationship between soil respiration and net primary productivity in many ecosystems (Raich & Schlesinger, 1992, Olson et al., 1983), drought conditions in our sites could have indirectly reduced soil respiration by limiting primary productivity of wetland vegetation.

Differences in seasonal patterns in soil respiration between the two sites in 2017 suggest that hydrology and temperature interact to determine seasonality of soil respiration. At A3A there was no standing water during the study period in 2017 and the highest soil respiration rate occurred in July, when soil temperatures were highest. This pattern is similar to that observed in a mitigation wetland dominated by upland species, where the highest soil CO_2 flux (>400 mg m^{-2} h^{-1}) occurred under low water levels in the summer (Winton & Richardson, 2015). In contrast, soil respiration at A2S increased from May to July and then plateaued through the fall, despite a decline in temperature. The decline in water table depth, from standing water in May to no visible water table in September likely contributed to this pattern. Standing water during May and July created anaerobic soil conditions, which could reduce soil respiration due to oxygen limitation, whereas in September, aerobic soils would promote soil respiration despite lower temperature (Winton & Richardson, 2015).

Overall, OM addition had a small effect on soil respiration rates in this study. This is in contrast to findings by Winton & Richardson (2015), who observed an increase in soil respiration with OM loading. There was a doubling of soil respiration in OM amended plots in September 2017, when soils were moist, but there was no standing water. This supports the hypothesis that OM addition will increase soil respiration in aerobic soils due to the OM providing C substrate that feeds microbial activity (Balch et al., 1979). The observation that OM addition had the most impact in A2S when there was no standing water, but the system was not drought stressed, is also consistent with the study by Winton & Richardson (2015), who also observed the most significant effect of OM loading when the water table was low.

In A3A, however, soil respiration was never significantly different in compost and control plots despite the absence of standing water. One explanation is that the higher species richness at this site could reduce the impact of OM addition. Several studies have found that vegetation composition impacts GHG fluxes by controlling C input and influencing organic matter quality (Treat et al., 2015; Turetsky et al., 2014; Bhullar et al., 2014; Inglett et al., 2012; Reddy & DeLaune, 2008; Ding et al., 2003; Brix et al., 2001). Therefore, organic matter differences related to vegetation cover could override any effects of OM addition. A greenhouse project looking at the relationship between species composition and soil respiration could be an important next step to more fully understand how soil respiration rates respond differently to OM addition in wetlands with different plant communities.

These findings highlight the fact that the impact of OM addition on soil respiration is dependent on local weather and vegetation features. The effectiveness and trade-offs associated with OM addition as a management technique in created wetlands are likely to be depended on interactions between multiple wetland features. Therefore, a generalization of these findings to other systems or even the same system in different years or seasons could be difficult.

4.3 Ecosystem C Flux (NPP, ER, and GPP)

Vegetation in a wetland ecosystems is a key factor determining GPP, NPP, and ER. At HANA, total vegetation cover was similar between the two wetlands, however, vegetation cover at A2S was dominated by *Phalaris arundinacea and Typha* spp., whereas A3A was more diverse (Table 2). These differences in species composition did not result in significant differences in ecosystem CO_2 exchange. This is different from studies conducted in other wetland systems, for

example in fens, variations in photosynthesis and respiration were found between poor fens dominated by *Sphagnum spp.* and rich fens dominated by sedges (Glenn et al., 2006). One explanation for our finding is that at our sites, variation in NPP, GPP, and ER were largely driven by temporal patterns associated with seasonal and inter-annual variation in temperature and rainfall. For example, during 2016, drought conditions resulted in very low NPP at both sites. Multiple studies ranging from Everglades marshes to Alaskan peatlands have also found that drought conditions decrease in NPP and cause ecosystems to shift from a C sink to a C source (Olefeldt et al., 2017; Malone et al., 2013). Drought conditions can lead to vegetation stress and lower rates of photosynthesis, reducing C uptake. Previous studies have also found that dry conditions have a negative effect on photosynthetic rate (Lafleur et al., 1997) while high standing water has a positive effect on photosynthetic rates (Malone et al., 2013; Adkinson et al., 2011).

Interactions between hydrological regime and seasonal patterns in temperature and plant growth contributed to a significant site by date interaction for NPP. In A2S, the highest NPP was observed in May and then NPP declined through the summer and into the fall. This decline in NPP was driven by both a decline in GPP and an increase in ER and tracked the decrease in water level across the summer. It is also consistent with the rapid early season growth observed in the dominant plant species *Phalaris arundinacea* and *Typha* spp. (Williams, unpublished data). Studies have shown that the highest rate of photosynthesis occurs under high water conditions (Malone et al., 2013; Adkinson et al., 2011) and that high rates of respiration occur under more aerobic conditions when the water table is low (Yang et al., 2013; Sulman et al., 2010; Sulman et al., 2009; Olson et al., 1983). Therefore, high water levels coupled with rapid early season plant growth had a positive effect on C uptake at this site. In contrast, at A3A, where soils were never inundated, NPP was highest in July due to high photosynthetic rates. Adkinson et al. (2011) also found a similar trend in July and attributed it to increases in vegetation cover and photosynthetic rate during the growing season. In A3A, ER was also highest in July, reducing overall C accumulation rates during mid-summer. This pattern matches observations of soil respiration at this site, with maximum respiration when temperature was highest. Additionally, carbon inputs to wetlands soils can be increased during warm periods when rates of photosynthesis are at the maximum (Crafts-Brandner & Salvucci, 2002; Chapin &

Shaver, 1996) and this C input combined with aerobic conditions and high temperatures can increase soil respiration (Bubier & Crill 2003).

Organic matter addition resulted in only a few changes in ecosystem CO_2 exchange during the study period. In A3A, NPP was lower in compost plots compared to control plots in August 2016. Problems with dark chamber measurements limits identification of the cause of this change, however, increased C availability could have increased ecosystem respiration, resulting in a decrease in NPP. In A2S, compost addition increased GPP and ER in September (2017) resulting in no change in NPP. This suggest that under moderate temperature and water conditions organic matter addition promotes both plant growth and soil and plant respiration, resulting in no net change in CO_2 exchange.

4.4 Methane emissions

Water conditions, both the amount and timing of rainfall as well as site differences in hydrology resulted in variability in CH_4 emissions. Low emissions in August 2016 and higher emissions in October of 2016 as well as all of 2017 can be attributed to rainfall patterns, with fewer days since rain predicting higher emissions. Measurements in 2017 suggest that in years with typical rainfall, CH_4 emissions are higher in the inundated soils found at A2S. However, high rates of ebullition in A2S, possibly caused by soil disturbance during chamber sampling, prevented accurate measurements CH_4 emissions at that site during most of 2017. Methane production and consumption is known to be particularly sensitive to the location of the water table. Methanogenesis only occurs in anaerobic soils, which are found below the water table, whereas methanotrophy requires oxic conditions, present at the soil water interface and the rhizosphere. This means that inundated conditions generally enhance CH_4 production (Olefeldt et al., 2017; Bansal et al., 2016; Whalen, 2005; Bubier & Crill, 2003; Griffis et al., 2001), while no standing water table can result in an increase in CH_4 oxidation and shift in decomposition towards aerobic processes that yield CO_2 (Olefeldt et al., 2017; Hou et al., 2013; Sulman et al., 2010; Ise et al., 2008; Whalen, 2005).

Organic matter addition did not have a significant effect on CH_4 emissions in this study. This is consistent with field measurements done in a mitigation wetland in Virginia, which found that heavy OM loading increased soil CO_2 fluxes, but did not affect CH_4 emissions (Winton & Richardson, 2015). It does conflict with the findings of Ballentine et al. (2015) who found that

OM addition in restored freshwater wetlands in western New York resulted in a 20% increase in potential net of CH_4 production. The lack of standing water at both sites during much of the study may have limited CH_4 production, restricting any observations of the effect of OM addition on CH_4 emissions.

Higher rates of CH_4 emission in the light compared to the dark were observed at A2S, but not A3A, suggesting that differences in vegetation between the sites may influence CH_4 transport. A closer look at individual chambers measurements shows that transect 1 and 9 in A2S had the largest differences in CH_4 flux between light and dark conditions. At the time of flux measurements, these locations also had the highest percent cover of *Typha latifolia* (30 and 10% respectively). Overall, A2S had higher percent cover of *Typha* spp., especially *Typha latifolia*, compared to A3A, which had no *Typha latifolia* and only a small amount of *Typha angustifolium* (Table 2, appendix B). These differences in the abundance of *Typha* species is likely to be an important factor contributing to the observed differences in CH_4 emissions under light and dark conditions. *Typha* spp. are known to transport gases through pressurized ventilation, a process that that is sensitive to light and results in CH_4 emissions that are highest under high light and lowest at night or in the dark (Chanton et al. 1993). In contrast, other wetland species use passive transport, which is not influenced by light intensity (Brix et al. 2001; King et al., 1998; Chanton et al., 1993). Ding et al. (2003) found that different plant types differ in their ability to transport CH_4 to the atmosphere. This was supported by Bhullar et al. (2013), who examined the plant transport ability of 20 forbs and graminoids species in European wetlands and found that 30-100% of the total CH_4 flux was transported by plants, with graminoid species transporting more CH_4 than forbs. Therefore, differences in vegetation are likely to have played an important role in determining the light sensitivity and magnitude of plant mediated CH_4 transport at the wetland sites.

4.5 Soil Incubations

Anaerobic CH_4 and CO_2 production rates were higher in A2S than A3A, suggesting that there are large differences in C metabolism at the two wetlands. Soils in A2S were moist during sampling and regularly experienced inundated conditions that create an anaerobic environment, whereas A3A was completely dry during soil sampling and is rarely flooded. This means that the microbial community in A3A may not be adapted to metabolize C under anaerobic conditions,

resulting in low CO_2 production and negligible CH_4 production rates under anaerobic conditions in the laboratory. Extremely low CH_4 production in A3A supports the hypothesis that A3A lacks a methanogen community. Previous studies have also shown similar variability in CH_4 production potential when soils collected from diverse wetland types are incubated under identical temperature and moisture conditions, with soils collected from aerobic habitats producing very little CH_4 when exposed to anaerobic conditions (Hodgkins et al., 2014; Boon et al., 1997). Organic matter lability can also impact CO_2 and CH_4 production potential in wetlands with different vegetation cover. Hodgkins et al. (2014) found that production of both gases and the CH_4:CO_2 ratio increased with organic matter lability in a thawing permafrost peatland. High nitrogen availability can also inhibit CH_4 production by stimulating denitrifiers, who outcompete methanogens for C substrate (Kim et al., 2015; Bodelier, 2011) and produce intermediate products (NO_2^-, NO and N_2O) that have been shown to be toxic to methanogens (Bodelier, 2011; Roy & Conrad, 1999). Further analysis of soil chemistry and microbial community composition could help explain the observed trends in anaerobic C gas production rates.

There were no significant effects of OM addition on CH_4 and CO_2 production rates and their ratio, however, there were trends towards higher production rates in soils from plots with added OM. Interaction between *in situ* soil chemistry and the composition of the added OM, particularly its low C:N (Williams unpublished data), may have contributed to the small response to OM amendment at both sites. The soil amendment study by Ballantine et al. (2015) showed that some, but not all types of soil amendment increase respiration rates in laboratory incubation, suggesting that the chemistry of the soil amendment can impact the response of the microbial community. While Ballantine et al. (2015) observed higher CH_4 production potential in OM amended soils, their incubations were conducted under field moisture conditions and they concluded that moisture differences between control and treatment plots may have been an important factor driving this pattern.

5. Conclusions

The results of this study show that precipitation patterns and hydrologic regime are key drivers of CH_4 and CO_2 fluxes in created wetlands. Variability in precipitation, particularly inter-annual differences in the timing and amount of rainfall, was the dominant factor explaining variability in all measured gas fluxes. This study also shows that differences in hydrology that occur when creating wetlands can change their potential to produce CO_2 and CH_4 under both field and laboratory conditions. Consideration of hydrology should therefore be a priority when planning created wetlands, to ensure ecosystem functions are resilient to climate fluctuations across seasons and years. Further, water availability was also a key determinant of whether organic matter amendment resulted in significant changes in GHG emissions, with the largest changes in CO_2 fluxes observed when soil moisture was high, but soils were not inundated. This highlights the importance of considering hydrological regime when predicting C sequestration and GHG flux responses to management approaches.

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APPENDICES

Appendix A. Summary of soil physiochemical features across each season of 2016 in compost and control plots at each site (average ± SE, n=4).

	May				July			
Factor	**Control**		**Compost**		**Control**		**Compost**	
	A3A	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**
µgNH_4-N/g dried soil	18.9±4.4	57.9±8.3	12.04±3.8	33.4±9.1	7.5±3.02	42.2±26.2	15.9±2.7	37.8±4.7
µgNO_3-N/g soil	18.5±5.8	0.2±0.1	25.6±1.4	0.4±0.2	66±4.4	86.4±18.9	69.3±9.2	179.9±19.9
mg P/kg dried soil	105.9±9.3	80 ±6.9	100.9±1	88.5±17.2	94.5±4.5	105.4±13.9	107.7±1.2	115.1±9.8
Organic matter (OM)%	12.4±1.2	16.3±0.9	13.6±0.5	12.3±1.3	12.3±0.7	16.8±2.1	13.9±1.1	25.4±2.6
Moisture Content (MC)%	13.6±4.3	27.4±6	24.6±1.3	26.5±2.2	5.1±1.2	12.3±0.03	5.7±1.1	17.5±1.9
pH	7.9±0.05	7.3±0.1	7.9±0.07	7.6±0.1	8.1±0.1	7.1±0.07	8.2±0.05	7.1±0.1
Bulk Density	0.9±0.1	0.9±0.1	0.9±0.04	0.9±0.06	0.8±0.05	0.7±0.03	0.8±0.04	0.5±0.07
Soil Porosity	63.1±4.1	64.8±3.2	63.6±1.6	63.9±2.3	69.7±2.2	73.5±1.2	69.9±1.7	82±2.6

	August				September			
Factor	**Control**		**Compost**		**Control**		**Compost**	
	A3A	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**
µgNH_4-N/g dried soil	19.2±6.2	18.3±5.3	17.8±3.8	18.3±2.5	38.5±5.4	39.5±8.5	29.7±4.5	70.6±3
µgNO_3-N/g soil	76±10.5	128.5±50.9	89.7±2.4	128.4±15.6	12.6±3.6	17.1±8.5	29.1±14	32±9.3
mg P/kg dried soil	107.1±2.9	87.7 ±4.8	113.3±8.1	99.3±10.3	117.9±13.2	101.4±3.5	121.9±9.1	138.5±11.4
Organic matter (OM)%	13.4±1.8	14.7±0.8	15.7±2.1	17.1±1.5	12.8±0.7	15.9±0.9	17.7±2.1	19.1±3.4
Moisture Content (MC)%	5.1±0.86	7.1±1.7	5.3±1.1	5.5±0.6	9.7±1.2	13.6±0.8	8.2±0.9	8.9±2.5
pH	7.3±0.08	6.9±0.09	7.3±0.008	7±0.08	7.4±0.04	7.1±0.03	7.5±0.04	7.2±0.09
Bulk Density	0.8±0.04	0.7±0.03	0.73±0.1	0.6±0.05	0.8±0.05	0.8±0.02	0.6±0.03	0.7±0.04
Soil Porosity	71.3±1.6	74.3±1	72.6±4.4	76.8±1.8	68.4±2.2	68.6±1.2	75.8±1.3	73.4±1.5

	November			
Factor				
	Control		**Compost**	
	A3A	**A2S**	**A3A**	**A2S**
µgNH_4-N/g dried soil	9.2±2	26±4	31.4±24.8	38.5±2
µgNO_3-N/g soil	32.1±7.2	19.6±4.5	31.9±3.8	12.8±5.1
mg P/kg dried soil	102.5±9.3	81 ±1.2	102±1.9	94.7±2.9
Organic matter (OM)%	12.1±0.8	15.7±1.4	15.1±1.5	15.7±0.5
Moisture Content (MC)%	20.4±0.9	31.5±4.1	22.4±1.4	29.3±0.8
pH	7.6±0.2	7.4±0.2	7.8±0.2	7.4±0.2
Bulk Density	0.9±0.05	0.8±0.07	0.8±0.08	0.7±0.09
Soil Porosity	62.3±2.6	69.3 ±3.2	68.6±2.9	71.9±3.4

Appendix B. Summary of the percent cover of major plant species (>1% cover) and total percent vegetation cover in control and compost plots at both sites. Data are averages from big chamber bases in 2017 (average ± SE, n=4).

	July				September			
Species	**Control**		**Compost**		**Control**		**Compost**	
	A3A	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**	**A3A**	**A2S**
Phalaris arundinacea	0	38.8±14.7	4.8±2.1	16.3±4.3	0	45±16.6	0	22.5±14.5
Typha angustifolia	0.3±0.3	0	1±1	0	0	0.3±0.3	1.3±1.3	5±0.8
Typha latifolia	0	1 ±0.6	0	11±6.7	0	0.8±0.3	0	4.3±1.4
Lythrum salicaria	0	0	0	0.3±0.3	0.5±0.5	0	0.3±0.3	2.5±2.5
***Alisma* spp.**	0	1±0.4	0	3.8±2.4	0	0.3±0.3	0	0.3±0.3
Eupatorium perfoliatum	7 ±4.5	0	8.8±4.1	0	2.8±1	0	0.5±0.3	0
***Polygonum* spp.**	5.3±4.9	0	0	0	0.3±0.3	0	0	0
Persicaria punctata	7.5±4.3	0	13.8±6.8	0	0	0	0	0
Juncus effusus	3.8±3.8	0	0.5±0.5	0	8.8±8.8	0	0.3±0.3	0
***Solidago* spp.**	0	0	0	0	3±2.5	0	1±1	0
Total percent cover	52±6.9	42±14.6	30±9.3	33±6.1	30±6.3	66±7.1	13±2.6	33±14.2

Appendix C. Results of Tukey's HSD (honest significant difference) test of differences in NPP, ER, and GPP between measurement dates in control plots in 2017. Significant p-values are bolded.

Site	NPP			GPP			ER		
	Date		*p*	Date		*p*	Date		*p*
A3A	May	Jul.	**0.04**	May	Jul.	**0.01**	Jul.	May.	0.25
	Sep.	Jul.	**0.05**	Sep	Jul.	**0.03**	Jul.	Sep.	0.52
	May	Sep.	0.98	May	Sep.	0.88	Sep.	May.	0.83
A2S	Sep.	May	**0.02**	Sep.	May	0.08	Sep.	May	**0.02**
	Jul.	May	0.09	Jul.	May	0.17	Sep.	Jul.	0.09
	Sep.	Jul.	0.65	Sep.	Jul.	0.93	Jul.	May	0.51