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Carbon dioxide (CO2) and methane (CH4) fluxes from agricultural drainage canals at the Timberlake Observatory for Wetland Restoration in North Carolina's coastal plain

Cover Page Footnote

Hannah Schleupner is an undergraduate at Hollins University. Though currently undecided, she will likely pursue a major in biology and a minor in social justice. Katherine Juarez is a fourth-year student at Wake Forest University pursuing a degree in computer science with a minor in biology. Mary Jane Carmichael is a Visiting Assistant Professor of Biology and Environmental Studies at Hollins University in Roanoke, Virginia. She is the faculty sponsor of this research and can be reached via email at carmichaelm@hollins.edu. The authors would like to thank Joseph White for field assistance, Scott Cory for assistance with statistical analyses, and William K. Smith and members of the Fall 2017 Biogeochemistry course at Hollins University for advice and helpful discussion.

1. Introduction

Wetlands represent the largest of all natural and anthropogenic methane (CH4) sources (Myhre et al., 2013; Schlesinger & Bernhardt, 2013). Natural and agricultural wetlands comprise 3.6% of Earth's surface, yet account for ca. 35-40% of CH4 in the atmosphere (Yavitt, 2010). CH4 has a global warming potential ca. 28-32x that of CO₂ over a 100-year period (Myhre et al., 2013), emphasizing the importance of understanding the processes and dynamics that control the flux of this potent greenhouse gas (GHG) to the atmosphere.

The exchange of GHGs in wetlands takes place across three primary interfaces, the water-atmosphere (Carmichael et al., 2018; Helton et al.2014; Poindexter et al., 2016), sediment-atmosphere (Chanton et al., 1989; Morse et al., 2012), and plant-atmosphere interfaces (Carmichael et al., 2018; Rusch & Rennenberg, 1998; Schütz et al., 1991). Fluxes across any of these interfaces can be influenced by a variety of factors, including environmental conditions such as atmospheric pressure (Clements & Wilkening, 1974; Mattson & Likens, 1990), hydrologic controls such as soil moisture content (Davidson et al., 2004) and the position of the water table (Strack & Zuback, 2013), and soil nutrient content, particularly carbon quality (Corteselli et al., 2017; Joabsson et al., 1999) and quantity (Schimel, 1995).

A wetland is defined as a transitional region between terrestrial and aquatic systems, in which the water table sits near, level to, or slightly above the land surface (Cowardin et al., 1979). In general, there are three important defining characteristics of a wetland environment: (1) the land periodically supports predominantly hydrophytes, (2) the substrate is predominately undrained hydric soil, and/or (3) the substrate is nonsoil and is saturated with water or covered by shallow water at some time during the annual growing season (Cowardin et al., 1979). A majority of research regarding GHG fluxes from wetlands is conducted in large-scale, permanently flooded, and/or easily classified wetlands. In contrast, cryptic wetlands – wetlands that may be small-scale, seasonally inundated, and/or otherwise difficult to identify or characterize on a landscape– are not studied to the same degree (Carmichael et al., 2014; Yavitt, 2010). This imbalance is likely the result of the difficulty inherent in locating and classifying these unique environments in a landscape – obstacles that may also explain the inadequate knowledge of the extent of

cryptic wetlands globally (Yavitt, 2010). Therefore, a gap currently exists in literature regarding the relative importance of cryptic wetlands (Martinson et al., 2010) in global biogeochemical cycles.

Agricultural drainage canals meet the criteria necessary for classification as a wetland (Cowardin et al., 1979), and may also be considered as cryptic wetlands due to the localized small-scale of these ecosystems within a landscape. However, even though they are individually small, the collective imprint of drainage canals across a landscape, especially in regions where land use is dominated by agriculture, may be large. Thus, agricultural drainage canals may represent an unrecognized source in the annual flux of GHG gases to the atmosphere from wetland ecosystems. The present study is a preliminary attempt to quantify the role of agricultural drainage canals in CO₂ and CH₄ fluxes to the atmosphere from a wetland ecosystem.

2. Materials and Methods

Site description

Due to the potential for highly productive croplands, large areas of North Carolina's Albemarle-Pamlico Peninsula (Figure 1) were converted from wetlands to farmland in the 1970's (Carter, 1975). However, due to the low-lying elevation of the region (Titus & Richman, 2001), land in the Albemarle-Pamlico Peninsula drains poorly and farmland must be intensively managed, often through the installation of extensive drainage infrastructure (i.e. canals and ditches) and pump station systems to prevent soil waterlogging and declines in crop productivity. Although individually small, the collective area of agricultural drainage infrastructure across the Albemarle-Pamlico Peninsula is extensive.

The Timberlake Observatory for Wetland Restoration (hereafter TOWeR) is a former tract of farmland located in Tyrrell County, North Carolina (35°54'22"N, 76°09'25"W, Figure 1) that was under active restoration during the early-mid 2000s (Ardón et al., 2010). This 4,200 acre site consists of ca. 634 acres of former agricultural land that was drained by 24 acres of vee-ditches and a pump station (Needham, 2006). Although a majority of the former drainage infrastructure at

TOWeR was reclaimed during the wetland restoration process, agricultural drainage canals still connect the restored wetland to the surrounding landscape, and ultimately the Albemarle Sound.



Figure 1: Location of the Timberlake Observatory for Wetland Restoration in relation to the state of North Carolina and the Albemarle-Pamlico Peninsula. The white outline in the map inset denotes the location of the Timberlake Observatory for Wetland Restoration in Tyrrell County, NC. Image was created using Google Earth (copyright by DigitalGlobe).

Four separate locations within the study site (Figure 2a) were chosen for sampling fluxes of CH₄, and CO₂ from agricultural drainage canals (Figure 2c). These sites were selected to measure GHG fluxes at multiple locations influenced by different land-use practices along the primary drainage canal at TOWeR. The Inflow site was chosen to measure GHG fluxes where water from adjacent agriculture fields flows into the wetland during heavy rains. The Wetland and Outflow sites were chosen to determine how the wetland might act as a filter and impact water-atmosphere GHG fluxes. The Roadside site was chosen to determine how surface water runoff from the adjacent highway might impact GHG fluxes from drainage canals.



Figure 2: (a) Sample sites within the Timberlake Observatory for Wetland Restoration, including sites from this study (squares) and Wetland data from Carmichael et al. (2018) (circle) for comparison purposes; (b) deployed floating static flux chambers for the measurement of water-atmosphere greenhouse gas fluxes; (c) representative image of a wetland drainage canal. The image in panel (a) was created using Google Earth (copyright by DigitalGlobe).

Site mesoclimate and environmental measurements

Environmental variables were continuously measured at each sampling location (Figure 2a) in July 2016 and compared to historical data from the State Climate Office of North Carolina's Climate Retrieval and Observations Network of the Southeast (CRONOS) Database monitoring station #311949 located within 2 km of TOWeR in the Gum Neck Community of Tyrrell County, North Carolina. Air temperature and relative humidity were measured continuously at 2m above ground using a HOBO Pro V2 sensor and data logger (Model U23–001, Onset, Bourne, MA) shielded from direct sunlight and the nighttime sky.

Daily water quality measurements were taken in the wetland at each site as described in Carmichael & Smith (2016). Salinity was monitored using a YSI EcoSense EC300A portable conductivity, salinity, and temperature meter (YSI, Yellow Springs, OH). Surface water pH was monitored using a YSI EcoSense pH100A portable pH, mV, and temperature meter. All instruments were calibrated in the field prior to measurements. In addition to mesoclimate and water quality measurements, and water depth at each site was measured (Carmichael et al., 2018).

Water-atmosphere greenhouse gas fluxes

Water-atmosphere GHG fluxes were measured using a static chamber approach, following a protocol previously used at TOWeR (Carmichael et al., 2018; Helton et al., 2014). Floating static flux chambers (Figure 2b) were constructed from 10L gas sampling bags as described in detail in Helton et al. (2014). Static flux chambers were deployed at three sites (Inflow, Outflow, and Roadside) at TOWeR (Figure 2a) over a 24 h period in July 2016. Data for the Wetland site are included in this manuscript with permission from Carmichael et al. (2018) for comparison. At the beginning of each sampling interval, air temperature, barometric pressure, and wind speed were recorded using a Kestrel 4000 weather and environmental meter (Kestrel Instruments, Boothwyn, PA). Triplicate 10 mL gas samples were collected from each chamber as described in Helton et al. (2014) and Carmichael et al. (2018) at three time points over a 24 h incubation: 0, 8, and 24 hours.

Gas analyses

All gas samples were stored at room temperature for less than one week before analysis via gas chromatography at the Duke River Center. Gas samples were analyzed for CO₂ and CH₄ concentrations at the Duke River Center following the protocol outlined in Carmichael et al. (2018), Carmichael & Smith (2016), Helton et al. (2014), and Morse et al. (2012). Samples were injected by a Tekmar 7050 Headspace Autosampler into a Shimadzu 17A gas chromatograph with an electron capture detector and flame ionization detector (Shimadzu Scientific Instruments, Columbia, MD) retrofitted with six-port valves and a methanizer to allow the determination of the three gases from the same sample. Ultra-high purity N₂ was used as the carrier gas, and a P5 mixture served as the make-up gas for the electron capture detector. A Nafion tube (Perma Pure, Toms River, NJ) and counter-current medical breathing air were used to remove water vapor from the sample stream. Gas concentrations were determined by comparing the peak areas of samples and certified primary standards (range of standards 100–10,000 μ L L⁻¹ for CO₂ and 0.3-5000 µL L⁻¹ for CH4; Airgas, Morrisville, NC) using GCsolution software (Shimadzu Scientific Instruments).

Water-atmosphere greenhouse gas flux calculations

Under ideal conditions in a static chamber incubation, gases either accumulate or are consumed linearly over time (Livingston & Hutchinson, 2009); GHG fluxes are determined by regression analysis of the change in gas concentration over time in the chamber. Static flux chambers are sensitive to disturbance, so rigorous quality control measures (see description below) must be applied. Measured gas concentrations were initially converted from ppmv to $\mu g m^{-3}$ using the ideal gas law and field measurements of air temperature and barometric pressure. Quality control measures, as described in detail in Carmichael et al. (2018), Helton et al. (2014), and McInerney & Helton (2016), were then applied to the data set.

For GHG flux calculations, we began by calculating the average of all sample replicates that were within 10% of one another (McInerney & Helton, 2016). Next, these values were used to calculate the minimum detectable concentration difference (MDCD) for each sampling date (Yates et al., 2006). Incubations that did not exceed the MDCD were excluded from the analysis. Gas fluxes are reported

as a flux per unit exchanging surface area. Therefore, some additional transformations were required before regression analyses could be completed (Carmichael et al., 2018). In calculating water-atmosphere fluxes, the volume to surface area ratio of the static flux chambers obtained by Helton et al. (2014) was used for conversions. Once these conversions were completed, linear regression was used to calculate GHG fluxes. An incubation met the assumption of linearity when $r^2 >$ 0.85; all incubations below this value were discarded from the analysis (Carmichael et al., 2018).

Statistical analyses

Data were tested for normality using a Shapiro-Wilk test. A one-way analysis of variance was then used to compare GHG fluxes among sites (threshold for significance, P<0.05). When necessary, multiple comparisons were conducted using Tukey-Kramer HSD tests. Statistical analyses were completed using Sigma Plot v. 12 (Systat Software, San Jose, CA).

3. Results

Site mesoclimate and environmental measurements

Mesoclimate data indicate that the daily temperature profile in July 2016 was similar to both the 10-year weather averages (average maximum daily temperature, $30.77\pm1.99^{\circ}$ C; average daily temperature, $25.90\pm2.08^{\circ}$ C; average minimum daily temperature, $21.02\pm2.37^{\circ}$ C) and the 30-year climate normal (average maximum daily temperature, 30.67° C; average daily temperature, 25.50° C; average minimum daily temperature, 20.28° C) for Tyrrell County, North Carolina. Fresh surface water conditions (salinity = 0.1 ± 0.0 ppt) and relatively constant surface water pH (pH = 4.69 ± 0.07) were maintained throughout the study period. Mean surface water depth at the Inflow, Outflow, Roadside, and Wetland were 0.42 ± 0.01 m (range, 0.39 - 0.44 m), 1.2 ± 0.2 m (range, 0.8 - 1.7 m), 0.37 ± 0.05 m (range, 0.24 - 0.51 m), and 0.31 ± 0.03 m (range, 0.19 - 0.45 m) respectively.

Water-Atmosphere greenhouse gas fluxes

The mean water-atmosphere GHG fluxes at the Inflow, Outflow, and Roadside ranged from $144.1 \pm 11.6 - 189.3 \pm 33.4 \text{ mg CO}_2 \text{ m}^{-2}\text{h}^{-1}$ and $3.1 \pm 0.6 - 12.1 \pm 6.2 \text{ mg CH}_4 \text{ m}^{-2}\text{h}^{-1}$ (Figure 3). For CO₂, there was no significant difference in water-atmosphere fluxes between the Inflow, Outflow, and Roadside; however, all sites had significantly lower fluxes of CO₂ when compared to the Wetland (P<0.001). For CH₄, the only significant difference was between the Roadside and Wetland, with the Wetland site having a significantly larger flux (ca. $10 \times$, P<0.05). The Inflow, Outflow, and Roadside were statistically indistinguishable.



Figure 3: Water-atmosphere greenhouse gas fluxes of CO_2 (a) and CH_4 (b). Asterisks indicate a significant difference between the mean greenhouse gas flux at a given sample site compared to the Wetland. Error bars represent standard error.

4. Discussion

GHG fluxes across the water-atmosphere interface have historically been understudied (Bastviken et al., 2004; Stanley et al., 2016). In this study, GHG emissions were measured across the water-atmosphere interface in agricultural drainage canals, a form of cryptic wetland that is common in cropland of low-lying coastal regions. Data from this study show that including agricultural drainage canals in the measured GHG fluxes from TOWeR increases site CO₂ and CH₄ emissions by ca. 1% each.

Greenhouse gas fluxes from TOWeR

The present study augments the existing pool of research regarding GHG fluxes from TOWeR across the three primary pathways of gas exchange in wetlands: the water-atmosphere, sediment-atmosphere, and plant-atmosphere interfaces. GHG flux via the water-atmosphere interface at TOWeR was previously studied by Carmichael et al. (2018) and Helton et al. (2014). As stated before, GHG flux data from Carmichael et al. (2018) was used as a point of comparison for the drainage canal fluxes reported in the present study. In Helton et al. (2014), a site located upstream of the Inflow site in this study received a high concentration of agricultural runoff and was found to have consistent CH₄ fluxes of < 8 mg m⁻² h⁻¹ between May and October 2012. The Inflow site of the present study had a stronger CH₄ flux of 12.1 ± 6.2 mg m⁻² h⁻¹. Helton et al. (2014) did not report values for CO₂ fluxes.

GHG flux across the sediment-atmosphere interface at TOWeR was studied by Morse et al. (2012). Data from sites within the regularly flooded portion of the restored wetland showed mean GHG fluxes of 150 mg $CO_2 \text{ m}^{-2} \text{ h}^{-1}$ and 1.2 mg CH₄ m⁻² h⁻¹ (Morse et al., 2012). While the present study reports similar CO₂ fluxes to Morse et al. (2012), CH₄ exchanges in this study appear to be stronger than those across the sediment-atmosphere interface.

GHG exchanges via standing dead trees, a pathway of gas flux across the plantatmosphere interface, were studied by Carmichael et al. (2018), with results showing a mean CH₄ flux of 0.4 ± 0.1 mg m⁻² h⁻¹ and a mean CO₂ flux of 114.6 ± 23.8 mg m⁻² h⁻¹. A comparison of data from Carmichael et al. (2018) to data from the present study indicates that CH₄ and CO₂ fluxes across the water-atmosphere interface at TOWeR represent the larger source of these GHGs to the atmosphere.

Results indicate that taking CO₂ and CH₄ fluxes from agricultural drainage canals into account during the calculation of total GHG flux from TOWeR increases site CO₂ and CH₄ emissions by ca. 1% each. Thus, including GHG fluxes

from these cryptic wetlands is an important consideration in calculating total GHG emissions from the site.

Patterns in water-atmosphere greenhouse gas fluxes at TOWeR

In this study, CH₄ and CO₂ fluxes from the drainage canal sites were lower than those from the Wetland. This disparity most likely stems from differences in vegetation, C quality, and sediment decomposition rates between these sites. The drainage canals at TOWeR lack abundant aquatic vegetation, which has been shown to increase the availability of high-quality carbon substrates in wetland sediments (Fonesca et al., 2017). This high-quality C can be classified as labile organic matter, which is more readily decomposed by sediment microbial communities (Barré et al., 2016), thus stimulating decomposition pathways and the evolution of CO₂ and CH₄ (Corteselli et al., 2017). In fact, higher CH₄ fluxes have been observed in vegetated drainage ditches compared to those that lack vegetation (Schrier-Uijl et al., 2010; Schrier-Uijl et al., 2011). Thus, the presence of vegetation in the wetland proper likely increased C-gas emissions, as compared to the sparsely-vegetated agricultural drainage canals.

Among the three drainage canal sites, the Inflow showed the greatest CO₂ and CH₄ emissions. Although we did not measure porewater concentrations of nitrogen (N) and phosphorous (P) in this study, the observed trend might be explained by the proximity of the Inflow site to agricultural runoff containing nutrients. Studies have shown that additions of specific amounts of N and P to water can lead to increased CH₄ emissions, especially in the presence of carbon (Kim et al., 2015). Juutinen et al. (2018) found that CH₄ flux in peatlands increased with the addition of an N/P/K fertilizer. However, N and P additions to soil have also shown widely varying effects on CH₄ evolution, including stimulation of CH₄ oxidation, a process which may result in decreased CH₄ fluxes to the atmosphere (Veraart et al., 2015). Similarly, the response of soil respiration (i.e. CO₂ flux) to nutrient addition is highly variable and inconsistent both spatially and temporally (Cleveland & Townsend, 2006; and as reviewed in Schlesinger & Andrews, 2000 and Raich & Schlesinger, 1992).

Conclusions

Cryptic wetlands have traditionally been understudied (Yavitt, 2010), though recent experimental evidence indicates the importance of including these wetlands in predictive models of ecosystem carbon-dynamics (Ullah & Moore, 2011; Martinson et al., 2010; Creed et al., 2003). In this study, including GHG fluxes from one type of cryptic wetland (i.e. previously-omitted agricultural drainage infrastructure) in the calculation of total GHG fluxes from TOWeR increased sitebased CO₂ and CH₄ fluxes. Although the localized spatial footprint of agricultural drainage infrastructure may be individually small, the collective impact of these human-engineered systems has the potential to be large, particularly in low-lying regions (i.e. < 2 m elevation) where land use is dominated by agriculture. As such, there is a need to further elucidate the processes and dynamics that influence GHG emissions from these cryptic wetland environments.

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