Methane and Nitrous Oxide Fluxes from Water, Plants, and Soils of a Constructed Treatment Wetland in Phoenix, AZ

by

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ABSTRACT

Constructed treatment wetlands (CTW) have been a cost-efficient technological solution to treat different types of wastewater but may also be sources of emitters of methane (CH_4) and nitrous oxide (N_2O). Thus, my objective for this dissertation was to investigate CH_4 and N_2O fluxes via multiple pathways from the Tres Rios CTW located in Phoenix, AZ, USA. I measured gas fluxes from the CTW along a whole-system gradient (from inflow to outflow) and a within-marsh gradient (shoreline, middle, and open water sites). I found higher diffusive CH_4 release in the summer compared to spring and winter seasons. Along the whole-system gradient, I found greater CH_4 and N_2O emission fluxes near the inflow compared to near the outflow. Within the vegetated marsh, I found greater CH_4 emission fluxes at the vegetated marsh subsites compared to the open water. In contrast, N_2O emissions were greater at the marsh-open water locations compared to interior marsh. To study the plant-mediated pathway, I constructed small gas chambers fitted to *Typha* spp. leaves. I found plant-mediated CH_4 fluxes were greater near the outflow than near the inflow and that CH_4 fluxes were higher from lower sections of plants compared to higher sections. Overall, *Typha* spp. emitted a mean annual daily flux rate of 358.23 mg CH_4 m^{-2} d^{-1}. Third, using a 30-day mesocosm experiment I studied the effects of three different drydown treatments (2, 7, 14 days) on the fluxes of CH_4 and N_2O from flooded CTW soils. I found that CH_4 fluxes were not significantly affected by soil drydown events. Soils that were dry for 7 days shifted from being N_2O sources to sinks upon inundation. As a result, the 7-day drydown soils were sinks while the 14-day drydown soils showed significant N_2O release. My results emphasize the importance of

studying ecological processes in CTWs to improve their design and management strategies so we can better mitigate their greenhouse gas emissions.

DEDICATION

Para mi mamá Guadalupe, mi papá Jorge, y mi hermana Maricarmen.

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CHAPTER 1

INTRODUCTION

1. BACKGROUND

The study of wetlands has allowed ecosystem ecologists to expand on ecological theories such as succession and energy flow (Clements 1916, Lindeman 1942), pulse dynamics (Odum et al. 1995), and systems ecology (Hopkinson 1992). From the first official wetland classification protocol by Cowardin et al. (1979) to Mitsch and Gosselink (2007), a reasonable consensus has developed that defines wetlands based on three main features: standing water or waterlogged soils, anoxic conditions, and hydrophytic vegetation.

These three features also represent the conditions necessary for enhanced biogeochemical cycling in wetlands. These features and structures, in combination with other factors such as temperature, water regime, and oxygen, set the necessary conditions for specific ecological processes to occur. For example, nitrogen cycling in wetlands includes the denitrification process, which may generate the desired ecosystem service of water quality improvement only if it is via permanent removal of nitrogen to N_2 gas (Vymazal 2007, Baron et al. 2013). Similarly, many wetlands accrete organic soils, a process that underlies the ecosystem service of carbon sequestration (Brix et al. 2001, Whiting and Chanton 2001).

Wetlands ecosystem functions, such as nutrient cycling, may generate important ecosystem services both locally and globally (Costanza et al. 1997, Mitsch and Gosselink 2007). Many of these services are a result of biogeochemical processes, in which wetland ecosystems are sinks or transformers of carbon and nutrients (Zedler and Kercher 2005). After the loss of more than 50% of the world's natural wetlands, and the continuous

alteration of the remaining wetlands, many efforts have arisen to protect and restore these ecosystems. Additionally, there has been an increase in establishment of constructed wetlands that are designed to provide specific services (Spieles and Mitsch 2000, Mitsch 2005). Certain structures, functions, and environmental conditions must be present in these wetlands to carry out the ecological processes that will ultimate generate the desired ecosystem services (Fig. 1). But in many cases, wetlands are being constructed to obtain the desired ecosystem service of water purification through nutrient sequestration and transformation (Jenkins et al. 2010) while other outcomes, services, or disservices, are not considered (Mitsch et al. 2013).

The ecological processes responsible for producing many wetland ecosystem services may also produce a disservice: the production of greenhouse gases (GHG) such as nitrous oxide (N_2O), methane (CH_4), and carbon dioxide (CO_2) (Mitsch and Gosselink 2007, Imfeld et al. 2009). The combination of aerobic and anaerobic conditions in wetland soils support the coupled processes of nitrification and denitrification, both of which produce N_2O that is ultimately emitted (Zumft 1997, Kowalchuk and Stephen 2001) (Risgaard-Petersen et al. 2003, Elgood et al. 2010, Garcia-Lledo et al. 2011). Likewise, anaerobic conditions support the production of CH_4 by methanogenic Archaea (Inamori et al. 2007, Mander et al. 2011). According to a recent Intergovernmental Panel on Climate Change (IPCC) report, CO_2, N_2O, and CH_4 emissions have increased 40%, 150%, and 20%, respectively, since the 1950s (IPCC 2013). Wetland ecosystems are the top natural source of CH_4 (40%) and N_2O (10%) to the atmosphere, and these emissions are predicted to continue to increase (Beaulieu et al. 2011, IPCC 2013). Moreover, although their atmospheric concentrations are much lower than CO_2, both N_2O and CH_4

have a higher global warming potential (GWP).

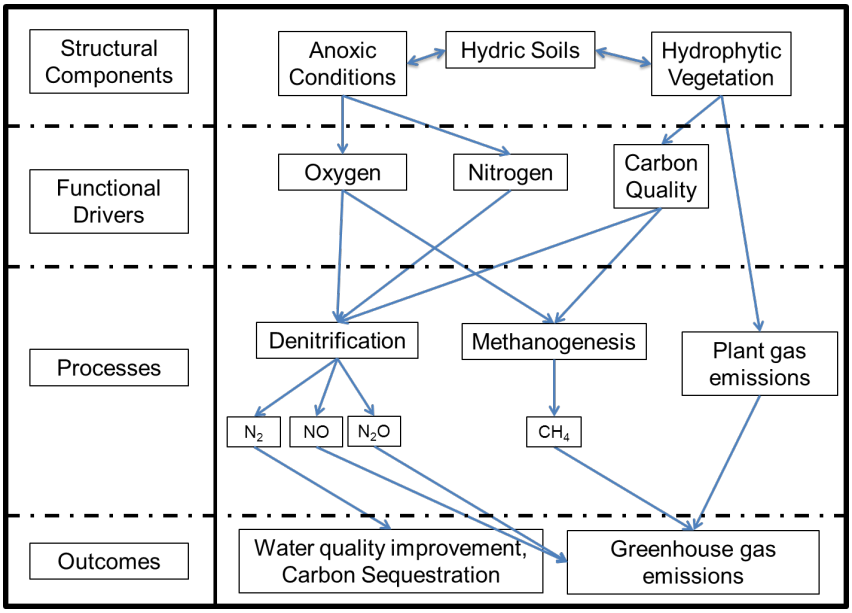


Figure 1. Representation of a wetland ecosystem with its structural components, functional drivers, and processes that ultimately are responsible for outcomes—the production of the services and disservices (arrows indicate influence to the next step).

2. CONSTRUCTED WETLANDS

Natural wetlands have been used for wastewater treatment to reduce the eutrophication of adjacent aquatic systems for decades (Odum et al. 1977, Tilton and Kadlec 1979, Brodrick et al. 1988, Hosomi et al. 1994). The technology to construct wetlands for the purpose of treating water originated in Europe in 1952 and North America in the 1970s (Bastian and Hammer 1993). Today, constructed treatment wetlands (CTW) are often an attractive, feasible, and cost-efficient alternative to technological solutions (Spieles and Mitsch 2000, Huang et al. 2010). Constructed treatment wetlands are being used to treat both point and non-point sources of pollutants, including domestic and industrial wastewater, stormwater runoff, and agricultural runoff (Kadlec and Wallace 2008). There are three categories of CTW: free-water surface

(FWS); horizontal subsurface flow (HSSF); and vertical subsurface flow (VSSF). Free-water surface wetlands are most common type of CTW used for the tertiary treatment of municipal or domestic wastewater due to their design which include slow water flow, shallowness, emergent vegetation, and microbial activity (Vymazal et al. 1998, Kadlec and Wallace 2008).

For the purpose of this study, I will focus on ecosystem-scale research at a FWS CTW in Phoenix, AZ. The Tres Rios CTW was designed and built as a cost-effective method to remove excess nitrogen from wastewater leaving the 91st Avenue wastewater treatment plant before it is discharged into the Salt River. The CTW was completed in 2010 and it started receiving effluent that had already undergone partial tertiary treatment in the spring of 2011 (Sanchez et al. 2016). The Tres Rios CTW includes two flow-regulating, deep-water basins that distribute effluent flow rates into three vegetated wetland cells (Fig. 2). These wetlands were planted with six species of native vegetation.

Key ecosystem processes have been monitored in the largest vegetated wetland (Cell 3) by ASU's Wetland Ecosystem Ecology Lab since Summer 2011, and I performed my dissertation research in this same wetland. Cell 3 has 21 ha of open water and 21 ha of vegetated marsh (Fig. 2). Depths throughout this marsh are approximately 25 cm and the open water areas are about 1.5 meters deep. The vegetation planted in the Cell 3 originally consisted of six native species of emergent macrophytes, but is now dominated by two species of *Schoenoplectus* and two species of *Typha* (Weller et al. 2016).

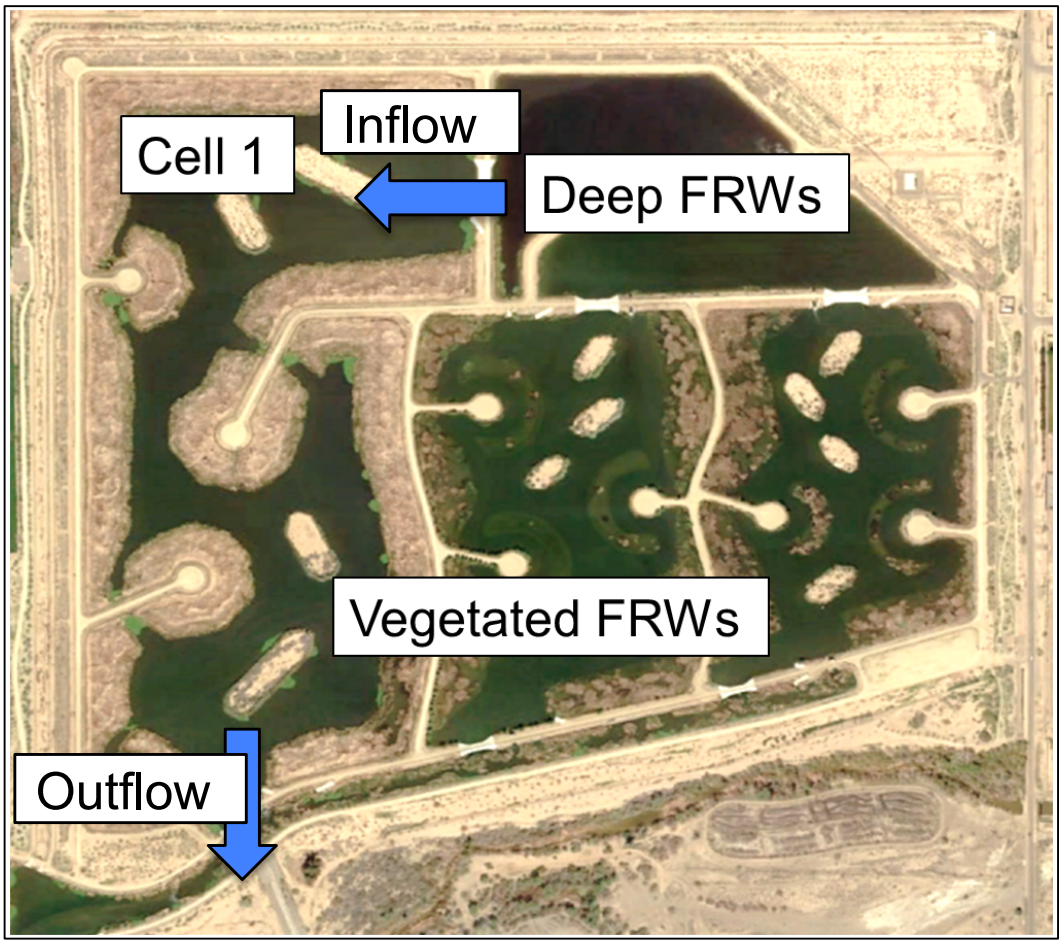


Figure 2. Aerial image of Tres Rios CWS indicating the location of the two deep water flow regulating wetlands (Deep FRWs), the three vegetated flow regulating wetlands (Vegetated FRWs), and Cell 1. The blue arrows indicate the inflow and the outflow of the Cell 1.

3. DISSERTATION OBJECTIVES

The overall objective of my dissertation was to quantify greenhouse gas fluxes from the Tres Rios CTW. I had three specific objectives: (1) to quantify fluxes of N_2O and CH_4 across the water-air interface; (2) to quantify fluxes of CH_4 and N_2O from wetland plants; and (3) to investigate the effects of different hydrologic management strategies on the CH_4 and N_2O gas fluxes from Tres Rios wetland soils.

4. APPROACH

To achieve my objectives, I used a combination of field sampling campaigns (Specific Objectives 1 and 2) and a greenhouse mesocosm experiment (Objective 3). The field campaigns consisted of a robust and well-replicated study design with two the marsh transects that are part of the long-term monitoring being conducted at Tres Rios (Weller et al. 2016). One transect was located near the effluent inflow and one near the outflow. These two transects represent a whole-system inflow-outflow gradient. The two marsh transects were approximately 50-60 meters long and were perpendicular to the shoreline, ending at the open-water interface. Along each transect, I established three sub-sites (shoreline, middle, and open water) to spatially represent the within-marsh wetland gradient. Additionally, I designed an experiment that combined the ecological background of the system with different potential management implications.

5. STRUCTURE OF THE DISSERTATION

My dissertation is made up of three research chapters, followed by a conclusion chapter. In Chapter 2, I focus on the diffusion pathway of CH_4 and N_2O fluxes at both within-marsh and whole-system scales (Specific Objective 1). Chapter 3 quantifies the role that the dominant plant species in the system, *Typha* spp., plays in greenhouse gas fluxes from the CTW (Specific Objective 2). These measurements were made along the same two transects and at the same locations as those I present in Chapter 2. Chapter 4 uses a greenhouse mesocosm experiment to explore the effects of different hydrological regime on greenhouse gas fluxes from Tres Rios wetland soils (Specific Objective 3). I designed an experiment that included soil-drying events of several different durations, as well as a control that mimicked the permanently flooded conditions that characterize Tres

Rios CTW management by the City of Phoenix. In the final synthesis chapter, I integrate the findings from Chapters 2 - 4 and present the general conclusions and implications of my dissertation research.

6. PRODUCTS OF DISSSERTATION

CHAPTER 1

Ramos, J., Chapman, E. J., Childers, D. L., Hall, S. J., Weller, N. *In prep*. Temporal and spatial patterns of methane and nitrous oxide diffusive fluxes from a constructed treatment wetland in Phoenix AZ USA. –*Wetland Ecology and Management*.

CHAPTER 2

Ramos, J. and Childers, D. L. *In prep.* Greenhouse gas fluxes from *Typha* spp. in a constructed treatment wetland using a new vegetation gas chamber AZ. - *Aquatic Botany*

CHAPTER 3

Ramos, J., Childers, D.L., Palta, M. M. *In prep*. How experimental drying and re-wetting influence methane and nitrous oxide fluxes from continuously inundated soils taken from a constructed treatment wetland – *Biogeochemistry*

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CHAPTER 2

TEMPORAL AND SPATIAL PATTERNS OF METHANE AND NITROUS OXIDE DIFFUSIVE FLUXES FROM A CONSTRUCTED TREATMENT WETLAND IN PHOENIX AZ USA

ABSTRACT

Many constructed treatment wetlands (CTW) systems featuring free-water surface flow have been developed to remove nutrients from wastewater effluent, but they also may emit methane (CH_4) and nitrous oxide (N_2O), two potent greenhouse gases. We measured diffusive CH_4 and N_2O fluxes from a CTW in Phoenix, AZ, USA along a whole-system gradient (inflow and outflow transects) and a within-marsh gradient inside each transect (shoreline, middle, and open water subsites). From 2012 to 2014, we used floating chambers to collect gas samples. We found significantly higher CH_4 release in the summer compared to spring and winter seasons. Along the whole-system gradient, we found significantly greater CH_4 and N_2O emission fluxes near the effluent inflow compared to near the outflow. Within the vegetated marsh, we found greater CH_4 emission fluxes at the interior marsh subsites compared to the open water subsite. In contrast, N_2O emissions were significantly greater at the marsh-open water location compared to interior marsh subsites. Stepwise multiple linear regressions revealed that NO_3^- and dissolved organic carbon (DOC) explained some of the variability of CH_4 emission fluxes (R^2=0.24, $F_{2, 104}$=17.65, p<0.0001), whereas temperature and DOC explained variability in N_2O emission fluxes (R^2=0.36, $F_{2, 100}$=29.16, p<0.0001). Results indicate that gas diffusive fluxes were related to proximity to the effluent inflow and to

location within the vegetated marsh, as well as to water column characteristics. Thus, the design of CTW as well as environmental conditions both may influence CH_4 and N_2O emissions.

1. INTRODUCTION

Wetlands have been used for decades for wastewater treatment in order to reduce the eutrophication of adjacent aquatic systems (Odum et al. 1977, Tilton and Kadlec 1979, Brodrick et al. 1988, Hosomi et al. 1994). The first research on constructed treatment wetlands (CTW) technology originated in Europe in the 1950s; these systems became popular in North America around the 1970s (Bastian and Hammer 1993, Kadlec and Wallace 2008). Since the 1980's, CTW are a cost-efficient technological solution to treat different types of wastewater from municipal, industrial, stormwater, and agricultural sources among others (Spieles and Mitsch 2000, Kadlec and Wallace 2008, Huang et al. 2010). Since then, there has been an increase in the diversity of designs and management schemes for constructed wetlands to provide the important ecosystem service of water purification from excess nutrients (Kadlec and Wallace 2008, Vymazal 2011). However, the ecological processes that so effectively treat wastewater in constructed wetlands may also result in these constructed ecosystems being sources of climate-warming greenhouse gases.

Biogeochemical processes by microorganisms, adsorption on nutrients onto soils, and plant mediation contribute to the transformation of nutrients in CTW (Zedler and Kercher 2005, Mitsch and Gosselink 2007). Hence, constructed wetlands are used to obtain the desired ecosystem service of water purification. Possible undesired outcomes

include the production and emission of greenhouse gases (Jenkins et al. 2010, Mitsch et al. 2013, Mander et al. 2014). Just like natural wetlands, constructed wetlands are generally sinks of carbon dioxide (CO_2) due to their high rates of carbon fixation (primary productivity), but they may also be sources of methane (CH_4) and nitrous oxide (N_2O) (Bridgham et al. 2006). According to the IPCC (2013), N_2O and CH_4 emissions have increased 150% and 20%, respectively, since the 1950s. Additionally, CH_4 and N_2O have a 28-fold and 298-fold global warming potential, respectively, over a 100-year period compared with the warming potential of CO_2 (IPCC 2013). Thus, our objective for this study was to investigate CH_4 and N_2O emission and uptake fluxes diffusing across the water-air interface in a constructed treatment wetland system located in an urban arid region—Phoenix, AZ USA.

Free-water surface (FWS) wetlands of the CTW are the most common for tertiary treatment from municipal wastewater treatment plants (Kadlec and Wallace 2008). These specific CTW are usually permanently flooded with a slow horizontal water flow and are dominated by rooted emergent vegetation (Kadlec and Wallace 2008). Having FWS wetlands permanently flooded sets the prime anoxic conditions for denitrification and inadvertent CH_4 and N_2O production (Mitsch and Gosselink 2007, Imfeld et al. 2009). Permanently flooded soils combined with a ready supply of labile organic carbon (C) are optimal conditions to encourage methanogenesis, making these wetlands potentially large sources of CH_4 (Inamori et al. 2007, Laanbroek 2010, Mander et al. 2011). Similarly, permanently flooded soils encourage the anaerobic process of denitrification, which converts nitrate (NO_3^-) into dinitrogen gas (N_2). This is a critical process for complete N removal from water flowing through the CTW. However, N_2O production and release

may result from incomplete denitrification or as a by-product of nitrification, which is not likely to occur under permanently flooded conditions (Liikanen et al. 2006). In addition to the permanently flooded conditions, when a wetland is created to treat wastewater, high-nutrient wastewater will influence rates of microbial processes and hence CH_4 and N_2O dynamics. For example, many studies have observed an increase of CH_4 and N_2O emissions when nutrient-rich wastewater is treated in constructed wetlands (Tanner et al. 1997, Johansson et al. 2003, Mander et al. 2005b, Teiter and Mander 2005, Sovik et al. 2006).

In these vegetated FWS CTW, CH_4 can be released via three pathways: diffusion, ebullition (bubble-mediated), and plant-mediated transport (Whiting and Chanton 1993, Johansson et al. 2004b, Sovik et al. 2006). Nitrous oxide (N_2O) was recorded to emit from CTW via diffusion in the field (Johansson et al. 2003, Sovik and Klove 2007) and in a mesocosm study using CTW soils (Inamori et al. 2007). Baulch et al. (2011) noted N_2O ebullition fluxes but found them to be an insignificant source compared to N_2O diffusion fluxes from stream ecosystems. In this study we narrowed our focus on the diffusion pathway, because it allowed us to study a flux pathway for both CH_4 and N_2O gases while using a single method adapted to quantify fluxes from vegetated and open water areas of the CTW (Johansson et al. 2003, Johansson et al. 2004b, Mander et al. 2005a, Sovik and Klove 2007, Mander et al. 2008).

Few studies have only focused on water budgets, evapotranspiration, vegetation dynamics and nutrient uptake of CTW in hot and arid regions (Sánchez-Carrillo et al. 2004, Kadlec 2006, Sanchez et al. 2016, Weller et al. 2016). Because higher temperature enhances microbial processes, including those that result in gas production of CH_4 and

N_2O via methanogenesis and denitrification, it is important to study how a constructed treatment wetland in a hot and arid region will influence greenhouse gas fluxes (Kadlec 2009). Currently, most of our knowledge on CH_4 and N_2O fluxes from constructed treatment wetlands is based on short-term studies in temperate or mesic regions. The latest literature reviews of CH_4 and N_2O dynamics from constructed treatment wetlands lack long-term studies of hot or arid regions (Ebie et al. 2014, Mander et al. 2014, Jahangir et al. 2016). Our Tres Rios FWS CTW wetland system provided a platform for understanding how the combination of permanently flooded soils, high-nutrient wastewater loading, and [often] hot and arid conditions influenced biogeochemical processes and the production and emission of CH_4 and N_2O.

2. METHODS

2.1. Study site

Our research was conducted at the Tres Rios CTW in Phoenix, AZ (USA; 33°23' N, 112°15'W) in the arid and hot Sonoran Desert. Air temperature ranges from 44.4° C in summer months to 1.2° C in winter months (City of Phoenix Water Services Department, 2015). Annual precipitation averages 230 mm, with peak rain falling during the summer monsoon season (July to September) or the winter frontal season (December to March).

Tres Rios CTW has been in operation since 2010 when it started receiving treated wastewater from the 91st Ave Wastewater Treatment Plant—the largest in the city. The CTW consists of two flood-regulating basins and three vegetated surface-flow treatment wetlands (Fig. 3). The purpose of the flood-regulating basins is to store and regulate water flow into the treatment wetlands. This study was completed in the largest vegetated

surface-flow wetland, which was planted in 2009 and was the first cell to receive effluent in 2010. The L-shaped Cell 1, with roughly 21 hectares of vegetated marsh and 21 hectares of open water and upland islands, received an average of 95,000 to over 270,000 m^3 d^{-1} of effluent, depending on the time of year (Sanchez et al. 2016). Seven native emergent macrophytes were originally planted and have been left relatively unmanaged in the system: *Schoenoplectus acutus, Schoenoplectus americanus, Schoenoplectus californicus, Schoenoplectus maritimus, Schoenoplectus tabernaemontani, Typha domingensis,* and *Typha latifolia*. The marginal vegetated marshes extend 50–60 meters from the shoreline to the open-water areas. Water depths in the marsh are consistently about 25 cm while open water depths are 1.5 - 2 meters. By design, water residence time is 4 days, after which water is released through the outflow to the Salt River.

2.2. Study design

We established our study in two of the marsh transects used in the vegetation and nitrogen monitoring study at Tres Rios CTW (Weller et al. 2016). The two transects were within the vegetated marshes; one near the effluent inflow and one near the outflow (Fig. 3). These two transects represented a whole-system inflow-outflow gradient. The two marsh transects were approximately 50-60 meters long and were perpendicular to the shoreline, ending at the open water interface. Along each transect, we established three sub-site sampling locations (shoreline, middle, and open water) to spatially represent a within-marsh wetland gradient.

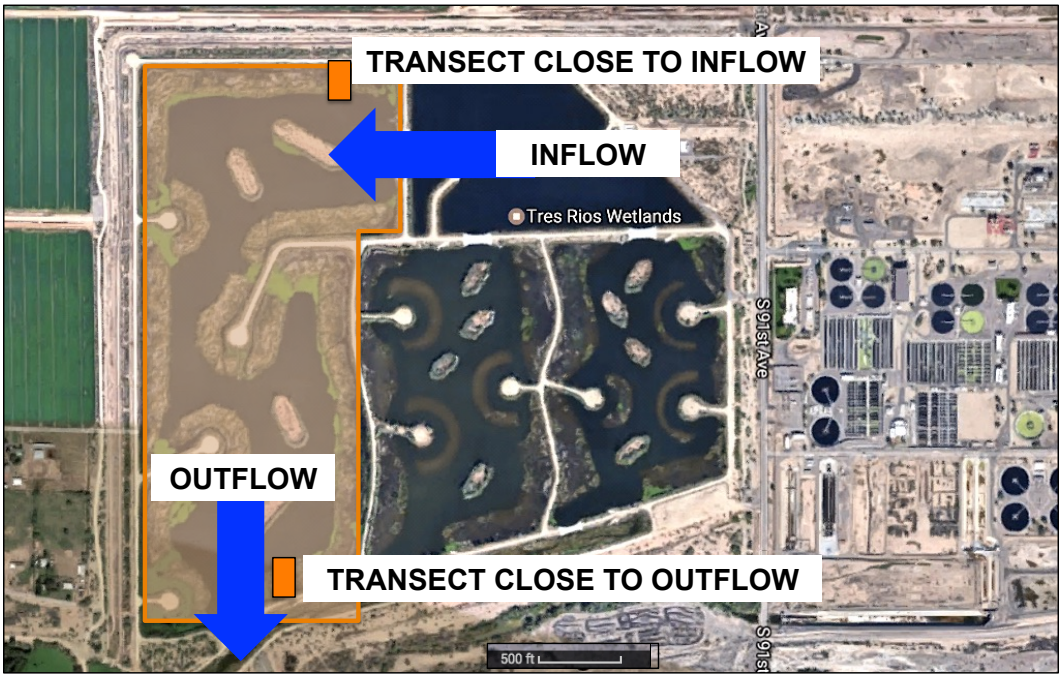


Figure 3. Aerial view of Tres Rios CTW (Cell 1 shaded in orange) illustrating the whole-system flow of the water (blue arrows), and the location of the two 50-transects (orange rectangles).

We measured CH_4 and N_2O gas fluxes across the water-air interface every two months from March 2012 to January 2014. Similar to a warm and tropical wetland study and due to the characteristics of the regional climate we classified seasons as spring (March), summer (May and July), fall (September and November), and winter (January) (Gondwe and Masamba 2014). We sampled at the three sub-sites along both transects using a modified version of the soil static chamber technique to capture the diffusion of trace gases at the water-atmosphere interface (Hutchinson and Mosier 1981, Hall and Matson 2003, Harrison and Matson 2003). Opaque PVC chambers (12 x 25 cm, ~5.9 L) previously used in terrestrial and aquatic gas flux studies were adapted to float on the water surface with floating foam rings (Harrison and Matson 2003, Hall et al. 2008).

Starting with the inflow transect, we deployed three floating chambers and simultaneously sampled 45-minute gas fluxes from them, three times during the day (8:00 AM, 10:00 AM, and 12:00 PM). We sampled gas fluxes along the outflow transect the following day. When deploying the three chambers at each sub-site, we tethered them to previously inserted stakes, and separated them by at least 1 meter. Once the chambers were deployed, we mixed the air three times inside the chamber to ensure a well-mixed air sample. Then, we collected gas samples from each floating chamber every 15 minutes for 45 minutes (t_0, t_{15}, t_{30}, t_{45}) using 20 ml plastic syringes. Gas samples were immediately injected into 10 mL glass vials that were previously sealed with inert stoppers and silicone, capped with aluminum caps, then flushed and evacuated with N_2. All gas samples were analyzed using a Varian CP-3800 Gas Chromatograph (GC). The GC was calibrated in the laboratory using certified CH_4 and N_2O standards (Matheson Tri-Gas® 2016).

During our first year of study (March 2012 to March 2013), we did not observe any significant differences among sampling times (8:00 AM, 10:00 AM, and 12:00 PM) and between the shoreline and middle marsh sub-site locations within the vegetated marsh area. This led us to modify our study design and continue the study from May 2013 until January 2014 with only two daytime sampling points (8:00 AM and 12:00 PM) and only two location subsites within the transect gradient (shoreline and open-water).

Because non-linear changes in gas concentrations fluxes are often observed in floating chambers, we calculated CH_4 and N_2O fluxes using the new Hutchinson Mosier R-package (HMR) procedure which is available as an add-on package (Pedersen et al.

2010) in R software (Version 2.15.1; R Development Core Team, 2012). The HMR package allowed us to calculate fluxes from both linear and non-linear concentration curves using a regression-based extension of the Hutchinson and Mosier model (Hutchinson and Mosier 1981, Pedersen et al. 2010). This approach has been found to be appropriate for analyzing CH_4 and N_2O gas fluxes collected using static chambers (Thomsen et al. 2010, Schelde et al. 2012, Audet et al. 2014, Pangala et al. 2014).

2.3 Environmental parameters

We designed our study to compliment ongoing bimonthly measurements of plant biomass, nutrient uptake, and water budgets in the same 42 ha system and along two of the same transects (Sanchez et al. 2016, Weller et al. 2016). We used various water quality parameters collected as part of this larger effort, namely temperature (°C), dissolved organic carbon (DOC), ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), pH, and % dissolved oxygen, as variables to explain patterns in both CH_4 and N_2O fluxes.

2.4 Statistical analysis

Our experimental design allowed us to examine temporal patterns in gas flux within a given day, by seasons, and at two different spatial scales—across the entire system (between the inflow and outflow transects) and within the vegetated marsh proper (along the marsh transects). Statistical analyses were performed using SPSS (Version 23). To better understand and evaluate both emission and uptake fluxes, we differentiated methane (CH_4) and nitrous oxide (N_2O) fluxes into emission fluxes (positive fluxes) and uptake fluxes (negative fluxes) (Tanner et al. 1997). Next, the fluxes were log-transformed to reduce skewedness and achieve normal distribution prior to analysis (Trudeau et al. 2013, Chmura et al. 2016). Because the uptake fluxes for CH_4 and N_2O

were negative values, in order to complete the log-transformation we added the lowest CH_4 and N_2O uptake flux value respectively to all of the CH_4 and N_2O uptake fluxes. The formula to log-transform CH_4 uptake fluxes was

$$log\ CH_4\ uptake = log(-CH_4\ flux + 2.2)$$

and

$$log\ N_2O\ uptake = log(-N_2O\ flux + 117)$$

for N_2O uptake fluxes. To test if there were any seasonal differences in CH_4 and N_2O fluxes, we used univariate analysis of variance (ANOVA) followed by multiple post-hoc comparisons using Tukey HSD analysis. To test if the CH_4 and N_2O fluxes were different between the inflow and outflow (at the whole system gradient), we used t-tests. To test for differences in CH_4 and N_2O fluxes among the three transect sub-sites (at the marsh open water gradient) we used ANOVA followed by multiple post-hoc comparisons using Tukey HSD analysis. Finally, we also used ANOVA to test if there were any differences among the sampling times (8:00 AM, 10:00 AM, and 12:00 PM).

To identify environmental factors that may explain variability in CH_4 and N_2O fluxes, we used stepwise multiple linear regression analysis. Water quality data were collected only at the shoreline and open water transect locations (Sanchez et al. 2016) thus, only CH_4 and N_2O gas flux data from those locations were used in this analysis. Since we did not include gas-flux data from the within-marsh middle subsite in this multiple linear regression analysis, the constant values added before the log transformation to the CH_4 and N_2O uptake (negative fluxes) values were different for both CH_4 and N_2O. The formula used to log-transform the CH_4 uptake fluxes was

$$log\ CH_4\ uptake = log(-CH_4\ flux + 0.5)$$

and

$$log\ N_2O\ uptake = log(-N_2O\ flux + 43)$$

for N_2O uptake fluxes. We performed separate multiple linear regression analyses for log CH_4 emission, log CH_4 uptake, log N_2O emission, and log N_2O uptake. The independent variables used as predictors in each of the stepwise multiple linear regression analysis were log-nitrate (NO_3^-), log-dissolved organic carbon (DOC), log-pH, log-temperature, and log-% oxygen. All statistical tests were evaluated at the 5% significance level.

3. RESULTS

The majority of measured diffusive CH_4 and N_2O gas fluxes observed were CH_4 and N_2O emission (positive) fluxes and not uptake (negative) fluxes. For example, we observed a greater number of CH_4 emission fluxes (n=167) than uptake fluxes (n=5). Similarly, we observed a greater number of N_2O emission fluxes (n=162) than uptake fluxes (n=8). We found seasonal patterns for diffusive CH_4 emission fluxes but not for N_2O fluxes from March 2012 to January 2014. We also discovered patterns of CH4 and N2O diffusive fluxes from the whole-system gradient and at the within-marsh gradient.

3.1 CH_4 fluxes

Diffusive CH_4 emission fluxes (n=167) ranged from 0.03 to 13.00 mg CH_4 m^{-2} h^{-1}, with an average of 2.34 (S.E.=0.25) mg CH_4 m^{-2} h^{-1}. Overall, we observed significantly higher mean CH_4 emission fluxes during the summer (3.36 mg CH_4 m^{-2} h^{-1}) compared to spring (1.95 mg CH_4 m^{-2} h^{-1}) and winter (1.29 mg CH_4 m^{-2} h^{-1}) seasons (Fig. 4a); however, the fall season mean CH_4 emission fluxes (2.32 mg CH_4 m^{-2} h^{-1}) were not significantly different than other seasons. At the whole-system scale, average CH_4

emission fluxes throughout the entire study period were significantly higher at the inflow (2.89 mg CH_4 m^{-2} h^{-1}) compared with the outflow (1.78 mg CH_4 m^{-2} h^{-1}; Fig. 4b). Within the vegetated marsh transects we found that average CH_4 emission fluxes were significantly higher at the shoreline and middle marsh locations (4.07 and 4.10 mg CH_4 m^{-2} h^{-1}, respectively) compared to the open-water location (0.53 mg CH_4 m^{-2} h^{-1}, Fig. 4c). Five methane uptake fluxes occurred mainly in the fall and winter season (on in the summer) and ranged from –2.17 to –0.13 mg CH_4 m^{-2} h^{-1} with an average of –0.66 mg CH_4 m^{-2} h^{-1}.

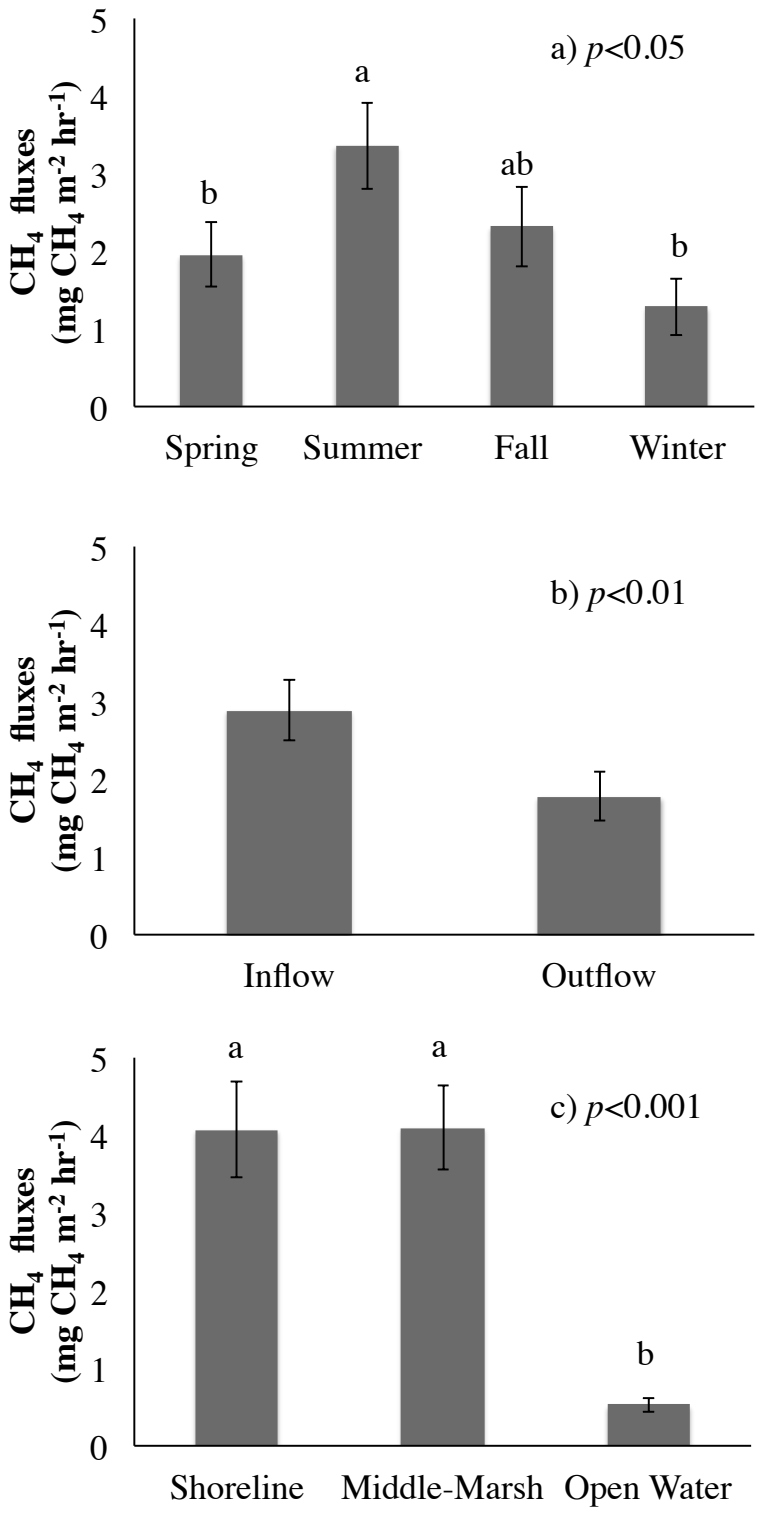


Figure 4. Diffusive methane (CH_4) emission fluxes over a) seasonal patterns, b) whole system gradient (inflow and outflow transects), and c) vegetated-shoreline to open-water gradient (shoreline, middle, and open water subsites) from Tres Rios CTW.

3.2 N_2O fluxes

Diffusive N_2O emission fluxes (n=162) ranged from 10.10 to 501.37 μg N_2O m^{-2} h^{-1} with an average of 183.64 μg N_2O m^{-2} h^{-1}. Overall, we did not observe any significant differences in N_2O emission fluxes among seasons (p=0.09, Fig, 5a). At the whole-system scale, average N_2O emission fluxes were significantly higher (p<0.01) near the inflow compared with near the outflow (202.52, 158.82 μg N_2O m^{-2} h^{-1}, respectively, Fig. 5b). Along the marsh transects the average N_2O emission fluxes were significantly higher (p<0.001) at the open-water locations (234.4 μg N_2O m^{-2} h^{-1}) when compared to the shoreline and middle marsh locations (117.22, 138.06 μg N_2O m^{-2} h^{-1}, respectively, Fig. 5c). Nitrous oxide uptake fluxes (n=8) ranged from –116.35 to –4.43 μg N_2O m^{-2} h^{-1} with an average of –29.25 μg N_2O m^{-2} h^{-1}.

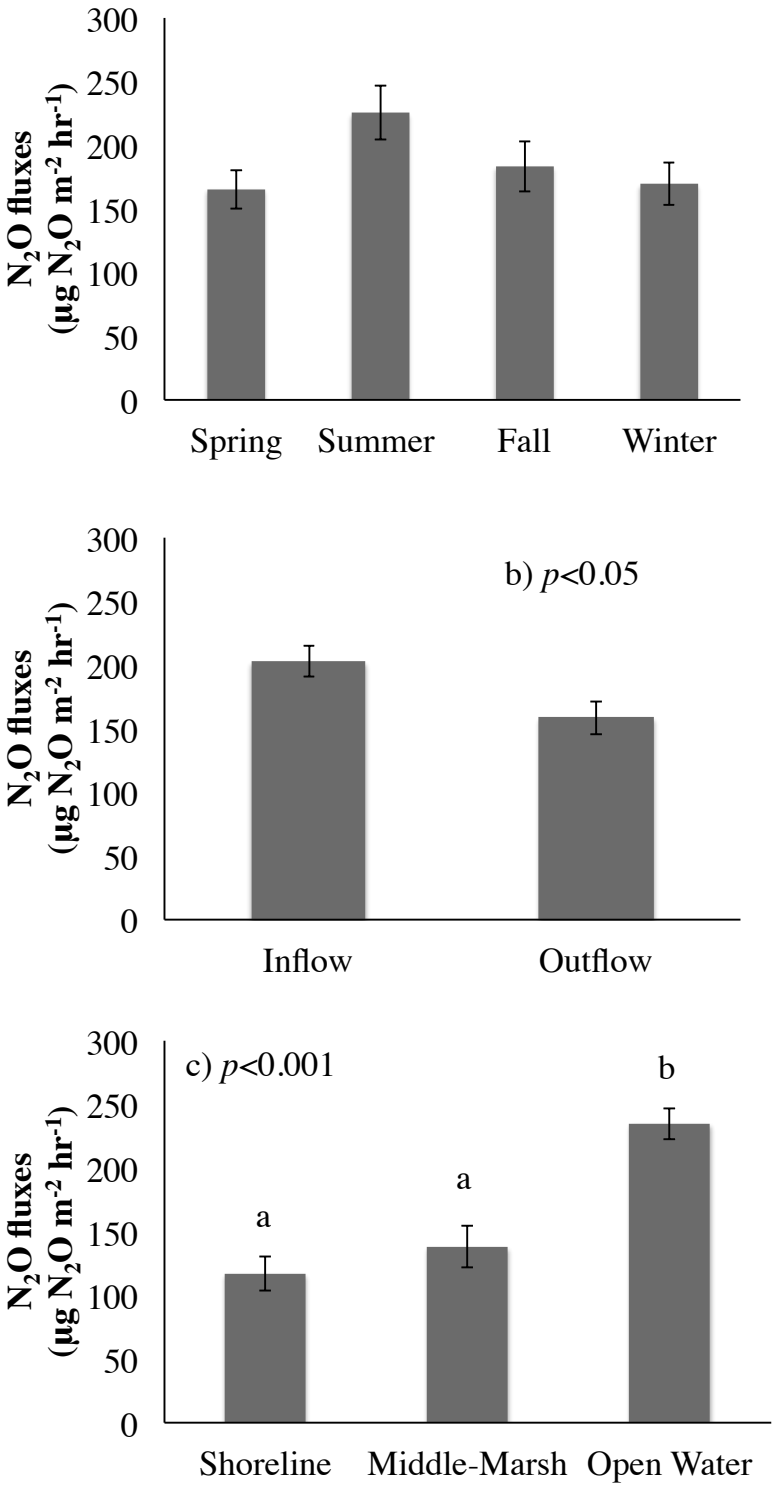


Figure 5. Diffusive nitrous oxide (N_2O) emission fluxes over a) seasonal patterns, b) whole system gradient (inflow and outflow transects), and c) vegetated-shoreline to open-water gradient (shoreline, middle, and open water subsites) from Tres Rios CTW.

3.3 Environmental controls over CH_4 and N_2O fluxes

The correlation tests showed that diffusive CH_4 emission fluxes (n=107) were significantly negatively correlated with NO_3^- (r= 0.47, p <0.0001), temperature (r= 0.23, p <0.05), and % oxygen (r= -0.31, p <0.01) in the water column. However, the stepwise multiple linear regression analysis revealed that the best model included NO_3^- and dissolved organic carbon. The model explained 24% of the variation in CH_4 emission flux from Tres Rios CTW ($F_{2, 104}$ = 17.65, p<0.0001). The significant standardized coefficients for the model of the CH_4 emission fluxes were 0.53 for NO_3^- (p <0.0001) and 0.20 for dissolved organic carbon (p <0.05).

The correlation tests of the diffusive N_2O emission fluxes (n=103) showed that they were significantly negatively correlated to DOC (r= 0.33, p <0.0001), and significantly positively correlated to NO_3^- (r= 0.40, p <0.0001), temperature (r^2= 0.57, p <0.0001), and % oxygen (r= 0.38, p <0.0001) in the water column. The stepwise multiple linear regression revealed that the best model included only the temperature and DOC variables. The model explained 36% of the variability of N_2O emission fluxes from Tres Rios CTW ($F_{2, 100}$=29.16, p<0.0001). The significant standardized coefficients of N_2O emission fluxes were 0.52 for temperature (p <0.0001) and 0.22 for DOC (p <0.05). Due to the limited number of CH_4 and N_2O uptake fluxes (negative) there were insufficient data to perform a regression analysis.

4. DISCUSSION

Free water surface CTW are systems that have been engineered and constructed to improve the quality of wastewater from point and nonpoint sources of water pollution

(Kadlec and Wallace 2008, Vymazal 2011). Recently, it has been found that the CTW designs that achieve the desired ecosystem service of water quality improvement have the potential to contribute to greenhouse gas production, of CH_4 and N_2O (Mitsch et al. 2012). We found that in the first four years its operation, Tres Rios CTW has also been producing and emitting CH_4 and N_2O gases. We show that patterns of diffusive CH_4 and N_2O emission gas fluxes depend on the gradients within the engineered system and environmental variables.

4.1 Diffusive CH_4 emission and uptake fluxes

Our observed range of CH_4 emission fluxes fall on the low end of the reported range in the Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands (Ebie et al. 2014). They reported that FWS CTW, such as the Tres Rios CTW, have CH_4 emissions fluxes within a range of 0.15 – 181.0 (S.E.=10.7) mg CH_4 m^{-2} h^{-1}, while we observed a range of 0.03 – 13.00? mg CH_4 m^{-2} h^{-1} (S.E.=0.25). Our average CH_4 emission fluxes of 2.34 mg CH_4 m^{-2} h^{-1} are comparable to other recent reviews of average CH_4 emission fluxes (5.9 and 4.7 mg CH_4 m^{-2} h^{-1}, respectively) by Mander et al. (2014) and Jahangir et al. (2016). Though CH_4 uptake fluxes are seldom observed from constructed wetland ecosystems (VanderZaag et al. 2010), some studies have reported uptake fluxes similar to the ones we observed. Our uptake fluxes are at the low end of the reported ranges from other CTW studies. For example, compared to our greater uptake value of -2.17 mg CH_4 m^{-2} h^{-1}, Johansson et al. (2004) and Tanner et al. (1997) reported uptake values of -15.62 mg CH_4 m^{-2} h^{-1} and -3.12 mg CH_4 m^{-2} h^{-1}, respectively.

4.2 Seasonal patterns of diffusive CH_4 emission fluxes

At Tres Rios FWS CTW, the summer average diffusive CH_4 emission fluxes (3.36 mg CH_4 m^{-2} h^{-1}) were no different than the mean measured fluxes in the fall season and 2-3 times higher than the mean fluxes measured in the spring and winter seasons. Though our average diffusive CH_4 emission fluxes are in the middle range of other FWS CTW reporting seasonal patterns, our results match the pattern of greater average diffusive CH_4 emission fluxes during the hotter and warmer seasons of the year. Wild et al. (2002) and Van der Zaag et al. (2010) reported significant seasonal patterns of greater average diffusive CH_4 emission fluxes during the summer months from two FWS CTW. In Germany, Wild et al. (2002) reported highest fluxes in summer at 1.47 mg CH_4 m^{-2} h^{-1}. Van der Zaag et al. (2010) in Canada also observed significantly higher average diffusive CH_4 emission fluxes (19.95 mg CH_4 m^{-2} h^{-1}) in summer compared to ???. Similarly, Johansson et al. (2004b) observed higher summer diffusive CH_4 emission fluxes in a two-year study from a horizontal subsurface flow (HSSF) CTW in Sweden (2004b). They found the average 1998 and 1999 summer diffusive CH_4 emission fluxes (6.33 and 8.00? mg CH_4 m^{-2} h^{-1}, respectively) to be 10-50 times higher compared to mean spring and fall fluxes (Johansson et al. 2004b). When comparing average CH_4 emission fluxes from a Finish HSSF CTW, Liikanen et al. (2006), found diffusive CH_4 emission fluxes to be low in winter and higher in autumn. Their 1992 and 2002 average summer diffusive CH_4 fluxes (16.58 and 5.79 mg CH_4 m^{-2} h^{-1} respectively) were higher than our reported summer fluxes but smaller than some of the FWS CTW (VanderZaag et al. 2010). Liikanen et al. (2006) stated that this specific observation was different from the literature because their highest observed diffusive CH_4 flux in the fall season was driven by the high rates of decomposition of plant biomass produced during the previous summer.

These seasonal observations are to be expected due to the fact that as temperature increases in the environment, the level of methanogenic activity also increases. In this study, we saw an increase in the production and emission of CH_4 in the hotter and warmer months in the CTW that has been observed in other wetland soil systems (Moore and Dalva 1993, Macdonald et al. 1998, Inamori et al. 2007).

4.3 Spatial patterns of diffusive CH_4 emission fluxes at whole-system

At the whole-system gradient of Tres Rios FWS CTW, our mean diffusive CH_4 emission fluxes were significantly higher at the transect near the effluent inflow (2.89 mg CH_4 m^{-2} h^{-1}) compared to the fluxes measured at the transect near outflow (1.78 mg CH_4 m^{-2} h^{-1}). In this study, we present first significantly different results of diffusive CH_4 emission fluxes from a FWS CTW at the whole-system gradient. Only one other similar pattern has been observed in a FWS CTW. In Japan, Tai et al. (2002) observed higher mean diffusive CH_4 emission fluxes from the section nearest to the inflow (503 mg CH_4 m^{-2} h^{-1}) compared to the last section near the outflow of their CTW (94 mg CH_4 m^{-2} h^{-1}). Though there is a great difference between their values, they did not perform statistical analysis in their study so it is not possible to note if they were significantly different from each other. Also, unlike Tres Rios FWS CTW, with secondarily treated water, (Tai et al. 2002) FWS CTW is using only primarily treated water. In northern Europe, Sovik et al. (2006) did not show any whole system significant patterns when comparing the CH_4 emissions between the inflow and the outflow of other FWS CTW. However, they did find significantly higher CH_4 emission fluxes at the inflow compared to the outflow sections from two different types of constructed treatment wetlands, horizontal subsurface flow (HSSF) and overland and groundwater flow (OGF) CTW (Sovik et al.

2006). Likewise, these patterns have on many occasions been reported in other horizontal subsurface flow and vertical subsurface flow constructed treatment wetland studies (Tanner et al. 1997, Mander et al. 2005a, Mander et al. 2005b, Picek et al. 2007). Though Sovik et al. (2006) argued that FSW are expected to emit less CH_4 due to higher CH_4 oxidation rates in their designed open water areas, we believe that the higher NO_3^- content near the inflow could have driven higher CH_4 emissions fluxes (Tanner et al. 1997, Tai et al. 2002, Mander et al. 2003).

4.7 Spatial patterns of diffusive CH_4 emission fluxes within marsh-open water gradients

With respect to the vegetated marsh to open water gradient, CH_4 emission fluxes were significantly higher in the vegetated shoreline and middle subsites areas (4.07 and 4.1 mg CH_4 m^{-2} h^{-1}, respectively compared to the open water subsite 0.53 mg CH_4 m^{-2} h^{-1}). Whiting and Chanton (1993) first observed that wetland sites with vegetation had the highest CH_4 emissions compared to sites with no vegetation. In a FWS CTW, Johansson et al. (2004) reported some of their maximum fluxes observed in areas vegetated with *Typha latifolia* (e.g. 52.45 mg CH_4 m^{-2} h^{-1}). However, the average fluxes measured from their open-water sites (10.20 mg CH_4 m^{-2} h^{-1}) were higher than the mean CH_4 emission fluxes found in the *Typha latifolia* vegetated areas (6.79 mg CH_4 m^{-2} d^{-1}). Similarly, an experimental set up of a horizontal subsurface flow CTW, Maltais-Landry et al. (2009b) also found higher CH_4 emission fluxes in areas with no plants (3.62 mg CH_4 m^{-2} h^{-1}) compared to those with *Typha latifolia* (11.95 mg CH_4 m^{-2} h^{-1}). These authors hypothesize (or show?) that in the presence of vegetation, oxygen is supplied to the roots oxidizing sediments near them, thus reducing CH_4 emission from the vegetated areas

(Tanner et al. 1997, Johansson et al. 2004b). From the review of many constructed treatments in northern Europe, Sovik et al. (2006) did not find any significant differences between their vegetated and open water subsites in their three FWS CTW. Our study illustrates the potential effect that the presence of vegetation might have on CH_4 production and emission by the being the provider of an abundance of root litter and root-derived soluble organic compounds. These two components are known to fuel the methanogenesis process in wetland sediments (Bendix et al. 1994b, Johansson et al. 2004b).

4.4 Diffusive N_2O emission and uptake fluxes

The observed N_2O emission fluxes from Tres Rios CTW overlaps the range reported by the Supplement to the 2006 IPCC Guidelines for National Greenhouse Gas Inventories: Wetlands (Ebie et al. 2014). According to the IPCC Supplement, the range for FWS CTW is 9–650 (S.E. = 0.03) μg N_2O m^{-2} h^{-1}, while we observed a range of 10–501 (SE= 0.009) μg N_2O m^{-2} h^{-1} (Ebie et al. 2014). Our average N_2O emission fluxes of 183 μg N_2O m^{-2} h^{-1} are also comparable to other recent reviews of mean N_2O emission fluxes from free water surface flow (FWS) CTW (130 and 110 μg N_2O m^{-2} h^{-1}, respectively) by Mander et al. (2014) and Jahangir et al. (2016). Our observed uptake fluxes of N_2O ranged from -116.35 to -4.43 μg N_2O m^{-2} h^{-1} (average of -29.25 μg N_2O m^{-2} h^{-1}) and fall between previous records of other uptake fluxes (-10.3 and 350 μg N_2O m^{-2} h^{-1}) observed in other FWS CTW with *Typha latifolia* as the dominant emergent vegetation (Wild et al. 2002, Johansson et al. 2003). One of the reasons the compared fluxes may be so different from each other is because Wild et al. (2002) only sampled for N_2O fluxes in the colder season from September 1998 to May 1999 in Germany, while

completed a two-year study and observed the greater uptake fluxes during the hot and warm months of July and September in Arizona, USA.

4.5 Seasonal patterns of diffusive N_2O emission fluxes

As in other studies, we also did not observe any significant seasonal patterns regarding the N_2O fluxes from our two-year study from Tres Rios CTW (Fey et al. 1999, Mander et al. 2003, Mander et al. 2005b, Sovik et al. 2006, Sovik and Klove 2007). Only a handful of studies reported seasonal patterns; for example, Liikanen et al. (2006) reported higher N_2O fluxes in the spring and summer seasons, and similarly, Inamori et al. (2008) observed higher N_2O fluxes during the hot summer season compared to the fall season. They attributed the higher N_2O fluxes to the warmer environmental temperature of the CTW stimulating microbial activity and the lower fluxes in the fall season to the senescence of the emergent vegetation of the wetland (Inamori et al. 2008). Huttunen et al. (2002) also reported great differences in the N_2O fluxes with fluxes ranging from -30 to 940 μg N_2O m^{-2} $d^{-1,}$ with the highest emission fluxes observed at the driest site during the summertime.

4.6 Spatial patterns of diffusive N_2O emission fluxes at whole-system

At the whole-system gradient of Tres Rios FWS CTW, our mean diffusive N_2O emission fluxes were significantly higher at the transect near the effluent inflow (202.52 μg N_2O m^{-2} h^{-1}) compared to the fluxes measured at the transect near outflow (158.82 μg N_2O m^{-2} h^{-1}). In this study, we present significantly different results of diffusive N_2O emission fluxes from a FWS CTW at the whole-system gradient. This whole system gradient pattern of higher N_2O emissions at the inflow compared to the outflow has been observed in a variety of different type of constructed treatment wetlands. From another

FWS CTW like Tres Rios CTW, Johansson et al. (2003) found average N_2O emission fluxes to be four times higher from the sampling sites closest to the inflow (185 μg N_2O m^{-2} h^{-1}) compared to the emission fluxes from the sampling sites closest to the outflow (41.9 μg N_2O m^{-2} h^{-1}) of the free water surface constructed wetland. Similarly, Mander et al. (2005b) observed N_2O emission fluxes eight times higher at the inflow subsites (350 μg N_2O m^{-2} h^{-1}), compared to the outflow subsites (40 μg N_2O m^{-2} h^{-1}) of their horizontal subsurface flow CTW. These same patterns have been observed in other HSSF in Estonia (Mander et al. 2005a, Mander et al. 2005b) and in created riparian marshes in Ohio, USA (Hernandez and Mitsch 2006). Similar to Mander et al. (2005b) and Hernandez and Mitsch (2006), we can relate our greater N_2O emission fluxes to the observed higher NO_3^- concentrations found in the inflow compared to the those in the outflow during our study period (Weller et al. 2016).

4.7 Spatial patterns of diffusive N_2O emission fluxes within marsh-open water gradients

Along the marsh transects we found that average N_2O emission fluxes were significantly higher at the non-vegetated open water locations (234.4 μg N_2O m^{-2} h^{-1}) when compared to the shoreline and middle marsh locations (117.22, 138.06 μg N_2O m^{-2} h^{-1}, respectively). Conflicting results regarding these gradients have been observed in other free-water surface flow constructed treatment wetlands. Johansson et al. (2003) found higher average N_2O emission fluxes of 157 μg N_2O m^{-2} h^{-1} in the non-vegetated open areas and 110 μg N_2O m^{-2} h^{-1} in the *Typha latifolia* vegetated areas. Yet, Strom et al. (2006) found the opposite pattern, they found greater N_2O emission fluxes in the *Typha latifolia* vegetated areas (205.83 μg N_2O m^{-2} h^{-1}) compared to the non-vegetated

subsites (10.83 μg N_2O m^{-2} h^{-1}). In an experimental set up of a horizontal subsurface flow CTW, Maltais-Landry et al. (2009b) found higher N_2O emission fluxes in areas with no plants (0.03 μg N_2O m^{-2} h^{-1}) compared to those with *Typha latifolia* (0.04 μg N_2O m^{-2} h^{-1}). Similar to the whole-system gradient pattern, higher N_2O fluxes were observed in the NO_3^--rich open water subsites compared to lower N_2O emission fluxes from the lower NO_3^- concentration in vegetated subsites (Weller et al. 2016). Given that there are higher and lower fluxes from vegetated and non-vegetated areas in constructed treatment wetlands, the results of the emergent vegetation effect on N_2O fluxes remain poorly understood (Maltais-Landry et al. 2009b).

4.8 Environmental controls over CH_4 and N_2O emission fluxes

The NO_3^- and DOC concentrations in the water column were the two environmental factors associated with measured diffusive CH_4 emission fluxes, although only a small portion of the variance was explained. Though an increase of nutrient-rich wastewater has been demonstrated to increase the productivity of greenhouse gases such as CH_4 and N_2O (Ebie et al. 2014, Mander et al. 2014), a few exceptions have been observed in which there was not a clear effect on CH_4 emission or uptake fluxes when high levels of nitrogen were added to a lakeside vegetated wetland (Siljanen et al. 2012). In contrast to our observed negative correlation between DOC and CH_4 emission fluxes, we were expecting a positive relationship, as DOC is known to support higher CH_4 emission fluxes. Dissolved organic carbon can initially be decomposed aerobically to CO_2 but in the permanently flooded wetland soils such as those in Tres Rios it can degrade anaerobically to CH_4 (Ström et al. 2006, Mander et al. 2014). However, another study did not find a relationship between CH_4 fluxes and DOC (Sovik et al. 2006).

The factors that best explained the variability of the N_2O emission fluxes were DOC concentration and water column temperature, which were negatively and positively correlated (respectively) to the N_2O emission fluxes. Similar to the CH_4 flux, we were expecting a positive correlation of what? with N_2O emission fluxes since it has been noted to act as a substrate for N_2O production in wetland ecosystems (Huang et al. 2004, Liu et al. 2016). Others have not found clear relationships between N_2O and DOC, noting that the quality of the DOC should also be investigated to better understand the role of different carbon-influenced N_2O production and uptake (Saari et al. 2009). The significant correlation with water-column temperature does agree with the findings of other studies indicating that N_2O fluxes are temperature depended in water-logged ecosystems (Goodroad and Keeney 1984, Maag and Vinther 1996). In the CTW literature, however, there is still an unclear relationship between temperature and N_2O emission fluxes (cf. in Jahangir et al. 2016).

Little variation was explained by the measured environmental factors in both of the models. The large amount of unexplained variation is likely driven by two methodological factors. First, the gas samples were obtained a week before the water quality parameters and thus might not reflect the exact environmental conditions when gas samples were collected. Second, the temporal and spatial scales associated with our study might be insufficient to capture the micro-scales at which CH_4 and N_2O are produced, emitted, and/or consumed.

4.9. Conclusions

Our study reports for the first time greenhouse gas fluxes from a FWS CTW designed to remove excess nutrients from secondarily treated water in an arid region.

Previous reviews and synthesis on the greenhouse gases from CTW did not include any information from an arid region (Ebie et al. 2014, Mander et al. 2014, Jahangir et al. 2016). Specifically, we report information on diffusive CH_4 and N_2O emission and uptake fluxes over a two-year period, finding seasonal patterns for CH_4 but not N_2O emission fluxes. The diffusive CH_4 and N_2O fluxes from Tres Rios CTW also showed large spatial variations, indicating that the production and emission of these gases can be influenced by spatial configuration between the inflow and the outflow, the location of the planted emergent vegetation, and environmental factors in these two spatial gradients. CTW are designed and constructed mainly to provide the ecosystem service of water purification by enhancing the ecological processes that remove excess nutrients, yet we should also investigate unexpected potential disservices from the CTW design, such as the production and emission of CH_4 and N_2O. For example, at Tres Rios CTW Weller et al. (2016) observed the greatest nitrogen removal during the growing season (March–September) and noted that the emergent wetland vegetation in the system contributed as much as 50% of whole-system nitrogen removal. In our study, we showed that the highest diffusive CH_4 emission fluxes were observed during the summer season (May–September) and were highest from the areas planted with emergent wetland vegetation that had lower NO_3^- concentration in the water column. Similarly, adding N_2O emission fluxes as another potential disservice adds another level of complexity because N_2O flux emissions showed the opposite pattern from CH_4 emission fluxes: the greatest N_2O fluxes occurred in open water, non-vegetated sites. Though we were unable to explain much of the variation of both CH_4 and N_2O flux variations, we provide important primary information on temporal and spatial patterns of greenhouse gas fluxes from a FWS CTW.

We also recognize that measuring only diffusive fluxes of CH_4 can underestimate the actual fluxes since the ebullition and plant-mediated pathways can sometimes contribute 0-90% of the total methane emissions from wetland ecosystems (Sovik et al. 2006, Carmichael et al. 2014). We aimed with this study to provide a full assessment and make a better estimate of CH_4 and N_2O fluxes from a new constructed treatment wetland. As the use of constructed treatment wetlands increases around the world, combined with new attention to improving national and global accounting of greenhouse gas budgets, it is important to perform multi-temporal and multi-spatial studies such as this one in CTW in other bioclimatic regions. It is important to incorporate the production and emission of CH_4 and N_2O gases to CTW studies focused on nitrogen-removal functions. This information will help scientists, engineers and managers adopt more efficient design and construction strategies of CTW with low greenhouse gas emissions.

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CHAPTER 3

GREENHOUSE GAS FLUXES FROM TYPHA SPP. IN A CONSTRUCTED TREATMENT WETLAND USING A NEW VEGETATION GAS CHAMBER

ABSTRACT

Emergent vegetation such as *Typha* spp. are frequently planted in constructed treatment wetlands (CTW) to reduce nutrient pollution. However, *Typha* spp. can also be a significant pathway, even sometimes the dominant pathway, of greenhouse gas fluxes from CTW. Our primary objective was to investigate the methane (CH_4) and nitrous oxide (N_2O) fluxes from *Typha* spp. in the Tres Rios CTW in Phoenix, AZ. We constructed small gas chambers specially fitted to *Typha* spp. to collect and measure gas samples directly from the vegetation along two transects, at two sites along each transects, and at two heights of *Typha* spp. plants. We made bimonthly measurements in the morning and in the afternoon from July 2014 to July 2015. Plant-mediated CH_4 fluxes during the study period ranged from -3.96 to 100.06 mg CH_4 kg^{-2} h^{-1} ($\bar{x}$=9.23, S.E.=3.18). Plant-mediated N_2O fluxes ranged from -8.89 to 1.76 mg N_2O kg^{-2} h^{-1} ($\bar{x}$=-0.62 S.E.=0.52). Methane fluxes were significantly greater at the transect near the outflow compared to the inflow transect but not different among seasons, sites, or sampling times. Additionally, CH_4 fluxes were significantly higher at lower sections of *Typha* spp. stems? compared to the higher sections of the plant. Methane emission fluxes from *Typha* spp. were better predicted by O_2 and dissolved organic carbon, while uptake fluxes were better predicted by NO_3^- in the water column. *Typha*-mediated N_2O emissions were better predicted by water column temperature.

1. INTRODUCTION

The presence of vegetation in wetlands can affect nutrient cycling through three pathways: (1) alteration of oxygen availability and redox conditions in soils; (2) as a source of labile carbon for microbial nutrient transformations; and (3) direct plant uptake of nitrogen (Engelhardt and Ritchie 2001). Microbial processes in wetland soils can produce greenhouse gases such as methane (CH_4, via methanogenesis) and nitrous oxide (N_2O, via nitrification and denitrification). These gases can then be emitted via three pathways: (1) ebullition (bubbles from wetland soils that float to the water surface); (2) diffusive movement of gases through the water to the surface; and (3) plant-mediated transport (Smart and Bloom 2001, Bridgham et al. 2013). Plant-mediated transport is the direct emission from soils to the atmosphere through the aerenchyma, which is a unique adaptation by macrophytic wetland vegetation to survive in water-saturated wetland soils that allows plants to transport oxygen from stomata to roots (Armstrong 1979, Mendelssohn et al. 1981, Mendelssohn et al. 1995).

Since the first observations of emission via plant-mediated transport of methane (CH_4) and nitrous oxide (N_2O), wetland vegetation is now recognized as an important pathway of greenhouse gas emissions from wetlands (Dacey and Klug 1979, Hutchinson and Mosier 1979, Whiting and Chanton 1993, Chang et al. 1998, Smart and Bloom 2001, Bridgham et al. 2013). Similarly, it is through aerenchyma tissue that significant quantities of greenhouse gases may pass from the root zone to the atmosphere (Reddy et al. 1989, Smart and Bloom 2001, Carmichael et al. 2014). These gas flows can be driven solely by a concentration gradient and diffusion through the aerenchyma; additionally, some plant species such as *Typha latifiolia* and *Typha dominguesis* are capable of

convective flows, which depend on the micrometeorological conditions around the leaves and stomata (Bendix et al. 1994a, Jackson and Armstrong 1999).

Different types of constructed treatment wetlands (CTW) are becoming a common treatment method of removing excess nutrients from treated secondarily wastewater (Kadlec and Wallace 2008). Free-water surface flow (FWS) CTW are commonly used to treat domestic wastewater with the purpose of removing excess nutrients such as nitrogen. Generally, the FWS are planted with emergent wetland macrophytes because these plants play an important role in removing excess nutrients via uptake from the FWS waters (Brix 1994, Weller et al. 2016). As emergent macrophytes contribute to the efficiency of CTW, it is important to study plant-mediated transport of CH_4 and N_2O from the emergent vegetation as well (Li et al. 2016). Plant-mediated transport has been reported in some cases to be up to 90% and 87% of the total wetland ecosystem-level CH_4 and N_2O flux, respectively (Yan et al. 2000, Carmichael et al. 2014). Also, we still need to refine greenhouse gas budgets to better inform policy makers (Robertson et al. 2000, Smart and Bloom 2001).

The majority of studies that investigate the role of plants in CH_4 and N_2O fluxes compare gas fluxes from chambers positioned on top of vegetated and non-vegetated areas of the wetland and attribute the observed differences to the vegetation (Johansson et al. 2004a, Hernandez and Mitsch 2006). Other studies build individual gas chambers specifically for the vegetation of interest and cover the plant completely to record the flux from the individual plant (Jorgensen et al. 2012, Emery and Fulweiler 2014). The former is the recommended design to obtain the most accurate representation of the greenhouse gas emissions from wetlands ecosystems because it includes all fluxes from soil, water,

and plants and it covers all three potential pathways of emission (Whiting and Chanton 1996). Though this method is recommended, if we are interested in specific sources of emissions (e.g., CWS component or plant species), it is important to follow up with individual and vegetation-specific gas chambers (Li et al. 2016). Additionally, when studies attempt to capture the plant-mediated pathway of CH_4 and N_2O from plants, they have conducted these studies in boreal and arctic ecosystems and temperate latitudes where vegetation is small enough to fit the chambers without disturbing them (Carmichael et al. 2014, Li et al. 2016). Procedures include cutting the plant and only sampling the gases emitted from the incomplete part of the stem, or bending and folding the plant to fit in the chamber (Brix et al. 2001, Duan et al. 2005, Dingemans et al. 2011).

Here, we test and present CH_4 and N_2O gas fluxes measured using a modified individual vegetation-gas chamber specific to a common wetland emergent plant, *Typha* spp. The cattail (*Typha* spp.) is one of the most commonly used emergent vegetation in constructed wetlands because its fast growing and ability to play an important role in nutrient uptake from wastewater (Vymazal 2013, Weller et al. 2016). Direct greenhouse gas emissions from *Typha* spp. have been reported, but methods used have overestimated or underestimated fluxes by either capturing other emission pathways and making inferences regarding the plant-mediated pathway or disrupting the airflows inside the aerenchyma (Yavitt and Knapp 1995, Yavitt 1997, Duan et al. 2005, Wang et al. 2008, Koebsch et al. 2013).

Our objective for this study was to investigate the CH_4 and N_2O fluxes from macrophytic vegetation (*Typha* spp.) planted in a FWS CTW system located in an arid region. This study contributes to a more comprehensive quantification of CH_4 and N_2O

gases from constructed treatment wetlands in general. Additionally, through the use of a novel gas flux chamber adapted to *Typha* spp. method, we demonstrate the feasibility of a new chamber to measure greenhouse fluxes from macrophytic vegetation in a range of ecosystems.

2. METHODS

2.1. Study site

The Tres Rios Constructed wetland is located in the City of Phoenix, AZ, USA (33°23' N, 112°15'W). This region is characterized by monthly mean temperatures ranging from 10.8°C in winter months to 35.2°C in summer months. Average temperature during our 12-month study period was 23.4°C, with the maximum temperature recorded in the summer month of July of 2014 (44.3°C) and minimum temperature recorded in January of 2015 (-1.6°C). Tres Rios is a FWS CTW that has been in operation since 2010, when it started receiving treated wastewater from the city's 91st Ave Wastewater Treatment Plant. The Tres Rios CTW consists of two flood-regulating basins that store and regulate water flow into the vegetated surface-flow wetlands. We studied Cell 1, the largest of the three vegetated surface-flow wetlands, which is 42 ha in size, with 21 ha of open water and 21 ha of fringing vegetated marsh along the margins (Fig. 6). The emergent vegetation was planted in 2009 and it was the first cell to receive water in 2010. During the study period, the Tres Rios CTW Cell 1 received an average of 95,000 to over 270,000 m^3 d^{-1} of effluent, depending on the time of year (Sanchez et al. 2016).

The CTW that we studied is 42 ha in size, with 21 ha of open water and 21 ha of fringing vegetated marsh along the margins. This vegetated marsh extends roughly 50–60 meters towards the open water regions. Seven native emergent macrophytes were originally planted: *Schoenoplectus acutus, Schoenoplectus americanus, Schoenoplectus californicus, Schoenoplectus maritimus, Schoenoplectus tabernaemontani, Typha domingensis,* and *Typha latifolia*. Since 2011, *Typha* spp. has accounted for a steadily increasing area of the emergent vegetation in the marshes (Weller et al. 2016). For this reason, we focused our measurements of plant-mediated CH_4 and N_2O flux on this specific genus of wetland emergent vegetation.

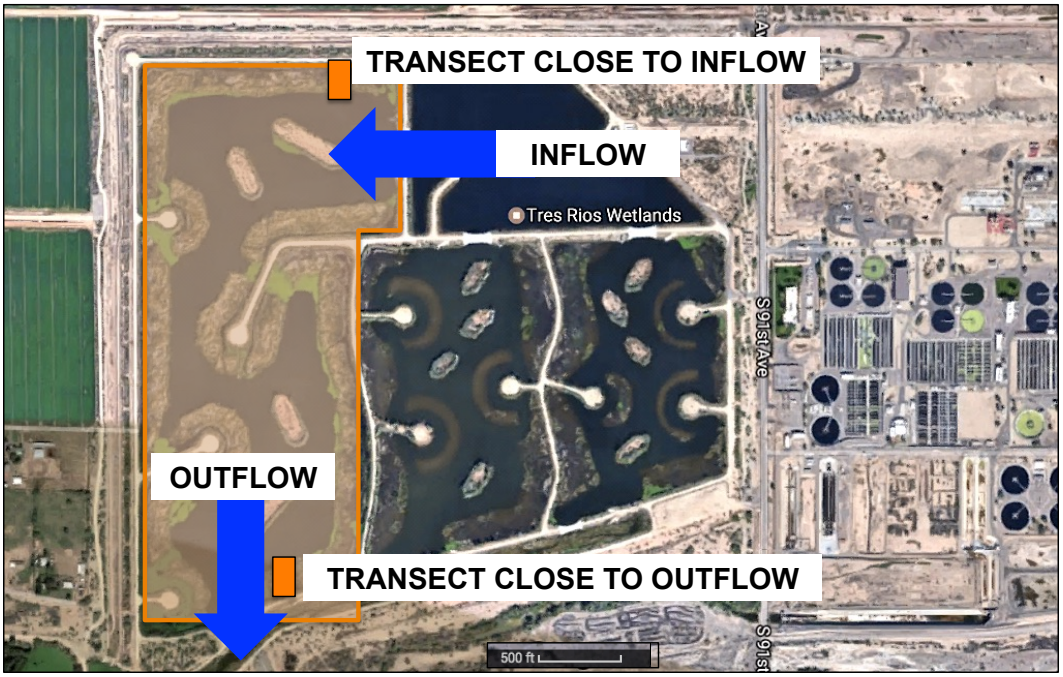


Figure 6. Aerial view of Tres Rios CTW (Cell 1 shaded in orange) illustrating the whole-system flow of the water (blue arrows), and the location of the two 50-transects (orange rectangles).

2.2. Study design

We established two marsh transects—one near the effluent inflow and one near the outflow, following the logic that plants along the former transect may receive more nutrients. These two transects spatially represent the inflow-outflow gradient of the whole system. The two transects were approximately 50-60 meters long and ran perpendicular from the shoreline to the open water. Along each transect, we established two sites (shoreline and open water) to spatially represent the shoreline-to-open-water gradient. The logic for this is that nutrient concentrations along these marsh transects decrease to near zero from the open water to the shoreline (Sanchez et al. 2016). The *Typha* spp. plants that we sampled at the shoreline sites were located about 5 meters from the shore while the plants in the open water sites were approximately 1 meter from the open water.

We measured N_2O and CH_4 gas fluxes from two different heights (high and low sections of individual *Typha* plants) at two different times of the day (Fig. 7). The lower sampling point was always about 0.5 m from the water surface and the higher sampling point was always about 2 m above the soil surface. We measured gas fluxes at each subsite on each transect in the morning (8AM) and in the afternoon (12PM). We conducted the field campaigns over the course of a year to cover a wide range of environmental conditions: cool winter periods of Arizona (November 2014 and January 2015) and the transition period to higher temperature and increased plant activity (July 2014, May 2015, July 2015).

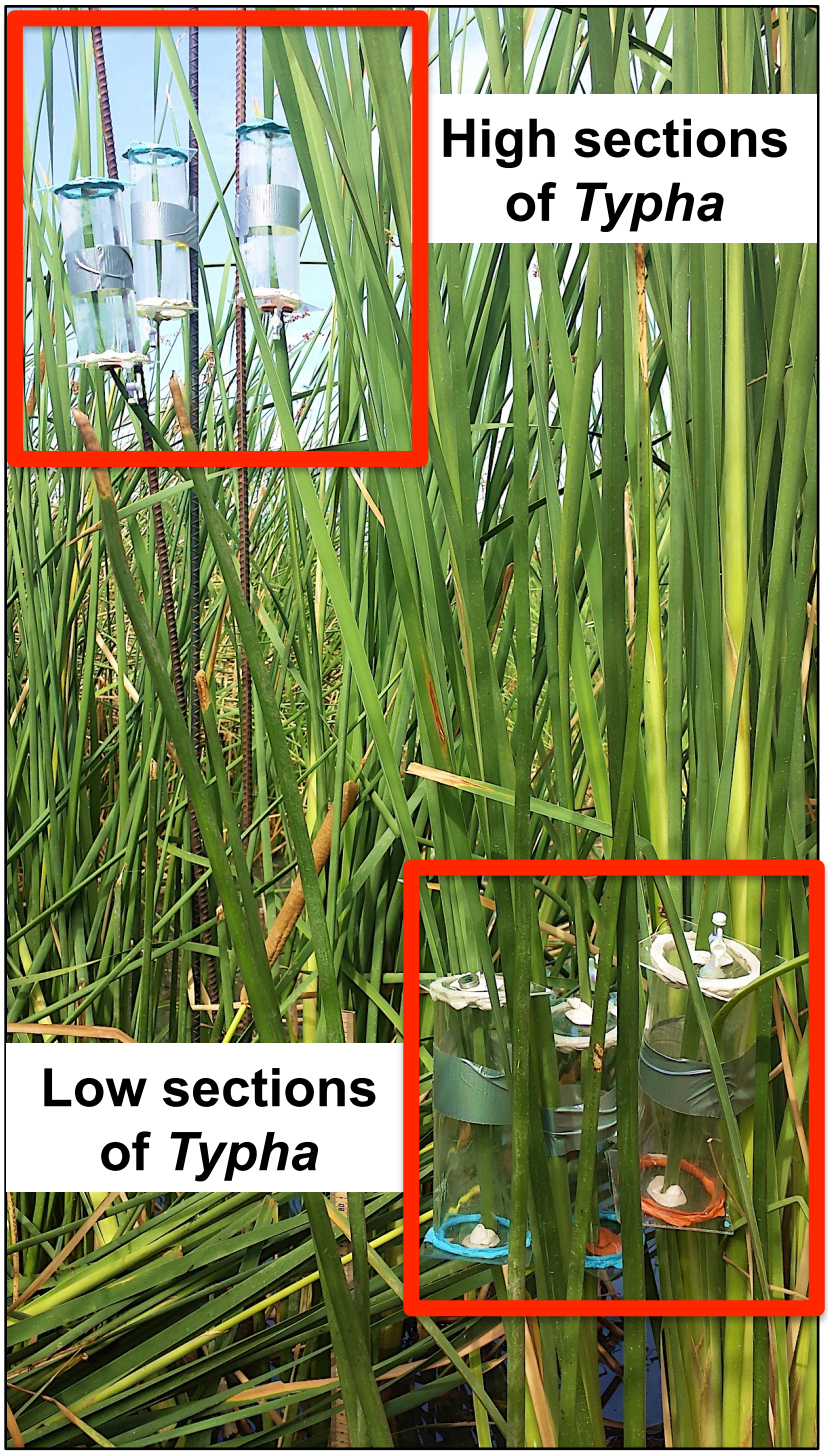


Figure 7. Specially adapted gas chamber in use on high and low sections of *Typha* spp. during the May 2015 afternoon sampling event.

The gas flux chambers were constructed from clear acrylic cylinders (7 x 20 cm, 0.76 L) with both ends sealed with an acrylic lid with an opening approximately 1 x 2 cm wide for the intact plant leaves. After sliding the chambers over the plant leaves, these openings were sealed securely with solid adhesive putty. Lower chambers were held in place by the plants themselves, while the higher chambers were supported by 3-meter rebar staked next to the plant. Six chambers were deployed simultaneously on six different plants at each site during each daytime sampling period. Once sealed, four gas samples were collected from each chamber every 15 minutes over a 45-minute period using 20-mL plastic syringes. We immediately injected all gas samples into manually pre-evacuated, silicone-sealed, 10-mL glass vials with inert stoppers sealed with aluminum caps. After gas-flux sampling was complete, the leaf sections that were enclosed in the chambers were cut, placed in ziplock®/paper bags, and transported to the lab. Once in the lab, the leaves were oven dried and weighed to determine dry plant biomass that was inside the chamber during the gas-flux sampling. Gases were analyzed for CH_4 and N_2O concentration using a Varian CP-3800 gas chromatograph (GC). The GC was calibrated in the laboratory using certified CH_4 and N_2O standards (Matheson Tri-Gas® 2016).

Because non-linear change in gas concentrations over time is often observed when using chambers, CH_4 and N_2O fluxes were calculated using the HMR procedure, which is available as an add-on package (Pedersen 2011) in the R software (version 2.15.1; R Development Core Team 2012). The HMR package allowed us to calculate fluxes from both linear and non-linear concentration changes using a regression-based extension of the Hutchinson and Mosier Model (Hutchinson and Mosier 1981).

2.3 Environmental parameters

We designed our study to be paired with the ongoing bimonthly biomass, nutrient budget, and water budget studies of the Tres Rios CTW (Sanchez et al. 2016, Weller et al. 2016). Our plant-mediated gas sampling always occurred 1-2 days before the collection of the water quality samples and a week before the biomass sampling to minimize any disturbance to the wetland vegetation and soils. Within transects, triplicate water samples were collected at the shoreline and open-water sites. Water samples were centrifuged and analyzed for nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) on a Lachat Quick Chem 800 Flow Injection Analyzer (0.85 µg NO3-N/L and 3.01 µg NH4-N/L). Dissolved organic carbon (DOC) concentration was analyzed using a Carbon/Nitrogen Analyzer (Shimadzu TOC-V). Water temperature and dissolved oxygen were measured using a YSI multi-probe (YSI Pro2030) and pH with the EcoSense pH100 instrument.

2.4 Statistical analysis

Statistical analyses were performed using the SPSS software version 23. We tested for significant differences among seasons (Summer, Fall, Spring, Winter), between the inflow and the outflow transects (whole-system perspective), and between the sites along each transect (shoreline and open-water). To achieve a normal distribution, the flux data for CH_4 and N_2O were log transformed. We used univariate analysis of variance (ANOVA) to test for a season effect. We performed t-tests to compare gas fluxes from the different transects (inflow and outflow), within-transect sites (shore and open water), sampling times (8AM and 12PM), and plant sections (low and high). Environmental variables were screened for correlations and multicollinearity. Nitrite and ammonium

were not included in the multiple linear regression analysis because of their high correlation with nitrate (r^2>0.7). We used linear regression analysis to identify environmental factors that might explain variability in plant-mediated fluxes of CH_4 and N_2O. Only the significant CH_4 and N_2O fluxes were used in these analyses. All statistical tests were evaluated at the 5% significance level ($p \leq 0.05$).

3. RESULTS

We examined the trace-gas emissions of the predominant emergent vegetation in an aridland constructed treatment wetland and we analyzed the CH_4 and N_2O fluxes—both emission and uptake—from *Typha* spp. over a year-long period. We observed 49 plant-mediated CH_4 fluxes that ranged from -3.96 to 100.14 mg CH_4 kg^{-2} h^{-1} ($\bar{x}$=9.23, S.E.=3.18). We found 13 uptake fluxes and 36 emission fluxes. Uptake CH_4 fluxes ranged from -3.96 to -0.06 mg CH_4 kg^{-2} h^{-1} ($\bar{x}$=-1.03, S.E.=0.29) and emission fluxes ranged from 0.12 to 100.14 mg CH_4 kg^{-2} h^{-1} ($\bar{x}$=12.94, S.E.=4.18). The 21 observed plant-mediated N_2O fluxes ranged from -8.89 to 1.76 mg N_2O kg^{-2} h^{-1} ($\bar{x}$=-0.62 S.E.=0.52). We observed 11 uptake fluxes and 9 emission fluxes. Uptake N_2O fluxes ranged from -8.89 to -0.06 mg N_2O kg^{-2} h^{-1} ($\bar{x}$=-1.47, S.E.=0.87) and emission N_2O fluxes ranged from 0.09 to 1.74 mg N_2O kg^{-2} h^{-1} ($\bar{x}$=0.41, S.E.=0.16).

3.1 Seasonal and daytime dynamics and spatial patterns

We found no significant differences among the seasons or between the daytime sampling times of any of the plant-mediated CH_4 and N_2O uptake and emission fluxes. At the whole-system scale, only mean CH_4 emissions throughout the entire study period were significantly higher along the outflow transect compared with the inflow (p<0.01,

$\bar{x}$=17.99, S.E.=5.76, $\bar{x}$=1.46 S.E.=0.66 mg CH_4 kg^{-2} h^{-1}, respectively, Fig. 8c). We found no significant differences in plant-mediated CH_4 and N_2O uptake and emission fluxes within the vegetated marsh proper (i.e., at the shoreline versus the open-water locations).

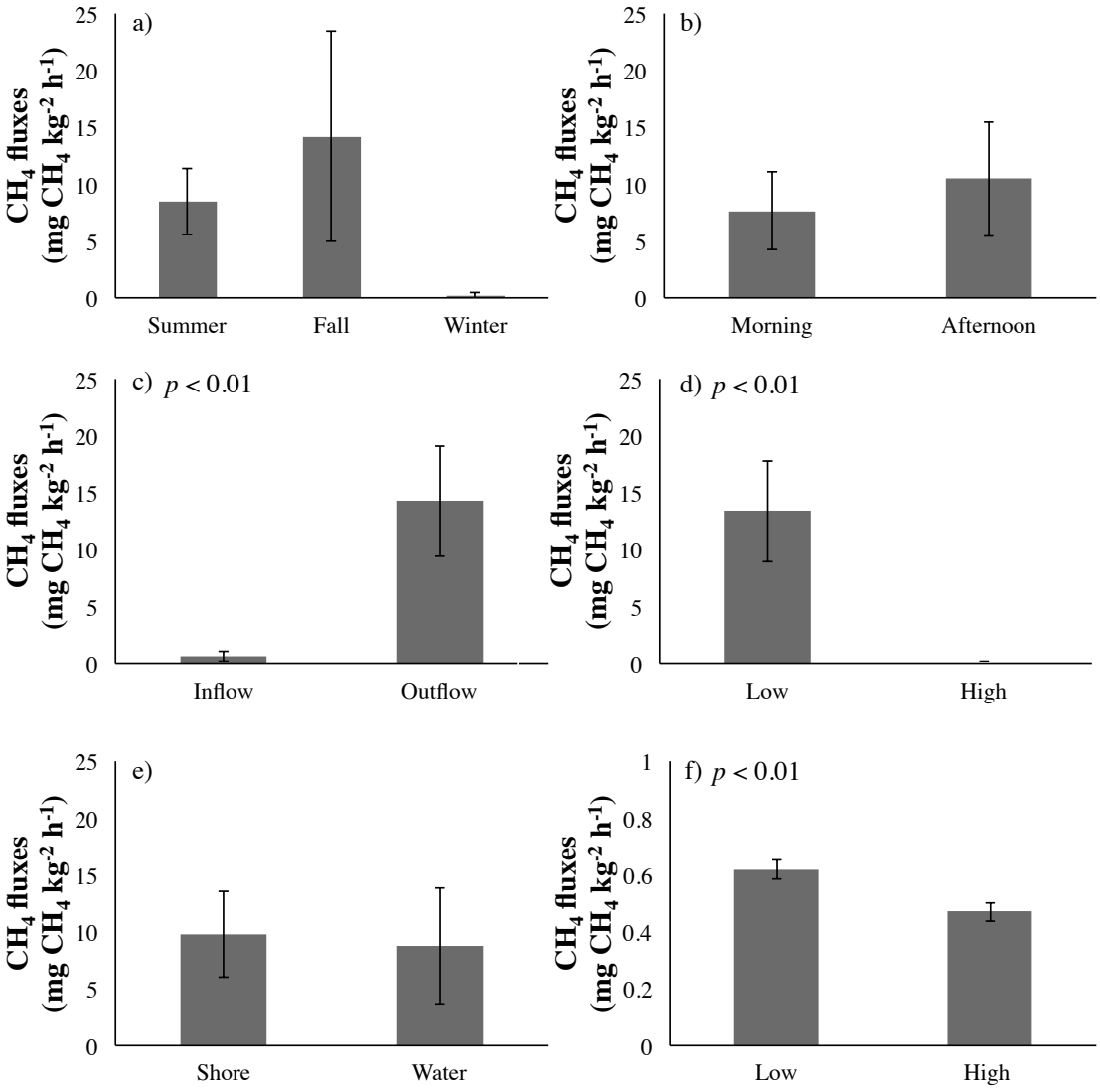


Figure 8. Observed plant-mediated CH_4 emission fluxes (mg CH_4 h^{-1} kg^{-1} of plant biomass) by a) season, b) time of day, c) transect (p<0.01), d) plant sections (p<0.01), and e) subsite. Graph f) shows the significantly greater amount in biomass found in the lower parts of the *Typha* spp. leaves compared to the higher parts of the leaves (p<0.01).

3.2 Fluxes from high and low sections of Typha spp.

We found significantly higher CH_4 emission fluxes from leaves located near the lower sections of the *Typha* spp. plants compared with fluxes from higher sections of the leaves (p<0.01, $\bar{x}$=17.1 S.E.=5.36, $\bar{x}$= 0.43 S.E.= 0.1 mg CH_4 kg^{-2} h^{-1}, respectively, Fig. 8d). The higher CH_4 emission fluxes from the lower sections of the *Typha* spp. coincide with the significantly greater biomass per unit leaf area of the leaves near the lower section of the plants ($\bar{x}$=0.58 S.E.=0.04 gr) compared to the higher section of the plants ($\bar{x}$= 0.46 S.E.= 0.04 gr, Fig. 8f). We did not find any differences when analyzing CH_4 uptake fluxes and N_2O emission or uptake fluxes between the low and high sections of *Typha* spp. plants.

3.3 Environmental parameters as predictors of CH_4 and N_2O fluxes

Pearson correlations showed that plant-mediated CH_4 emission fluxes (n=35) were significantly negatively correlated to O_2 (r^2= -0.42, p <0.01) and positively correlated to dissolved organic carbon (DOC) (r^2= 0.34, p <0.05) in the water column. The stepwise multiple linear regression analysis revealed that the best model included only O_2 explaining only 18% (adjusted R^2) of the variation in plant mediated CH_4 emission fluxes from *Typha* spp. ($F_{1, 33}$ = 7.22, p<0.05, Table 1). The significant standardized coefficients for the model of the CH_4 emission fluxes for O_2 was -0.42 (p <0.05). For plant-mediated CH_4 uptake fluxes (n=11), the Pearson correlations showed a positive correlation with NO_3^- concentration (r^2= 0.66, p <0.05) in the water column. The stepwise multiple linear regression analysis revealed that the best model included only NO_3^- concentration, which explained 38% (adjusted R^2) of the variation in plant mediated

CH_4 uptake fluxes from *Typha* spp. ($F_{1,9} = 7.17$, $p<0.05$, Table 1). The significant standardized coefficient for the model of CH_4 uptake fluxes for NO_3^- was 0.66 ($p<0.05$).

Pearson correlations showed that plant-mediated N_2O emission fluxes (n=8) were significantly positively correlated to temperature in the water column ($r^2= 0.73$, $p<0.05$). The stepwise multiple linear regression analysis revealed that the best model included only temperature as a predictor, explaining 46% (adjusted R^2) of the variation in plant-mediated N_2O emission fluxes from *Typha* spp. ($F_{1,6} = 6.86$, $p<0.05$, Table 1). The significant standardized coefficient for the model of the CH_4 emission fluxes for O_2 was 0.75 ($p<0.05$). Pearson correlations and multiple linear regression analysis were performed for plant-mediated N_2O uptake fluxes, but no model produced significant results.

Table 1. Results of multiple linear regression analysis of plant mediated CH_4 and N_2O fluxes environmental variables measured at the same subsite of the plants sampled at that time.

	CH_4 emission fluxes	CH_4 uptake fluxes	N_2O emission fluxes	N_2O uptake fluxes
Adjusted R^2	0.18 ($F_{1,33} = 7.22, p<0.05$)	0.38 ($F_{1,9} = 7.17, p<0.05$)	0.46 ($F_{1,6} = 6.86, p<0.05$))	N/A
DOC (mg/L)				
Nitrate NO3 (mg/L)		0.66 ($p<0.05$)		
Temperature (°C)			0.75 ($p<0.05$)	
Oxygen (%)	-0.42 ($p<0.05$)			

4. DISCUSSION

Since vegetation is known to have an effect on the dynamics of carbon and nitrogen cycling in wetlands, it is also important to study the role of plants as conduits of CH_4 and N_2O gases between soils and the atmosphere (Strom et al. 2005, Strom et al. 2007, Carmichael et al. 2014). This study reveals new information on plant-mediated transport

and emphasizes the need to further examine greenhouse gas fluxes of CH_4 and N_2O from CTW as they continue to develop globally (Sovik et al. 2006, Jahangir et al. 2016). This study adds to our understanding of the contribution of vegetation to global CH_4 and N_2O budgets and the need to reduce model uncertainty (Huang et al. 2013, Kirschke et al. 2013, Carmichael et al. 2014). Most studies that have reviewed greenhouse gas emissions from CTW have shown higher emissions in vegetated areas compared with non-vegetated areas. For this reason it is important to refine our understanding of the spatial and temporal CH_4 and N_2O flux patterns from CTW, specifically from a common emergent wetland plant—*Typha* spp. In this study, due to the variety of results reporting plant-mediated CH_4 and N_2O fluxes in the CTW literature, we sought to better understand the relative contribution of plant-mediated CH_4 and N_2O fluxes to the atmosphere and potential environmental controls on them (Huang et al. 2013, Carmichael et al. 2014).

4.1 CH_4 and N_2O emission fluxes

We report direct plant-mediated CH_4 and N_2O fluxes measured directly from a common wetland emergent plant that is present in natural and constructed wetlands. Previous plant-mediated flux studies have only reported CH_4 and N_2O fluxes from growing seasons or single dates (Yavitt and Knapp , Brix et al. 1996, Hu et al. 2016). Long-term studies of direct plant-mediated CH_4 and N_2O from macrophytic vegetation such as *Typha* spp. are still uncommon in the literature and more is needed if we are to improve our global greenhouse budget models (Yavitt and Knapp 1995, Huang et al. 2013, Carmichael et al. 2014). Though we did not find significant differences among seasons, our results are comparable to the few observations of plant-mediated pathway of CH_4 from *Typha* spp. Similar to Sebacher et al. (1985), our study observed greater CH_4

emission fluxes during the summer and fall seasons and lower plant-mediated CH_4 emission fluxes from *Typha* spp. during the winter months. Our study will add to Sebacher et al. (1985) only two single fluxes in the late spring season and in the winter season. Specifically, the study reports one late-spring daytime CH_4 emission flux over two hours scaled up to a day from a *Typha latifolia* in the southern USA of 9.8 mg CH_4 d^{-1} (Sebacher et al. 1985). They also reported that during the winter months, the flux had decreased to a low of 1.0 mg CH_4 d^{-1} due to the *T. latifolia* turning brown (Sebacher et al. 1985).

In terms of N_2O emission fluxes from vegetated constructed treatment wetlands, few studies have carried out long-term studies and fewer have observed seasonal patterns (Mander et al. 2005b, Picek et al. 2007, Sovik and Klove 2007). For example, Picek et al. (2007) aimed to quantify CH_4 and N_2O emissions from a CTW but was only able to measure CH_4 emissions and did not quantify plant-mediated fluxes. We observed mostly N_2O uptake fluxes, which contrasts with Sovik et al. (Sovik and Klove 2007), who only observed two uptake fluxes over the course of a year and did not investigate the role of vegetation. Tetier and Mander (2005b) did observe and quantify multiple N_2O emission and uptake fluxes from three CTW, recording the highest emission flux in the winter months, but did not address the plant-mediated pathway nor the vegetation over the three year period. Yang and Silver (2016) observes significant differences of gross N_2O production between winter and summer seasons and among three different vegetated plots in a salt marsh ecosystem. However, similar to previous studies, they did not report season al variation in gross N_2O production nor the role of plant community composition in N_2O production (Yang and Silver 2016).

It is important to note that plant-mediated emission fluxes of CH_4 and N_2O do not just reflect gas transport from roots through the leaves to the atmosphere. Just recently, the production of both CH_4 and N_2O gases inside the leaves has been hypothesized and observed in some plants. For example, Keppler et al. (2006) recorded the first observation of aerobic CH_4 production from plant biomass and has received more attention as a potential source and emission of CH_4 as reviewed by Keppler et al. (2009) and Bruhn et al. (2012). Similarly, just recently it has been found that N_2O emission directly from the leaves could result from the nitrate assimilation process within the leaves and not only as a result from plant-mediated transported N_2O from the rhizosphere (Smart and Bloom 2001, Hakata et al. 2003, Yu and Chen 2009). Our methods did not attempt to address this mechanism, which warrants further investigation from commonly planted vegetation in new and restored, aquatic and terrestrial ecosystems since it has been found to occur in both (Yu and Chen 2009).

4.2 CH_4 and N_2O uptake fluxes

The few plant-mediated CH_4 uptake fluxes observed within the leaves could be explained by a phenomenon observed in another *Typha* spp., *T. latifolia* (Bendix et al. 1994a). They discovered that CH_4 can enter the middle-aged leaves of the *T. latifolia* plant via uptake, circulate towards the below ground/water parts of the plant, and vent back into the atmosphere through another leaf of the plant (Bendix et al. 1994a). This process can explain the numerous uptake fluxes observed in our study, especially because the majority of our observed uptake fluxes occurred during the hot and sunny days of the summer season, similar to the conditions under which Sebacher et al. (1985) and Bendix et al. (1994a) observed uptake fluxes. The conditions of these hot and sunny days are

known to stimulate the pressure-induced gas flow from the green leaves through the rhizome, and back through the outer leaves and it has also been observed in other emergent wetland vegetation (Dacey 1981).

Very few studies have reported direct plant-mediated N_2O uptake. Li et al. (2011) summarized existing hypotheses to explain observations: N_2O absorption and metabolism within leaves (Grundmann et al. 1993), transportation from the leaves into the soils, which act as N_2O sinks (Henault et al. 1998, Verchot et al. 1999). In their study, Li et al. (2011), observed direct plant-mediated N_2O uptake fluxes using vegetation-only gas chambers from wheat, cotton, and maize plants. Though they could not point to the exact mechanisms behind their observations, they did conclude that their uptake fluxes usually occurred during dry soil conditions. Our site, the Tres Rios CTW is managed to maintain permanently flooded soils, so our N_2O uptake fluxes match those observed in other waterlogged soil conditions (Blackmer and Bremner 1976, Goossens et al. 2001, Butterbach-Bahl et al. 2004, Mander et al. 2005b).

4.3 Fluxes from low and high sections of Typha spp.

Significantly greater CH_4 emission fluxes from the lower parts of the plant compared to the higher parts of the plant confirm Yavitt and Knapp's (1995) findings on leaf or air space CH_4 concentrations inside *Typha latifolia* leaves. Similar to our study, they found CH_4 emission fluxes from the tip and the base of the leaf, but higher emission fluxes from the middle part of the leaf (Yavitt and Knapp 1995). These observations confirm a plant-mediated diffusion pathway for CH_4 since CH_4 concentrations are on a low to high gradient from the base to the tip of the plant. But, since *Typha* spp. can emit gases first through the molecular diffusion pathway, then through an additional process of

throughflow convective flow (i.e. pressurization), emission fluxes should be influenced by more than just leaf CH_4 concentration *Typha* spp. Yavitt and Knapp (1995) explain that these factors can include (1) changes in the gas concentration gradient, (2) the rate of gas flow from the plant to the atmosphere, and (3) the resistance along the flow path. Regarding N_2O emissions, studies have identified aerenchymous plants (*Oryza sativa*) as effective conduits of N_2O (Mosier et al. 1990) but have not attempted to identify specific sections of other wetland plants as the source or sinks. Yu and Chen (2009) summarized that the most common mechanism behind N_2O emission fluxes are similar to those of CH_4, via molecular diffusion from the soils to the atmosphere, but less common by convection (Heincke and Kaupenjohann 1999). To investigate which specific components of the plants influence plant-mediated N_2O fluxes, Yu and Chen, did investigate which parts of terrestrial plants (soybean and what) emitted more N_2O (Yu and Chen 2009). They found the highest emission fluxes from the leaves of the plants and attributed the emission fluxes to leaf nitrate assimilation processes, which ultimately produce and are responsible for the emitted N_2O. Both plant-mediated N_2O emission and uptake fluxes from the leaf, but just like the studies in terrestrial plants, we show that N_2O fluxes from wetland plants is a dynamic process that necessitates further investigations.

4.4 Time of day CH_4 and N_2O fluxes

Because plant-mediated gas transport can be influenced by the transpiration activity within the plants (both CH_4 and N_2O can be transported through the convective pathway), we were expecting different plant-mediated CH_4 and N_2O fluxes between the morning and the afternoon. Studies did observe higher emission fluxes in the afternoon compared to morning and later in the afternoon (Chanton et al. 1993, Yavitt and Knapp

1995). Ferch and Romheld (2001) found plant-mediated N_2O emission fluxes to increase from the morning to the afternoon and attributed to their observed increase in transpiration during the same time. Though we did not observe significant differences of plant-mediated fluxes between our morning and afternoon sampling times, we did record our higher CH_4 and N_2O emission fluxes in the afternoon. We attempted to capture the difference between times of day, but because access the Tres Rios CTW was restricted to 7:00 AM to 2:00 PM, sunlight was probably not that different between our sampling times since sunrise had already triggered photosynthesis and transpiration processes within *Typha* spp.

4.5 Whole system CH_4 and N_2O spatial dynamics

Our study is the first one to show significant differences in plant-mediated CH_4 emission fluxes from *Typha* spp. from a whole-system perspective of a FWS CTW in an aridland region. In a review by Sovik et al. (Sovik et al. 2006), they showed similar patterns of higher CH_4 emission fluxes from constructed wetlands but these only include flux measurements using static chambers over the surface of the wetlands which could not differentiate among plant-mediated fluxes, ebullition and diffusion pathways (Sovik et al. 2006). Additionally, these findings were also found to be significantly different in horizontal subsurface flow and overland and groundwater flow wetlands in temperate regions but not in any FWS or in warm or arid regions (Sovik et al. 2006).

4.6 Environmental controls over plant-mediated CH_4 and N_2O emission fluxes

The two environmental factors from the water column in Tres Rios CTW that best explained some of the variability measured in *Typha* spp. plant-mediated CH_4 emission fluxes were O_2 and DOC. The negative correlation between % O_2 in the water column

and the plant-mediated CH_4 emission fluxes confirms previous observations of higher CH_4 emission fluxes were found in areas with low O_2 and high carbon content, two indicators of methanogenesis activity. In this context, like Jorgensen and Elberling (2012) note, plant-mediated CH_4 emission fluxes can be observed when CH_4 is present in the root zone of the vegetated areas of the wetlands. Once it is produced, wetland vegetation such as *Typha* spp. can serve as a passive (diffusion) or active (convection) pathway for CH_4 from the root area to the atmosphere (Mosier et al. 1990). The positive correlation between DOC in the water column and CH_4 emission fluxes demonstrates the positive relationship between the roots and CH_4 emissions observed in previous studies (Hu et al. 2016). It is known that wetland vegetation and their associated roots stimulate the production of CH_4 via the input of labile carbon in root exudates, and also serve as CH_4 gas conduits from the root area to the atmosphere (Hu et al. 2016). However, DOC was ultimately not included in the best multiple regression model that explained 18% of the plant-mediated CH_4 emission fluxes. However, most studies show a positive relationship between CH4 emission fluxes and increased NO_3^- loading (see review by Mander et al. 2014), the observed relationship between *Typha* spp. CH_4 plant-mediated uptake fluxes and NO_3^- has been hypothesized before in tidal and marsh ecosystems (Holm et al. 2016). Holm et al. (2016) explained this because, as it has been observed to repress CH_4 emissions in forest ecosystems (Mosier et al. 1990), it might be suppressing the production of CH_4 in the *Typha* spp. root area by NO_3^- increasing alternate and more efficient electron acceptors (Reddy and DeLaune 2008).

The plant-mediated N_2O emissions observed from *Typha* spp. are similar to those observed by previous studies in temperate regions (Jorgensen and Elberling 2012,

Jorgensen et al. 2012, Bansal et al. 2016). Comparable to our permanently flooded system of Tres Rios CTW, Chan et al. (1998) concluded that it was the water saturated soils that promoted plant-mediated N_2O emission fluxes. Additionally, our positive correlation between plant-mediated N_2O emission fluxes and temperature agrees with the literature because microbiological processes regulate the production of N_2O in soil that ultimately could have been transported from the roots into the atmosphere (Keeney et al. 1979). However, in a review of several CTW and riparian buffer zones, Teiter and Mander (2005b) did not find any significant correlations between N_2O fluxes and soil/water temperature in vegetated and non-vegetated sections of these constructed systems. Other positive relationships between N_2O emission fluxes and temperature have been observed; however, most of them are representative of wetlands and marsh ecosystems in temperate and tundra biomes (Zhu et al. 2008, Dunmola et al. 2010, Schaufler et al. 2010).

4.7 A Tres Rios CTW plant-mediated CH_4 annual emission flux estimate

The biomass of *Typha* spp. throughout the Tres Rios CTW has been measured monthly (??) since its inception (Weller et al. (2016). This effort provides us the means to translate our fluxes to a whole-system, seasonal estimate of CH_4 and N_2O emissions to the atmosphere. We multiplied biomass of *Typha* spp. (Weller et al. 2016) at the times of our sampling campaigns by mass-specific CH_4 and N_2O flux to obtained areal flux rates. Our highest CH_4 emission fluxes coincided with the highest *Typha* spp. biomass values. Similarly, our lowest CH_4 emission fluxes were observed when the *Typha* spp. biomass was lowest. Averaging the CH_4 emission flux rates over one year (July 2014-July 2015), fluxes from *Typha* spp. yielded a mean annual daily flux rate of 358.23 mg CH_4 m^{-2} d^{-1}.

This estimate falls within the range (43 to 6000 mg CH_4 m^{-2} d^{-1}) that Knapp and Yavitt (1995) reported for nine USA sites with *Typha* spp, and is similar to the global mean CH_4 emission rate for wetland marshes of 238 mg CH_4 m^{-2} d^{-1} reported by Aselmann and Crutzen (1989).

Our data are not sufficient to estimate plant-mediated N_2O flux emission over the year because we only observed representative fluxes during the month of July 2015. If we convert our July 2015 mass-specific emission flux estimates to areal rates using Weller et al. (2016) biomass, we obtain a mean emission rate of 9.55 mg N_2O m^{-2} d^{-1}. Our summer estimate is lower than N_2O emissions reported during the summer in salt marshes (15.6 mg N_2O m^{-2} d^{-1}) and much lower than those in managed temperate grasslands (84 mg N_2O m^{-2} d^{-1}) (Yang et al. 2011, Yang and Silver 2016).

4.8 Conclusions

Our results confirm that *Typha* spp. can be a major contributor of CH_4 and N_2O from CTW via plant-mediated pathways (Sebacher et al. 1985, Knapp and Yavitt 1992, Whiting and Chanton 1993, Smart and Bloom 2001, Yu and Chen 2009). Though there is an extensive body of literature examining the role of vegetation on CH_4 and N_2O fluxes from wetlands, ours is the first from a desert region. The recent reviews examining greenhouse gas emissions by Carmichael et al. (2014), Huang et al. (2013), and Yu and Chen (2009) noted that few such studies have been conducted in tropical region wetlands and none in wetlands located in desert regions. Similarly, our observations of both CH_4 and N_2O uptake fluxes are in accordance of other observations in other studies but there are many mechanistic processes yet to be investigated with higher temporal resolutions and using isotope-based methods. Additionally, most studies that have reported CH_4 and

N_2O fluxes from vegetation used whole-ecosystem chambers, comparing areas with and without vegetation (Duan et al. 2005, Laanbroek 2010). Though these results are valuable to understand the sink or source behavior of individual vegetated ecosystems or ecosystem types, they do not reveal the specific contribution of fluxes from emergent wetland plants. For instance, to calculate the annual plant-mediated CH_4 emission flux from wetlands to the atmosphere, as Carmichael et al. (2014) points out, scientists combine the herbaceous-dominated wetlands mapped in the Global Roads Open Access Data Set (Center for International Earth Science Information Network-CIESIN-Columbia University 2013) with individual CH_4 emission flux estimates extracted directly from *Typha* spp. reported from just two literature sources (Yavitt and Knapp 1995, 1998). The most current global N_2O budget from wetland ecosystems that included the effect of vegetation was done by Murray et al. (2015), but it focused on estuarine environments and their associated vegetation, such as mangroves and seagrass. Along with Carmichael et al. (2014) and Murray et al. (2015), our study contributes much-needed understanding of the contribution to CH_4 and N_2O fluxes by specific wetland vegetation during different seasons. Additional data such as these will allow us to further refine global CH_4 and N_2O budgets by adding a dynamic plant-mediated metric.

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CHAPTER 4

HOW EXPERIMETNAL DRYING AND RE-WETTING INFLUENCES METHANE AND NITROUS OXIDE FLUXES FROM CONTINUOUSLY INUNDATED SOILS TAKEN FROM A CONSTRUCTED TREATMENT WETLAND

ABSTRACT

Wetlands are particularly effective at treating municipal wastewater because these ecosystems have both oxic, hypoxic, and anoxic environments, which enhances nitrogen (N) removal via several biogeochemical processes. When constructed treatment wetlands (CTW) are built to remove N from wastewater but are operated with a permanently flooded hydrologic regime, they may become large producers of greenhouse gases such as methane (CH_4) and nitrous oxide (N_2O). Using a 30-day mesocosm experiment we studied the effect of adding three different dry periods (2, 7, 14 days) on the fluxes of CH_4 and N_2O from wetland soils collected from a permanently flooded aridland CTW located in Phoenix, AZ, USA. We found that CH_4 fluxes were not significantly affected by soil drydown events. Soils that were dry for 7 days shifted from being N_2O sources to sinks after being re-inundated ($F_{1, 3} = 4.88$, $p<0.05$). We identified that this shift occurred within the first five minutes after the re-wetting event ($F_{1, 2} = 3.64$, $p=0.05$). As a result, the 7-day drydown soils were N_2O sinks cumulatively while the 14-day drydown soils showed significant cumulative release of N_2O ($F_{1, 3} = 4.02$, $p<0.05$). These results suggest that drying and re-inundating continuously flooded CTW soils will influence greenhouse gas fluxes differently depending on the duration of the dry period and the re-wetting event.

1. INTRODUCTION

Worldwide, the use of constructed wetlands to treat wastewater (CTW) has been increasing (Vymazal 2017). These "working ecosystems" are a cost-efficient alternative to reduce excess nutrients from various anthropogenic activities (Vymazal 2005). These CTW are most effective at nutrient processing and removal when certain physical, chemical, and biological conditions, mainly for soil microbial communities, are met; this is particularly true for nitrogen (N). For example, to permanently remove N from the overlying water, cycles of soil drying and re-wetting are necessary to couple nitrification (an aerobic process) and denitrification (an anaerobic process); (Firestone and Davidson 1989). However, pulsing hydrologic regimes—i.e., alternating wet and dry— can generate conditions that lead to the excess production of greenhouse gases, such as methane (CH_4) and nitrous oxide (N_2O). Both are potent greenhouse gases that have 25- and 298-fold greater global warming potentials (GWPs), respectively, than CO_2 (on a time horizon of 100 years;(IPCC 2013)). Additionally, since CTW are developed to treat water containing excess nutrients, such as N, an excess of this nutrient can stimulate microbial activity that can promote even higher fluxes of CH_4 and N_2O (Martikainen et al. 1993, Huang et al. 2005). Because of these unintended greenhouse gas emissions attributable to the utilization of CTW worldwide, it is important that management decisions are based on sound biogeochemical and ecological knowledge, and that those decisions consider the various trade-offs of alternative strategies—including different hydrologic regimes.

Methanogenesis is a microbially driven respiration process, in which CO_2 is used as an electron acceptor and CH_4 is produced. This process occurs in wetland soils under low-redox conditions and produces further reduced conditions with redox potential <-200 mV (Le Mer and Roger 2001, Mitsch and Gosselink 2007). Methanogens consume labile carbon, such as acetate (CH_3COOH), as well as H_2 and CO_2, which are produced in the anoxic conditions of flooded wetland soils by other anaerobic and fermentative bacteria (Bridgham et al. 2013). Methane production pathways include the fermentation of acetate (the most common pathway) and the reduction of CO_2 with H_2. The latter process is typically responsible for 25%-30% of total CH_4 production by wetland soils (Inglett et al. 2013). Methane that is produced in wetland soils can be oxidized by methanotrophs in oxic regions within the soils and in the water column (Boon and Lee 1997). If not oxidized, it leaves soils via diffusion to the water column or atmosphere, via ebullition through water column, and via plant-mediated transport from the roots into the atmosphere (Bridgham et al. 2013). Because anoxia and low redox are associated with permanently flooded conditions, studies have suggested that a pulsing hydrology that stimulates oxygenation of wetland soils can minimize CH_4 emissions from wetlands (Altor and Mitsch 2006, Sha et al. 2011).

The microbially mediated cycling of N in wetlands occurs mainly via ammonification (production of ammonium, NH_4^+ during breakdown of organic matter), followed by the aerobic process of nitrification ($NH_4^+ \rightarrow NO_2 \rightarrow NO_3^-$) and finally the anaerobic process of denitrification ($NO_3^- \rightarrow NO_2^- \rightarrow NO^- \rightarrow N_2O \rightarrow N_2$). Denitrification is the only process capable of permanently removing nitrogen from wetland ecosystems via N_2. Because of this, CTW are designed to optimize conditions that promote the

denitrification process. It is important to note, however, that due to limited oxygen diffusion into soils, nitrification (the aerobic process that generates the NO_3^- to be denitrified) is usually the limiting step of permanent N removal in continuously flooded CTW (Tanner and Kadlec 2003). Because of this, in order to enhance nitrification, oxygenation of wetland soils may be carried out via multiple means: plant mediated gas transport and modification of hydrological regimes, among others. Plants transport O_2 to the roots located in the submerged wetland soils promoting more oxic environments (Conrad 1993, Whalen 2005). It is reported that CTW managed for periodically aerated soils have higher nitrogen removal rates than continuously flooded CTW (Cottingham et al. 1999, Higgins 2003). Specifically, Maltais-Landry et al. (2009a) showed that artificial aeration of wetland soils in mesocosms via air appears to have a greater impact on the aeration and nitrification process than those compared to solely vegetation wetland soils in mesocosms. It is the oxic environments that allow the nitrification process to occur and transform ammonium (NH_4^+) into the readily available NO_3^-. The anaerobic process of denitrification commonly occurs at redox potentials between +120 and +250 mV and can be limited by the supply of labile organic matter, lack of available NO_3^-, or oxic conditions (Firestone and Davidson 1989). Additionally, in flooded wetland soils, soil properties such as bulk density, particle density, and organic matter content play an important role in the denitrification process by providing suboxic pore spaces necessary to promote hot spots of denitrification (Palta et al. 2014). These two processes, nitrification and denitrification, can produce N_2O as a by-product compound during the transformation process. However, higher rates of production and emission of N_2O are more commonly associated with denitrification than nitrification (Firestone and Davidson

1989). A drying pulse to permanently flooded soils may not only benefit achieving the goal of a more efficient permanent removal of nitrogen, but it may also stimulate the production and emission of N_2O via both nitrification and denitrification (Hernandez and Mitsch 2006, 2007, Vymazal 2007). Because of this, it is important to study the various trade-offs associated with alternative hydrological strategies when managing permanently flooded CTW.

Intermittent hydrological loading is often applied in CTW with the aim of mimicking the pulsing hydrological cycles of natural floodplains, watersheds, and wetlands (Junk et al. 1989, Odum et al. 1995, Odum 2000, Day et al. 2003, Altor and Mitsch 2008). Such intermittent loading is commonly used in vertical subsurface flow and horizontal subsurface flow CTW as it increases the aeration and nitrification rates, making them more effective at permanent N removal (Vymazal 2005, Langergraber et al. 2008). Fewer studies have examined the effects of pulsing hydrology in free-water surface (FWS) CTW, because they are constructed and designed to maintain permanently flooded conditions (Mander et al. 2011). These permanently flooded conditions promote the anaerobic process of denitrification to remove excess nitrate from their wastewater (Kadlec and Wallace 2008, Vymazal 2013). Two *in situ* studies examined the effects of intermitted loading in agricultural and stormwater treatment FWS; both showed improvements in wastewater purification when water inflows were pulsing (Carleton et al. 2001, Lu et al. 2009). However, neither these two studies nor a recent review of intermittent loading in CTW considered the influence of a pulsing hydrology on CH_4 and N_2O fluxes (Mander et al. 2011).

Given the continuing increases in atmospheric concentrations of greenhouse gases, CTW should be designed to minimize CH_4 and N_2O emissions (Mander et al. 2011, Mitsch et al. 2013). Because hydrology drives redox conditions in wetland soils, the focus of this study was to understand how hydrologic pulsing influenced soil conditions and processes that produce CH_4 and N_2O. Thus, the primary objective of this study was to examine the effects of three wet-dry-wet regimes on CH_4 and N_2O fluxes from wetland soils taken from a permanently flooded FWS CTW in Phoenix, AZ. We expected higher CH_4 fluxes from permanently flooded conditions compared to those exposed to a wet-dry-rewetting hydrological regime. We expected this because the oxic conditions stimulated by soil drying precludes methanogenesis and may stimulate CH_4 oxidation, thus overall reducing CH_4 emissions. We hypothesized higher N_2O fluxes from permanently flooded conditions compared to those exposed to a wet-dry-rewetting hydrological regime since a pulsing hydrology will stimulate coupled nitrification-denitrification. Our study aims to better understand the effects of a dynamic hydrology on the CH_4 and N_2O fluxes from CTW, to inform better CTW management practices.

2. METHODS

We conducted this study using wetland soil cores from the Tres Rios CTW in Phoenix, AZ, USA. The CTW has is located west of downtown and has been receiving treated wastewater since 2010 from one of the largest wastewater treatment plans in Phoenix. The CTW is a FWS wetland and comprises three vegetated surface-flow cells of which the largest one, from where we obtained the soil cores, has been permanently flooded since 2010. The depth within the vegetated-marsh portions of the cell is

approximately 25 cm and its vegetation has been shifting to a dominant presence of the macrophytic *Typha* spp. (Weller et al. 2016). Several studies have shown that the vegetated marsh area of the CWT plays an important role in removing nearly all of the inorganic N supplied from the effluent of the wastewater treatment plant (Sanchez et al. 2016, Weller et al. 2016). Additionally, the hot and dry conditions of arid region of Phoenix, AZ, generate a transpiration-driven "biological tide" that drives large volumes of water into the marsh areas, which stimulate soil microbes to process nutrients found in the water (Sanchez et al. 2016).

2.1 Mesocosm experiments

Mesocosms experiments have been used to study how wetland and marsh hydrology affect microbial processes, ecosystem respiration, eutrophication, and greenhouse gas emissions (Garnet et al. 2005, Bhullar et al. 2013, Langley et al. 2013, Mueller et al. 2016). For example, in a year-long mesocosm experiment, Rhymes et al. (2016) showed that lowering the water table in wetland soils by 10 cm decreased denitrification rates and CH_4 fluxes while stimulating higher N_2O fluxes. In a three-year mesocosm experiment, Updegraff et al. (2001), showed that when the water table in wetland soils was increased by only 1 cm, CH_4 fluxes remained constant for the first year but significantly increased in the 2nd and 3rd years of the experiment. They suggested that the effect of variable water tables (+1, -10 and, -20 cm) on temperate peatland soils becomes more important in the long term as it builds appropriate conditions for methanogenesis (Updegraff et al. 2001). Specific to CTW, experimental mesocosms studies have predominantly focused on studying the effects of vegetation community composition on CH_4 and N_2O fluxes (Inamori et al. 2007, Zhang et al. 2013, Chang et al.

2014, Zhao et al. 2016). However, these studies did not include the effects of pulsing hydrologic regimes on the fluxes of these two important greenhouse gases.

2.2 Experimental setup

Twenty-four intact soil cores were collected from the Tres Rios CWS in June 2015 from a previously vegetated area located near the water inflow to the wetland. We collected intact soil cores using opaque polyvinyl chloride (PVC) cylinders 66 cm high and 10 cm diameter, extracting approximately 20 cm of wetland soil. The soil cores were immediately transported back to a greenhouse on the campus of Arizona State University (Tempe, AZ, USA) and allowed to stabilize for eight days before the experiment began (Fig. 9). The 24 cores were randomly assigned to four treatments: control (permanently flooded) and three wet-dry-rewet (WDW) periods: a 2-day dry period, a 7-day dry period, and a 14-day dry period. On average, the soil cores were 21 cm deep with an average of 3.5 cm of unconsolidated organic matter layer on the surface. When flooded, water level was kept at an average of 10 cm above the organic matter layer. On each day of the experiment, we replaced one-third of the surface water in the mesocosms that were flooded (including the controls) with water from the Tres Rios CTW to mimic the water residence time rate of the system.



Figure 9. Experimental set up inside the greenhouse of the 24-wetland soil core mesocosms.

2.3 Daily gas flux sampling and analysis

After letting the soil cores stabilize for eight days (June 19 – June 25), we began measuring initial CH_4 and N_2O fluxes using a continuous flow-through Gasmet DX4040 FTIR gas analyzer (Gasmet Technologies Inc, 2013). We measured headspace gas concentrations in the mesocosms approximately every six seconds for two minutes using a fitted PVC lid adapted with two syringes connected to hoses that connected to the gas analyzer. We made the Gasmet measurements between 12:00 PM and 4:00 PM. Because of a mechanical failure of the Gasmet on Day 5, we collected headspace gas manually for the rest of the experiment. This switch from the continuous flow-through Gasmet

analyzer to manual gas sampling created a data gap from Day 5 to Day 7. With the switch to manual gas sampling, we constructed new PVC lids for the mesocosms that included 7.6 cm battery-powered fans to mix the headspace air during gas sampling. Before the daily sampling events, the fan-adapted-lids were carefully set on top of each core and gases were extracted at 0, 10, and 20 min time points using 20 mL plastic syringes. We also collected gas samples from the mesocosms after the re-wetting events to evaluate how this would influence CH_4 and N_2O fluxes on short time scales. After each of the drying periods, we used water from the Tres Rios CTW to return water level to the original level in the treatment mesocosms; this was done carefully to minimize disturbance of the soil surface. Immediately after adding the water, we sampled the headspace gas in each mesocosms twice—at 0 and 5 minute time points. The cores were then uncapped and vented for five minutes before being sampled again at 10, 15, and 20 minutes after re-wetting.

The gas flux data collected with the Gasmet DX4040 FTIR were processed using the Calcmet Lite software (Calcmet Lite v2.0). All gas samples collected using the manual gas sampling technique were immediately placed from the syringe into manually pre-evacuated, silicone-sealed, 10 mL glass vials with inert stoppers sealed with aluminum caps. Samples were analyzed using a Varian CP-3800 Gas Chromatograph. The GC was calibrated in the laboratory using certified CH_4 and N_2O standards (Matheson Tri-Gas® 2016). Gas fluxes were calculated as the change in concentration within each mesocosm over a 20-min period, corrected for molecular weight of the gas, area of the surface of the mesocosm, and volume of the headspace in the mesocosm (Hall et al. 2008). In this

study, we considered positive fluxes as emission fluxes and negative fluxes as uptake fluxes.

2.4 Mesocosm water and soil characteristics

To investigate how wet-dry-wet treatments affected water quality, we collected water samples from the mesocosms periodically throughout the experiment. Water from all 24 mesocosms was sampled on Day 1 and at the end of their respective post-rewetting treatment. The control soil cores were sampled on all days in which any other treatment was sampled. The 2-day dry period soil cores were sampled again on day 22 (24 hours after re-wetting) and on day 27 (seven days after re-wetting). The 7-day dry period soil cores were sampled again on Day 17 (24 hours after re-wetting) and on day 22 (seven days after re-wetting). The 14-day dry period soil cores were sampled again on day 24 (24 hours after re-wetting) and on day 29, (seven days after re-wetting), the end of experiment. Water was collected using acid-washed 100 ml glass flasks, filtered, and frozen in centrifuge tubes. These were then analyzed for NO_3^-, nitrite (NO_2^-), and, NH_4^+ via flow injection analysis on a Lachat QC 8000 Quickchem Flow Injection Analyzer.

Water temperature (°C) was measure using a YSI multi-probe (YSI Pro2030). Redox potential and pH were measured with an EcoSense pH100 handheld instrument. These three physicochemical water characteristics were measured daily by inserting the probes in flooded mesocosms. When the surface water was removed from the treatment mesocosms during their respective dry periods, the Eh (redox) probe would be the inserted about 2 cm into the wet unconsolidated organic layer. Soil characteristics, including bulk density, particle density, and organic matter content, were measured at the end of the experiment. Bulk density was calculated using the Blake and Hartge (1996a)

method and particle density was determined using a modified pycnometer method (Blake and Hartge 1996b). To determine organic matter content, 10 g of oven-dried soils were combusted at 550 °C for 4 hours, then re-weighed to determine mass loss (Heavenrich and Hall 2016).

2.5 Statistical analysis

Repeated-measures ANOVA analyses were used for CH_4, N_2O, redox, pH and temperature testing for treatment, time, and interactions effects. To test for the effects of the re-wetting event, we sliced ANOVA analyses on CH_4 and N_2O daily fluxes on the dry day before re-wetting and on the 5 min and 15 min after the re-wetting event. To test for differences in the final mean cumulative CH_4 and N_2O fluxes among all treatments, we performed an ANOVA analysis followed by post-hoc comparisons using Tukey HSD analysis. These mean cumulative fluxes were calculated first by adding the daily fluxes from each individual mesocosm followed by averaging the total cumulative flux from the six replicates of each treatment (one control and three dry treatments). Finally, we also used an ANOVA analysis to test for differences in the nitrogen concentrations and soil properties among the control and treatment mesocosms. Fluxes met the normality assumptions and all statistical tests were performed in R version 3.3.1, SPSS 23.0 software, and considered significant at $\alpha \leq 0.05$ (Maltais-Landry et al. 2009b, R Core Team 2016).

3. RESULTS AND DISCUSSION

3.1 CH_4 fluxes

Methane (CH_4) fluxes from the control, the 2-day dry, and 14-day dry treatments were consistently emission (positive) fluxes throughout the experiment, and all total cumulative emissions were approximately 250 g CH_4 m^{-2} (Fig. 10a, b, d). The 7-day dry treatment showed a similar pattern, but with a large emission flux immediately after re-wetting (Fig. 10c). This large emission increased the mean cumulative CH_4 emissions from the 7day treatment to 1078.76 g CH_4 m^{-2} (Fig. 10c). We found no significant differences when we tested for treatment, time, and interaction effects among and within. The CH_4 fluxes were not significantly different among the three time points before and after the re-wetting event. We did not observe any significant differences among treatments in the final mean cumulative CH_4 gas emissions (Fig. 11a). Overall, we were surprised by these findings because we expected that altering the hydrology by lowering the water table enough to expose the soil to the atmosphere in the treatment mesocosms was going to inhibit methanogenesis and promote CH_4 oxidation, ultimately decreasing CH_4 flux emissions. These results suggest that though continuously soil mesocosms from Tres Rios CTW were exposed to wet-dry-wet conditions, the conditions may have remained sufficiently suboxic to maintain CH_4 emissions.

Our results indicate that redox, a driver of methanogenesis, was altered in the treatments compared to the redox in the control. The redox potential was significantly different among all treatments ($F_{1, 3} = 36.58$, $p<0.001$) and its significant interaction with time, indicates that redox changed over time and it changed in different ways ($F_{1, 3} = 4.02$, $p<0.01$, Fig. 14a). The paired t-tests / post-hoc pairwise comparisons revealed that the 14-day treatment was significantly different than the control and the other treatments ($p<0.001$). Though the redox potentials differed among treatments, the data show that

they were rarely above the value in which methanogenesis is inhibited (-200 mV) (Fig. 14a). These conditions contributed to a continuous reducing environment, in which CH_4 was similarly and continuously produced in the control and treatment mesocosms. Ultimately we can conclude, that though our experiment succeeded in altering the redox environment, redox potentials did not reach high enough values (>200 mV) to reduce the production and emission of CH_4 from the treatment mesocosms.

Though not significant, the large variability in the net cumulative CH_4 emission fluxes in the 7-day dry treatment mesocosms could be the result of the re-wetting event. This large variability in the data was driven by the CH_4 fluxes immediately (0-5 mins) after the re-wetting event (Fig. 10c). We suggest that the physical act of the re-wetting event may have disturbed the soils, thus stimulating the release of concentrated CH_4 gases via diffusion or ebullition from the anoxic microsites in the 7-day mesocosms (Van der Nat and Middelburg 2000). This pattern has been observed previously in a mesocosm experiment in which a rapid change in the water table may have resulted in fast CH_4 and N_2O emissions from a lakeshore wetland (Dinsmore et al. 2009).

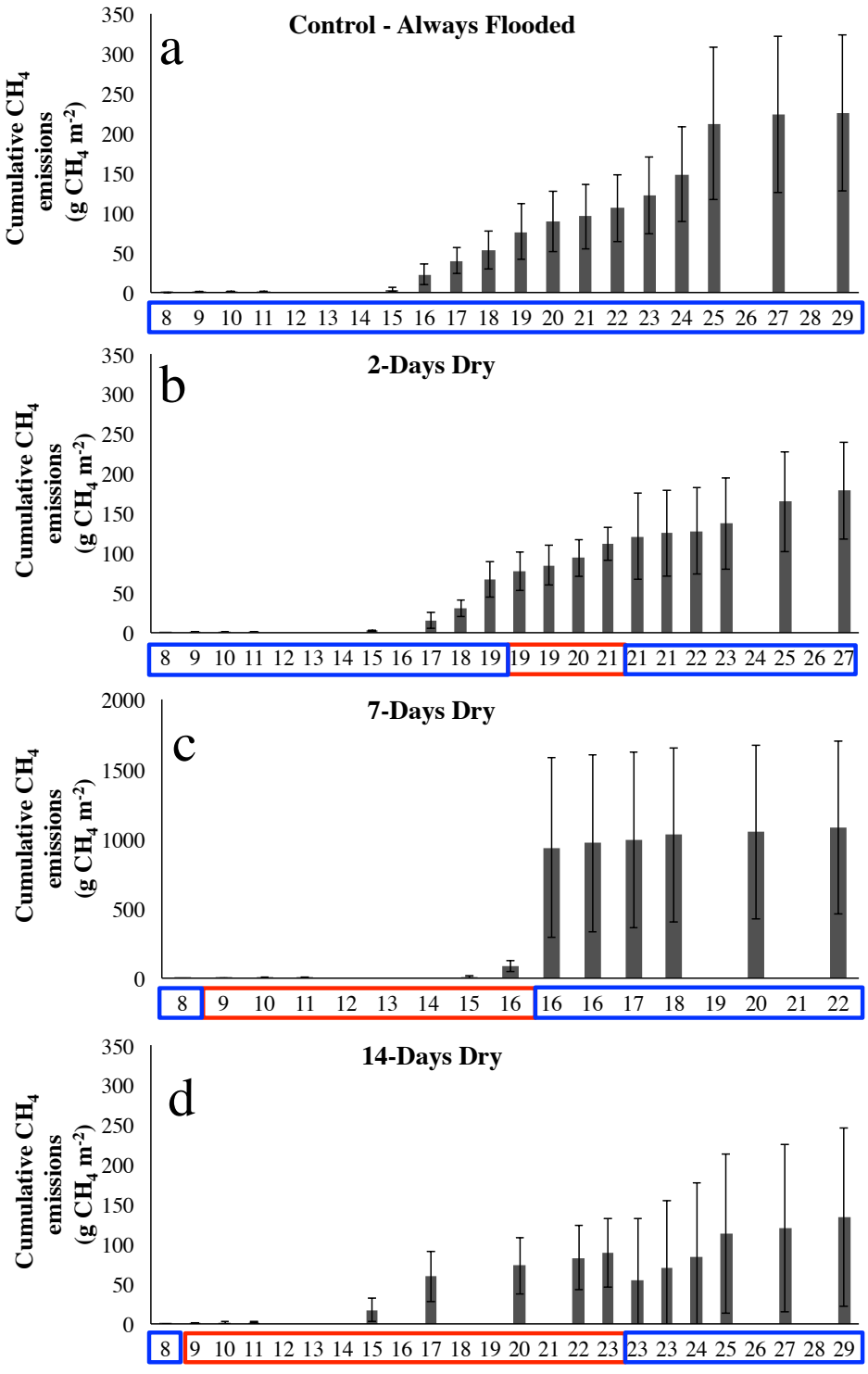


Figure 10. Total cumulative methane emissions (g CH_4 m^{-2}) of the a) control, b) 2-days dry period, c) 7-days dry period, and d) 14-days-dry period treatments. The blue color in the x-axis represents wet conditions and red color represents dry conditions of the wetland soil mesocosms.

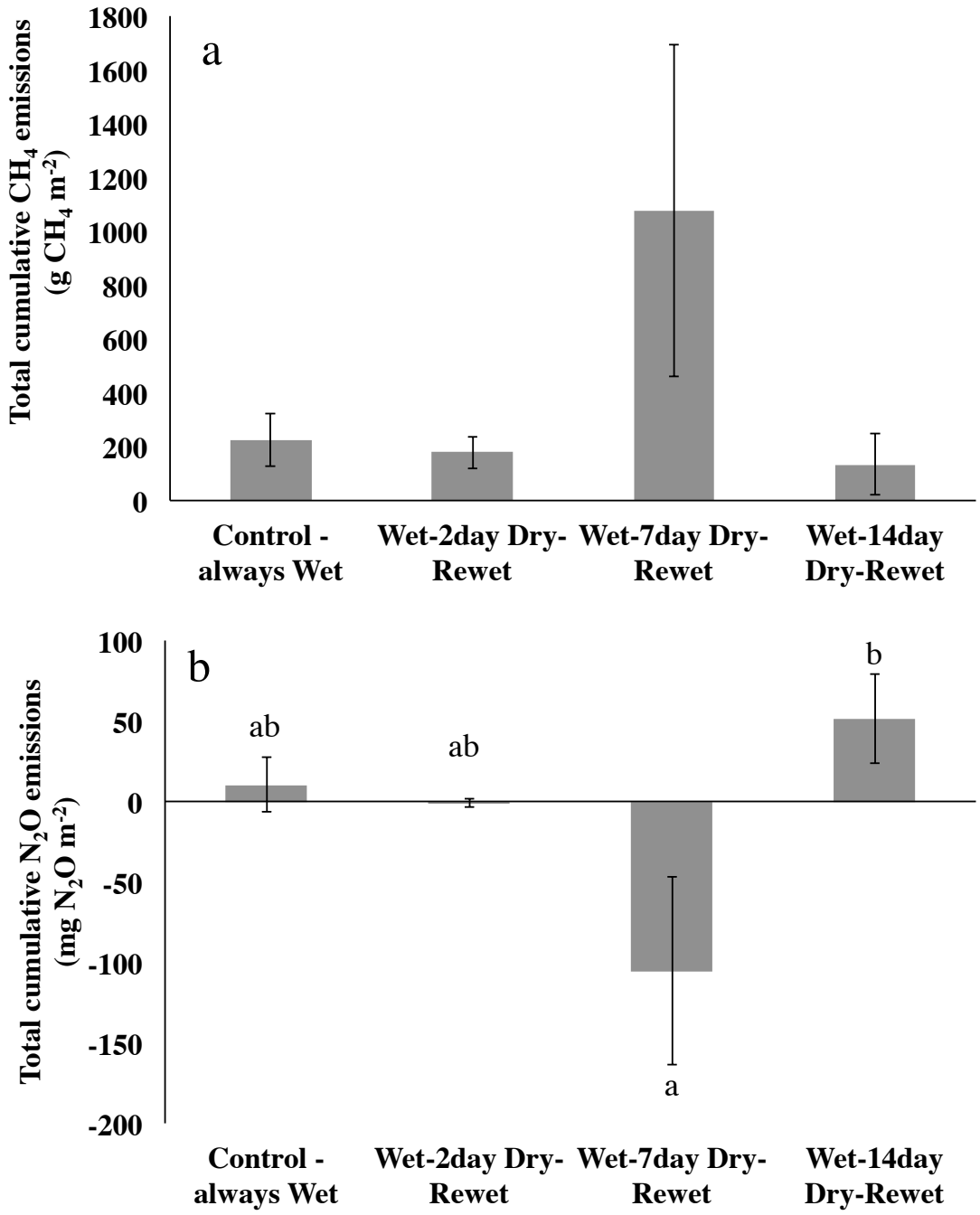


Figure 11. a) No significant differences were found among the total cumulative CH_4 fluxes from the treatments. b) The cumulative gas fluxes for N_2O showed that the 7-day treatment acted as a sink and was significantly different compared to the 14-day treatment that acted as an N_2O source.

3.2 N_2O fluxes

The N_2O fluxes showed a combination of uptake (negative) and emission (positive) fluxes in the control and all treatments (Fig. 12). With the repeated measure analysis we found that the N_2O daily fluxes from the 7-day treatment were significantly different over the duration of the experiment, shifting from sink to source. ($F_{1, 3} = 4.88$, $p<0.05$, Fig. 12c). Using the sliced ANOVA analysis, we identified that this shift occurred within the first five minutes after the re-wetting event ($F_{1, 2} = 3.64$, $p=0.05$, Fig. 13). The mean N_2O fluxes in the dry day prior to re-wetting were emission fluxes (25.21 mg N_2O m^{-2}) compared to the mean uptake N_2O fluxes (-119.58 mg N_2O m^{-2}) within the first five minutes after the re-wetting event (Fig. 13).

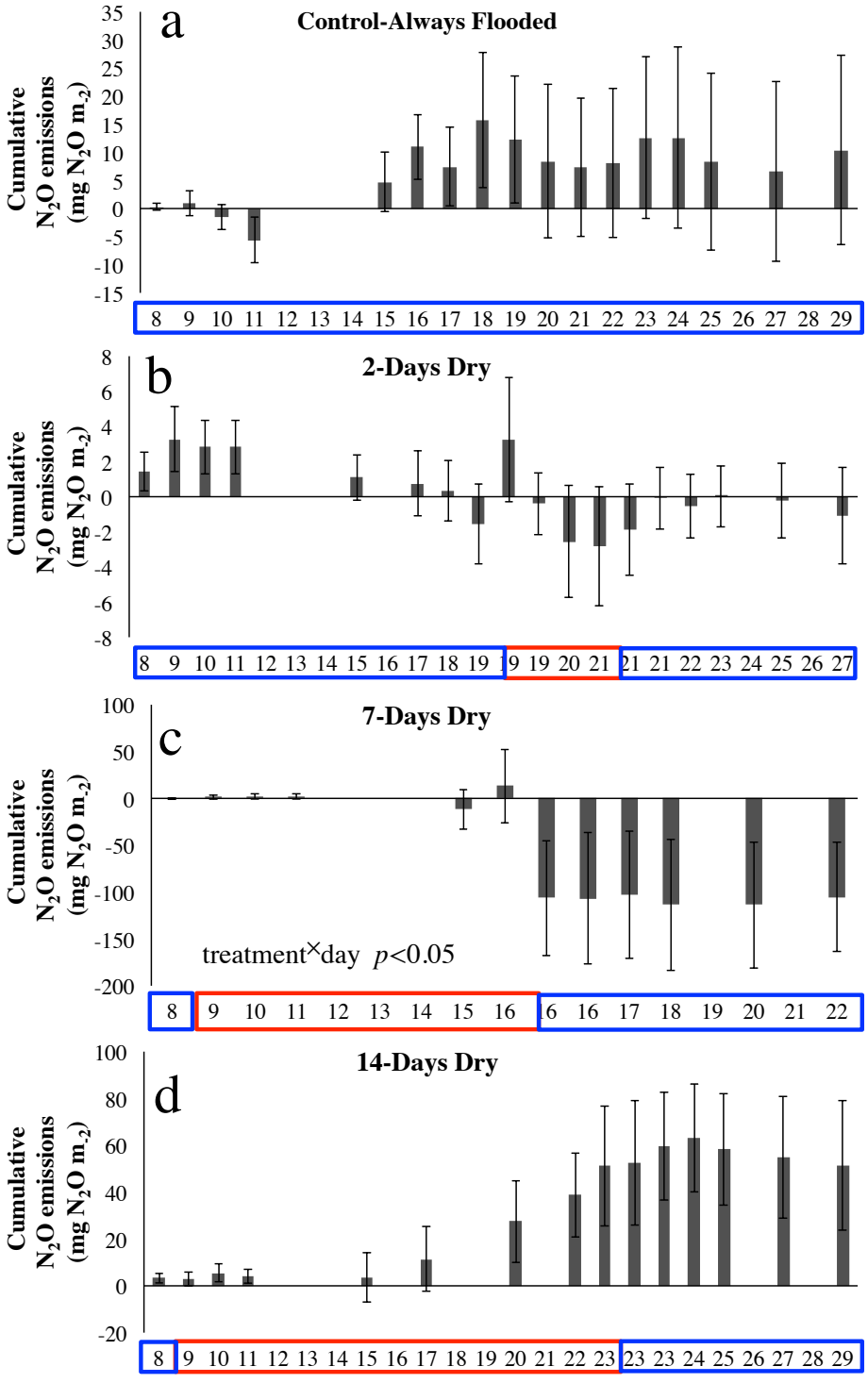


Figure 12. Total cumulative methane emissions (mg N_2O m^{-2} d^{-1}) of the a) control, b) 2-days dry period, c) 7-days dry period, and d) 14-days- dry period treatments. The blue color in the x-axis represents wet conditions and red color represents dry conditions of the wetland soil mesocosms.

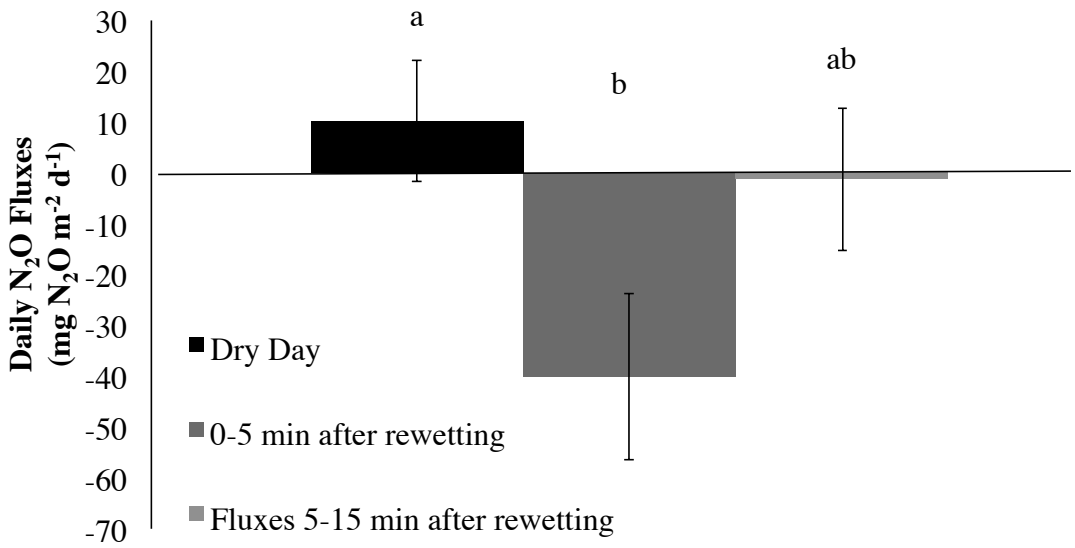


Figure 13. Mean (error bars are S.E.) N_2O daily fluxes immediately before and after (0-5 min and 5-15 min) the watering event on the 7-day dry treatment.

We hypothesized that altering permanently flooded soils with an artificial aeration event would cause a decrease in N_2O emissions compared to the permanently flooded soils. We expected a change because pairing aerobic and anaerobic conditions would promote the coupling of nitrification and denitrification processes and achieve a permanent removal of NO_3^- via production of N_2, thus mitigating N_2O emissions. Previous studies in wetland soils have observed the highest removal of NO_3^- and soil denitrification rates to be associated with paired nitrification-denitrification activity (Palta et al. 2014). Our study shows that the longest treatment (the 14-day dry period) had the biggest influence in N_2O fluxes (Fig. 11b). The 7-day dry period showed the highest N_2O uptake rates, whereas the 14-day dry period treatment exhibited the highest N_2O emission rates (Fig. 11b). Both of these observations can be related to microbial production of N_2O during the N transformations in the nitrification and denitrification processes of the N cycle (Firestone and Davidson 1989, Wrage et al. 2001). The nitrification process is the

oxidation of NH_4^+ or NH_3 in the presence of oxygen to the form of nitrogen used in denitrification, NO_3^-. Following nitrification, the denitrification process is carried out as the reduction of NO_3^- to N_2O and N_2 by facultative anaerobic bacteria. The production and emission of N_2O is higher during denitrification because the N_2O reductase can be inhibited by several factors, such as low pH and high oxygen availability (Wrage et al. 2001).

The redox conditions after the re-wetting event in the 14-day dry treatment mesocosms indicate the presence of the necessary anoxic conditions for NO_3^- removal via denitrification, with N_2O production (Table 2, Fig. 14a). Hernandez and Mitsch (2006) observed similar results when they noted an increase of N_2O fluxes after a re-flooding event in a constructed marsh wetland. They attributed the emissions to the combination of available carbon, nitrogen, and the anoxic conditions created by the flooding event. Similar to their results, we also continued to observe high N_2O fluxes three days after the flooding event, but a decrease by the seventh day (Hernandez and Mitsch 2006).

Hernandez and Mitsch (2006) and Hefting et al. (2003) have previously observed N_2O emission fluxes in constructed and forested wetlands when the soils were exposed and in shallow water tables. They attributed their results to the soils being saturated but without actual standing water, which sets suboptimal conditions for denitrification in which N_2O is the predominant end product (Yoshinari 1990, Hefting et al. 2003, Hernandez and Mitsch 2006). The combination of at least three large N_2O emission fluxes in the 14-day treatment mesocosms (Fig. 12d) and an increase of NO_3^- concentration (Table 2) right after the re-wetting event suggest that the N_2O could be a by-product of the nitrification process in this treatment. Right after the re-wetting event,

we continued to see N_2O emission fluxes, a drop in the redox potential, and a decline of NO_3^- concentration in the water column (Fig. 12d, Fig. 14a, Table 2). This suggests that when re-wetting occurred, the restored anoxic conditions stimulated NO_3^- removal via denitrification.

3.3 Water and soil properties

In the 14-day treatment, the redox potential significantly increased during the dry period compared to the redox potential during the beginning, re-wetting, and end of the experiment flooded conditions ($F_{1, 3} = 15.66$ $p<0.001$, Fig. 14a). The redox potential was not significantly different during the experiment among the control and the 2-day and 7-day treatments (Fig. 14a). Similarly, in the 14-day treatment, NO_3^- concentration was significantly higher the day after the re-wetting event compared to the rest of the duration of the experiment ($F_{1, 2} = 10.66$, $p<0.001$, Table 2). Temperature and pH levels were not significantly different among the wet-dry-rewet time points throughout the duration of the experiment (Fig. 14b, c). At the end of the experiment, the mean bulk density and the mean particle density of the wetland soil mesocosms were similar (Table 3). The mean organic matter content of the soil mesocosms from the 2-day treatment was significantly lower (9.89 g, $F_{1, 2} = 7.80$, $p<0.01$) compared to the control, 7-day and 14-day treatments (23.13, 25.59 and 27.15.g respectively, Table 3).

The values for bulk density (0.11-0.2 g/cm^3) and particle density (1.82-2.17 kg/L) are within the ranges of characteristic of rich organic wetland soils reported in the literature (Skopp 2000, Redding and Devito 2006, Mitsch and Gosselink 2007) The similarity of the soils among the mesocosms supports the fact that trends observed were in response to the wet-dry-wet treatments applied to the soil mesocosm and not an artifact of different

soil properties. Though the organic matter content for was lower from the 2-day dry treatment, it did increase CH_4 and N_2O as expected (Table 3). The control, 7-day, and 14-day dry treatments had organic matter content values at the end of the experiment within the range of typical wetland organic soils (>20-35%) as reported in the literature (Mitsch and Gosselink 2007).

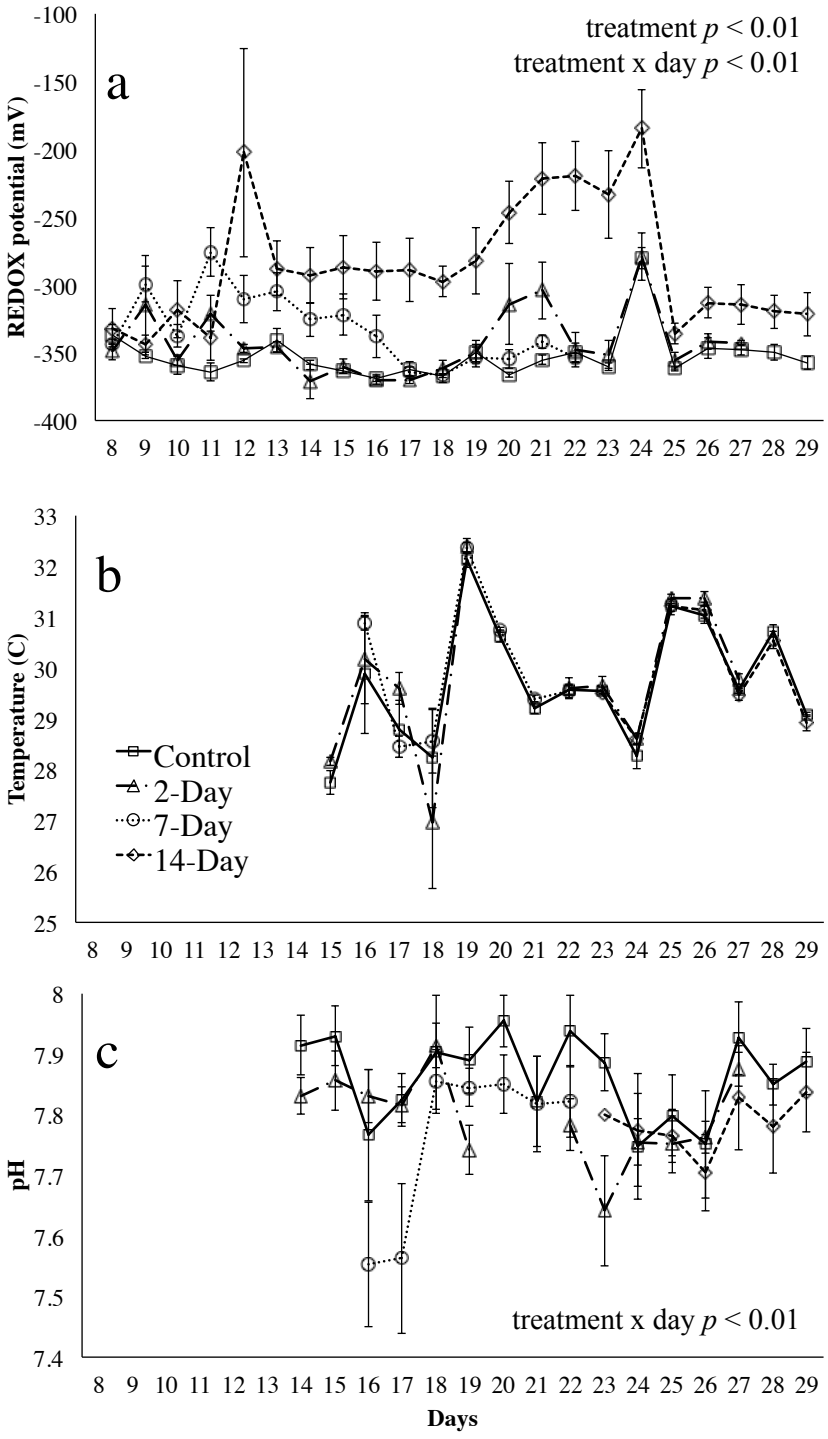


Figure 14. Mean (error bars are S.E.) redox potential (a), temperature (b), and pH (c) of the 24 wetland soil mesocosms over the course of the experiment.

T**able 2**. Water column nitrate (NO_3^-) concentrations of the control and treatment mesocosms.

	Nitrate mg/L (NO_3^-)					
Treatments	**Days of Experiment**					
Control	**1**	**17**	**22**	**24**	**27**	**29**
	0.01 (0.01)	0.03 (0.01)	0.03 (0.02)	0.02 (0.01)	0.01 (0.005)	0.04 (0.01)
2-Days Dry	**1**		**22 re-wet**		**27 end**	
	0.08 (0.07)		0.04 (0.02)		0.03 (0.01)	
7-Days Dry	**1**	**17 re-wet**	**22 end**			
	0.05 (0.04)	0.07 (0.03)	0.09 (0.04)			
14-Days Dry	**1**			**24 re-wet**		**29 end**
	0.08 (0.06)			0.63 (0.16)**		0.02 (0.009)

Table 3. Bulk density, particle density, and organic matter content at the end of the experiment of the control and treatment mesocosms.

	Control	**2-Days Dry**	**7-Days Dry**	**14-Days Dry**
Bulk Density (g/cm^3)	0.12 (0.01)	0.2 (0.08)	0.14 (0.01)	0.11 (0.01)
Particle Density (kg/L)	2.04 (0.16)	1.82 (0.24)	2.17 (0.29)	1.56 (0.2)
Organic Matter Content (%)	23.13 (3.18)	9.89 (2.01)*	25.59 (1.84)	27.15 (6.62)

3.4 Comparison with in-situ Tres Rios CTW diffusion fluxes

The mean CH_4 emission fluxes from the control wetland soil mesocosms ranged from -0.52 to 1.59 mg CH_4 m^{-2} h^{-1}. From the three treatment mesocosms, the mean CH_4 emission fluxes ranged from 0.01 to 13.75 mg CH_4 m^{-2} h^{-1}. These are on the lower range but still comparable to the diffusion CH_4 emission fluxes observed from an in-situ study using floating chambers ranging from -2.17 to 13.00 mg CH_4 m^{-2} h^{-1} (Ramos 2016). Our CH_4 emission values from the treatment mesocosms are similar to CH_4 flux rates measured during the flood-pulse regulated growing season in a created wetland in Ohio,

USA (3.4–7.7 mg CH_4 m^{-2} h^{-1})(Altor and Mitsch 2006). Other studies report mean similar CH_4 emission fluxes from systems exposed to a fluctuating hydrology: Amazon floodplains (3.83 – 10.13 mg CH_4 m^{-2} h^{-1}), China marshes (1.8-15.2 mg CH_4 m^{-2} h^{-1}), Venezuela floodplains (0.6-1 mg CH_4 m^{-2} h^{-1}), and in the Mississippi river delta (7.8 ± 5.9 mg CH_4 m^{-2} h^{-1}) (Alford et al. 1997, Smith et al. 2000, Ding et al. 2004, Melack et al. 2004)

The mean N_2O fluxes from our control wetland soil mesocosms ranged from -0.13 to 0.13 μg N_2O m^{-2} h^{-1}. In treatment mesocosms the N_2O fluxes ranged from -0.38 to 0.38 μg N_2O m^{-2} h^{-1}. These are on the lower range but still within the diffusion N_2O emission fluxes observed from in-situ using floating chambers ranging from -116.35 to 501.37 μg N_2O m^{-2} h^{-1} (Ramos 2016). Other mesocosm studies investigating the effect of vegetation of constructed treatment wetlands in microcosms have reported higher N_2O emission fluxes than our mesocosm study but still within our observed in-situ fluxes from Tres Rios CTW. Sun et al. (2013), Zhang et al. (2013) and Chang et al. (2014) observed ranges of 1.1-4.8, 6.69-9.36, and 37-77 μg N_2O m^{-2} h^{-1} N_2O emissions, respectively. Their higher N_2O fluxes may be attributed to the presence of emergent vegetation in their in-situ experimental plots, which is known to stimulate and serve as a conduit for N_2O from the soils (Chang et al. 1998, Ramos 2016)

The absence of emergent vegetation in the soil mesocosms may have underestimated both the CH_4 and N_2O fluxes from the experiment. In wetland ecosystems, wetland vegetation adapted to flooded soils can act as a conduit for trace gases such as CH_4 and N_2O. Previous studies show that emergent wetland vegetation, such as the dominant vegetation in Tres Rios CTW, *Typha* spp. can drive the flow of gases directly from the

wetland soils into the atmosphere through their aerenchyma (Knapp and Yavitt 1995, Yavitt and Knapp 1998, Ramos 2016). This is particularly important during the flooded conditions because it is when the aerenchyma system is actively transporting oxygen from the leaves into the root system and transporting back trace gases from the wetland soils into the atmosphere. Future mesocosm research focusing on the relationship between hydrological regimes and greenhouse gas emissions should include the dominant vegetation of the system to account for all possible emission pathways (Waddington et al. 1996, Chang et al. 1998, Altor and Mitsch 2006).

3.5 Conclusions

It is important to continue to study the effects of hydrology in wetlands because it is the single most important driver of all biotic and abiotic processes in wetland ecosystems (Mitsch and Gosselink 2007). In most cases, maintaining a dynamic hydrology is often designed into CTW to improve their abilities to purify water. Since we started investigating the water budget, vegetation community dynamics, and greenhouse gases from the permanently flooded Tres Rios CTW, we aimed to investigate the effect of a dry pulse in terms of greenhouse gas emissions (Ramos 2016, Sanchez et al. 2016, Weller et al. 2016). Only two other studies have investigated hydroperiod in relationship to water quality improvement but both did not include the effect it may have on the production and emission of CH_4 and N_2O gas fluxes. We demonstrated that adding a pulse to permanently flooded wetland soils in which we remove the surface water and expose the soils to the atmosphere does influence CH_4 and N_2O fluxes. In terms of CH_4 emissions, as most of the constructed treatment wetland literature suggests (Mander et al. 2011), we were expecting a decrease in CH_4 emissions due to the inhibition of methanogenesis and

methane oxidation that would result in the exposure of the soils to the atmosphere. We did not observe any significant differences on CH_4 gas fluxes among our control and treatments. We did observe a large variability in CH_4 fluxes in the re-wetting event that has been observed in other cases and it should be studied more in the future (Van der Nat and Middelburg 2000, Dinsmore et al. 2009). We also hypothesized that with the addition of the dry pulse to permanently flooded wetland soils we would see a decrease in N_2O flux emissions due to the complete coupling of the aerobic and anaerobic conditions for nitrification and denitrification processes (Hernandez and Mitsch 2006, Maltais-Landry et al. 2009a). Our experiment revealed that the soils that were exposed to the atmosphere the longest also cumulatively emitted more N_2O gases. Similar results have been observed in other types of constructed wetlands as well as in laboratory experiments (Regina et al. 1998). Theoretically, the combination of aerobic and anaerobic conditions should link the nitrification and denitrification processes to achieve complete removal of NO_3^- with N_2 as the end product. However, the nitrogen cycle in wetland ecosystems depends on many more factors than just oxygen availability, such as labile carbon, pH, temperature, and soil properties, among others (Groffman et al. 2006, Palta et al. 2014). More detailed studies should be developed to find the right set of conditions for constructed treatment wetlands to deliver their desired services of clean water while minimizing the unintended disservice of CH_4 and N_2O emissions.

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CHAPTER 5

CONCLUSION

The first research on CTW technology was conducted in Europe in the 1950s; these systems began to be constructed in North America around the 1970s (Bastian and Hammer 1993, Kadlec and Wallace 2008). Since the 1980's, CTW have been increasingly used as an alternative, cost-effective technological solution to treat different types of wastewater (Spieles and Mitsch 2000, Kadlec and Wallace 2008, Huang et al. 2010). A diversity of designs and management schemes allow CTW to provide a variety of important ecosystem services (Kadlec and Wallace 2008, Vymazal 2011). However, the ecological processes that make CTW so effective at treating wastewater may also result in these ecosystems being sources of climate-warming greenhouse gases. As with natural wetlands, CTW are generally sinks of carbon dioxide (CO_2) due to their high rates of carbon fixation (primary productivity), but they are often sources of methane (CH_4) and nitrous oxide (N_2O); (Bridgham et al. 2006, IPCC 2013). The goal of dissertation research was to quantify the production of these dangerous greenhouse gases, CH_4 and N_2O, from the water, plants, and soils of a constructed treatment wetland in Phoenix, AZ. In this conclusion chapter, I present a summary table (Table 4), highlight the main contributions from each chapter and synthesize the major results of my doctoral studies and research.

5.1 Diffusive fluxes from Tres Rios CTW

After studying the diffusive CH_4 and N_2O fluxes from Tres Rios CTW for two years, I found that they were continuously emitted year round with significantly higher emissions in the summer season compared to spring and winter seasons. Spatially, at the

whole-system scale, the gas fluxes for CH_4 and N_2O were significantly greater near the effluent inflow to the CTW. Similarly, at the vegetated marsh gradient, I found greater CH_4 emission fluxes at the interior vegetated marsh subsites compared to the open water subsites. In contrast, N_2O emissions were significantly greater at the open water subsites compared to interior vegetated marsh subsites. The environmental factors that explained the CH_4 flux variability within are nitrate (NO_3^-) and dissolved organic carbon (DOC). The variability of N_2O emission fluxes was best explained by DOC and temperature. These results confirm that the Tres Rios CTW is an ecosystem in which CH_4 and N_2O do diffuse from the water surface. The magnitude of the fluxes depends on the factors that will first and establish the conditions in the CTW for the production of these gases. For example, factors such as nitrogen, carbon, and temperature are needed to stimulate and produce CH_4. These were respectively provided spatially and temporally by the wastewater, the established vegetation in the marsh areas, and the summer season thus, influencing greater CH_4 fluxes.

5.2 Plant-mediated fluxes from Tres Rios CTW

I examined the temporal and spatial dynamics of plant-mediated CH_4 and N_2O fluxes across the same gradients as in Chapter 2. To achieve this, I constructed and adapted individual vegetation-gas chambers specifically to fit the dominant macrophytic wetland emergent vegetation in Tres Rios CTW, *Typha* spp. I found that opposite to the diffusive fluxes, the plant-mediated CH_4 fluxes were significantly greater at the transect near the outflow (Table 4). Additionally, the vegetation-gas chambers confirmed that different sections of the macrophytic vegetation emit more CH_4 than other parts. In our

system, the *Typha* spp. mediated CH_4 fluxes were significantly higher in the lower sections of plant compared to the higher sections. We found that plant-mediated fluxes could be explained by the surrounding water quality parameters such as % oxygen, NO_3^- and temperature. These relationships confirm that microbially driven processes that produce CH_4 and N_2O in the submerged and near the roots of *Typha* spp. do influence the gas content transported into the atmosphere. As Carmichael et al. (2014) and Murray et al. (2015), this study contributes to the much needed understanding of the key contribution of CH_4 and N_2O from wetland vegetation to further refine global CH_4 and N_2O budgets by adding a more robust and dynamic plant-mediated metric.

5.3 Soil fluxes from a mesocosm experiment from Tres Rios CTW

Because hydrology drives the aerobic versus anaerobic conditions in wetland soils, I focused this study to understand how a drying and re-wetting pulse influenced soil conditions and processes that ultimately affect CH_4 and N_2O fluxes. Surprisingly, I found that CH_4 fluxes were not significantly affected by soil drydown events. These observations combined with the environmental conditions of the soils (i.e. redox) suggest that despite experiencing a drydown period, the soil conditions remained suitable for CH_4 production and emission. On the other hand, soils that were dry for 7 days shifted from being N_2O sources to sinks after being re-inundated. We identified that this shift occurred within the first five minutes after the re-wetting event. As a result, the 7-day drydown soils were N_2O sinks cumulatively while the 14-day drydown soils showed significant cumulative release of N_2O at the end of the experiment. These results suggest that drying and re-inundating continuously flooded CTW soils will influence greenhouse gas fluxes differently depending on the duration of the dry period and the actual re-wetting event.

5.4 Dissertation Contributions and Synthesis

Previous reviews and synthesis on the greenhouse gases from CTW have not included any information regarding diffusive or plant-mediated CH_4 and N_2O fluxes from arid regions (Ebie et al. 2014, Mander et al. 2014, Jahangir et al. 2016). My dissertation as a whole contributes to this much-needed greenhouse gas information from CTW that is now included as part of the IPCC reports (Ebie et al. 2014). The information in my dissertation will definitely broaden the range of diffusive and plant-mediated CH_4 and N_2O fluxes needed if we are to improve our global greenhouse budget models (Yavitt and Knapp 1995, Huang et al. 2013, Carmichael et al. 2014). Finally, my studies have contributed new information on the effects of different management strategies to mitigating the unnecessary disservices (greenhouse gas emissions) when using CTW to treat wastewater. I showed that not all applications of a dry period to continuously flooding soils will reduce greenhouse gas emissions. For example, in my study, CH_4 emissions were not affected and the two longest dry periods resulted in soils becoming both cumulative sinks and sources of N_2O, depending on length of the dry period. The study revealed that though the treatment with the longest wet-dry-rewet period promoted the appropriate anaerobic conditions for NO_3^- removal via denitrification, it was also the treatment with the highest cumulative N_2O flux emission.

As a whole, the chapters of my dissertation highlighted several pathways from which the unexpected disservice of the production and emission of greenhouse gases such as CH_4 and N_2O from CTW. When scaling up my emission fluxes to the respective areas of open water (21 ha) and vegetated areas (21 ha) of the Cell 1 at Tres Rios from my field studies (Chapter 2, 3), I am able to demonstrate that of the amount of CH_4 emitted per

hour from the whole Cell 1 of Tres Rios, 76% were emitted via the plant-mediated pathway and 24% were emitted via the diffusion pathway (Table 4).

T**able 4.** Total contribution of diffusion and plant-mediated CH_4 fluxes from Cell 1 Tres Rios CTW.

Diffusion emission fluxes	CH_4 fluxes (g CH_4 h^{-1})	% Contribution of CH_4 emission fluxes
Open Water	111	
Vegetaed areas	854	
Total diffusion fluxes Cell 1	965	23%
Plan-mediated emission fluxes	CH_4 fluxes (g CH_4 h^{-1})	
Typha spp.	3134	
Total *Typha* spp. fluxes from Cell 1	3134	76%

Though the results from the experiment can not be compared to the field studies because of the experimental set up in the greenhouse and the absence of vegetation in the wetland soils, we can draw conclusions that continuously flooded soils can be great sources of CH_4 regardless of the proposed hydroperiods at Tres Rios CTW. On the other hand, N_2O emissions might be exacerbated by short-term dry-periods in currently continuously flooded the Tres Rios FWS CTW design. If we were to add the soil fluxes from the experiment (Chapter 4) as part of the Tres Rios CTW greenhouse inventory, the highest proportion of CH_4 emitted would shift to the pathway from fluxes, especially for CH_4.

Strategies to manage existing CTW currently do not account for mitigating the production and emission of greenhouse gases from them. Therefore, studies that

incorporate strategies to mitigate the production and emission of CH_4 and N_2O while still achieving their desired ecosystem service of clean water need to be pursued. My study adds to a growing body of knowledge of Tres Rios CTW that has examined some of these aspects, such as vegetation dynamics and water budget, with the hope of completing a full-scale CTW study (Sanchez et al. 2016, Weller et al. 2016). As the use of CTW increases around the world combined with new attention to improve national and global accounting of greenhouse gas budgets, it is important to perform multi-temporal and multi-spatial studies as shown in my dissertation from many types of CTW in many bioclimatic regions. New knowledge on how to improve the CTW design, to provide the ecosystem service of N removal intersects with the production and emission of CH_4 and N_2O will help scientists, engineers and managers adopt more efficient design and construction strategies.

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BIOGRAPHICAL SKETCH

Jorge Ramos Holguín was born in El Paso, Texas on July 21, 1984. He received his elementary education at the María Martínez, Juan de Dios Peza, and El Instituto México in Ciudad Juárez, México. His secondary education was completed at El Instituto México and high-school education at the Preparatoria El Chamizal in Ciudad Juárez, México. In 2002, Jorge entered the University of Texas at El Paso, El Paso, Texas, majoring in Environmental Sciences with a concentration in Biology. Upon graduation in 2006, he joined the Ecological Society of America as the SEEDS Student coordinator in Washington, D.C. and helped the organization promote the science of ecology and recruit students through fellowships, conferences, and national and international field trips. In September of 2007, he entered the College of Forest Resources at the University of Washington, Seattle, Washington, and graduated with a Master of Science in Forest Resources in 2010. At the end of his MS studies, he was awarded a three-year fellowship from the National Science Foundation (NSF) and pursued his doctoral studies in Environmental Life Sciences program at the School of Life Sciences of Arizona State University. During his PhD studies, Jorge was also awarded the NSF GK-12 Sustainability Science for Sustainable Schools fellowship to participate in sustainability outreach and curriculum development activities with three local high-schools in Phoenix, Arizona. Throughout his education, Jorge served as a graduate student mentor for the ESA SEEDS program and the Society for the Advancement of Chicanos/Latinos and Native Americans in Science (SACNAS) at the local and national level. He was a member of the American Geophysical Union, ESA, International Network of Next-Generation Ecologists, SACNAS, and the Society of Wetland Scientists. Jorge is now the Manager for the Oceans and Climate team inside the Center for Oceans at Conservation International in Washington, D.C. He is managing the Blue Carbon Initiative, leading the International Blue Carbon Scientific Working Group, and globally supporting both coastal adaptation and mitigation projects in the America's division.