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Temperature Effects on Greenhouse Gas Production From Treatment Wetland Soils Along a Nutrient Gradient

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ABSTRACT: It is generally accepted that increased temperatures are positively correlated with microbial respiration rates, causing greater greenhouse gas (GHG) emissions (CO_2 and CH_4) from wetlands. The goal of this study was to understand the interacting effects of temperature and nutrient concentrations on GHG emissions from wetland soils. Complementary field studies and a laboratory study were completed within Cell 1 of the Orlando Wetlands Park (Christmas, FL). Four sampling locations were established along a transect and sampled in summer and winter for the field studies. Soils (0-10 cm) were incubated under anaerobic conditions for 48-hours at ambient or elevated temperatures and GHG flux was measured. Surface water nutrients and soil physiochemical properties were also analyzed. Carbon dioxide and methane production both differed through an interaction between season and site ($p = 0.04$ and $p < 0.001$, respectively) with higher rates in the summer and at sites with the higher soil organic matter; water nutrients did not have a significant effect on GHG emissions. The laboratory study used only one soil sample, but varied water nutrients and temperature (3 x 3 factorial design) using four replicates per treatment and incubating under anaerobic conditions for 10 days. Temperature had a significant effect on both CO_2 and CH_4 production (both $p < 0.001$), but water nutrients did not have an effect, presumably due to existing high nutrient levels within the soil porewater. These results highlight the importance of soil properties (organic matter content and porewater nutrients) when determining the influence of temperature and water nutrients on GHG production.

KEYWORDS: wetland; treatment wetland; greenhouse gasses; eutrophication; climate change; biogeochemistry; soil

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1. INTRODUCTION

Excess amounts of greenhouse gasses (GHGs), particularly carbon dioxide (CO_2) and methane (CH_4), alter the energy balance of the Earth's climate system and are major drivers of climate change. Since the beginning of the Industrial Revolution, atmospheric CO_2 has increased from 278 ppm to 406 ppm (NOAA, 2017), while CH_4 has increased from 722 ppb to 1803 ppb throughout the same time scale (Ciais et al., 2013; Ballantyne et al., 2012). The largest proportion of these emissions are from anthropogenic manipulations, such as the burning of fossil fuels and land use change (IPCC, 2007; Johnson et al., 2007). Carbon dioxide accounts for approximately 65% of GHGs in the atmosphere. Although CH_4 accounts for a smaller percentage, it absorbs 30 times the infrared radiation absorbed by CO_2, contributing to increased warming (Houghton, 2001).

Greenhouse gas emissions from wetlands are primarily mediated by microbial respiration and decomposition. Wetlands are often inundated or saturated, causing microbially-mediated decomposition rates to be comparatively slower than adjacent upland environments (Reddy and DeLaune, 2008). This lack of oxygen, the most preferred electron acceptor for microorganisms, causes facultative aerobic bacteria and anaerobic bacteria to utilize alternative electron acceptors, resulting in different end products (Table 1). The rate at which these alternate electron acceptors are consumed depends on their relative abundance, the quantity of organic compounds (electron donor), and the microbial populations present (Reddy and DeLaune, 2008).

Many scientists expect increasing temperatures resulting from climate change will increase soil microbial respiration rates (Brooks et al., 1997; Butterbach-Bahl et al., 2011; Dalal and Allen, 2008; Holst et al., 2008). This increase in respiration rates is particularly important in wetland soils, which serve as a vast reservoir of accumulated carbon (C), nitrogen (N), and phosphorus (P) by incorporating dead plant biomass and other detritus into the soil matrix (Larmola et al., 2013). If warmer temperatures accelerate decomposition in wetland soils, a greater efflux of CO_2 and CH_4 is expected, creating a positive GHG feedback loop that could promote further warming.

Concurrently, anthropogenic activities, predominantly agricultural fertilizer use, cause eutrophication (bodies of water receiving excess nutrients). Most notable are N and P, primarily due to human activities such as agricultural fertilizer use and point-source pollution (Webber, 2010). This excess of typically limiting nutrients promotes the prolific growth of algae and other vegetation, the potential for harmful algal blooms, a decrease in water transparency, changes in fish communities, and hypoxic waters that result in "dead zones" (Ansari, 2011). As the human population continues to rise (increasing from 6.5 billion people in 2005 to a projected 9.2 billion in 2050 (UNPD, 2008)), fertilizer use is predicted to increase by 40 percent between 2002 and 2030 (FOA, 2000), thus contributing to amplified eutrophication. Nitrate (NO_3^-), ammonium (NH_4^+), and soluble reactive phosphorous (SRP) are the most bioavailable forms of N and P and greatly contribute to anthropogenic eutrophication (McCormick and Laing, 2003).

Treatment wetlands are constructed ecosystems dominated by aquatic vegetation that use natural processes to remove pollutants (Reddy and DeLaune, 2008). These systems can mitigate anthropogenic eutrophication by utilizing wetland vegetation, soils, and associated microbial communities to improve water quality (Chen, 2001; Kadlec, 2008, 2009; Marimon and Chang, 2016). They are commonly constructed for several different purposes, such as treating anthropogenic wastewater (domestic, agricultural, and industrial), reducing metals, and treating pathogens by biological means (Kadlec, 2009) at an inexpensive cost. However, it is not fully understood how both temperature and bioavailable nutrient concentrations interact to affect GHG emissions from treatment wetland soils. The goal of this study is to scrutinize the effect of climate change scenarios (increased temperatures and eutrophication) on CO_2 and CH_4 production in treatment wetland soils in order to assess the role of treatment wetlands to future GHG emissions. We hypothesized that GHG flux will be positively correlated with increased temperature and bioavailable nutrient concentrations.

2. METHODS

2.1 Site Description

The Orlando Wetlands Park (OWP), located in Christmas, Florida, spans 1,220 acres (Kadlec and Wallace, 2008). The OWP is designed to use wetland macrophytes to promote N and P removal and polish off tertiary treated domestic wastewater from the City of Orlando before entering the St. Johns River (Sees and Turner 1997). The Florida Department of Environmental

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Protection (FDEP) permits annual discharge limits of 2.31 mg L^{-1} for total nitrogen (TN) and 0.200 mg L^{-1} for total phosphorous (TP). In 2015, the OWP's annual discharge concentration for TN and TP were 0.95 mg L^{-1} and 0.075 mg L^{-1}, respectively, demonstrating the functionality of the constructed wetland (Rothfeld, 2015).

The OWP is divided into seventeen cells and three distinct wetland communities: deep marsh, mixed marsh, and a 90-acre lake. The deep marsh encompasses cells 1–12 and is strategically planted with cattails (*Typha latifolia*) and bulrush (*Scirpus spp.*) due to their ability to take up nutrients in wastewater (US. EPA. 1993; Figure 1). Average water depth of the deep marsh is 1 to 1.5 meters and is inundated year-round. The OWP is an excellent location to test our hypotheses due to the clear nutrient gradient that develops as water flows throughout the park from the inflow to the outflow. Based on data from the park's management, Stratum 1 (Cells 1, 2, 11, and 12) has been shown to be the most efficient system at polishing off NO_3^-, NH_4^+, and SRP within the surface water. Cell 1 served as our sampling location, as it contained an influent structure (source of nutrient-rich water entering the OWP), which served as a proxy for a eutrophic system, and because it was a part of the most successful stratum, meaning a large nutrient gradient should be present.

2.2 OBSERVATIONAL FIELD STUDY

2.2.1 Soil Sampling

We established four sampling sites along a 660 meter transect spanning the length of Cell 1 (Figure 1), spaced ~165 m apart. Sampling locations contained similar vegetation, including cattail (*Typha latifolia*) and bulrush (*Scirpus spp.*). Three soil cores were taken at each of the four sites via the push-core method, and each soil core was extruded in the field into two depth intervals: 0–10 cm and 10–20 cm. Soil samples were placed in sealed polyethylene bags. Surface water samples (1 L) were collected at each site in acid-washed polyethylene bottles to determine ambient concentrations of NH_4^+, NO_3^-, and SRP via U.S. EPA methods 50.1 Revision 2.0, 353.2 Revision 2.0, and 365.1 Revision 2.0, respectively (U.S. EPA, 1993). Sampling occurred twice along this transect (June 27, 2016 and January 20, 2017) to capture seasonal variability. Segmented samples were placed on ice and immediately transported back to the laboratory, where field replicates were homogenized to provide a representative sample of site conditions at each depth.

2.2.2 Greenhouse Gas Production (CO_2 and CH_4)

Eight (8) replicate bottle incubations were created within 24 hours of sample collection by weighing 15 g field-moist soil, exclusively from the 0–10 cm depth interval, into 100 mL serum bottles. The bottles were capped with a rubber septa and aluminum crimp, evacuated to -75 mmHg, and purged with 99% O_2-free N_2 gas for three minutes in order to mimic the anaerobic conditions of the site. Fifteen (15) mL of site water was filtered via Supor 0.45 µm membrane filters, purged with N_2 gas, and injected into each bottle. Four bottles from each site were randomly assigned to each of two treatments—ambient and elevated temperature. Ambient temperature was defined as the atmospheric temperature during the time of sampling; elevated temperature was based on a four degree Celsius increase from the ambient temperature. The four degree Celsius increase was chosen for the elevated temperature due to the IPCC report "Climate Change 2007: Impacts, Adaptation, and Vulnerability," which concludes that the global average temperature is predicted to increase 1.4–5.8°C by 2100 (Parry et al., 2007). During the summer sampling, ambient temperature was 32°C and elevated was 36°C. During winter, ambient temperature was 22°C and elevated was 26°C. Bottles were incubated continuously in an orbital shaker at 150 rpm at their respective temperature treatment for 48 hours. Carbon dioxide and CH_4 samples were extracted from the bottle headspace using gas-tight syringes at 6, 12, 24, and 48 hours and analyzed using a GC-2014 Gas Chromatograph (Shimadzu Instruments, Kyoto, Japan) equipped with a flame ionization detector (FID), and a Hayesep N 80/100 Mesh 1/8in X 1.5M stainless pre-conditioned column. Breathing grade air was used as the carrier gas. The temperature of the injection port was sufficient to sterilize the syringe between injections. Respiration rates at each temperature were calculated as linear slopes obtained by repeated measures of CO_2 and CH_4 concentrations over time. The fraction of CO_2 and CH_4 dissolved in the liquid phase was calculated via the use of Henry's Law.

2.3 CONTROLLED LABORATORY STUDY

2.3.1 Soil Sampling

A laboratory study was conducted to determine the interactive effects of temperature and nutrient

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concentrations on greenhouse gas emissions, independent of other environmental variables. Fourteen (14) cores were collected within a 5 m^2 area in Cell 1 (28˚34'20.2"N and 81˚00'33.2W) and extruded to a depth of five (5) centimeters. Soils were stored in sealed polyethylene bags on ice and immediately transported to the laboratory, where samples were homogenized to eliminate spatial variability. In addition to soil cores, 1 L of surface water was collected in an acid-washed polyethylene bottle.

2.3.2 Greenhouse Gas Production (CO_2 and CH_4)

To determine the interactive effects of nutrient concentrations and temperature on greenhouse gas emissions, soil bottles were randomly assigned to one of three temperature treatments and also one of three nutrient treatments in a 3 x 3 factorial design. Again, there were four replicates per treatment, totaling 36 bottles. The same preparations for bottle incubations were followed for the controlled laboratory study as described in section 2.2.2 of this paper. Three temperature treatments were established (25˚C, 30˚C, and 35˚C) and were randomly assigned to one of three different aqueous nutrient treatments: high, medium, or low concentrations of ammonium, phosphate, and glucose. Ammonium was chosen as the N source because it is the predominate form available in anaerobic wetland soils. Phosphate was used for the P source because it is the bioavailable form of P (Reddy and DeLaune, 2008). To isolate the effects of the N and P treatments and avoid impeded microbial metabolism due to C limitations, all three nutrient treatments received a non-limiting amount of glucose as a C source. The phosphate solution was created using 1000 ppm Phosphate Standard and the ammonium solution was made from Ammonia Standard, 1000 ppm NH_3, both by Ricca Chemical Company. The glucose solution was made from D(+)-Glucose monohydrate (manufactured by Acros Organics) from Fisher Scientific (Table 2). The 'High' nutrient concentrations were defined as the highest level of the respective nutrient that has ever been loaded into the OWP, which was 2.2 mg L^{-1} and 0.5 mg L^{-1} for TN and TP, respectively (Rothfeld, 2015). 'Medium' nutrient concentrations were half of the high concentrations; 'low' nutrient concentrations received pure nanopure water (Table 2). All four replicates per treatment were incubated continuously in an orbital shaker at 150 rpm at their respective temperatures for ten days, while CO_2 and CH_4 production was measured at intervals of 24, 48, 72, 144, 192, and 240 hours.

2.4 Soil and Water Properties

Within the laboratory, the chemical and physical properties of the soils at each depth segment from both sections 2.2.1 and 2.3.1 were analyzed, including bulk density, percent organic matter (OM), percent moisture content, pH, extractable nutrients, total carbon (TC), TN, and TP. Surface water and porewater were filtered through Supor 0.45 μM filters and acidified with double-distilled sulfuric acid (DDI H_2SO_4) for preservation and analyzed for NO_3^-, NH_4^+, and SRP concentrations. Samples were stored at 4˚C and all analyses were performed within three weeks of sampling.

Moisture content and bulk density were determined by drying a subsample at 70˚C for three days until a constant weight was achieved. Dried soils were ground using a SPEX Sample Prep 8000M Mixer/Mill (Metuchen, NJ). Ground soils were analyzed for TC and TN using a Vario Micro Cube CHNS Analyzer (Elementar Americas Inc., Mount Laurel, NJ). Percent OM was determined by loss on ignition (LOI), while dry soils were combusted at 550 ˚C for 5 hours and final weight was subtracted from initial weight. Total P was determined by digesting the resulting ash from LOI in 50 mL of 1 N HCl on a hot plate for 30 minutes at 65.5 ˚C . The TP solution was then filtered through a Whatman #41 filter paper (Andersen, 1976). Extractable porewater nutrients (NH_4^+, NO_3^-, and SRP) were processed by placing 2.5 g of wet soil into a 40 mL centrifuge tube with 25 mL of 1 M KCl and shaking the solution for an hour at 150 rpm. Samples were then placed in a centrifuge for 10 minutes at 4000 rpm and 10˚C, filtered through Supor 0.45 μM filters, and acidified with DD H_2SO_4 for preservation. Surface water, porewater, TP, and extractable nutrients samples were analyzed using an AQ2 Automated Discrete Analyzer (Seal Analytical, Mequon, WI) according to EPA method 365.1 Rev. 2 (U.S. EPA, 1993).

2.5 Microbial Biomass Carbon

Microbial Biomass C (MBC) was determined in all depth segments by chloroform fumigation (Vance et al., 1987). Fumigate and non-fumigate duplicates were prepared by placing 2.5 g of wet soil in 40 mL centrifuge tubes. The fumigated samples were exposed to volatilized chloroform for 24 hours in a glass vacuum desiccator in order to detect the organic C within the microbial

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cell wall. After 24 hours, the fumigated samples were extracted with 25 mL of 0.5M K_2SO_4, placed in an orbital shaker for 1 hour at 150 rpm and 25°C, then centrifuged for 10 minutes at 4000 rpm and 10°C. The sample was vacuum-filtered through Supor 0.45 μM filters, and acidified with DD H_2SO_4 for preservation. The non-fumigate samples served to quantify dissolved organic C and were prepared in the same way, except for not being exposed to the chloroform fumigation. Total organic carbon (TOC) was found by using a Shimadzu TOC-L Analyzer (Kyoto, Japan) for both treatments. Microbial biomass C was calculated by the difference between the fumigated samples and the non-fumigated samples.

2.6 Statistics

In order to determine the temperature and nutrient effects on GHG production, statistical analysis was conducted in R (R Foundation for Statistical Computing, Vienna, Austria). Datasets met the assumptions of homogeneity of variance, which was tested via use of the Levene's test ($\alpha = 0.05$). Normality was verified by the Shapiro-Wilks test ($\alpha = 0.05$); datasets that did not meet the assumptions of normality were logarithm-transformed to verify normality. A two-way ANOVA was used to determine the significance of predictor variables temperature, season, and site (distance from inflow) in predicting CO_2 and CH_4 production ($\alpha = 0.05$). Akaike's Information Criterion (AIC) was used to implement a model simplification approach, which determined that the most appropriate predictor variables were season, site (distance) and the interaction between them for the field study. The same approach was implemented in the controlled laboratory study, which determined that temperature was the only significant predictor variable to determine GHG emissions, which excluded nutrient availability. Tukey post-hoc tests were used to determine significance of pairwise comparisons, also using $\alpha = 0.05$.

3. RESULTS

3.1.1 Observational Field Study Greenhouse Gas Production

Carbon dioxide production differed by season and site ($p < 0.001$, $p = 0.052$), as well as the interaction between the two predictor variables ($p = 0.043$). CO_2 production ranged from 0.226 ± 0.059 μg CO_2-C g^{-1} d^{-1} at site 4 during the winter to 17.3 ± 7.23 μg CO_2-C g^{-1} d^{-1} at site 2 during the summer (Figure 2). In general, summer CO_2 production was greater than winter CO_2 production. Though site was a significant predictor of CO_2 production, a clear trend of decreasing CO_2 production with increasing distance from the inflow was only evident during the winter sampling. Temperature did not have a significant effect on CO_2 production, and was thus not incorporated into the model, but CO_2 production was positively correlated to CH_4 production, TN, TC, MBC, percent OM, and moisture content, while CO_2 production was negatively correlated to bulk density and C/N ratio (Table 3).

Similar to the results of CO_2 production, CH_4 production differed by both season and site, and there was a significant interaction between the two variables (all $p < 0.001$). Sites 2 and 3 were not significantly different from each other; all other sites differed from each other. Winter CH_4 production was lower than summer CH_4 production. Methane production ranged from 0.002 ± 0.0001 μg CH_4-C g^{-1} d^{-1} during the winter at site 3 to 0.411 ± 0.030 μg CH_4-C g^{-1} d^{-1} at site 3 during the summer (Figure 3). Temperature treatment (ambient and elevated) was excluded from the model, as they were not significant predictor variables for CH_4. Methane was significantly correlated to CO_2 production, TN, TC, MBC, percent OM, and moisture content, while negatively correlated to bulk density and C/N ratio (Table 3). In addition to soil physiochemical properties, CH_4 production was significantly correlated to surface water NH_4^+ and porewater NH_4^+.

Though there were observable differences in soil physiochemical properties and microbial biomass C (MBC) at each site, low replication prevented analysis via ANOVA due to the soils being homogenized for a representative sample. However, all soil physiochemical properties were correlated to each other (Table 3), as well as MBC. All correlations were positive except for bulk density, which was significantly negatively correlated to all other physiochemical properties and microbial biomass C.

3.1.2 Observational Field Study Nutrient Analysis

Surface water NO_3^- was highest at site 1 in winter with 1.13 mg N/L and lowest at sites 2 and 4, with no NO_3^- detected. Porewater NO_3^- was only detected in site 3 at 0.015 mg N/L in the winter and varied from 0.264 mg N/L at site 1 to 0.032 mg N/L at site 4 in the summer. Surface water NO_3^- varied from 0.136 mg N/L in site 1 to 0.011 mg N/L in site 3 during summer.

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Surface water NH_4^+ was found to be highest in site 2 with 0.163 mg N/L and lowest in site 1 with 0.091 mg N/L during the winter. Surface water NH_4^+ increased during the summer and varied from 0.338mg N/L in site 1 to 0.124 mg N/L at site 4. Porewater NH_4^+ was highest in site 1 with 2.23 mg N/L and lowest in site 4 with 0.393 mg N/L in winter. During summer, there was a large spike in porewater NH_4^+ which ranged from 12.4 mg N/L in site 1 to 3.07 mg N/L in site 4.

Surface water SRP showed an opposite trend during winter and had the highest concentration in site 4 (0.424 mg P/L) and the lowest in site 1 (0.270 mg P/L). In summer, surface water SRP was greatest within site 3 at 0.392 mg P/L and lowest in site 4 at 0.116 mg P/L. Winter porewater SRP was greatest in site 1 with 1.77 mg P/L and lowest in site 4 with 0.574 mg P/L. Summer porewater SRP was greatest in site 4 with 1.314 mg P/L and lowest in site 2 with 0.429 mg P/L.

Overall, the clear nutrient gradient we expected to find between the inflow and outflow of cell 1 was only observed for winter porewater SRP with nutrient concentrations decreasing with distance from the inflow. Other nutrients that had the highest concentrations in site 1 and lowest in site 4, but no linear decrease, were: winter surface water NO_3^-, winter surface water NH_4^+, summer surface water SRP, summer porewater NO_3^-, and summer and winter porewater NH_4^+. From this information, soil nutrients were determined to have a greater effect on GHG emissions from wetland soils compared to surface water nutrients because microbial populations cannot utilize the nutrients suspended within the surface water as readily.

3.2 Controlled Laboratory Study

Temperature treatments had a significant relationship with CO_2 production ($p < 0.001$). Carbon dioxide production increased with increasing temperature, ranging from 0.966 ± 0.037 μg CO_2-C g^{-1} d^{-1} at the lowest temperature treatment (25˚C) to 1.82 ± 0.040μg CO_2-C g^{-1} d^{-1} at the highest temperature treatment (35˚C) (Figure 4a). Each temperature treatment differed from every other treatment. Temperature was also the only predictor of CH_4 production ($p < 0.001$), increasing with increasing temperature. The 25˚C treatment produced 0.149 ± 0.011 μg CH_4-C g^{-1} d^{-1}, while the 35˚C produced 0.423 ± 0.014 μg CH_4-C g^{-1} d^{-1} (Figure 4b). None of the concentrations of nutrients added had a significant effect on either CO_2 or CH_4 production, and thus were excluded from the model.

4. DISCUSSION

4.1 CO_2 and CH_4 Emissions

Several controlled laboratory studies and field studies have been conducted to determine the effects of temperature on GHG emissions (Brooks et al., 1997; Butterbach-Bahl et al., 2011; Chin et al., 1999; Dalal and Allen, 2008; Fang and Moncrieff, 2001; Holst et al., 2008; Inglett et al., 2012), while other studies have studied bioavailable nutrients as a factor for stimulating GHG emissions from soils (Bridgham and Richardson, 1992; Niu et al., 2010; Peng et al., 2011; Shi et al., 2014). The goal of our study was to use natural field conditions concomitant with a controlled laboratory study to determine if temperature and nutrient availability work synergistically to control GHG emissions from wetland soils. In general, temperature was positively correlated to CO_2 and CH_4 emissions, though the interaction with nutrient availability was less clear due to our experimental considerations (see Bioavailable Nutrients discussion below).

In a controlled laboratory study, Inglett et al. (2012) reported an increase in both emissions with increased temperature under aerobic and anaerobic conditions, using different soil types incubated at 10, 20, and 30˚C. In our study, though not statistically significant, three out of the four sites had higher CO_2 production at 32˚C than at 36˚C, suggesting that a maximum temperature threshold may occur that impedes microbial respiration. O'Connel (1990) also found in a laboratory study that the optimal temperature for microbial decomposition was approximately 30˚C and any temperature above that threshold decreased respiration rates. However, this trend was not applicable to CH_4 production, which suggests that methanogens (microbes that produce CH_4 as a metabolic byproduct in anaerobic conditions) are more stimulated by increased temperatures compared to other soil microbes. After a 30-day incubation period of anoxic rice field soils, Chin et al. (1999) found that at 30˚C methanogenic archea predominated, whereas at 15˚C, the archeael community was significantly more diverse. These findings suggest that methanogenic microbes become more dominant as temperature continues to rise.

In a laboratory setting, Fang and Moncrieff (2001)

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found an exponential increase in CO_2 emissions with increased temperature, up to 32°C, for upland soils with different soil moisture contents and incubation periods. This observation correlates to the findings of our laboratory study, suggesting that temperature is a factor determining GHG flux rates, but there are more variables that regulate CO_2 production in wetland soils. Chapuis-Lardy et al. (2007) concluded that microbial activity, root respiration, soil organisms, and fungi were all sources of GHGs in soils. Additionally, Ludwig et al. (2001) found that soil moisture, pH, soil temperature, and bioavailable nutrients were also factors determining GHG emissions from soils.

Based on the significant correlations with OM and both GHG emissions in this study, our results suggest that one of the most important factors when considering GHG emissions from treatment wetland soils is the content of OM in the soils (Table 3). As OM content of the soils increases, microbial and plant communities will also increase in biomass due to the high bioavailable forms of C and N. In turn, as microbial communities increase in both biomass and activity, more soil OM will be decomposed and CO_2 and CH_4 will be produced at faster rates. By way of example, the soil from site 4 during the summer consisted of only 3.67% OM and thus had the lowest emissions for both GHGs under both ambient and elevated temperatures, while the highest soil OM content was found in site 3 (24.3%) and had the highest CH_4 emission and the second highest CO_2 emission for each temperature treatment. For the same season, site 4 also had the lowest microbial biomass (14,467.17 mg/kg) and site 3 had the highest (81,613.03 mg/kg). This trend was also found during the winter with site 1 having the highest soil OM content of 8.42% and the highest emissions for both GHGs under both temperature treatments. The lowest soil OM content for winter was in site 2 (1.58%) but had the second highest emissions for both GHG under both temperature treatments, although almost negligibly higher than sites 3 and 4 (Figure 2 and 3), which could potentially be explained by the sites' high moisture content (41.8%) (McKenzie et al., 1998).

The observed differences in soil OM along the field transect may be because Cell 1 was 'demucked' in 2001, where approximately 46 cm of sediment was removed in order to rejuvenate the hydraulic flow of surface water. We found that approximately 24 cubic yards of white builders sand was deposited and spread within Cell 1 in order to serve as an identifiable layer within the soil profile. The sand serves to quantify the rate of OM accumulation over time after the demucking procedure (personal communication, M. Sees). We found this sand layer approximately 18 to 25 cm below the surface layer for a majority of the samples. Additionally, the OWP has historically always had surficial soils consisting of fine sand underlain by clayey soils (US. EPA. 1993). We suspect that this presence of fine sand is the primary reason why many of our soil samples contained minimal OM, compared to other freshwater marshes. Our results can be applicable to wetlands with low rates of vegetative senescence and thus slow soil OM accumulation rates, or to wetlands dominated by sandy soils.

4.2 Bioavailable Nutrients

For the controlled laboratory study, we observed a general increase in CH_4 as nutrient concentrations and temperatures increased. Within the observational field study, porewater and surface water NH_4^+ were the only observed nutrients to be positively correlated with CH_4. There is much discrepancy as to how NH_4^+ affects CH_4 emissions (Oertel et al., 2016). The negative correlation between the two can be explained at the biochemical level (Dunfield and Knowles, 1995; Gulledge and Schimel, 1998) and also at the microbial community level (Bodelier and Laanbroek, 2004). However, at the ecosystem level, increased availability of NH_4^+ generally increases plant growth.

Carbon dioxide and CH_4 emissions generally increased with increasing nutrients, but the effect was not statistically significant in either of our laboratory or field studies. We hypothesize there was not a significant nutrient effect on GHG emissions in the observational field study because the soils at each site differed significantly from each other, particularly in terms of soil OM content. We likewise did not see a nutrient effect in our controlled laboratory study, likely due to high initial concentrations of NO_3^-, NH_4^+, and SRP in the soil porewater (1.28 mg/g, 94.2 mg/g, and 13.5 mg/g, respectively). Since the soil microbes had access to this nutrient-rich porewater under all treatment conditions, the N and P added within the surface water were less important as these nutrients were already likely to be non-limiting.

5. CONCLUSION

The emissions of CO_2 and CH_4 from treatment wetland

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soils increased with increasing temperatures between 25 and 35˚C. The same general trend of increasing GHG production with increasing temperature was also observed in the field study, but the effect was not significant. Bioavailable nutrients in the surface water (NH_4^+, NO_3^-, and SRP) did not show any statistical significance when considering GHG emissions for any of the studies conducted; however, general increases in emissions were observed with increasing nutrients for each study. We suspect we did not observe a nutrient effect on GHG emissions for the field study because differences in soil properties (specifically, OM content) were so significant that surface water nutrient concentrations were not the limiting factor for soil microbial activity. Similarly, we did not see a nutrient effect in the controlled laboratory study due to the soils already being highly eutrophic, as exemplified by high porewater nutrient concentrations. In order to isolate the effect of bioavailable nutrients on GHG emissions from wetland soils, another laboratory study should be conducted with oligotrophic soils in order to isolate the effects of both nutrient concentration and temperature.

According to our findings, microbial communities that have CO_2 as a byproduct of respiration may have a temperature threshold around 30–35˚C, where metabolic processes are not stimulated at or above this range. This finding can be used to make accurate predictions for future climate change models (specifically for the C budget) when trying to understand the C flux from wetlands under climate change scenarios. Alternatively, a temperature threshold did not exist for CH_4, showing that this microbial community may have increased metabolic rates as temperature continues to rise. Though CO_2 was still the predominant GHG being produced for both studies and all temperature and nutrient treatments, the global warming potential of CH_4 must be taken into consideration when making predictions for future atmospheric temperatures. Another study should be conducted in order to identify different microbial communities within the soil, using qPCR before and after an extended incubation period at the same temperature and nutrient treatments selected in the controlled laboratory study to determine how these communities change under both treatments.

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Figure 1: Schematic of the OWP indicating cell numbers and flow trains.

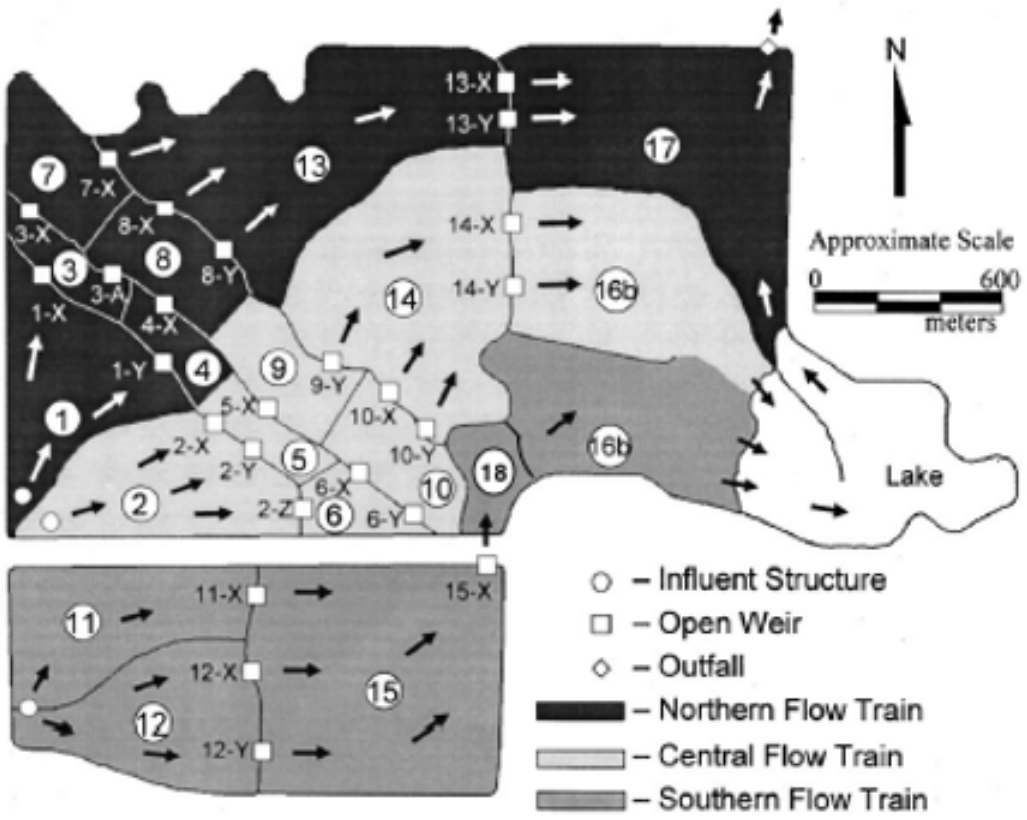

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Figure 2: Carbon dioxide flux for the observational field study for both seasons and temperature treatments. Error bars represent standard error of the mean. Ambient and elevated temperatures for winter were 22˚C and 26˚C, respectively. Ambient and elevated temperatures for summer were 32˚C and 36˚C, respectively. Error bars with the same letter are not significantly different.

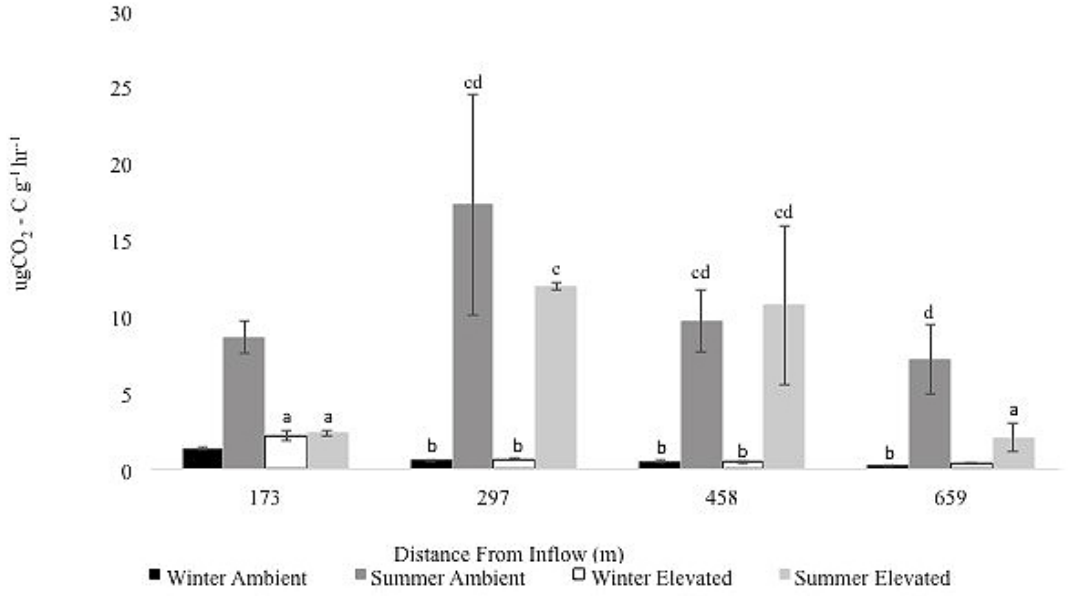

Figure 3: Methane flux for the observational field study for both seasons and temperature treatments. Ambient and elevated temperatures for winter were 22˚C and 26˚C, respectively. Ambient and elevated temperatures for summer were 32˚C and 36˚C, respectively. Error bars represent standard error of the mean. Error bars with the same letter are not significantly different.

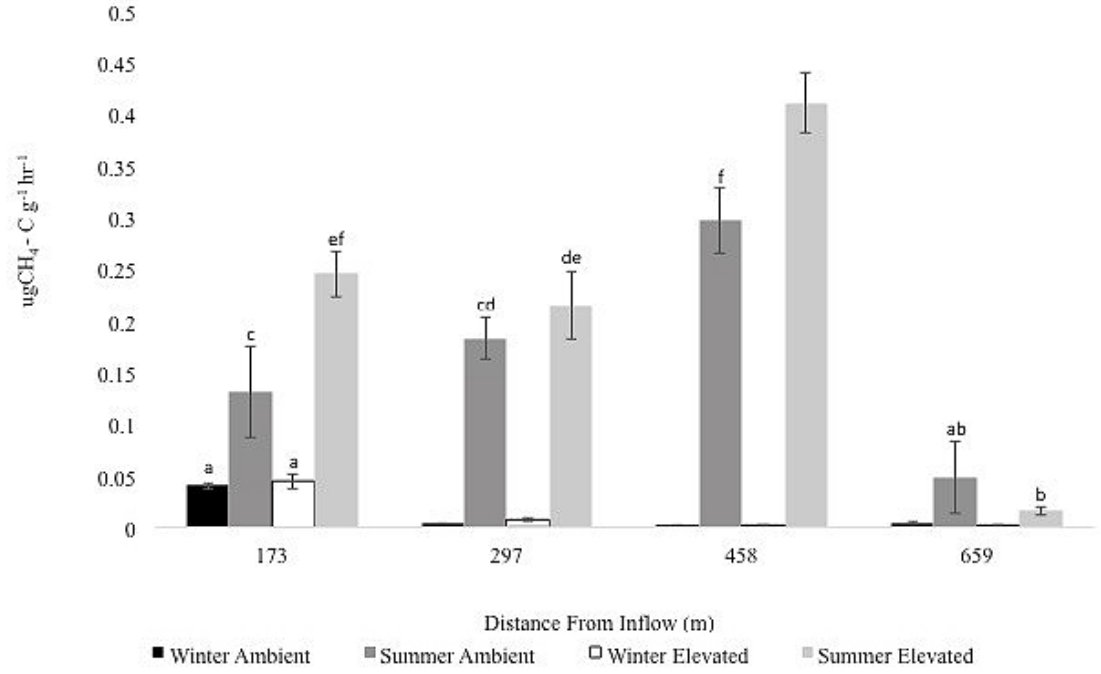

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Figure 4a: Carbon dioxide flux for the controlled laboratory study with respect to the added nutrient concentration. Error bars represent standard error of the mean. Error bars with the same letter are not significantly different.

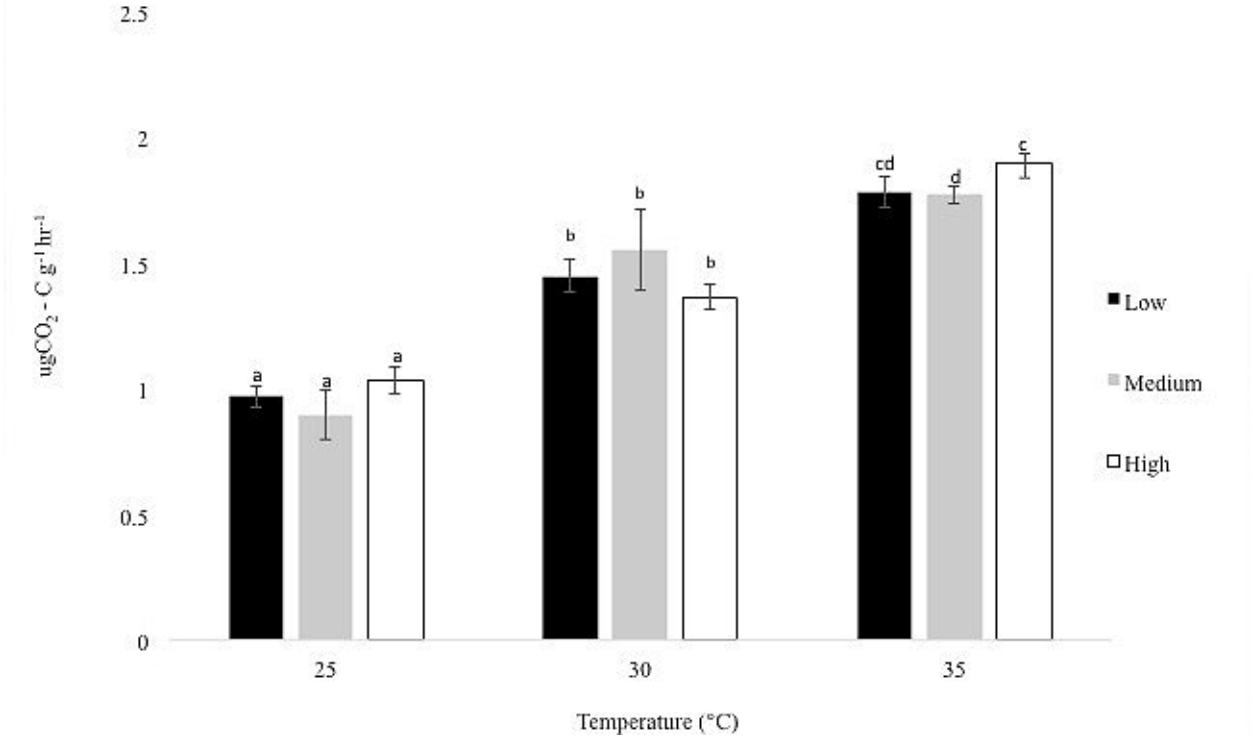

Figure 4b: Methane flux for the controlled laboratory study with respect to the added nutrient concentration. Error bars represent standard error of the mean. Error bars with the same letter are not significantly different.

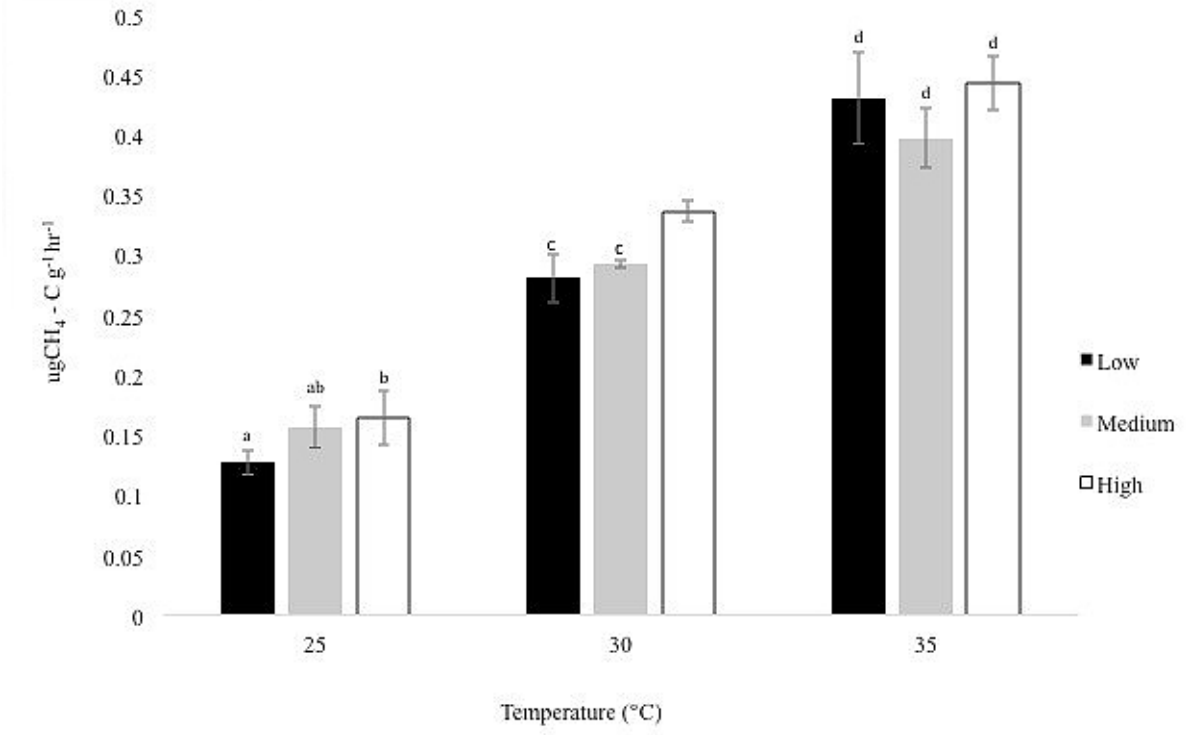

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Table 1: Electron acceptors used for microbial respiration and their end products. Metabolic energy yield decreases with each subsequent pathway.

Sequence of Reduction	*Electron Acceptor*	*Electron Donor*	*End Products*
1	O_2	Organic Matter	$CO_2 + H_2O$
2	NO_3^-	Organic Matter	$CO_2 + N_2O/N_2$
3	Mn^{4+}	Organic Matter	$CO_2 + Mn^{2+}$
4	Fe^{3+}	Organic Matter	$CO_2 + Fe^{2+}$
5	SO_4^{2-}	Organic Matter	$CO_2 + H_2S$, S
6	CO_2/H_2	Organic Matter	CH_4

Table 2: Nutrient concentrations added to 1 L of nanopure water to mimic surface water nutrient concentrations.

	High	*Medium*	*Low*
Phosphate (mL)	0.4	0.2	0
Ammonium (mL)	1.1	0.55	0
Glucose (g)	0.146	0.145	0.144

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Table 3: Correlations of soil physiochemical properties, soil/water nutrients, and MBC to both GHG emissions for both seasons of the observational field study. Italic values denote significance. Critical value is 0.497 and n = 16.

	CO_2 Flux	CH_4 Flux
CO_2 Flux		
CH_4 Flux	*0.712*	
TP (mg/g)	-0.292	-0.277
TN (%)	*0.812*	*0.915*
TC (%)	*0.800*	*0.912*
C/N Ratio	*-0.595*	*-0.569*
MBC	*0.873*	*0.937*
pH	0.207	0.249
Organic Matter	*0.707*	*0.878*
Bulk Density	*-0.732*	*-0.834*
Moisture Content	*0.758*	*0.850*
Surface Water NO_3^-	-0.351	-0.299
Surface Water NH_4	0.221	*0.558*
Surface Water SRP	-0.225	0.197
Porewater NO_3^-	0.255	0.477
Porewater NH_4	0.427	*0.669*
Porewater SRP	-0.449	-0.282

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