The Effects of Organic Matter Amendments and Migratory Waterfowl on Greenhouse Gas and Nutrient Dynamics in Managed Coastal Plain Wetlands

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environmental Science and Policy in the Graduate School of Duke University

2016
ABSTRACT

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Abstract

Wetland ecosystems provide many valuable ecosystem services, including carbon (C) storage and improvement of water quality. Yet, restored and managed wetlands are not frequently evaluated for their capacity to function in order to deliver on these values. Specific restoration or management practices designed to meet one set of criteria may yield unrecognized biogeochemical costs or co-benefits. The goal of this dissertation is to improve scientific understanding of how wetland restoration practices and waterfowl habitat management affect critical wetland biogeochemical processes related to greenhouse gas emissions and nutrient cycling. I met this goal through field and laboratory research experiments in which I tested for relationships between management factors and the biogeochemical responses of wetland soil, water, plants and trace gas emissions. Specifically, I quantified: (1) the effect of organic matter amendments on the carbon balance of a restored wetland; (2) the effectiveness of two static chamber designs in measuring methane (CH\textsubscript{4}) emissions from wetlands; (3) the impact of waterfowl herbivory on the oxygen-sensitive processes of methane emission and coupled nitrification-denitrification; and (4) surface water nitrogen (N) exports caused by prescribed draw down of a waterfowl impoundment.

The potency of CH\textsubscript{4} emissions from wetlands raises the concern that widespread restoration and/or creation of freshwater wetlands may present a radiative forcing hazard. Yet data on greenhouse gas emissions from restored wetlands are sparse and
there has been little investigation into the greenhouse gas effects of amending wetland soils with organic matter, a recent practice used to improve function of mitigation wetlands in the Eastern United States. I measured trace gas emissions across an organic matter gradient at a restored wetland in the coastal plain of Virginia to test the hypothesis that added C substrate would increase the emission of CH₄. I found soils heavily loaded with organic matter emitted significantly more carbon dioxide than those that have received little or no organic matter. CH₄ emissions from the wetland were low compared to reference wetlands and contrary to my hypothesis, showed no relationship with the loading rate of added organic matter or total soil C. The addition of moderate amounts of organic matter (< 11.2 kg m⁻²) to the wetland did not greatly increase greenhouse gas emissions, while the addition of high amounts produced additional carbon dioxide, but not CH₄. Thus I found little evidence to support the concern that widespread use of organic matter amendments in restored wetlands poses a CH₄ emissions hazard.

I found that the static chambers I used for sampling CH₄ in wetlands were highly sensitive to soil disturbance. Temporary compression around chambers during sampling inflated the initial chamber CH₄ headspace concentration and/or lead to generation of nonlinear, unreliable flux estimates that had to be discarded. I tested an often-used rubber-gasket sealed static chamber against a water-filled-gutter seal chamber I designed that could be set up and sampled from a distance of 2 m with a
remote rod sampling system to reduce soil disturbance. Compared to the conventional design, the remotely-sampled static chambers reduced the chance of detecting inflated initial CH$_4$ concentrations from 66 to 6%, and nearly doubled the proportion of robust linear regressions from 45 to 86%. The new system I developed allows for more accurate and reliable CH$_4$ sampling without costly boardwalk construction.

I explored the relationship between CH$_4$ emissions and aquatic herbivores, which are recognized for imposing top-down control on the structure of wetland ecosystems. The biogeochemical consequences of herbivore-driven disruption of plant growth, and in turn, mediated oxygen transport into wetland sediments, were not previously known. Two growing seasons of herbivore exclusion experiments in a major waterfowl overwintering wetland in the Southeastern U.S. demonstrate that waterfowl herbivory had a strong impact on the oxygen-sensitive processes of CH$_4$ emission and nitrification. Denudation by herbivorous birds increased cumulative CH$_4$ flux by 233% (a mean of 63 g CH$_4$ m$^{-2}$ y$^{-1}$) and inhibited coupled nitrification-denitrification, as indicated by nitrate availability and emissions of nitrous oxide. The recognition that large populations of aquatic herbivores may influence the capacity for wetlands to emit greenhouse gases and cycle nitrogen is particularly salient in the context of climate change and nutrient pollution mitigation goals. For example, our results suggest that annual emissions of 23 Gg of CH$_4$ y$^{-1}$ from ~55,000 ha of publicly owned waterfowl impoundments in the Southeastern U.S. could be tripled by overgrazing.
Hydrologically controlled moist-soil impoundment wetlands provide critical habitat for high densities of migratory bird populations, thus their potential to export nitrogen (N) to downstream waters may contribute to the eutrophication of aquatic ecosystems. To investigate the relative importance of N export from these built and managed habitats, I conducted a field study at an impoundment wetland that drains into hypereutrophic Lake Mattamuskeet. I found that prescribed hydrologic drawdowns of the impoundment exported roughly the same amount of N (14 to 22 kg ha\textsuperscript{-1}) as adjacent fertilized agricultural fields (16 to 31 kg ha\textsuperscript{-1}), and contributed approximately one-fifth of total N load (~45 Mg N y\textsuperscript{-1}) to Lake Mattamuskeet. Ironically, the prescribed drawdown regime, designed to maximize waterfowl production in impoundments, may be exacerbating the degradation of habitat quality in the downstream lake. Few studies of wetland N dynamics have targeted impoundments managed to provide wildlife habitat, but a similar phenomenon may occur in some of the 36,000 ha of similarly-managed moist-soil impoundments on National Wildlife Refuges in the southeastern U.S. I suggest early drawdown as a potential method to mitigate impoundment N pollution and estimate it could reduce N export from our study impoundment by more than 70%.

In this dissertation research I found direct relationships between wetland restoration and impoundment management practices, and biogeochemical responses of greenhouse gas emission and nutrient cycling. Elevated soil C at a restored wetland
increased CO$_2$ losses even ten years after the organic matter was originally added and intensive herbivory impact on emergent aquatic vegetation resulted in a ~230% increase in CH$_4$ emissions and impaired N cycling and removal. These findings have important implications for the basic understanding of the biogeochemical functioning of wetlands and practical importance for wetland restoration and impoundment management in the face of pressure to mitigate the environmental challenges of global warming and aquatic eutrophication.
Dedication

This work is dedicated to the late Prof. Thomas J. Crowley, who inspired my interest in climate science. He died of cancer in 2014.
Contents

Abstract .............................................................................................................................. iv

List of Tables .................................................................................................................... xiv

List of Figures .................................................................................................................. xvi

Acknowledgements .......................................................................................................... xxi

1. Introduction .................................................................................................................. 1

   1.1 Problem Statement ................................................................................................. 1

   1.2 Scope of Inquiry ..................................................................................................... 4

   1.3 Research Objectives .............................................................................................. 6

2. The effects of organic matter amendments on greenhouse gas emissions from a
mitigation wetland in Virginia’s coastal plain ................................................................. 9

   2.1 Introduction ............................................................................................................. 9

   2.2 Methods .................................................................................................................. 11

      2.2.1 Site Description ............................................................................................... 11

      2.2.2 Site Characterization ....................................................................................... 13

      2.2.3 Greenhouse Gas Sampling .............................................................................. 14

      2.2.4 Hydrologic Data .............................................................................................. 16

      2.2.5 Statistical Analyses ......................................................................................... 16

      2.2.6 Carbon Balance ............................................................................................... 17

2.3 Results ..................................................................................................................... 17

   2.3.1 GHG Fluxes ...................................................................................................... 17

      2.3.1.1 CO2 Flux ...................................................................................................... 17
5.2.5.4 Atmospheric Deposition ................................................................. 100
5.2.6 Nitrous Oxide Emission ........................................................................ 100
5.2.7 Early Drawdown Simulation ................................................................. 102
5.3 Results ...................................................................................................... 103
5.4 Discussion ................................................................................................ 111
  5.4.1 Nitrogen vs Phosphorus ........................................................................ 111
  5.4.2 Drawdown Timing ................................................................................ 112
  5.4.3 Role of Waterfowl ................................................................................. 115
  5.4.4 Implications for Lake Mattamuskeet .................................................... 117
6. Conclusions ................................................................................................ 118
  6.1 Summary of research findings .................................................................. 118
  6.2 Integration and implications of research findings ...................................... 121
Appendix A ...................................................................................................... 125
References ....................................................................................................... 130
Biography ......................................................................................................... 149
List of Tables

Table 1: Summary of linear regression and ANOVA tests for differences and trends in log-transformed carbon dioxide (CO\textsubscript{2}) emissions between and across gradient of plots treated with different levels of organic matter (OM) at the Charles City Wetland in Charles City County, Virginia. Values that meet \( p < 0.05 \) are bolded .................................................. 19

Table 2: Summary of monthly averages (±SE) soil temperature (at 5 cm depth), hydrology and soil carbon emissions from the Charles City Wetland in Charles City County, Virginia. Methane (CH\textsubscript{4}) values reported below our minimum detection threshold (0.037 mg m\textsuperscript{-2} hr\textsuperscript{-1}) are the results of averaging many zero fluxes with a few low values. All data collected in 2012 except for October, 2011 and January, 2013 .......... 23

Table 3: Review of methane (CH\textsubscript{4}) emissions rates in kg CH\textsubscript{4}-C ha\textsuperscript{-1} y\textsuperscript{-1} from natural and restored forested wetlands of the Southeastern United States......................................................... 30

Table 4: Comparison of microtopographic and growing season hydrologic conditions at the Charles City Wetland in Charles City County, Virginia between 2005 (Bailey et al) and 2012 (this study)......................................................................................................................... 33

Table 5: Mean (±standard error of the mean) chemical composition of soils collected from paired exclosure and waterfowl-accessible plots during spring 2013 and 2014 at Mattamuskeet Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA ........................................................................................................... 69

Table 6: Measured methane flux from emergent wetlands suitable for waterfowl in the conterminous U.S (adapted from 2). Excludes data from forested wetlands, open water wetlands and tundra systems .............................................................................................................. 79

Table 7: Waterfowl counts from aerial surveys conducted at Lake Mattamuskeet in North Carolina, U.S.A. by the U.S. Fish and Wildlife Service (Wires, 2015) ................................................. 98

Table 8: Comparison of wetland size, drawdown volume, pre-drawdown surface water nutrients, nutrient export, and pre-drawdown peak bird use across 2 years at Mattamuskeet National Wildlife Refuge in North Carolina, USA and one year at Backus Lake in Michigan, USA (Kadlec, 1962) ........................................................................................................ 104

Table 9: Observed and simulated total nitrogen (TN) export from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. Percent change refers to difference between simulated and observed N export for a given year. ......................................................................................................................... 108
Table 10: Land use of the watershed draining into Lake Mattamuskeet in North Carolina, U.S.A................................................................. 109

Table 11: Comparison of the estimated relative importance of nitrogen sources to Lake Mattamuskeet in North Carolina, U.S.A. Reported percentage shares of total load are based on mean values............................................................................................................................. 110

Table 12: Waterfowl counts from ground surveys conducted at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. by the U.S. Fish and Wildlife Service (Wires, 2015) ........................................................................................................................................ 116

Table 13: Estimate of the extent of impoundment habitats similar to those at Mattamuskeet National Wildlife Refuge. Freshwater moist soil and/or emergent marsh waterfowl impoundment areas within National Wildlife Refuges (NWR)1 and under state management in Waterfowl/Wildlife Management Areas (WMA) of the Southeastern U.S. region, including Alabama2, Arkansas3, Delaware4, Florida5, Georgia6, Kentucky7, Louisiana8, Maryland9, Mississippi10, North Carolina11, South Carolina12, Tennessee13, Virginia14. The total acreage reported here represents a fraction of total suitable waterfowl habitat as it excludes flooded agricultural lands, brackish impoundments, forested wetlands, ponds, reservoirs, lake margins and unmanaged natural marshes, as well as all privately owned wetlands. ........................................ 125
List of Figures

Figure 1: Mean (±SE) carbon dioxide flux from the organic matter experimental plots at the Charles City Wetland in Charles City County, Virginia across nine sampling dates from September, 2011 to January, 2013. Different dash patterns represent loading rates of organic matter in kg m\(^{-2}\).................................................................18

Figure 2: Mean carbon dioxide flux from a range of organic matter treatments as a function of soil temperature at 5 cm depth from experimental plots at the Charles City Wetland in Charles City County, Virginia across eight sampling dates from November, 2011 to January, 2013. Error bars represent standard errors of the mean..........................19

Figure 3: Actual carbon dioxide flux compared to linear model predictions at the Charles City County Wetland in Charles City County, Virginia for: A) data across five sampling dates from May 2012 to January 2013 and multiple regression predictions based on soil temperature (5 cm depth), soil volumetric water content, and total soil carbon (top 5 cm); and B) data from 22 July 2012 and linear predictions based on total soil carbon (top 5 cm) and total soil nitrogen at 20 cm depth. Dashed curves represent 95 percent confidence intervals for the regression line. Dashed horizontal line indicates mean carbon dioxide flux value........................................................................................................21

Figure 4: Methane flux (CH\(_4\)) rates as a function of soil volumetric water content measured from the organic matter experimental plots at the Charles City Wetland in Charles City County across five sampling dates from May 2012 to January 2013 ..........23

Figure 5: Carbon dioxide (CO\(_2\)) and methane (CH\(_4\)) flux from soil across five levels of organic matter loading rates estimated from sampling on 7 May and 26 September, 2012 at the Charles City Wetland in Charles City County, Virginia, USA. Note: CH\(_4\) was converted to CO\(_2\)-equivalents by multiplying by 45—its 100-year sustained global warming potential following Neubauer and Megonigal (2015). Error bars represent standard errors of the mean .................................................................25

Figure 6: Water level as recorded by five 1.5 meter loggers placed in 1.5 m wells (W1 through W5) at the Charles City Wetland in Charles City County, Virginia, USA from 22 February, 2012 to 21 January, 2013. Positive values indicate standing water. Overlaid precipitation data are from a station in nearby James City County, Virginia (National Climate Data Center)........................................................................................................27

Figure 7: Linear regressions of mean (±SE) total carbon in top 10 cm of soil in 2011 across organic matter amendment plots at the Charles City Wetland in Charles City County, Virginia, USA. 2005 data from Bailey et al. (2007).................................................................28
Figure 8: Illustrations of the water-filled-gutter static chamber design that allows use of remote rod sampling system (RRSS): Photograph of chamber being deployed using RRSS in the Charles City Wetland in Charles City County, Virginia (a); schematic of chamber disassembled to reveal water fillable gutter on rim of collar that creates an air tight seal, internal fan to mix headspace air and thermocouple to monitor internal chamber temperature (b); schematic of chamber assembled (c) ................................. 40

Figure 9: Examples of gas data illustrating unsuccessful use of a rubber-gasket static chamber design on 21 October 2011(a) and successful use of water-filled-gutter static chamber design with remote rod sampling system on 26 September 2012 (b) ............... 44

Figure 10: Tukey boxplots of log-transformed initial CH4 concentrations in chamber headspace (a) and r-squared value of linear regression line used to estimate CH4 flux (b) from 67 incubations using the rubber-gasket static chamber design taken from Sept. 2011 through Feb. 2012, and 110 incubations using the water-filled-gutter with remote rod sampling system method taken from May 2012 through Jan. 2013. Dashed lines mark ambient CH4 concentration (a) and r-squared threshold of 0.9 (b) ........................................ 45

Figure 11: Tukey boxplot of log-transformed initial CH4 concentration in chamber headspace from 10 incubations using remote rod sampling system to setup the chamber cap to minimize disturbance and from 10 incubations for which the chamber cap set up by hand. Dashed line marks ambient CH4 concentration .......................................................... 48

Figure 12: Conceptual diagram of hypothetical effects of waterfowl on cycling of CH4 and N in wetlands. H1: Dashed arrows trace potential impact of herbivory disrupting plant-mediated efflux of biogenic gases, which would force CH4 to diffuse or ebulliate slowly through surficial sediments where CH4 oxidation is most effective. H2: Split arrows trace potential impact of herbivory starving wetland sediments of plant-transported O2, which would enhance the anaerobic process of CH4 production, while inhibiting aerobic processes of CH4 oxidation and nitrification. H3: Solid bold arrows trace potential impact of enhanced N inputs from waterfowl feces, which could stimulate N cycling and inhibit CH4 production by increasing availability of Nitrate, an efficient alternate electron acceptor ................................................................. 56

Figure 13: Exclusion of overwintering waterfowl increases emergent plant regrowth, associated oxygen transport and reduces methane flux. (A) Waterfowl mega-flock at Mattamuskeet National Wildlife Refuge in North Carolina, USA. (B) Total ducks (pink circles) and swans (yellow triangles) counted at the Refuge during Midwinter Waterfowl Inventories by U.S. Fish and Wildlife Service 1961 to 2012. (C) Dense stands of Spike Rush (E. quadrangulata) in May within white quadrangles which delineate extent of exclosures (only installed from November through March) to prevent grazing by
waterfowl. (D) Fall: healthy emergent vegetation prior to senescence and transport of carbohydrates into roots and tubers. (E) Winter: Tundra Swan (C. columbianus) grazing and grubbing for macrophytes. Where herbivores are excluded by experimental exclosures, plant roots and tubers remain intact. (F) Spring: differential regrowth of emergent vegetation and associated differences in gas exchange. Grazed wetland areas (left side) are slow to regrow and lack oxygen inputs via plant aerenchyma, whereas areas protected from waterfowl herbivory (right side) regenerate emergent vegetation and oxygen transport more rapidly. Differences in plant-mediated oxygen inputs lead to differences in methane flux as indicated by arrow width.

Figure 14: Photos of static chamber for measurement of trace gas emissions deployed at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge, North Carolina, USA.

Figure 15: Mesocosms containing soil and plants from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA used for waterfowl feces addition experiment in the Duke University Greenhouse.

Figure 16: Two seasons of estimated overwintering swan and duck use of experimental plots at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA. Use estimates are generated from counts of birds captured within plots by time-lapse cameras shooting at 5-minute intervals during daylight hours. Approximately 100,000 images were analyzed. Because no images were captured at night when birds may have been present and active in plots these should be considered conservative estimates. Each line represents a separate plot.

Figure 17: Waterfowl grazing impacts on vegetation and oxygen-sensitive soil-nitrogen processes. Paired Tukey box plots (whiskers represent 1.5 of interquartile range) of field data collected from bird-impacted (brown) and exclosure (green) plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA. (A) Belowground biomass in top 15 cm of soil (p = 0.002); (B) E. quadrangulata emergent stem density (p < 0.001); (C) plant-available nitrate/nitrite in the surface soil (0 to 5 cm) (p = 0.03); and (D) nitrous oxide flux (p = 0.02). P-values are generated from Welch’s two-sample t-tests of data log-transformed to better meet assumptions of normality and variance homogeneity. All data are from May 2014 except belowground biomass (April 2013).

Figure 18: Effects of waterfowl on seasonal methane flux and long-term cumulative emission in context of site conditions. (A) Seasonal mean (±standard error) methane (CH4) flux measured in bird-affected (brown) and exclosure (green) plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA from December 2012 through June 2014. (B) Tukey boxplots (whiskers represent 1.5 of interquartile range) of
cumulative CH4 emitted over the course of the study based on extrapolation of flux between sampling periods. We found mean cumulative CH4 flux to be significantly higher from “bird” plots (p = 0.026; Welch’s one-tailed t-test). Data were log-transformed to conform to the assumption of variance homogeneity. (C) Water level (blue curve) and water temperature (red curve) are the principle abiotic factors influencing seasonal fluctuations in CH4 flux over the course of the experiment. Gray shading delineates peak waterfowl seasons. Maximum depth of water level recorders is approximately 120 cm. ..........................................................74

Figure 19: Potential methane production of soils collected from experimental plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA June 2014. Overwintering waterfowl were excluded from “exclosure” plots but had unrestricted access to control (“birds”) plots. We detected no statistically significant treatment effect (p = 0.43; Welch’s t-test). ..........................................................75

Figure 20: Relationships between mean porewater methane (CH4) concentration and mean CH4 flux measured in experimental plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA during high CH4 flux sampling dates (April, May and June of 2013; April and May of 2014). Error bars represent standard errors of the mean 76

Figure 21: Tukey’s boxplots of methane (CH4) flux from wetland mesocosms over two sampling dates in response to waterfowl feces addition. Letters indicate significant differences in mean CH4 flux based on analysis of variance and Tukey’s honest significant differences test. ..........................................................77

Figure 22: Wetland loss and recovery of waterfowl populations have led to an increase in waterfowl density over the past 50 years. (A) Mass of overwintering ducks (Drilling et al. 2002), Tundra Swan (Cygnus columbianus) (Limpert and Earnst 1994) and Snow Goose (Chen caerulescens) (Mowbray et al. 2000) at Mattamuskeet National Wildlife Refuge based on midwinter aerial counts by the U.S. Fish and Wildlife Service from 1961 to 2012 (Wires 2015) with positive exponential and moving 5-year mean trend lines. (B) Wetland land cover on the North Carolina coastal plain in pre-1900s (North Carolina Department of Environment, Health 1991), 1955, 1981 (Cashin et al. 1992), 1994, 2001 (Carle 2011), and 2010 (O’Driscoll 2012) with negative exponential trend line for modern data. (C) Exponentially increasing trend and individual points (triangles) in waterfowl density index are based on ratios of waterfowl mass and wetland area from (B) and (A), respectively. ..........................................................83

Figure 23: Landcover map of the Lake Mattamuskeet watershed in North Carolina, USA. Red dot marks field site at Marsh Impoundment 10 North.................................................91
Figure 24: Water level at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge as measured hourly by water level recorders during the months surrounding spring drawdown in 2013 and 2014. Gray shading highlights period of prescribed drawdown by Refuge management.

Figure 25: Tukey boxplots comparing surface water ammonia/ammonium (NHx), total nitrogen (TN), nitrate/nitrite (NOx) and total phosphorus (P) concentrations during the first half of two growing seasons at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA. Whiskers include points within 1.5 times the interquartile range; outliers are individual points. Letters indicate results of Tukey’s test of honest significant differences at \( \alpha = 0.05 \). Differences in spring nutrient concentrations were not statistically significant in all cases, as tested by ANOVA and Tukey’s Honest Significant Differences Test, but some true differences are likely obscured by low statistical power due to small sample sizes (\( n = 4 \)). There were no significant differences among NO3 data. TN data for 2013 is modelled from NHx (see 5.2.4.1 Impoundments).

Figure 26: Tukey boxplots of nitrous oxide (N2O) emissions from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. Whiskers include points within 1.5 times the interquartile range; outliers are individual points. N2O emissions were almost entirely less than the minimum detectable threshold of 0.013 mg N2O m\(^{-2}\) h\(^{-1}\) until after spring drawdown oxidized surface soils around 23 May 2014.

Figure 27: Submerged aquatic vegetation cover at Lake Mattamuskeet in North Carolina, U.S.A. (A) plotted over time, and (B) spatially mapped with the highest coverage areas shaded green indicating a major reduction over in a recent three-year period.
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1. Introduction

1.1 Problem Statement

Wetland ecosystems provide many valuable ecosystem services, including carbon (C) storage, improvement of water quality, and provision of habitat for wildlife (Costanza et al. 1997). Policies, such as section 404 of the Clean Water Act in the U.S., mandate wetland restoration, and agencies, such as the U.S. Fish and Wildlife Service and The Ramsar Convention, provide management support to ensure that important wetlands provide habitat for desired wildlife (North American Waterfowl Management Plan Committee 2012, Matthews 2013). Thus, wetlands are frequently restored or managed with a singular practical goal, and yet prevailing practices inevitably produce unrecognized or undervalued co-benefits and/or costs. In mandated wetland restoration, the most important outcome for most interested parties is to meet regulatory criteria to earn financially valuable mitigation credits (Zedler and Callaway 1999). While criteria are designed to lead to provision of valued ecosystem services, such as wildlife habitat (Hansson et al. 2005), in some cases, unintended consequences, such as nutrient export (Ardón et al. 2010a) or greenhouse gas emission (Morse et al. 2012) have been documented. National Wildlife Refuges and Ramsar sites are evaluated primarily on their capacity to support populations of wildlife, such as waterfowl (North American Waterfowl Management Plan Committee 2012, Matthews 2013), but relatively little research has investigated the water quality (Kadlec 1962, Brandvold et al. 1976, Post et
al. 1998) or greenhouse gas cycling functions of these constructed and managed ecosystems provide.

In light of global warming (Myhre et al. 2013), the potential for managed wetlands to produce greenhouse is an especially pressing concern. The widespread creation and/or restoration of mitigation wetlands, for example, has been identified as a potentially important additional source of greenhouse gases (Bridgham et al. 2006). Wetlands are responsible for 20 to 40 percent of global emissions of the potent greenhouse gas, methane (CH$_4$), representing the largest source to the atmosphere (Bloom et al. 2010). The long-term rise in atmospheric CH$_4$ to 1.5 times the preindustrial level is attributed to releases from fossil fuel extraction (Myhre et al. 2013), but shorter term perturbations in recent decades are at least partly related to dynamics of the wetland CH$_4$ source (Nisbet et al. 2014). Even millennial-scale trends in global CH$_4$ have been partly attributed to the changing role of wetlands. After sea level reached contemporary levels following the last glacial maximum, wetland extents dramatically increased and they have since been among the most dominant sources of atmospheric CH$_4$ (Zimov and Zimov 2014). The problem remains that quantification of CH$_4$ or carbon balances for restored wetlands are rare (Morse et al. 2012). Furthermore, there has been little investigation into the greenhouse gas consequences of specific restoration practices (Bailey 2006).
Much like wetlands created through restoration programs, those managed to provide wildlife have rarely been investigated for their potential to produce greenhouse gases. North American waterfowl populations have recovered dramatically from lows of the 1930s, as a result of decades of prudent wetland habitat creation and management (North American Waterfowl Management Plan Committee 2012). But despite recent research demonstrating the pervasive influence of wildlife populations on carbon cycling (Schmitz et al. 2013), there is little understanding of the role of wildlife in the functioning of the Wildlife Refuge wetlands. For example, waterfowl are known to facilitate nutrient cycling and transport (Manny et al. 1994, Post et al. 1998) as well as alter the structure of emergent vegetation (Evers et al. 1998, Idestam-Almquist 1998, Froelich and Lodge 2000). Other than a few examples (Huang et al. 2005, Bodelier et al. 2006, Dingemans et al. 2011), wildlife impacts, such as herbivory, have yet to be linked to greenhouse gas cycling.

Effectively managed wetlands for waterfowl will also be critical under growing global environmental threats posed by climate change (Myhre et al. 2013) and aquatic eutrophication (Smith 2003). But fundamental gaps in our understanding of how current wetland management prescriptions contribute to the mitigation or exacerbation of global environmental challenges are an impediment to progress.
1.2 Scope of Inquiry

The primary purpose of this dissertation research is to contribute to the basic understanding of the biogeochemical consequences of wetland management practices and their implications for greenhouse gas emissions and nutrient pollution. Wetlands include a wide diversity of ecosystems (i.e. marshes, swamps, bogs, floodplain forests, constructed treatment wetlands, waterfowl impoundments etc.), which all share in common the state of hydrologic saturation, and, importantly, the anoxia it creates. This research focuses on highly oxygen-sensitive cycles of CH₄ and N.

CH₄ is the product of the least efficient form of anaerobic respiration; methanogenesis only proceeds efficiently if oxygen and alternative electron acceptors (nitrate, manganese, ferric iron and sulfate) are absent or have been reduced and depleted (Whalen 2005). CH₄ production is carried out by Archaea via two distinct pathways: 1) acetate-splitting ‘acetoclastic methanogenesis’; and 2) CO₂ reducing ‘hydrogenotrophic methanogenesis’ (Ye et al. 2012). Net CH₄ flux from wetland soils to the atmosphere is typically attenuated greatly by the consumption of CH₄ by aerobic methanotrophic bacteria in oxic surficial layers, microsites, and/or an oxidized rhizosphere (Le Mer and Roger 2001, Conrad 2007). The microbiological paradigm of ‘everything is everywhere’ suggests that CH₄ production and consumption should not be limited by the availability of producers and consumers (O’Malley 2007). This assumption is supported by the ability of incubated soils from diverse upland and arid
settings to have the capacity to produce CH$_4$ without any need for microbial inoculation or even additional carbon substrate input (Angel et al. 2011). Thus, while microorganisms are the machines that run the biogeochemistry of the world, this dissertation, like many biogeochemical investigations, leaves them sequestered in the microbial ‘black box.’ This work instead focuses on the physio-chemical conditions, such as hydrology, availability of oxygen and electron acceptors, quantity/quality of C substrate, which are understood to be the ultimate drivers of CH$_4$ dynamics in wetlands (Whalen 2005).

N cycling in wetlands is governed by coupled aerobic nitrification and anaerobic denitrification (Firestone 1982), which allow for the long-term removal of N from ecosystems. For this reason constructed wetlands are designed with oxic and anoxic components to facilitate the aerobic and anaerobic N cycle processes (Brix 1994). Since N-limitation is a characteristic of many aquatic ecosystems (Scott et al. 2007), reduction targets for N export from point and non-point pollution sources are a component of many efforts to mitigate aquatic eutrophication (Borsuk et al. 2003, Jha et al. 2010).

We focus our field investigations at two locations: 1) a restored mitigation wetland in Charles City, Virginia, U.S.A.; and 2) a constructed freshwater waterfowl impoundment at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. These wetlands serve as natural laboratories and sites for the implementation of experiments designed to test hypotheses about biogeochemical responses to
management prescriptions. At the restored wetland I studied the impact of prescribed organic matter amendments on C emissions. At the waterfowl impoundment I investigated the role of managed waterfowl populations and prescribed hydrologic manipulations on CH₄ emissions and N export.

1.3 Research Objectives

The overarching objective of this dissertation research is to address knowledge gaps in the scientific understanding of how wetland restoration practices and waterfowl management affect critical wetland biogeochemical processes related to greenhouse gas emission and nutrient cycling.

In Chapter 2 I investigate the effects of organic matter amendments on the carbon (C) balance of a restored wetland (Winton and Richardson 2015a). Carbon dense, high organic matter soils are a characteristic of wetlands. Yet in restored wetlands, soils typically lack sufficient organic matter, which has been blamed for the poor establishment of wetland vegetation (Stauffer and Brooks 1997, Bailey et al. 2007). In wetland restoration practice, organic matter can be added to enhance soil properties and microbial processes in order to accelerate restoration of ecosystem function (Bruland 2009), which would otherwise play out slowly via decade or century-scale successional processes (Odum 1969). The impact of the practice of adding supplemental organic matter on the emission of CH₄ has not been fully explored. All else being equal, one should expect that a soil with higher organic matter content
should have higher rates of respiration, oxygen demand, and therefore the potential for higher emissions of CH₄. I test the hypothesis that the addition of organic matter to a restored wetland will increase CH₄ emission, thus altering the carbon balance of restored wetlands (Bridgham et al. 2006).

In Chapter 3 I compare the effectiveness of two static chamber methodologies for the measurement of trace gases in wetlands (Winton and Richardson 2015b). Static chambers used for sampling CH₄ in wetlands are highly sensitive to soil disturbance. Temporary compression around chambers during sampling can inflate the initial chamber CH₄ headspace concentration and/or lead to generation of nonlinear, unreliable flux estimates that must be discarded (Nahlik and Mitsch 2010, Morse et al. 2012). I tested the hypothesis that a water-filled-gutter seal chamber design that could be set up and sampled from a distance of 2 m with an originally-designed remote rod sampling system would reduce soil disturbance-induced errors compared to a commonly-used rubber-gasket sealed static chamber (Weishampel and Kolka 2008).

In Chapter 4 I investigate the potential for managed populations of overwintering herbivorous waterfowl to disrupt the formation of an oxidized rhizosphere and alter dynamics of CH₄ emission and N cycling. Emergent aquatic vegetation is widely believed to enhance the emission of CH₄ (Sorrell and Boon 1992, Van Der Nat et al. 1998, Turetsky et al. 2014), but the effects of natural vegetation removal via herbivory has not been investigated. Nor has the herbivore-induced
disruption of the process of radial oxygen loss from plant roots (Armstrong 1964), which facilitates processes that occur along oxic-anoxic soil interfaces, such as N cycling (Reddy et al. 1989) and CH₄ oxidation (King 1994, Jespersen et al. 1998). I test the hypothesis that exclusion of waterfowl will lead to: 1) greater belowground biomass; 2) denser emergent plant stems; 3) lower CH₄ emissions; and 4) more nitrification.

In Chapter 5 I investigate the relative importance of hydrologically managed waterfowl impoundments as exporters of N to downstream waters. Hydrologic regimes for waterfowl impoundments are prescribed based on ‘the art’ of moist-soil management (Strader et al. 2005), which encourages long-term flooding followed by a pumping of surface waters. Since mineralization of N is accelerated by springtime warming, while N removal is often limited by oxygen availability for nitrification (Brix 1994), the timing of hydrologic draw down may be an important factor in determining N loading from drainage water. I compare N export from impoundments to other sources and test the hypothesis that earlier draw down of waterfowl impoundments would significantly reduce their importance as an N source.

In Chapter 6 I review the findings of this dissertation and discuss their implications for the understanding the biogeochemical functioning of managed wetlands.
2. The effects of organic matter amendments on greenhouse gas emissions from a mitigation wetland in Virginia’s coastal plain

2.1 Introduction

Despite making up only five to eight percent of world land cover (Mitsch and Gosselink 2007), wetland ecosystems play an important role in regulating the Earth’s climate. Wetland soils contain 16 to 33 percent of the earth’s soil carbon (C) pool of 2,500 Pg (Lal 2005; Bridgham et al. 2006) and emit 20 to 40 percent of methane (CH$_4$) (Bloom et al., 2010), an important greenhouse gas (GHG) (Myhre et al. 2013).

A review of North American wetland C exchange found that because of CH$_4$ emissions, most wetlands emit more GHG than they sequester on century timescales and therefore: “…that creating and restoring wetlands may increase net radiative forcing...’’(Bridgham et al. 2006). Others have claimed that because wetlands are sustainable ecosystems and persistent as C sinks, the widely-used 100-year time horizon is too short, and that: “…wetlands can be created and restored to provide C sequestration and other ecosystem services without great concern of creating net radiative sources on the climate due to methane emissions” (Mitsch et al. 2013). But errors in both the math and reasoning underpinning this latter view have been exposed (Neubauer 2014, Bridgham et al. 2014), which reaffirms the potential century-scale impact of restored and created wetland CH$_4$ emissions on regional climate budgets.
While this controversy over the C balance of wetland restoration and creation is partly a disagreement about the appropriate use and calculation of global warming potential, versus sustained flux models, which account for annual pulses of GHGs (i.e. Frolking et al., 2006; Neubauer, 2014), it also reflects the great uncertainty (100%) around wetland GHG flux estimates (Bridgham et al. 2006). It thus may be particularly difficult to make long-term assumptions regarding restored and created wetland GHG fluxes given their complex histories of human disturbance and intervention and that they routinely fail to achieve the same ecological function of reference ecosystems over short timescales (Zedler and Callaway 1999). An important remaining question is whether created or restored freshwater wetlands with mineral soils are in fact a sink or source of GHG over policy-relevant timescales?

In the eastern United States large areas of freshwater wetlands are created or restored as part of compensatory mitigation mandated by section 404 of the Clean Water Act, and they commonly suffer from an initial deficiency of soil organic matter (OM) (Stauffer and Brooks 1997, Whittecar and Daniels 1999) compared to natural wetlands (Bailey et al. 2007). Many studies have advocated for the amendment of created wetlands with OM in the form of salvaged topsoil or compost to help them achieve reference functionality (Stauffer and Brooks 1997, Whittecar and Daniels 1999, Bruland and Richardson 2004). Indeed, studies have found that moderate loading of compost OM into a created wetland increase woody plant development (Bailey et al. 2007) and soil functions, such as microbial decomposition and increased denitrification enzyme activity (Bruland and Richardson 2009, Sutton-Grier et al. 2009).
Few studies have measured GHG emissions from created or restored wetlands and fewer still have done so at sites amended with OM. It is unclear whether or not the practice of adding OM to created wetlands will have an effect on their radiative impact, though one study at a freshwater tidal wetland in Virginia may provide a clue. In this case wetland soil cores, when amended with leaf litter OM, tended to produce more CH₄ and CO₂ over unamended controls, while soils amended with compost OM produced more CO₂, but no additional CH₄ (Morrissey et al. 2014). These findings are suggestive, but need to be replicated at an independent site and confirmed in situ.

The purpose of this study is to investigate how a gradient of added compost OM affects GHG emissions from a created mitigation wetland. Included in our analysis is an estimate of how long it would take for our restored wetland to change from a GHG source to a sink, calculated as the radiative forcing switchover time following Frolking et al. (2006).

2.2 Methods

2.2.1 Site Description

The study took place within the 20.8-hectare Charles City Wetland Mitigation Site (CCW), which is located in Charles City County, Virginia, USA (37°20'37”N, 76°55'33”W), and owned by the Virginia Department of Transportation (VDOT) as part of its compensatory mitigation program (Bailey et al., 2007). Precipitation is the dominant hydrologic input and the CCW may hold up to 0.5 m of standing water
during cooler months (Bailey et al. 2007). Site history is described in detail by Bergschneider (2005) and Bailey et al. (2007), but briefly summarized here. Prior to restoration the site was covered by upland mixed hardwood forest that had been partially converted to agricultural field. The soil was mapped as a complex of Chickahominy (fine, mixed, semiactive, thermic Typic Endoaquults) and Newflat (fine, mixed, subactive, thermic Aeric Endoaquults) series (Bergschneider 2005). Mitigation efforts attempted to convert field and remnant forest to wetland status during the winter of 1997-1998 by excavating into the subsoil (E or Btg horizon) to the depth of the presumed seasonal high water table. After revegetation, many parts of the site were found to be covered in facultative or upland plant species with much less hydrophytic cover than desired for mitigation purposes, a result attributed to restoration activities in which topsoil was lost, leaving compacted, low organic matter (OM) subsoil at the surface. The addition of an OM source had been proposed as a method for improving function of mitigation wetlands (Stauffer and Brooks 1997), but no data existed regarding the quantity of added OM required to achieve sufficiently improved wetland function in this setting. With a goal of determining optimal OM amendment loads for the wetland, a research group from Virginia Polytechnic Institute and State University implemented a gradient experiment in 2001 with 4 replicate plots of 4 OM loading rates (plus control) in an experimental block. Municipal wood and yard waste compost was rototilled into the topsoil of 4.6 by 3.1 m plots at loading rates of 56, 112, 224 and 336 kg
m² (dry weight) in July, 2002. Control plots received only rototilling. Each plot was planted with five Pin Oak (*Quercus palustris*) and River Birch (*Betula nigra*) saplings, but otherwise the site was allowed to revegetate naturally from seed bank. In January, 2013 we found a mean count of 3.4 *Q. palustris* and 4.6 *B. nigra* survived in each 14.3 m² plot with some volunteer tree species, such as Red Maple (*Acer rubrum*) and Black Willow (*Salix nigra*), established sporadically.

### 2.2.2 Site Characterization

We measured the relative elevation of each plot near the gas collars used for measuring GHGs using a Topcon RL-H3A laser level and collected soil cores in each plot in September, 2011 using a 10-cm diameter soil-corer. Cores were split into 0 to 5 and 5 to 10 cm depth sections in the field. In the lab each core section was weighed wet and a subsample was weighed, oven-dried and re-weighed to estimate wet:dry ratios and calculate bulk density. Subsamples were analyzed for total carbon (C) and total (N) using a CE Instruments Flash Elemental Analyzer. We sampled soils again in September, 2012 using a punch tube and separated depth sections of 0 to 2 cm, 4 to 6, 9 to 11 and 19 to 21 cm in the field, and then composited corresponding depths from three replicate punches. These soils were analyzed for total C, total N (following the same method as above), digested following a nitric-perchloric acid method followed by colorimetric analysis of total phosphorus (P) using a Beckman DU-64 spectrophotometer, Meilich-3-extractable P, KCl-extractable nitrate/nitrite (NO₃⁻) and
ammonia/ammonium (NH₃) using a Lachat Quickchem 8000 autoanalyzer. We installed litter fall traps (approximately 1000 cm²) in each plot in September, 2012 and litter was collected during subsequent site visits.

2.2.3 Greenhouse Gas Sampling

We installed one 20-cm diameter PVC collar 10 to 15 cm into the soil in each plot for static chamber GHG gas sampling (Livingston and Hutchinson, 1995, Weishampel and Kolka 2008) in fall 2011. After observing that the close approach necessary for setup and sampling of PVC collars was generating unreliable CH₄ data due to soil disturbance, we redeployed 30-cm diameter static chamber collars affixed with a water-fillable gutter in the spring of 2012. To avoid soil disturbance and improve CH₄ data quality, we used a remote rod sampling system, which allows chambers to be set up and sampled from a distance of 2 m (Winton and Richardson 2015b) for subsequent trace gas sampling every two months from May, 2012 until January, 2013. We excluded from analysis unreliable CH₄ data generated by the original PVC collars, but include complete, unimpacted CO₂ data collected from these collars on October 2011 and February 2012. Sampling errors on September 12, 2012 impacted a large portion of CH₄ flux data, so we repeated measurements on September 26 for CH₄ analysis. CO₂ results were not affected and we averaged results from both September, 2012 dates for subsequent analysis.
Static chambers were opaque and total extracted gas volume was never greater than 5 percent of chamber headspace. We left any plants growing within chamber footprints intact unless they were long enough to interfere with chamber setup, in which case we clipped them. On each sampling date we collected headspace gas four times over the course of half-hour incubations from collars in each of the 20 plots. Following placement of the static chamber top on the collar we immediately extracted a 50-ml headspace sample via a plastic syringe and deposited it into a mylar gas-tight sample bag. We recorded ambient air temperature (T), internal chamber T, soil T at 5 cm depth for initial and subsequent samples taken approximately 5, 15 and 30 minutes following chamber setup. We transported gas bags to the Duke University Wetland Center laboratory and analyzed within one week of collection on a Varian 450 Gas Chromatograph (GC) equipped with a flame ionization detector and methanizer to analyze CH₄ and CO₂ concentrations synchronously. All samples were run in duplicate with the mean value used for gas flux calculations unless duplicate values differed by >10%, in which case the obviously outlying value was assumed to stem from analytical error and discarded. Flux was estimated by linear regression of sample concentrations as a function of time elapsed. If a threshold r-squared value of 0.90 was not met, we removed one outlying point if it improved fit to > 0.90 (approximately 5% of incubations), otherwise such estimates were treated as failed incubations and discarded. We estimate the minimum detectable flux for CO₂ was 52 mg m⁻² h⁻¹ and for CH₄ was
0.037 mg CH₄ m⁻² h⁻¹. We analyzed all samples for nitrous oxide, but found flux to be below minimum detection thresholds for approximately 90 percent of incubations, so we focus our results and discussion on CH₄ and CO₂.

2.2.4 Hydrologic Data

We measured soil volumetric water content (SVWC) in the top 5 cm using a Fieldscout 100 time domain reflectometry probe (Spectrum Technologies) starting in May, 2012. We recorded the mean of five measurements taken adjacent to each chamber collar. We installed five Odyssey loggers (Dataflow Systems, Christchurch, New Zealand), which monitored water level hourly starting in February 2012. We collected daily rainfall and air T data from nearby meteorological stations in Williamsburg and Norfolk, Virginia, which are publicly accessible via the National Climatic Data Center (Lawrimore et al. 2011).

2.2.5 Statistical Analyses

We used ANOVA and Tukey’s honest significant difference test to test for differences in gas flux between groups of plots with different OM treatments and linear regression to look for trends in gas flux across the OM gradient. We evaluated all data for normality by generating box-and-whisker, histogram and quantile-quantile plots and log-transformed data when necessary. We explored relationships between gas flux and potential explanatory variables using the Ecodist package (Goslee and Urban, 2007) and by building generalized linear models (GLM). We used these models to estimate daily
emissions of CO$_2$ and CH$_4$ and generate an annual flux budget from March 1, 2012 to February 28, 2013. We used JMP Pro 11 (SAS Institute Inc.) to plot GLM outputs. All other statistics were computed using the R programming language (R Core Team 2013) and in Microsoft Excel 2010.

**2.2.6 Carbon Balance**

We compare the relative radiative impacts of soil CH$_4$ and CO$_2$ fluxes by multiplying CH$_4$ by its 100-year sustained global warming potential of 45 (Neubauer and Megonigal 2015). We estimate radiative forcing switchover time, determined by the ratio of CO$_2$ stored : CH$_4$ emitted, (Frolking et al. 2006) for the CCW by assuming annual net ecosystem exchange (NEE) estimates (Bailey 2006) and annual CH$_4$ flux estimates generated in this study will be sustained over centuries. Bailey (2006) found NEE to be negative (net emission of CO$_2$) for most of the CCW plots because of rapid oxidation of added OM early in the experiment, therefore we only used his positive mean NEE values (net uptake of CO$_2$) from the lowest loading rates (141.1 and 29.9 g CO$_2$-C m$^{-2}$ y$^{-1}$) to generate a range of potential radiative forcing switchover times.

**2.3 Results**

**2.3.1 GHG Fluxes**

**2.3.1.1 CO$_2$ Flux**

We observed the highest CO$_2$ fluxes (>400 mg m$^{-2}$ h$^{-1}$) during warmer, drier months and these contrast with fluxes approaching minimum analytical detection limits
during cold, wet months (Fig. 1). CO₂ emissions from soil directly responded to increases in soil T (Fig. 2) and in general, the higher CO₂ emissions are associated with higher OM loading rates; linear regression of log-transformed CO₂ flux as a function of OM treatment shows significant positive relationships across all sampling months except September (Table 1). The relationship between OM and CO₂ emission is strongest during peak flux in July which is one of only two months (the other being January) where significant differences in CO₂ flux between OM treatments occur.

Figure 1: Mean (±SE) carbon dioxide flux from the organic matter experimental plots at the Charles City Wetland in Charles City County, Virginia across nine sampling dates from September, 2011 to January, 2013. Different dash patterns represent loading rates of organic matter in kg m⁻²
Figure 2: Mean carbon dioxide flux from a range of organic matter treatments as a function of soil temperature at 5 cm depth from experimental plots at the Charles City Wetland in Charles City County, Virginia across eight sampling dates from November, 2011 to January, 2013. Error bars represent standard errors of the mean.

Table 1: Summary of linear regression and ANOVA tests for differences and trends in log-transformed carbon dioxide (CO₂) emissions between and across gradient of plots treated with different levels of organic matter (OM) at the Charles City Wetland in Charles City County, Virginia. Values that meet \( p < 0.05 \) are bolded.

<table>
<thead>
<tr>
<th>Month</th>
<th>linear regression</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p-value</td>
<td>r-squared</td>
</tr>
<tr>
<td>Oct., '11</td>
<td>0.021</td>
<td>0.22</td>
</tr>
<tr>
<td>Feb., '12</td>
<td>0.93</td>
<td>-0.05</td>
</tr>
<tr>
<td>May, '12</td>
<td>0.028</td>
<td>0.24</td>
</tr>
<tr>
<td>July, '12</td>
<td>&lt;0.001</td>
<td>0.55</td>
</tr>
<tr>
<td>Sept., '12</td>
<td>0.133</td>
<td>0.12</td>
</tr>
<tr>
<td>Nov., '12</td>
<td>0.009</td>
<td>0.40</td>
</tr>
<tr>
<td>Jan., '13</td>
<td>0.043</td>
<td>0.21</td>
</tr>
</tbody>
</table>
A GLM with three parameters: soil T (coefficient = 0.058; p < 0.001), soil volumetric water content (SVWC; coefficient = -0.025; p < 0.001), and surface soil total C (coefficient = 0.031; p < 0.001), explains much of the variability ($r^2 = 0.75$) in log-transformed CO$_2$ flux across all sampling dates (Fig. 3A). Soil T and SVWC are the two most important terms in our GLM explaining log-transformed CO$_2$ flux variability across seasons, with r-squared values of 0.50 and 0.49 respectively. Soil T and SVWC are correlated with each other (r-squared of 0.40), but this relationship is driven by one sampling date in July when the site was both very warm and very dry. Including both soil T and SVWC improves model r-squared to 0.71. The third model parameter, total surface soil C partly reflects the amount of OM available to be decomposed. The effects of soil C on CO$_2$ flux become obvious when the site is sufficiently dry (i.e. July), but during wetter periods the importance of surface soil C is obscured. So while soil C is very weakly correlated with log-transformed CO$_2$ flux across all sampling dates (r-squared of 0.05), including it in the GLM helps improve fit (r-squared of 0.75) and reduces the Akaike information criterion.
Figure 3: Actual carbon dioxide flux compared to linear model predictions at the Charles City County Wetland in Charles City County, Virginia for: A) data across five sampling dates from May 2012 to January 2013 and multiple regression predictions based on soil temperature (5 cm depth), soil volumetric water content, and total soil carbon (top 5 cm); and B) data from 22 July 2012 and linear predictions based on total soil carbon (top 5 cm) and total soil nitrogen at 20 cm depth. Dashed curves represent 95 percent confidence intervals for the regression line. Dashed horizontal line indicates mean carbon dioxide flux value.

With T and soil moisture held relatively constant across the site during a given sampling date (relative to seasonal changes), we found surface soil C to be the most important parameter (coefficient = 25; p = 0.001) explaining CO$_2$ flux in July (r-squared of 0.52). The inclusion of total soil N at 20 cm depth (coefficient = 3900; p = 0.05) improved our GLM r-squared to 0.63 (see Fig. 3B) and it was not highly correlated with surface soil C (r-squared of 0.24).

Since we do not have high frequency SVWC and soil T data, we used highly correlated water level (r$^2$ = 0.93) and mean air T from the preceding 7 days (r$^2$ = 0.98; as measured in Norfolk, Virginia; National Climatic Data Center), as respective substitutes.
to model daily CO$_2$ flux from March 1, 2012 through February 28, 2013. We assume surface soil total C would remain constant over the year. From this model we estimate an annual CO$_2$ flux ranging from 0.44 to 0.64 kg CO$_2$-C m$^{-2}$ yr$^{-1}$ from the low to high end of the OM gradient.

2.3.1.2 CH$_4$ Flux

We find CH$_4$ fluxes consistently exceed minimum analytical detection only when soil T was at least 15 °C and some ponded water was present at the CCW (see Table 2). We identify a threshold of 50 percent SVWC, below which CH$_4$ was never greater than 0.13 mg CH$_4$ m$^{-2}$ h$^{-1}$ (Fig. 4). When conditions at the CCW are favorable for methanogenesis fluxes are highly variable across plots. We observed maximum CH$_4$ fluxes of approximately 3 to 5 mg m$^{-2}$ h$^{-1}$ during sampling in May and September 2012. But even on these high flux dates we are unable to detect any statistically significant patterns in CH$_4$ flux related to OM loading rate using linear regression (May, 2012: p = 0.16; and Sept. 2012: p = 0.21) and Tukey’s honest significant difference test (May, 2012: p = 0.92; and Sept. 2012: p = 0.60) when comparing the highest loading rate to control). Furthermore the non-significant linear regression coefficients were negative, with lower CH$_4$ flux associated with greater OM amendment, the opposite of what would be expected if added OM is fueling CH$_4$ production and emission.
Figure 4: Methane flux (CH$_4$) rates as a function of soil volumetric water content measured from the organic matter experimental plots at the Charles City Wetland in Charles City County across five sampling dates from May 2012 to January 2013.

Table 2: Summary of monthly averages (±SE) soil temperature (at 5 cm depth), hydrology and soil carbon emissions from the Charles City Wetland in Charles City County, Virginia. Methane (CH$_4$) values reported below our minimum detection threshold (0.037 mg m$^{-2}$ hr$^{-1}$) are the results of averaging many zero fluxes with a few low values. All data collected in 2012 except for October, 2011 and January, 2013.

<table>
<thead>
<tr>
<th>Month</th>
<th>Soil Temp. °C</th>
<th>Water Level cm</th>
<th>Soil volumetric water content %</th>
<th>CH$_4$ emissions mg·m$^{-2}$·h$^{-1}$</th>
<th>CO$_2$ emissions mg·m$^{-2}$·h$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct.</td>
<td>16.2±0.2</td>
<td>3.7±0.8$^1$</td>
<td>NA$^2$</td>
<td>NA$^3$</td>
<td>77±8</td>
</tr>
<tr>
<td>Feb.</td>
<td>7.3±0.3</td>
<td>5.9±0.9</td>
<td>NA$^2$</td>
<td>NA$^3$</td>
<td>47±5</td>
</tr>
<tr>
<td>May</td>
<td>18.1±0.1</td>
<td>5.3±0.6</td>
<td>65.6±1.7</td>
<td>0.82±0.20</td>
<td>117±21</td>
</tr>
<tr>
<td>July</td>
<td>24.6±0.08</td>
<td>-39.9±6.4</td>
<td>29.9±2.6</td>
<td>0.02±0.01</td>
<td>595±43</td>
</tr>
</tbody>
</table>

$^1$ Water depth measured by hand within plots rather than in wells; may not be comparable to other data  
$^2$ Not measured  
$^3$ Methodological issue led to unusable CH$_4$ data, therefore we omit from analysis. See methods section.
To model annual CH$_4$ flux we assume CH$_4$ will respond in a binary fashion to soil T and SVWC based on the thresholds we identified. We imputed mean measured CH$_4$ flux from CH$_4$-favorable sampling dates (1.1 mg CH$_4$ m$^{-2}$ hr$^{-1}$) for each day during which conditions met the threshold we identified (soil T > 15 °C and SVWC > 50 percent). If the soil T and SVWC thresholds were not both met, we assumed CH$_4$ flux would be equal to mean measured CH$_4$ flux from CH$_4$-unfavorable sampling dates (0.012 mg CH$_4$ m$^{-2}$ hr$^{-1}$). By running this model from March 1, 2012 through February 28, 2013 we estimate an annual flux of 3.1 g CH$_4$-C m$^2$ y$^{-1}$ from the CCW.
2.3.2 Carbon Balance

During the sampling dates when CH$_4$ flux was large enough to be detectable (May and September), its contribution to radiative forcing was relatively minor on average (less than one-third) when compared to soil CO$_2$ flux using a newly suggested 100-year sustained global warming potential of 45 for CH$_4$ (Neubauer and Megonigal 2015) (Fig. 5). If we make the same comparison using our modelled annual CO$_2$ and CH$_4$ flux estimates we find CH$_4$ contributes approximately 8 to 12 percent of the radiative forcing budget. The CO$_2$:sequestration:CH$_4$-flux ratio of CCW ranges from 125 to 26, corresponding to a radiative forcing switchover time range of less than 300 years following Neubauer’s (2014) model.

Figure 5: Carbon dioxide (CO$_2$) and methane (CH$_4$) flux from soil across five levels of organic matter loading rates estimated from sampling on 7 May and 26 September, 2012 at the Charles City Wetland in Charles City County, Virginia, USA. Note: CH$_4$ was converted to CO$_2$-equivalents by multiplying by 45—its 100-year
sustained global warming potential following Neubauer and Megonigal (2015). Error bars represent standard errors of the mean.

### 2.3.3 Hydrology and Soil Elevations

Water level data suggest that the hydrology of CCW is controlled by precipitation inputs with storm events and dry spells driving periodic fluctuations of more than 1 m in the water table (Fig. 6). Ponded water was present at the site 59 percent of the time from 22 February, 2012 to 21 January, 2013 and reached a maximum depth of 14 cm above the mean elevation of unamended plots. The distribution of plot elevations is approximately normally distributed with a standard deviation of 4 cm and two outliers: a 12 cm “hummock” and a -9 cm “hollow.” Pairwise comparison (ANOVA) of plots grouped by OM loading rate shows no significant differences in mean elevation, though there is a weak ($r^2 = 0.18$), but significant ($p < 0.05$) positive linear trend in elevation across the OM gradient.
2.3.4 Soil Nutrients

Total soil C data show that as much as 50% of the added OM has been lost since 2005 (Bailey et al. 2007), with the biggest loss observed in plots loaded with 112 and 224 kg m\(^{-2}\) OM. Nevertheless, the gradient, as originally established, persists (see Fig. 7), with total C in the top 10 cm of soil ranging from approximately 2 to 13 percent. Mean and standard error (SE) litter fall across the plots during the fall of 2012 was 0.37 ± 0.045 kg m\(^{-2}\), which assuming litter is 50% C by weight (Bocock 1964), represents an input of 0.19 ± 0.023 kg C m\(^{-2}\) y\(^{-1}\) to surface soils. Total soil C, N and P are generally higher in plots that received higher loading rates of OM, but decrease with depth such that
differences between loading rates are negligible at 10 and 20 cm depth. KCl-extractable 
NH₄ and NO₃ and Mehlich-3-extractable P follow roughly similar patterns.

Figure 7: Linear regressions of mean (±SE) total carbon in top 10 cm of soil in 
2011 across organic matter amendment plots at the Charles City Wetland in Charles 
City County, Virginia, USA. 2005 data from Bailey et al. (2007)

2.4 Discussion

2.4.1 Controls of Greenhouse Gas Emissions

Wetland GHG flux at the CCW is moderated by soil T because of the 
temperature dependence of soil respiration (Lloyd and Taylor 1994), as well as 
hydrologic dynamics. Hydrology is important because saturation inhibits aerobic 
decomposition and creates conditions favorable for CH₄ emission (Whalen 2005), as is 
illustrated by the soil volumetric water content threshold we identified (Fig. 4). In
addition to facilitating CH₄ flux, high water levels are also associated with lower rates of CO₂ emission because soil respiration is typically oxygen-limited in a wetland setting. Our hydrologic data are consistent with previous work indicating that the CCW is a groundwater recharge system with hydrologic inputs dominated by precipitation (Despres 2004).

Related to hydrology is the elevation of surface soil. While the site has very little relief, microtopographic features (< 10 cm) are related to OM loading rate and can be significant relative to water depth at the CCW. During OM addition to the CCW in 2001 there was difficulty in completely incorporating the highest OM loading rates into plots, which led to mounding (Daniels et al. 2005). Therefore we suspected elevation might be a confounding factor in predicting GHG flux since it correlates with OM loading rate, but when we included it as a predictor of GHG fluxes in our GLM tests it did not emerge as significant. Thus we conclude that differences in elevation across the OM gradient are not driving seasonal GHG patterns.

In addition to soil T and hydrology, surface soil C also correlates with CO₂ flux, which suggests that the addition of OM causes higher rates of aerobic decomposition and/or root respiration. We assume that soil N at depth correlates with CO₂ flux because a greater N pool in the rooting zone should stimulate higher rates of autotrophic and heterotrophic respiration related to N mineralization (Schlesinger 1997).
While we were able to find some relationships between soil C/N and CO$_2$ flux, CH$_4$ did not correlate with any of the soil chemical properties we measured. Hydrology and T both control rates of CH$_4$ production by dictating oxygen availability and demand (Whalen 2005), which explains why we found CH$_4$ flux to be very low during cold and/or dry periods. CH$_4$ flux variability is consistent with results from other forested wetlands of the Southeastern US but our annual CH$_4$ flux estimate was on the low end of the range of published estimates for analogous systems (Table 3).

**Table 3: Review of methane (CH$_4$) emissions rates in kg CH$_4$-C ha$^{-1}$ y$^{-1}$ from natural and restored forested wetlands of the Southeastern United States.**

<table>
<thead>
<tr>
<th>CH$_4$ flux</th>
<th>Location</th>
<th>Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>311</td>
<td>Ogeechee River, Ga. (west)</td>
<td>Natural</td>
<td>(Pulliam 1993)</td>
</tr>
<tr>
<td>297</td>
<td>Okefenokee Swamp, Ga.</td>
<td>Natural</td>
<td>(Flebbe 1982)</td>
</tr>
<tr>
<td>262</td>
<td>Creeping Swamp, NC</td>
<td>Natural</td>
<td>(Pulliam 1981)</td>
</tr>
<tr>
<td>107</td>
<td>Timberlake Restoration Preserve, NC</td>
<td>Restored</td>
<td>(Morse et al. 2012)</td>
</tr>
<tr>
<td>92</td>
<td>Ogeechee River, Ga. (east)</td>
<td>Natural</td>
<td>(Pulliam 1993)</td>
</tr>
<tr>
<td>72</td>
<td>Palmetto Peartree Preserve, NC</td>
<td>Natural</td>
<td>(Morse et al. 2012)</td>
</tr>
<tr>
<td><strong>31</strong></td>
<td><strong>Charles City Wetland, Va.</strong></td>
<td><strong>Restored</strong></td>
<td><strong>This study</strong></td>
</tr>
<tr>
<td>14</td>
<td>Timberlake Restoration Preserve, NC</td>
<td>Restored</td>
<td>(Morse et al. 2012)</td>
</tr>
<tr>
<td>0.5</td>
<td>Timberlake Restoration Preserve, NC</td>
<td>Restored</td>
<td>(Morse et al. 2012)</td>
</tr>
</tbody>
</table>

Our CH$_4$ flux shows no significant relationship with OM loading rate, suggesting that if excess nutrients and enhanced primary productivity are enhancing methane production, then the increase is being cancelled out by concomitant CH$_4$ oxidation. This result contrasts with findings from another study in which addition of OM to intact
wetland soil cores led to higher rates of potential net methane emissions compared to controls (Ballentine et al. 2015). Ballentine et al. (2015) found that adding OM created conditions more favorable for methanogenesis through an indirect mechanism—by increasing water retention. At the CCW, OM additions may have had the opposite effect on soil moisture because of a slight mounding effect as described earlier. Our data from the relatively drier months of May and July show weak (r-squared of 0.16 and 0.14, respectively), marginally significant (p < 0.09 and p < 0.11, respectively) relationships between SVWC and OM loading rate. Increasing soil C by adding OM does not necessarily provide additional C substrate for methanogens, but it may alter methane production and/or oxidation because of indirect hydrologic effects. Heavy OM addition may elevate the soil surface allowing for more oxic conditions (our study), or conversely, it may enhance water holding capacity facilitating anoxia (Ballantine et al. 2015).

The OM gradient at the CCW does not incorporate a test of the effects of different OM types and results of other studies conflict as to whether or not OM quality matters. Ballentine et al. (2015) found that several OM sources (straw, topsoil, straw + biochar, biochar) led to statistically indistinguishable increases in potential net CH₄ emissions over control. In contrast Morrissey et al. (2013) found added leaf litter led to increased CH₄ flux, but added compost did not. It is noteworthy that our results are consistent with one of the conclusions reached by Morrissey et al. (2013): that adding compost increases CO₂ flux, but not CH₄ flux.
2.4.2 Climate Impacts of Created/Restored Wetlands

The radiative forcing switchover time (Frolking et al. 2006) for CCW is uncertain because of high variability in NEE (Bailey 2006) and CH$_4$ flux data (this study). Furthermore, for this analysis we must assume that CH$_4$ emissions and NEE will remain constant over many decades. In reality NEE is likely to be dynamic over at least several decades of succession (Odum 1969). The temporal mismatch in our input data—NEE data from 2006 and CH$_4$ data from 2012—is a potential source of error. If the CCW ecosystem has become a more efficient C sink over the 6-year period as trees have matured, then we may be overestimating radiative forcing switchover time. Therefore it would take a long-term monitoring approach to improve the certainty of radiative forcing switchover time for the CCW. But despite the shortcomings mentioned above, we may conclude that CCW has a relatively short radiative forcing switchover time due to its low CH$_4$ flux. Importantly, CCW will likely become a net GHG sink more quickly than at least six out of eight wetlands analyzed by Neubauer (2014).

While we may be tempted to generalize the results from CCW to all other created/restored wetlands, we caution that wetlands are diverse, idiosyncratic ecosystems, especially those which have undergone intense transformations, as the CCW has. Many factors determine how much CH$_4$ is produced and emitted, including the quality of any added OM, type of plant cover, productivity and hydrology. Since all data were collected from a single site amended with a single OM type (compost), our
conclusions may or may not apply to other created/restored wetlands but our analytical approach for assessing radiative forcing switchover time should prove useful.

2.4.3 Temporal Changes at Charles City Wetland

We found micro-elevational differences between plots, described above, to be less pronounced in 2012 compared to conditions in 2005 reported by Bailey et al. (2007). The relationship between OM loading rate and elevation was far weaker in 2012 (see Table 4), which could be the result of settling or subsidence due to more rapid OM oxidation in elevated, high-OM plots. The higher rates of soil respiration that we and Bailey et al. (2007) detected coming from higher OM plots are consistent with an oxidation-subsidence explanation for the loss of elevation, as is the difference in total soil C between 2005 and 2012 we observed (see Fig. 7).

<table>
<thead>
<tr>
<th>Year</th>
<th>Rainfall (Apr. - Oct.; cm)</th>
<th>Elevation across OM loading rates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>total</td>
<td>depart. from normal</td>
</tr>
<tr>
<td>2005</td>
<td>69</td>
<td>-7.5</td>
</tr>
<tr>
<td>2012</td>
<td>82</td>
<td>+5.7</td>
</tr>
</tbody>
</table>

Our annual soil respiration budget does not fully account for the soil C losses in the 112 and 224 kg m$^{-2}$ plots we observe between 2005 and 2012, which were approximately 3.4 and 8.9 percent C, respectively (Fig. 7), corresponding to respective losses of 0.8 and 1.6 kg C m$^{-2}$ y$^{-1}$ over seven years from the top 10 cm of soil. These loss
rates are two to four times greater than our estimated annual soil respiration loss from these plots: 0.42 and 0.49 ± 0.032 kg CO$_2$-C m$^{-2}$ y$^{-1}$ respectively, but bracket estimates of approximately 1.3 and 1.4 kg CO$_2$-C m$^{-2}$ y$^{-1}$ respectively made by Bailey (2006). The relatively wet conditions in 2012 relative to 2005 (Table 4) could explain why we found soil respiration to be lower than Bailey (2006) since saturation inhibits soil respiration.

The CCW was relatively wet during the 2012 growing season when it received 82 cm of rain (7.5 percent above mean; National Climatic Data Center; Lawrimore et al. 2011) and held ponded water 52 percent of the time. This contrasts with conditions during the 2005 growing season when the CCW received 10 percent less rainfall than average (National Climatic Data Center; Lawrimore et al. 2011) and water was ponded just 25% of the time (Bailey et al. 2007). We also suspect that the rate of C loss has decreased as soil C content decreased leaving more recalcitrant materials over the past seven years. Some C loss may also be due to leaching of dissolved OM into lower soil layers and/or transport of particulate OM during floods.

The overall seasonal pattern in soil CO$_2$ flux we observe is similar to what Bailey (2006) reported from the CCW for 2005/2006 with peak respiration of greater than 400 mg m$^{-2}$ h$^{-1}$ during summer dry periods and low CO$_2$ flux of less than 100 mg m$^{-2}$ h$^{-1}$ during wet winter months. The positive relationship between CO$_2$ flux and soil OM loading rate is also consistent with Bailey’s (2006) results.
2.4.4 Conclusions

We found little evidence to suggest that added composted yard waste increases CH$_4$ emissions from CCW a decade after restoration. CH$_4$ emissions are only significant when soils are warm and water levels and soil moisture are high. Even when CH$_4$ flux is at its greatest magnitude, it still represents a relatively modest contribution to radiative forcing compared to soil CO$_2$ flux.

Yet even if CCW were to produce no CH$_4$, it would still be a net CO$_2$ source at high OM loading rates because of negative NEE (Bailey 2006), at least until the excess OM is respired. Therefore the addition of high levels of OM (>11.2 kg m$^{-2}$) to created wetlands may be detrimental to greenhouse gas budgets due to enhanced soil respiration. At the CCW, heavy additions of OM decompose over time while adding little in the way of tangible productivity increases (Bailey et al. 2007) or soil geochemistry improvement (Bruland and Richardson 2009), not to mention incurring greater material transport and associated construction costs.
3. A cost-effective method for reducing soil disturbance-induced errors in static chamber measurement of wetland methane emissions

3.1 Introduction

Methane (CH$_4$) is the second most important long-lived greenhouse gas (GHG) after carbon dioxide (Myhre et al. 2013), with wetlands representing the single-most important source to the atmosphere (Bridgham et al. 2006). As a result, the estimation of CH$_4$ flux has become an important component of many studies of wetland carbon biogeochemical cycling, with the non-flow-through-non-steady-state chamber method (Livingston and Hutchinson 1995), or “static chamber” method, most popular because of its simplicity and cost-effectiveness.

It is popular to use simple linear regression of chamber headspace concentrations as a function of time to estimate gas emissions rate (Weishampel and Kolka 2008), but the act of repeated sampling from static chambers can cause soil disturbance and significant deviations from linearity (Forbrich et al. 2010). Dissolved CH$_4$ within saturated or inundated wetland soil pores is particularly sensitive to pressure changes, which can lead to CH$_4$ ebullition (Strack et al. 2005). Ebullition can occur in absence of artificial disturbance, but disturbance-induced ebullition is typically the cause of nonlinear (based on an $r^2$ threshold of 0.9) or unusable CH$_4$ flux data. Studies do not always report how much data are discarded due to soil disturbance, but anywhere from 45% (Nahlik and Mitsch 2010) to 65% (Morse et al. 2012) of static chamber incubations...
can be affected. Loss of such a high proportion of data is not only resource-inefficient, but also poses the threat of introducing sampling errors and bias.

After observing persistent evidence that disturbance was affecting our CH₄ flux results using a rubber-gasket (RG) static chamber (Weishampel and Kolka 2008), we redesigned our static chambers to incorporate a water-filled-gutter (WFG) seal (Livingston and Hutchinson 1995, Wang et al. 2006, Krauss and Whitbeck 2011), which allows setup and sampling to be conducted from a distance of 2 m using a remote rod sampling system (RRSS) so as to minimize wetland soil disturbance (Fig. 8). Here we compare reliability data from our WFG static chamber with RRSS compared against those of a more conventional RG static chamber. We also present reliability data testing whether the WFG seal alone or remote setup using the RRSS in conjunction with the WFG is necessary to improve reliability.

Our findings may help improve field effort efficiency and eliminate boardwalk construction costs for those planning to utilize static chambers for estimation of CH₄ gas flux from wetlands.

3.2 Methods

The study took place within the 20.8-hectare Charles City Wetland Mitigation Site, which is located in Charles City County, Virginia, USA, and owned by the Virginia Department of Transportation as part of its compensatory mitigation program (Bailey et al., 2007). Precipitation is the dominant hydrologic input and the CCW may hold up to
0.5 m of standing water during cooler months (Bailey et al. 2007). The soil is mapped as a complex of Chickahominy (fine, mixed, semiactive, thermic Type Endoaquults) and Newflat (fine, mixed, subactive, thermic Aeric Endoaquults) (Bergschneider 2005). Site history is complex, with one unsuccessful restoration attempt for this formerly forested wetland being followed by an experimental addition of organic matter amendments to improve tree growth. For more detail on site characteristics, see Bruland and Richardson (2004), Bergschneider (2005), and Bailey et al. (2007).

In late summer 2011 we imbedded a 20 cm diameter PVC collar 10 to 15 cm into the soil in each of 20 plots (4.6 by 3.1 m) for RG static chamber trace gas sampling. We set up the RG static chambers by placing a PVC cap with a rubber gasket over beveled collar tops by hand. Mean assembled RG chamber height was approximately 13.5 cm corresponding to a volume of >4 l. We sampled gas using RG static chambers from 20 collars in September and October, 2011 and from 27 collars in February, 2012 for a total of 67 sets of gas measurements.

In spring of 2012 we imbedded 45 cm diameter plastic collars affixed with water-fillable gutters to a depth of 10 to 15 cm in the same 20 plots for WFG static chamber trace gas sampling using RRSS. Both WFG and RG chamber caps are equipped with an internal computer fan powered by a 9-volt battery to circulate chamber headspace as recommended by Christiansen et al (2011) and a thermocouple allowing for internal chamber temperature (T) to be recorded during each sample extraction. We coated the
WFG caps with reflective aluminum foil to minimize solar warming as recommended by the US Department of Agriculture (Parkin and Venterea 2010).

We set up and sampled WFG static chambers from a distance of approximately 2 m using our RRSS in which we fill the gutter with water using a 2 m polyethylene tube and place the plastic top using a 2 m rod. We then extracted air samples using a plastic syringe via the 2 m, 1 mm inner diameter plastic tube following flushing to mix tube air with chamber headspace (total tube volume is 1.6 ml—approximately 0.01 percent of headspace volume). Mean height of assembled WFG chamber was 23 cm with headspace volume ranging from approximately 10 liters during periods of high water to 16 liters during dry periods. We sampled CH4 gas using WFG static chambers from 20 collars in May, July, September (twice) and November, 2012 and February, 2013 for a total of 120 sets of gas measurements. During one of the two sampling dates in September 2012 we sampled 10 of the 20 WFG static chambers without using the RRSS for setup. Instead we simply walked up to fill the gutter and placed the chamber top by hand (the traditional method; required when using RG) to test for disturbance during WFG setup without RRSS.
Figure 8: Illustrations of the water-filled-gutter static chamber design that allows use of remote rod sampling system (RRSS): Photograph of chamber being deployed using RRSS in the Charles City Wetland in Charles City County, Virginia (a); schematic of chamber disassembled to reveal water fillable gutter on rim of collar that creates an air tight seal, internal fan to mix headspace air and thermocouple to monitor internal chamber temperature (b); schematic of chamber assembled (c)

After setup of both RG and WFG static chambers we immediately extracted a 50-ml headspace sample via a plastic syringe and deposited it into a mylar gas-tight sample bag. We recorded ambient air temperature (T), internal chamber T, soil T at 5 cm depth for initial and subsequent samples taken approximately 5, 15 and 30 minutes following chamber setup. Total extracted sample volume (200 ml) was never more than 5 percent of total headspace volume and acts to counterbalance the pressure buildup from emitted gases over 30 minutes of incubation. We transported gas bags to the Duke University Wetland Center laboratory and analyzed within one week of sampling on a Varian 450
Gas Chromatograph (GC) equipped with a flame ionization detector. We analyzed duplicates of all samples and when duplicate values differed by <10% we used the mean for flux calculations.

In addition to CH₄, we simultaneously measured carbon dioxide (CO₂) concentrations using a methanizer in our GC, allowing us to test for static chamber sampling effects on CO₂. Since CO₂ is approximately 50 times more soluble in water, it is far less susceptible to disturbance-induced ebullition effects and did not manifest obvious performance differences between RG and WFG chambers with or without the RRSS system. Therefore we limit the scope of this paper to the effects we observed on CH₄ reliability. CH₄ flux estimates at the CCW (described in detail in Winton & Richardson, 2015b) range from below detection limits to as high as approximately 5 mg m⁻² hr⁻¹.

We consider two performance metrics in our evaluation of chamber reliability: 1) the initial headspace CH₄ concentration (C_initial) as sampled immediately after chamber setup, with higher values indicative of more soil disturbance during chamber setup; and 2) r² of the calculated regression line.

We separately analyzed data from one of our two sampling dates in September, 2012 when 10 of 20 plots were sampled without using the RRSS for WFG static chamber setup, to evaluate the importance of the RRSS in improving data reliability over WFG static chambers set up by hand.
To test for differences in mean \( C_{\text{initial}} \) between RG (n = 67) and WFG with RRSS (n = 100) static chamber methods, and between WFG with and without the RRSS, we use Welch’s t-test on data log-transformed to better meet the assumption of a normal distribution. To test for differences in mean \( r^2 \) between RG and WFG with RRSS methods, we excluded 54 “no flux” regressions (Pedersen et al. 2010) that showed insignificant deviations from ambient CH\(_4\) concentration over the four sample points since relatively minor concentration differences stemming from experimental or analytical error often produced a low \( r^2 \) value unrelated to chamber or soil disturbance during setup or sampling. We used the non-parametric Mann-Whitney U test on rank-transformed \( r^2 \) absolute-values from the remaining linear regressions since the distributions of \( r^2 \) absolute-values do not adequately meet the normal distribution parameter required by t-tests.

Since we sampled from WFG chambers with the RRSS subsequent to our sampling from RG chambers (rather than a side-by-side comparison), it is necessary to investigate whether site conditions can explain differences in performance results. We consider water level and chamber T as potentially important site condition variables because they each have potential to influence the pressure on interstitial soil gases and ebullition. To evaluate the relative importance of site conditions on CH\(_4\) data quality, we compared the mean and standard deviation of standing water level and T between static chamber methods. We compared chamber T recorded between methods using a
Welch’s t-test. Conventional statistical tests are not appropriate for water level, which contains excessive zeros (indicating water below the soil surface), so we converted the water level data to a binary factor indicating the presence/absence of ponded water. We tested whether the presence/absence of ponded water or chamber design had a more important impact of log-transformed \( \mathrm{CH}_4 \) concentration using a 2-way analysis of variance (ANOVA) and Tukey’s honest significant differences test.

### 3.3 Results

Incubations from selected plots (sampled 21 October 2011 using RG; 26 September 2012 using WFG with RRSS) illustrate the differences in data reliability typically observed between the two sampling methods. RG static chambers often produced \( C_{\text{initial}} \) values that were inflated one to three orders of magnitude above ambient \( \mathrm{CH}_4 \) concentration with nonlinear \( \mathrm{CH}_4 \) accumulation over time (Fig. 9A). In contrast, WFG static chambers sampled using the RRSS regularly produced circum-ambient \( C_{\text{initial}} \) values and linear slopes (Fig. 9B).
Figure 9: Examples of gas data illustrating unsuccessful use of a rubber-gasket static chamber design on 21 October 2011 (a) and successful use of water-filled-gutter static chamber design with remote rod sampling system on 26 September 2012 (b)

Use of RG static chambers produced a $C_{\text{initial}}$ greater than 3.6 ppm (double ambient CH$_4$ concentration) in 44 out of 67 (66%) incubations and a $C_{\text{initial}}$ greater than 18 ppm (10 times ambient CH$_4$ concentration) in 30 out of 67 (45%) incubations compared to 6 out of 100 (6%) and 0 out 100 (0%) when using WFG static chambers with RRSS (see
Fig. 10A. Welch’s t-test measured significantly higher mean $C_{\text{initial}}$ values ($p < .0001$) from RG compared to WFG static chambers with RRSS.

Figure 10: Tukey boxplots of log-transformed initial CH4 concentrations in chamber headspace (a) and $r$-squared value of linear regression line used to estimate CH4 flux (b) from 67 incubations using the rubber-gasket static chamber design taken
from Sept. 2011 through Feb. 2012, and 110 incubations using the water-filled-gutter with remote rod sampling system method taken from May 2012 through Jan. 2013. Dashed lines mark ambient CH4 concentration (a) and r-squared threshold of 0.9 (b)

Use of RG static chambers produced an r² greater than 0.9 for just 29 out of 64 (45%) non “no flux” linear regressions compared to 42 out of 49 (86%) for those of the WFG static chamber with RRSS (see Fig. 10B). Mann-Whitney U test found significantly lower mean r² values (p < .0001) from RG compared to WFG static chambers with RRSS.

To determine if environmental site conditions found in 2011 versus 2012 can explain the differences in performance results between the RG compared to WFG static chambers with RRSS we compared water tables and soil temperatures. We found that chamber T was similar during RG and WFG static chamber sampling both years, with means (±standard deviation) of 21.6 (±3.3) and 20.5 (±7.0) degrees C respectively. A Welch’s t-test found the difference in mean chamber T to not be significant (p = 0.18). Water level data suggests that conditions may have been wetter during RG static chamber sampling compared to WFG, with mean (±standard deviation) standing water of 1.8 cm (±2.8) and 0.8 cm (±1.7) cm respectively. Ponded water was present for 32 of 67 (48%) RG chamber incubations compared to just 30 out of 100 (30%) of WFG chamber incubations. However, a 2-way ANOVA and Tukey’s honest significant differences test found that differences in $C_{\text{initial}}$ were related to sampling method (p = 0.0001) rather than the presence/absence of ponded water (p = 0.86).
We tested whether it was necessary to use our RRSS with the WFG by sampling half the WFG chambers without the RRSS in September 2012. In these 10 incubations the RRSS system never produced $C_{\text{initial}}$ values greater than 3.6 ppm. When we did not use the RRSS, in contrast, all $C_{\text{initial}}$ values were greater than 3.6 ppm with 5 out of 10 greater than 18 ppm (Fig. 11). The Welch’s t-test found that the RRSS produced significantly lower ($p < .001$) mean $C_{\text{initial}}$ values. When we used the RRSS, 9 out of 9 incubations that did not meet “no flux” criteria, produced $r^2$ values greater than 0.9 compared to just 7 out of 10 when the we did not use the RRSS for setup. While, the Mann-Whitney U test for a difference in mean $r^2$ values did not produce sufficient evidence to reject the null the comparison shows that more samples will be lost when the RRSS system is not used.
Figure 11: Tukey boxplot of log-transformed initial CH4 concentration in chamber headspace from 10 incubations using remote rod sampling system to setup the chamber cap to minimize disturbance and from 10 incubations for which the chamber cap set up by hand. Dashed line marks ambient CH4 concentration.

3.4 Discussion

In studies of CH4 flux from wetlands, static chamber design and setup can have a huge influence on data reliability, and therefore on the efficiency of data collection effort. Use of a RG-style static chamber (Weishampel and Kolka 2008) or any design that requires a close approach during chamber setup and sampling has the potential to cause soil compression resulting in high initial CH4 values. While disturbance may be imperceptible to a field observer, our data suggest it can artificially inflate C_{initial} and/or reduce linear regression r^2 such that a large portion of flux estimates must be discarded, thus reducing statistical power and introducing a potential source of bias.
We found that a WFG static chamber design used in conjunction with our newly developed RRSS produced more reliable and readily-useable CH$_4$ concentration data for flux estimates compared to other wetland studies that have reported static chamber CH$_4$ data loss (Nahlik and Mitsch 2010, Morse et al. 2012). Elevated $C_{\text{initial}}$ values, which we frequently detected when not using the RRSS, indicate that CH$_4$ has been purged from soil pores into the chamber headspace during setup. While this could be related to pushing on the PVC cap of RG static chambers to form a good seal, the results of our test of the RRSS for WFG static chamber setup suggest that elevated $C_{\text{initial}}$ can be caused by merely standing close to the chamber collars with either RG or WFG designs. Elevated $C_{\text{initial}}$ on its own may not necessarily lead to a decrease in $r^2$, but repeated approaches to the chamber during each of the four sampling time points, as we did before implementing the RRSS, significantly compromised regression linearity, thus leading to a rejection of a large number of measurements.

For decades researchers have discussed optimal chamber design for achieving precision and accuracy in the estimation of gas flux from soils (Hutchinson and Mosier 1981, Anthony et al. 1995, Conen and Smith 2000, Forbrich et al. 2010). The data we present here demonstrate that disturbance of wetland soil around static chambers can be effectively avoided by employing a WFG that allows for chambers to be set up and gas to be sampled using a RRSS to prevent soil disturbance. We suggest that disturbance is far more likely to impair data reliability than other potential sources of error endemic to
the static chamber method, such as slight variations in internal chamber pressure due to extracted sample volume.

Chambers measured continuously using laser off-axis integrated-cavity output spectroscopy (Mastepanov et al. 2008), automated flux chambers (Scott et al. 1999) and eddy covariance towers (Kormann et al. 2001) are viable alternative methods for estimating methane flux from wetlands while avoiding soil disturbance, but they can increase costs by two or three orders of magnitude and the latter may not be appropriate for small footprint applications. The manual static chamber method remains popular because of its efficiency and cost-effectiveness, yet in a wetland setting a standard practice to avoid soil disturbance during sampling is to construct boardwalks, which can be costly, labor-intensive, and logistically impractical. Our newly developed RRSS circumvents the need for boardwalks.
4. Wetland herbivores disrupt methane oxidation and nitrogen cycling

4.1 Introduction

Ecosystem structure is strongly influenced by herbivory in many contexts (Milchunas et al. 1988, Hulme 1996, Augustine and McNaughton 1998, Olff and Ritchie 1998, Malhi et al. 2016) and wetlands are no exception. Recent high-profile work in salt marshes demonstrated the impact of grazing snails on the extent of salt marshes (Silliman et al. 2005), and the ability of herbivores, such as waterfowl (anseniformes—ducks, geese and swans), to alter the density and composition of wetland vegetation has been recognized for decades (Anderson and Low 1976, Evers et al. 1998, Idestam-Almquist 1998, Froelich and Lodge 2000, Badzinski et al. 2006, Veen et al. 2013). Yet, the biogeochemical consequences of such intensive herbivory are largely unknown, especially as it relates to greenhouse gas fluxes and nitrogen (N) cycling.

Hydrophytes, the food source for aquatic herbivores, are notable for their ability to efficiently transport oxygen (O$_2$) through aerynchyma to their roots, as well as rooting zone soils in a process called radial O$_2$ loss, in which O$_2$ diffuses into soils from root tips (Armstrong 1964). Wetland plants utilize a variety of physiological adaptions to achieve O$_2$ transport through their stems, a trait ubiquitous among hydrophytes (Sculthorpe 1967) to overcome the stresses of hypoxia, such as the phytotoxicity of chemically reduced compounds (Penhale and Wetzel 1983, Lamers et al. 1998). The presence of an oxidized rhizosphere—essentially an extension of the oxic atmosphere into otherwise
hypoxic or anoxic wetland soils—creates a complex, high surface-area hyporrheic zone, which facilitates the adjacency of diverse redox conditions. Thus radial O$_2$ loss has profound influence on many wetland biogeochemical processes governed by oxidation-reduction reactions, for example, those involving iron (Green and Etherington 1977, Sundby et al. 1998), methane (CH$_4$) (King 1994, Jespersen et al. 1998) and N (Reddy et al. 1989).

Evidence for the importance of plant-mediated O$_2$ transport on wetland biogeochemical functioning has inspired a few studies of herbivory effects on CH$_4$ dynamics. Such investigations have focused on either the effects of sediment bioturbation on the potential for CH$_4$ oxidation to occur (Huang et al. 2005, Bodelier et al. 2006), or on physical leaf damage reducing gas flow resistance and increasing the stem efflux of CH$_4$ (Dingemans et al. 2011, Petruzzella et al. 2015). However, none of these studies address the potential biogeochemical consequences of aquatic herbivory preventing the formation of an oxidized rhizosphere and none consider the potential implications for other sensitive biogeochemical processes, such as N cycling.

The testing of herbivory effects is made difficult because it has to be layered atop the complicated role of plants (Fig. 1). Vegetation is integral to wetland biogeochemical processes because it provides the C substrate, in the form of detritus and exudates, that drive all forms of wetland soil heterotrophic respiration (Whiting and Chanton 1993, Ding et al. 2005, Laanbroek 2010). In addition, the plant-mediated gas transport process
flows both ways. Plants not only transport O$_2$ into wetland soils, but they also allow gas by-products of wetland sediment respiration to diffuse into capillary-like root networks and follow concentration gradients up to and out of gas-permeable aerenchymous stems (Dacey and Klug 1979, Boon et al. 1995, Nat and Middelburg 1998). Gas transport by plants has important implications for wetland CH$_4$ emissions and its quantification. In the absence of plant-stem efflux pathways CH$_4$ often builds up in wetland sediments until it can escape via ebullition (Glaser et al. 2004). Since bubble-formation is strongly influenced by temperature and pressure, ebullition is often episodic and driven by sediment disturbance or a drop in water level (Comas and Wright 2012). Episodic ebullition presents a serious problem for ground-based chamber measurements of wetland CH$_4$ emission, as such approaches depend on the assumption that short duration measurements can be accurately extrapolated to long time periods (Strack et al. 2005, Comas et al. 2011).

Ebullition-related uncertainties aside, experiments designed to quantify the effect of emergent plants on CH$_4$ flux have typically utilized clipping manipulations (Schimel 1995, Shannon et al. 1996, Kelker and Chanton 1997). The results of these clipping experiments affirm that the plant-efflux pathway is important for CH$_4$ dynamics, leading to the generally accepted paradigm that aerenchymous emergent wetland vegetation enhances CH$_4$ emission. A literature review (Turetsky et al. 2014) confirms that, indeed, CH$_4$ emissions tend to be greater from wetlands dominated by emergent
graminoid vegetation. While this pattern is compelling, it does not disentangle the multiple ways in which plants impact CH₄ emissions—via providing substrate, an efflux pathway and an oxidized rhizosphere. Furthermore, plant community structure is closely related to, if not determined, by abiotic conditions, such as hydrology, soil properties and climate, further muddying the underlying reasons for the observed correlation between CH₄ and plant community.

Even the clipping experiments that form the empirical foundation of the plant-CH₄ paradigm don’t adequately test the holistic role that plants play. Clipping of culms below water level and/or the sealing of exposed clipped culms shuts off the gas transport, which has an instantaneous effect on the amount of CH₄ leaving the plants and the amount of O₂ entering sediments—both essentially drop to zero. In contrast, the poising effects of an oxidized rhizosphere, such as elevated redox potential and the presence of oxidized iron, has long-lasting effects on the production and oxidation of CH₄. Because of poise and the gradual nature of alternative electron acceptor depletion, soil slurry lab incubations, for example, typically monitor CH₄ production over periods of weeks or months (Bodelier et al. 2000). Thus field clipping experiments only measure short-term CH₄ effects and cannot account for the temporally lagging role radial O₂ loss plays in CH₄ oxidation. No study has experimentally prevented vegetation from emerging (i.e. via removal of roots and tubers in winter) which would test how the lack of oxidized rhizosphere development might influence CH₄ emission and contribute to a
more complete understanding of role that emergent plants and their consumers play in wetland \( \text{CH}_4 \) emission.

In addition to herbivory effects, waterfowl may influence the biogeochemical functioning of wetlands by enhancing decomposition through digestion and excretion (Malhi et al. 2016), and/or by transporting nutrients across landscapes (Doughty et al. 2015). For example, a large flock of geese in New Mexico supplies nearly 40% of N inputs to a managed wetland where it roosts (Post et al. 1998). It would be reasonable to assume that such a bird-derived enhancement in wetland N inputs would accelerate the N removal process of coupled nitrification-denitrification, and if so, the bird-driven increase in the availability of nitrate, an efficient alternate electron acceptor, would suppress \( \text{CH}_4 \) production. These potential biogeochemical impacts of waterfowl fecal inputs have not been investigated.

In this study, we conceptually model the hypothetical impacts of waterfowl on \( \text{CH}_4 \) and N cycling (Fig. 12) and test three specific hypotheses through a combination of field, greenhouse and laboratory experiments. Hypothesis 1: herbivory will reduce the efflux of \( \text{CH}_4 \) through plant stems, thus increasing \( \text{CH}_4 \) oxidation in soils and suppressing \( \text{CH}_4 \) emission. Hypothesis 2a: herbivory will inhibit \( \text{O}_2 \) transport into wetland sediments, thus enhancing \( \text{CH}_4 \) production and limiting \( \text{CH}_4 \) oxidation leading to greater \( \text{CH}_4 \) emission. Hypothesis 2b: diminished \( \text{O}_2 \) transport into wetland sediments by plants will limit nitrification, leading to lower nitrate availability, denitrification and
nitrous oxide emission. Hypothesis 3: waterfowl feces will increase nitrate availability, which will inhibit CH₄ production and reduce CH₄ emission.

Figure 12: Conceptual diagram of hypothetical effects of waterfowl on cycling of CH₄ and N in wetlands. H₁: Dashed arrows trace potential impact of herbivory disrupting plant-mediated efflux of biogenic gases, which would force CH₄ to diffuse or ebulliate slowly through surficial sediments where CH₄ oxidation is most effective. H₂: Split arrows trace potential impact of herbivory starving wetland sediments of plant-transported O₂, which would enhance the anaerobic process of CH₄ production, while inhibiting aerobic processes of CH₄ oxidation and nitrification. H₃: Solid bold arrows trace potential impact of enhanced N inputs from waterfowl feces, which could stimulate N cycling and inhibit CH₄ production by increasing availability of Nitrate, an efficient alternate electron acceptor.

The biogeochemical processes we hypothesize to be altered by waterfowl—CH₄ emission and N removal—are of critical environmental importance. Wetlands are the greatest source of atmospheric CH₄, a potent greenhouse gas (Bridgham et al. 2006), and are also valued for their capacity to transform, sequester and remove excess nitrate through coupled aerobic/anaerobic nitrification/denitrification (Firestone 1982). Aquatic herbivory may thus serve an important, unrecognized role in climate regulation and the eutrophication of aquatic ecosystems.
An understanding of the influence of waterfowl on wetland biogeochemistry also has important implications for organizations that manage and conserve wildlife. For example, a primary function of the U.S. Fish and Wildlife Service is to manage wetland habitats to support populations of approximately 78 million North America waterfowl (North American Waterfowl Management Plan Committee 2012). An understanding of the interactions between the huge bird populations residing on federal land and the magnitude of CH$_4$ emission from publicly managed wetland habitats is needed in order to meet the Department of the Interior mandate to achieve carbon neutrality (Fish and Wildlife Service 2010).

We investigate herbivory effects in a managed freshwater waterfowl impoundment, which is a common habitat archetype in North America. Waterfowl impoundments are widespread and management practices prescribed by state governments or the U.S. Fish and Wildlife Service drive these ecosystems toward convergent hydrologic and vegetation-herbivore regimes (Strader et al. 2005). Therefore we hypothesize that waterfowl populations have the potential to collectively exert important effects on CH$_4$ and N cycling across a large portfolio of publicly-managed emergent freshwater waterfowl impoundments in the southeastern United States. We summarize the extent of these waterfowl habitats and estimate their annual CH$_4$ emission to provide a contextualized regional estimate of their aggregate contribution to greenhouse gas flux.
4.2 Methods

4.2.1 Field Site Description

We implemented our exclosure experiment at Marsh Impoundment 10 North (MI10N) (35°32′08″N, 76°04′24″W), an 88-ha wetland constructed specifically to provide habitat for migratory birds at Mattamuskeet National Wildlife Refuge (MNWR) in Hyde County, North Carolina, United States. Dominant plant species in MI10N include *Phragmites australis, Eleocharis quadrangulata, Panicum dichotomiflorum, Bacopa monieri* and *Alternanthera philoxeroides*. MI10N soil is mapped as Weeksville loam (95%) and Engelhard loamy very fine sand (5%) (Soil Survey Staff 2015). At the east end of the refuge, MI10N is closed to hunting and has restricted public access.

A network of canals, flow control structures and pumps allow MNWR staff to control water levels in MI10N and other impoundments, which they do, following an Annual Marsh/Water Management Plan prepared by the US Fish and Wildlife North Carolina Migratory Bird Field Office to meet habitat objectives for migratory waterfowl and shorebirds (John Stanton, personal communication). In June of 2013 pumps were run for approximately 8 days to draw down 16 cm of standing water. In May 2014 pumps were run for approximately 17 days to draw down 34 cm of standing water.

Aerial surveys on the Refuge and surrounding agricultural fields have recorded recent 5-year means of 180,000 ducks and 32,000 Tundra Swan (*Cygnus Columbianus*) (Wires 2015) (Figs. 13A, B). Increases in local waterfowl numbers reflect long-running
positive trends in North American populations attributed to decades of successful management efforts (e.g., habitat preservation, restoration and construction; hunting regulations). Peak waterfowl counts recorded by Refuge staff during bimonthly surveys at MI10N included 5036 C. columbianus and 4992 dabbling ducks (Wires 2015), corresponding to a density of roughly 110 herbivores ha⁻¹.

Figure 13: Exclusion of overwintering waterfowl increases emergent plant regrowth, associated oxygen transport and reduces methane flux. (A) Waterfowl mega-flock at Mattamuskeet National Wildlife Refuge in North Carolina, USA. (B) Total ducks (pink circles) and swans (yellow triangles) counted at the Refuge during Midwinter Waterfowl Inventories by U.S. Fish and Wildlife Service 1961 to 2012. (C) Dense stands of Spike Rush (E. quadrangulata) in May within white quadrangles which delineate extent of exclosures (only installed from November through March)
to prevent grazing by waterfowl. (D) Fall: healthy emergent vegetation prior to senescence and transport of carbohydrates into roots and tubers. (E) Winter: Tundra Swan (C. columbianus) grazing and grubbing for macrophytes. Where herbivores are excluded by experimental exclosures, plant roots and tubers remain intact. (F) Spring: differential regrowth of emergent vegetation and associated differences in gas exchange. Grazed wetland areas (left side) are slow to regrow and lack oxygen inputs via plant aerenchyma, whereas areas protected from waterfowl herbivory (right side) regenerate emergent vegetation and oxygen transport more rapidly. Differences in plant-mediated oxygen inputs lead to differences in methane flux as indicated by arrow width.

4.2.2 Exclosure Experiment

To test for waterfowl effects, we established eight 2x2 m plastic bird exclosures in October 2012 (Figs. 13C-F). Exclosures were removed during waterfowl absence from March to October, 2013 to allow other large-bodied fauna, such as turtles, wading birds, raccoon (Procyon lotor), nutria (Myocastor coypus), muskrat (Ondatra zibethicus), white-tailed deer (Odocoileus virginianus) and red wolf (Canis rufus), to have unimpeded access to plots. We initially established four bird-accessible plots and added an additional four in spring of 2013 for a balanced experiment of eight exclosure and eight ‘bird’ plots. We chose eight replicates in order to detect trends despite the high spatial variability of CH₄ emission within wetlands (Whalen 2005, Strack and Waddington 2008). We sited plots randomly within a zone dominated by the emergent Eleocharis quadrangulata, a species known to support an oxidized rhizosphere (Michaud and Richardson 1989) and to serve as a food item for waterfowl (Rodewald 2015). Within each plot we installed a permanent porewater well for interstitial water sampling and an enamel-coated 2 m rebar rod to serve as a base for static chamber gas sampling.
4.2.3 Static Chamber Trace Gas Sampling

The nature of the study required that we design and build static chambers with the following criteria in mind: 1) chambers should be tall so that emergent vegetation can be left intact during sampling as clipping can impact plant-mediated flux (Laanbroek 2010); 2) chambers should be transparent so that plants can be photosynthetically active during incubations as gas may be emitted via stomata (Laanbroek 2010); and 3) chamber setup and sampling should be possible without disturbing wetland sediments as this can cause ebullition that biases flux data (Winton and Richardson 2015b). We built static chambers from 1 m sections of 55 cm diameter TEDLAR plastic sleeves that pass 88% of photosynthetically active radiation with circular Plexiglas caps fringed with closed-cell foam to make an air-tight seal (Fig. 13). We affixed the cap with a 2 m sampling tube (1 mm inner diameter), a wide-bore closeable exhaust vent and a thermocouple for measuring internal chamber temperature (T). To set up the static chamber apparatus for trace gas sampling we attached the cap to the sleeve using binder clips, inserted a plastic brace into the base of the sleeve to maintain a cylindrical chamber headspace, and carried it into the wetland hanging from a 2 m PVC pole. With the exhaust vent open we lowered the chamber to rest on the rebar base using the PVC pole. As the chamber ‘skirt’ submerged, air displaced by standing water was able to escape via the exhaust vent. We closed the vent before extracting the
first headspace sample. We conducted chamber set up and sampling from a submerged cinder block platform to avoid disturbing sediments and driving ebullition into the chamber. Before extracting each sample, we flushed the sampling tube to mix this small volume (approximately 3 ml) with the larger headspace (approximately 160 L). We extracted one 60 ml headspace sample immediately following chamber set up and four additional samples at 5 to 8 minutes intervals for a total of 5 samples over an incubation of approximately 30 minutes. We recorded internal chamber T immediately following each extracted sample. We measured chamber height above water level to calculate cylindrical chamber volume. During sampling dates with low or no surface water (July and Sept. 2013, May and June 2014), use of the chamber described above was not possible because standing water is necessary to create a sealed headspace. Instead we used a permanent collar static chamber method, with a water-filled gutter and remote rod sampling system (Winton and Richardson 2015b). Because of smaller chamber volume (approximately 15 L), we extracted 4 headspace samples instead of 5.
Figure 14: Photos of static chamber for measurement of trace gas emissions deployed at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge, North Carolina, USA.

4.2.4 Trace Gas Analysis

We stored gas samples collected at the field site in labeled Mylar bags for transport back to the Duke University Wetland Center laboratory. We analyzed samples for CH₄ and nitrous oxide within one week of collection using a Varian 450 gas chromatograph equipped with a flame ionization detector and electron capture detector. We ran all samples in duplicate with the mean value used for gas flux calculations unless duplicate values differed by >10%, in which case the obviously outlying value was assumed to stem from analytical error and discarded. We determined concentration
of CH$_4$ and N$_2$O from simultaneous measurements of five standard concentrations (Airgas Specialty Gases) and controlled for drifting baseline by running pairs of check standards for every 10 samples. Flux was estimated by linear regression of sample concentration as a function of time elapsed. If a threshold r-squared value of 0.90 was not met, we removed one or two outlying point if it improved fit to > 0.90 (15% of incubations), otherwise such estimates were treated as failed incubations and excluded from subsequent analysis (3% of incubations). We estimate the minimum detectable flux for CH$_4$ was 0.037 mg CH$_4$ m$^{-2}$ h$^{-1}$. For all flux estimates less than 3 times this minimum detectable flux we instead imputed the minimum value for analysis (24% of incubations). We followed the same protocol for nitrous oxide, except we estimated a minimum detectable flux of 0.013 mg N$_2$O m$^{-2}$ h$^{-1}$.

4.2.5 Cumulative Methane Flux analyses

We estimate cumulative CH$_4$ flux from each experimental plot for the duration of the experiment by interpolating linearly between field measurements and then calculated area under the resulting curve (Wickland et al. 1999). We excluded curves with missing data points (due to failed incubations) from subsequent analysis. We log-transformed the remaining cumulative CH$_4$ flux data to better meet the assumption of normality and equal variance and tested the hypothesis that bird plots emitted more cumulative CH$_4$ than exclosure plots using a Welch’s t-test. To estimate the effect of bird herbivory and bird exclusion on cumulative flux we calculated the ratio of geometric
mean cumulative CH$_4$ flux from exclosure and bird plots. We report this ratio as the effect of herbivory on CH$_4$ flux. We used the same method to calculate the difference between 2013 and 2014 peak season cumulative CH$_4$ fluxes and to estimate the yearly contribution of the peak season compared to the rest of the year. To estimate annual CH$_4$ emission we calculated cumulative emissions from two 12-month periods of data that each cover one of the two early growing season CH$_4$ emission peaks in 2013 and 2014. We used the arithmetic mean of these two annual CH$_4$ flux estimates to report annual flux from the site with and without birds.

4.2.6 Dissolved Trace Gas Measurement

To evaluate soil CH$_4$ production and assess the role played by ebullition in CH$_4$ emission in our plots, we calculated dissolved trace gases using the headspace equilibrium method (Kampbell et al. 1989). Briefly, we injected 50 ml of water into mylar gas bags preloaded with 100 ml of dinitrogen. We vigorously shook all bags for five minutes to ensure equilibrium between liquid and gas phases at lab T and pressure and then extracted headspace gas for analysis by gas chromatograph. We used Henry’s law to estimate dissolved CH$_4$ in the water samples based on the concentration of CH$_4$ in headspace.

4.2.7 Extractable Nitrate

To evaluate the amount of oxygen available to surface sediments we tested surface soils for nitrate/nitrite, which is produced by the aerobic process of nitrification.
During each site visit, we collected surface soil (top 5 cm) from each plot in triplicate and made a field composite. We performed a potassium chloride salt extraction on a 5 g soil subsample. We analyzed the extracts for nitrate/nitrite using a Lachat Quickchem 8000 autoanalyzer. We weighed and then oven-dried the remaining soil to calculate a wet:dry ratio, which we used to estimate extractable nitrate/nitrite per dry g of soil.

4.2.8 Vegetation Measurements

To detect grazer effects on vegetation we conducted stem counts and measured belowground biomass. Each time we measured gas flux we used the static chamber footprint as a sample area to estimate emergent stem density in each plot. We counted emergent *E. quadrangulata* stems within the chamber footprint and converted to stems per m². In April 2013 we took 3 replicate 30 cm deep punch cores from each plot using a 10 cm diameter plunger corer. We split the cores into surface to 15 cm and 15 to 30 cm sections and passed them through a 2 mm sieve to extract coarse belowground biomass. We oven-dried and then weighed the belowground biomass.

4.2.9 Direct Herbivore Observations

To document and quantify herbivore activity in our un-exclosed plots, we deployed time-lapse cameras during the waterfowl seasons (November to March) of both study years. Cameras captured images of four plots every five minutes during daylight hours generating approximately 80,000 images. We counted swans and ducks present within plots and divided by 12 to yield an estimate of bird-use hours.
4.2.10 Mesocosm Experiment

To test for effects of bird nutrient inputs on CH₄ flux we implemented a mesocosm dosing experiment in the Duke University Greenhouse. In September, 2013 we collected 10 kg of soil from our field site at Mattamuskeet National Wildlife Refuge in each of 24 5-gallon (19 L) plastic buckets. We multiplied our bird-use estimates, derived from our direct camera trap observations, by published waterfowl defecation masses and rates (Hahn et al. 2007) to estimate a range of fecal mass that should be added to a mesocosm to simulate in situ loading. We collected waterfowl feces from Sylvan Heights Waterfowl Park in Scotland Neck, NC and in March 2014 and dosed one-third of mesocosms with approximately 30 g of feces, one-third with approximately 10 g of feces and left the final third as a negative control. We report results from June and July, 73 and 103 days after initial dosage, respectively.
Figure 15: Mesocosms containing soil and plants from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA used for waterfowl feces addition experiment in the Duke University Greenhouse.

4.2.11 Potential Methane Production Assay

To test whether bird activity increased the capacity for impoundment soil to produce CH₄, we set up a potential CH₄ production assay in vitro (Bodelier et al. 2000).

We collected surface soils (0 to 5 cm) from the field experiment plots in triplicate in June 2014 and placed approximately 20 g in each of three 150 ml sealed flasks for a total of 48
incubations. We added 100 ml of helium-sparged de-ionized water and replaced the flask headspace with dinitrogen to create an anoxic setting for the soil. We measured soil headspace for CH₄ concentration 15 to 19 times over a four week dark incubation period and estimated potential peak production rate by calculating the greatest slope of total flask CH₄ per unit time generated by at least three points that also produced an $r^2 > 0.9$.

4.2.12 Soil Chemistry

We collected surface soil from each field plot at MI10N during monthly trace gas sampling in spring and soil from 10-15 cm depth in May and July 2013. All soil samples were collected in triplicate, composited in the field and stored on ice for transport to the lab at Duke University. We measured all samples for total carbon and nitrogen using a Flash Elemental Analyzer (CE Instruments) and measured total phosphorus using the ascorbic acid-molybdate blue method (Wetzel and Likens 2000) following nitric-perchloric acid digestion. We analyzed digests of soils collected in 2014 (surface only) for total iron using the thiocyanate method (Kopáček et al. 2007). For data summary, see Table 5.

Table 5: Mean (±standard error of the mean) chemical composition of soils collected from paired exclosure and waterfowl-accessible plots during spring 2013 and 2014 at Mattamuskeet Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA

<table>
<thead>
<tr>
<th>Depth cm</th>
<th>Total C %</th>
<th>Total N %</th>
<th>Total P µg/g</th>
<th>Total Fe %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 5</td>
<td>4.75±0.18</td>
<td>2.75±0.14</td>
<td>367±22</td>
<td>7.49±0.28</td>
</tr>
<tr>
<td>10 to 15</td>
<td>3.72±0.17</td>
<td>1.47±0.08</td>
<td>202±20</td>
<td>-</td>
</tr>
</tbody>
</table>
4.2.13 Synthesis of Regional Waterfowl Impoundment Area

We generated an estimate of total area of public waterfowl impoundments that are analogous to our field site, MI10N, in terms of salinity and management goals for vegetation and waterfowl, and also located within the southeastern U.S. (Alabama, Arkansas, Delaware, Florida, Georgia, Kentucky, Louisiana, Maryland, North Carolina, Tennessee and Virginia). We reviewed Comprehensive Conservation Plan documents for 107 National Wildlife Refuges and requested data from all 13 state natural resources agencies. We counted freshwater impoundments managed for moist soil or emergent marsh vegetation specifically to provide habitat for wildlife. When possible, we excluded “green tree” reservoirs (forested), seasonally flooded agricultural lands and managed brackish marshes. No comprehensive data exists for vast areas of privately owned impoundments and state data was not available for Virginia and northern and southwestern portions of Louisiana, so our calculated total may underestimate the true area of managed emergent freshwater waterfowl habitats by as much as 50%.

4.2.14 Waterfowl Impoundment Methane Flux Estimate

Two recent literature syntheses provide potential estimates for waterfowl impoundment CH₄ flux (Bridgham et al. 2006, Turetsky et al. 2014). From the synthesis of Bridgham et al. (2006) we culled data from forested wetlands and any wetlands that do not provide suitable waterfowl habitat, included the estimate from our study (including data from bird and no-bird plots separately) and recalculated a mean to
represent a rate for waterfowl impoundments. We use Turetsky et al.’s reported daily mean of CH$_4$ flux for graminoid-dominated wetlands, as waterfowl impoundments are typically managed for grasses, sedges and rushes, and convert it to an annual mean using Bridgham et al.’s conversion factor of 0.36. Both approaches yielded similar results (43.0 and 41.4 g CH$_4$ m$^{-2}$ y$^{-1}$), so we averaged them and multiplied by our conservatively estimated area of freshwater waterfowl impoundments to yield our estimate of total annual CH$_4$ flux from these wetlands.

Because of the spatial and temporal variability of waterfowl flocks and their uncertain distribution across privately owned wetlands and ponds, it is difficult to estimate with certainty the grazing impact of the 10 million overwintering waterfowl in the southeastern U.S. (U.S. Fish and Wildlife Service 2015a). Nevertheless, if we coarsely assume that half of this population overwinters on the region’s 55,000 ha of publicly managed freshwater waterfowl impoundments (Table S1), then mean density is roughly 90 birds ha$^{-1}$, similar to peak density of 110 recorded at our study impoundment. Thus, as a first approximation, we conclude that waterfowl grazing intensity at our study site is similar to that of other similar habitats in the same region.
4.3 Results

Waterfowl grazed heavily at our field site; each 4 m² plot received a mean of 42 and 28 hours of swan and duck use, respectively per winter (Fig. 16).

Figure 16: Two seasons of estimated overwintering swan and duck use of experimental plots at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA. Use estimates are generated from counts of birds captured within plots by time-lapse cameras shooting at 5-minute intervals during daylight hours. Approximately 100,000 images were analyzed. Because no images were captured at night when birds may have been present and active in plots these should be considered conservative estimates. Each line represents a separate plot.

Impacts on aquatic vegetation are significant. We find that exclosures, as intended, prevent consumption of roots and tubers, leading to significantly greater belowground biomass (Fig. 17A) and dramatically denser and more rapidly emerging E. quadrangulata culms (Fig. 17B).
Figure 17: Waterfowl grazing impacts on vegetation and oxygen-sensitive soil-nitrogen processes. Paired Tukey box plots (whiskers represent 1.5 of interquartile range) of field data collected from bird-impacted (brown) and exclosure (green) plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA. (A) Belowground biomass in top 15 cm of soil (p = 0.002); (B) E. quadrangulata emergent stem density (p < 0.001); (C) plant-available nitrate/nitrite in the surface soil (0 to 5 cm) (p = 0.03); and (D) nitrous oxide flux (p = 0.02). P-values are generated from Welch’s two-sample t-tests of data log-transformed to better meet assumptions of normality and variance homogeneity. All data are from May 2014 except belowground biomass (April 2013).

Waterfowl exclusion is also associated with significant differences in O₂-sensitive CH₄ and N biogeochemical processes. We observe dramatically reduced CH₄ emission from exclosures, which emitted a mean of 69 g CH₄ m⁻² over the 21-month study,
whereas bird-grazed plots emitted a mean of 230 g CH$_4$ m$^{-2}$ over the same time period (a 233% increase associated with herbivore presence) (Figs. 18A, 18B). Extractable nitrate is greater in surface soils of ungrazed exclosures (Fig. 17C), indicating an enhancement in aerobic nitrification. Nitrous oxide emission from exclosures is also significantly greater (Fig. 17D), indicating that a lack of herbivore pressure enhanced the capacity of wetland soil to cycle N.

Figure 18: Effects of waterfowl on seasonal methane flux and long-term cumulative emission in context of site conditions. (A) Seasonal mean (±standard error) methane (CH$_4$) flux measured in bird-affected (brown) and exclosure (green) plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA from December 2012 through June 2014. (B) Tukey boxplots (whiskers represent 1.5 of interquartile range) of cumulative CH$_4$ emitted over the course of the study based on extrapolation of flux between sampling periods. We found mean cumulative CH$_4$ flux to be significantly higher from “bird” plots (p = 0.026; Welch’s one-tailed t-test). Data were log-
transformed to conform to the assumption of variance homogeneity. (C) Water level (blue curve) and water temperature (red curve) are the principle abiotic factors influencing seasonal fluctuations in CH4 flux over the course of the experiment. Gray shading delineates peak waterfowl seasons. Maximum depth of water level recorders is approximately 120 cm.

We observe that anaerobically incubated soils from grazed and un-grazed sediments did not exhibit significantly different rates of CH4 production (Fig. 19), and yet pore-water CH4 reach greater concentrations in grazed plots (Fig. 20), suggesting that differences in observed CH4 flux are driven by CH4 oxidation, rather than production.

Figure 19: Potential methane production of soils collected from experimental plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA June 2014. Overwintering waterfowl were excluded from “exclosure” plots but had unrestricted
access to control (“birds”) plots. We detected no statistically significant treatment effect ($p = 0.43$; Welch’s $t$-test).

Figure 20: Relationships between mean porewater methane (CH4) concentration and mean CH4 flux measured in experimental plots at Mattamuskeet National Wildlife Refuge in North Carolina, USA during high CH4 flux sampling dates (April, May and June of 2013; April and May of 2014). Error bars represent standard errors of the mean.

Our mesocosm investigation into the effects of fecal inputs did not yield any consistent significant differences in CH$_4$ emission (Fig. 21), indicating that waterfowl feces are not an important driver of CH$_4$ emission patterns.
Figure 21: Tukey’s boxplots of methane (CH4) flux from wetland mesocosms over two sampling dates in response to waterfowl feces addition. Letters indicate significant differences in mean CH4 flux based on analysis of variance and Tukey’s honest significant differences test.

We observe significant differences in CH4 dynamics between 2013 and 2014 related to site hydrology, which emphasizes the sensitivity of CH4 emission to the availability oxidants. The vast majority of CH4 (72 and 93% in 2014 and 2013, respectively) is emitted during a critical window of ideal conditions in late spring/early summer. With the site in continuous flood starting each fall and with emergent vegetation die-back in winter, the only source of O2 to wetland sediments for months leading up to these high-emissions windows is via the slow process of diffusion. As
sediment and surface water temperatures increase, so do respiration and biological O\(_2\) demand, while rates of O\(_2\) diffusion decrease. These processes work in concert to severely deplete sediments of O\(_2\) and alternative electron acceptors. In such reduced conditions, CH\(_4\) production is most efficient and CH\(_4\) oxidation is severely limited. As water levels drop at our field site during early summer, surface soils are eventually exposed and oxidized, which leads to an immediate decrease in CH\(_4\) emission by two orders of magnitude (Fig. 18A). Following the summer oxidation CH\(_4\) crash, the biogeochemical differences between grazed and ungrazed wetlands we observe earlier in the season disappears. These patterns of interaction between hydrology and CH\(_4\) further indicates that consumptive prevention of the development of plant-mediated O\(_2\) transport pathways is the process that drives the dramatic biogeochemical differences we observe at our field site.

The extrapolation of our observations across similar regional habitats is made difficult by the lack of previous measurements of CH\(_4\) emission from waterfowl impoundments. Therefore, we use literature syntheses of methane emissions rates from analogous freshwater emergent wetland habitats (Bridgham et al. 2006, Turetsky et al. 2014) (Table 6). Based on impoundment area reported by government management agencies, we estimate that 55,000 ha of public waterfowl impoundments in the southeastern U.S. (Appendix A) emit 23 Gg CH\(_4\) yr\(^{-1}\) without invoking any bird effects. Our experimental results of a 233\% increased cumulative CH\(_4\) emission suggest that if
we were to consider herbivory effects, the CH$_4$ emission from southeastern impoundments could be more than tripled to 76 Gg CH$_4$ yr$^{-1}$, equivalent to 6% of the 1238 Gg CH$_4$ yr$^{-1}$ emitted by landfills in this region (Environmental Protection Agency 2015).

**Table 6: Measured methane flux from emergent wetlands suitable for waterfowl in the conterminous U.S (adapted from 2). Excludes data from forested wetlands, open water wetlands and tundra systems**

<table>
<thead>
<tr>
<th>Habitat</th>
<th>State</th>
<th>Annual flux (g CH$_4$ m$^{-2}$ y$^{-1}$)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wetland</td>
<td>CO</td>
<td>10.3</td>
<td>(Smith and Lewis 1992)</td>
</tr>
<tr>
<td>Nuphar bed</td>
<td>CO</td>
<td>73.6</td>
<td>(Smith and Lewis 1992)</td>
</tr>
<tr>
<td>Spikerush</td>
<td>FL</td>
<td>10.7</td>
<td>(Bartlett et al. 1989)</td>
</tr>
<tr>
<td>Sawgrass</td>
<td>FL</td>
<td>14.1</td>
<td>(Bartlett et al. 1989)</td>
</tr>
<tr>
<td>Sawgrass/Spikerush/Perphyton</td>
<td>FL</td>
<td>16.4</td>
<td>(Bartlett et al. 1989)</td>
</tr>
<tr>
<td>Sawgrass</td>
<td>FL</td>
<td>26.2</td>
<td>(Bartlett et al. 1989)</td>
</tr>
<tr>
<td>Sawgrass</td>
<td>FL</td>
<td>38.9</td>
<td>(Burke et al. 1988)</td>
</tr>
<tr>
<td>Marsh (Marl)</td>
<td>FL</td>
<td>10.9</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Marsh (Marl)</td>
<td>FL</td>
<td>18</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Marsh (Peat)</td>
<td>FL</td>
<td>16.5</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Marsh (Peat)</td>
<td>FL</td>
<td>4.7</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Marsh (Peat)</td>
<td>FL</td>
<td>59.6</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Marsh (Peat)</td>
<td>FL</td>
<td>7.4</td>
<td>(Happell et al. 1993)</td>
</tr>
<tr>
<td>Wet Prairie/Sawgrass</td>
<td>FL</td>
<td>22.2</td>
<td>(Harriss et al. 1988)</td>
</tr>
<tr>
<td>Waterlilly Slough</td>
<td>FL</td>
<td>33.1</td>
<td>(Schipper and Reddy 1994)</td>
</tr>
<tr>
<td>Everglades - Cladium</td>
<td>FL</td>
<td>16.5</td>
<td>(Chanton et al. 1993)</td>
</tr>
<tr>
<td>Everglades - Typha</td>
<td>FL</td>
<td>52</td>
<td>(Chanton et al. 1993)</td>
</tr>
<tr>
<td>Freshwater Marsh</td>
<td>LA</td>
<td>91.4</td>
<td>(Alford et al. 1997)</td>
</tr>
<tr>
<td>Freshwater Marsh</td>
<td>LA</td>
<td>213</td>
<td>(DeLaune et al. 1983)</td>
</tr>
<tr>
<td>Freshwater Marsh</td>
<td>LA</td>
<td>18.7</td>
<td>(DeLaune et al. 1983)</td>
</tr>
<tr>
<td>Submerged</td>
<td>MN</td>
<td>14.4</td>
<td>(Naiman et al. 1991)</td>
</tr>
<tr>
<td>Submerged Tidal</td>
<td>NC</td>
<td>52.7</td>
<td>(Kelley et al. 1995)</td>
</tr>
<tr>
<td>Banks Tidal</td>
<td>NC</td>
<td>7.3</td>
<td>(Kelley et al. 1995)</td>
</tr>
</tbody>
</table>
### Waterfowl Impoundment

<table>
<thead>
<tr>
<th>Waterfowl Impoundment (birds excluded)</th>
<th>NC</th>
<th>96.9&lt;sup&gt;1&lt;/sup&gt;</th>
<th>this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typha Marsh - mineral soil</td>
<td>NY</td>
<td>125.3</td>
<td>(Yavitt 1997)</td>
</tr>
<tr>
<td>Typha Marsh - peat soil</td>
<td>NY</td>
<td>23.7</td>
<td>(Yavitt 1997)</td>
</tr>
<tr>
<td>Emergent Tidal Freshwater</td>
<td>VA</td>
<td>96.2</td>
<td>(Neubauer et al. 2000)</td>
</tr>
<tr>
<td>Emergent Peltandra</td>
<td>VA</td>
<td>56.4</td>
<td>(Wilson et al. 1989)</td>
</tr>
<tr>
<td>Emergent Smartweed</td>
<td>VA</td>
<td>30.2</td>
<td>(Wilson et al. 1989)</td>
</tr>
</tbody>
</table>

| n                                    | 30   |

| Arithmetic Mean                      | 43.0 |
| Arithmetic Standard Error            | 8.2  |
| Geometric Mean                       | 28.0 |
| Geometric Standard Error             | 2.6  |

### 4.4 Discussion

Our experimental results provide definitive field evidence that intense waterfowl grazing can exacerbate CH<sub>4</sub> emissions and inhibit N cycling in a waterfowl impoundment. Exclosure of waterfowl yielded effects consistent with our hypothesis that waterfowl herbivory would diminish O<sub>2</sub> transport into wetland sediments (Figs. 12, 13). While herbivory must also reduce the capacity for plant-mediated CH<sub>4</sub> emission and fecal nutrient inputs may not be entirely irrelevant to CH<sub>4</sub> dynamics, our data indicate that soil O<sub>2</sub> availability was a far more important factor governing CH<sub>4</sub> emissions (Fig. 18B). Differences we observed in porewater CH<sub>4</sub> concentration (Fig. 20), soil nitrate availability (Fig. 17C), and N<sub>2</sub>O emissions (Fig. 17D) are in congruence with our

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<sup>1</sup> See 4.2.5 Cumulative Methane Flux Analyses for details on how these numbers were calculated.
conclusion that herbivore-alteration of plant O\textsubscript{2} transport is the key mechanism driving CH\textsubscript{4} emission.

Our findings demonstrate that top-down trophic interactions (Silliman \textit{et al.} 2005) can control wetland biogeochemical cycling in areas that host large populations of aquatic herbivores, which alter plant-facilitated soil-atmosphere gas exchange. Herbivores can prevent the formation of an oxidized rhizosphere and fundamentally change wetland biogeochemical function.

This observation augments the paradigm of emergent vegetation increasing wetland CH\textsubscript{4} emissions (Laanbroek 2010, Turetsky \textit{et al.} 2014). Zones of emergent aquatic vegetation are hotspots for CH\textsubscript{4} emission, but herbivore disturbance can exacerbate emissions rather than reduce them, as might be intuited from clipping experiments (Schimel 1995, Shannon \textit{et al.} 1996, Kelker and Chanton 1997). Thus, other dominant wetland herbivores, such as nutria along North America’s Gulf Coast (Evers \textit{et al.} 1998) or moose in boreal regions (Pastor \textit{et al.} 1988), may provide similarly important and unrecognized top-down control on wetland biogeochemical function. Despite similar observations of trophic interactions leading to major changes in regional carbon budgets (Schmitz \textit{et al.} 2013), there is relatively little understanding of how CH\textsubscript{4} emission from wetlands will interact with changes in wildlife populations as brought on by management, habitat degradation, or climate warming.
Historic losses of North American waterfowl populations since pre-Columbian times have greatly reduced the pervasiveness of the herbivory effects on wetland biogeochemistry we document in this study. Yet, dramatic population recoveries over recent decades (Fig. 22A) combined with losses of roughly half of historic wetland cover, both nationwide (Dahl 2011), and on the North Carolina coastal plain in particular (North Carolina Department of Environment, Health 1991, Cashin et al. 1992, Carle 2011, O’Driscoll 2012) (Fig. 22B), have concentrated waterfowl in remaining habitats (Fig. 22C). Therefore, the biogeochemical effects of waterfowl herbivory in North American are probably stronger today than at any time in the past century, and they are likely to continue to strengthen as long as wetland degradation and waterfowl population recovery continue.
Figure 22: Wetland loss and recovery of waterfowl populations have led to an increase in waterfowl density over the past 50 years. (A) Mass of overwintering ducks (Drilling et al. 2002), Tundra Swan (Cygnus columbianus) (Limpert and Earnst 1994) and Snow Goose (Chen caerulescens) (Mowbray et al. 2000) at Mattamuskeet National Wildlife Refuge based on midwinter aerial counts by the U.S. Fish and Wildlife Service from 1961 to 2012 (Wires 2015) with positive exponential and moving 5-year mean trend lines. (B) Wetland land cover on the North Carolina coastal plain in pre-1900s (North Carolina Department of Environment, Health 1991), 1955, 1981 (Cashin et al. 1992), 1994, 2001 (Carle 2011), and 2010 (O’Driscoll 2012) with negative exponential trend line for modern data. (C) Exponentially increasing trend and individual points (triangles) in waterfowl density index are based on ratios of waterfowl mass and wetland area from (B) and (A), respectively.

The premium waterfowl habitats in the southeast, within which birds are most concentrated, are managed moist-soil impoundments. While these waterfowl
impoundments vary geographically in soil type and plant species, they are uniform with respect to the most important factors affecting CH$_4$ emission: hydrology and emergent vegetation cover. Under conventional moist soil management, impoundments experience long periods of inundation during the winter months (Strader et al. 2005), setting up the potential for extreme CH$_4$ emissions during the early part growing season, as we observe in our experiment. A management goal for these habitats: to produce aquatic plants preferred by waterfowl, guarantees that herbivory effects will be strong in the presence of dense herbivore populations. Given that the development of aerenchyma for facilitating O$_2$ transport to the rhizosphere through radial O$_2$ loss (Armstrong 1964) is ubiquitous among aquatic plants (Sculthorpe 1967), we should expect that overgrazing has the potential to influence CH$_4$ emissions across a wide geographic range of impoundment habitats.

State and federal wildlife managers have an opportunity to reduce CH$_4$ emissions through alteration of moist-soil impoundment management prescriptions and potentially offset portions of refuge or agency C footprints. Yet, our analysis shows that even if such practices were adopted en masse across all the publicly owned freshwater emergent waterfowl impoundments in the southeast, the impact on regional CH$_4$ emissions would be relatively small; the widely recognized CH$_4$ emitters (fossil fuel industry, cattle, landfills, rice paddies) still dominate global and national budgets (Myhre et al. 2013). Nevertheless, as governments and NGOs continue to investigate
opportunities for greenhouse gas mitigation through land management (Fish and Wildlife Service 2010), recognition of the importance of trophic interaction controls on wetland CH₄ will be critically important.

The implications of our finding that waterfowl herbivory can disrupt wetland N cycling may be of even greater importance for wildlife management, given that habitat degradation caused by eutrophication has a more immediate and localized effect on wildlife than does global warming. We found that the long-term N removal process of denitrification was limited at our field site proximally by low nitrate, and ultimately by herbivory and the lack of plant-mediated O₂ transport. Based on depth of water drawdown and surface water N concentrations we estimate that the impoundment exported 14 and 22 kg N ha⁻¹ to Lake Mattamuskeet in 2013 and 2014, respectively (Table 1), a range similar to N loading estimates for fertilized agricultural lands in the North Carolina coastal plain (Deal et al. 1986). Given that eutrophication of ecosystems is a growing concern globally (Smith 2003), as well as locally at Lake Mattamuskeet (North Carolina Department of Health Environment and Natural Resources 2013, U.S. Fish and Wildlife Service 2015b), it will be necessary for managers to understand the impact of herbivory and waterfowl impoundment habitats on nutrient loading in order to optimize landscape-scale habitat quality.

Since this study demonstrates some negative environmental consequences of high densities of herbivorous birds, we feel it necessary to mention that North American
waterfowl populations provide many important ecological functions and cultural values. Rather than advocate for a reduction in bird populations (which would be undoing nearly a century of conservation effort), we point out that the undesirable herbivory effects could likely be mitigated by simple changes to hydrologic management prescriptions of waterfowl impoundments. Earlier seasonal drainage and oxidation of the surface soils of our study impoundment in 2014, led to a roughly 80% reduction in CH$_4$ emissions compared to 2013. Earlier seasonal drainage could not only reduce CH$_4$, but also enhance coupled nitrification-denitrification, thus reducing the potency of impoundment habitats as exporters of N. Of course such changes in hydrologic management may impose biogeochemical (such as N$_2$O emissions) and/or habitat quality trade-offs. We recommend further research in managed waterfowl wetlands that investigate whether altered seasonal hydrologic regimes may improve greenhouse gas and biogeochemical performance while simultaneously meeting habitat and wildlife goals.
5. Waterfowl impoundments as sources of nitrogen pollution

5.1 Introduction


Wetlands are hotspots for nitrogen (N) cycling and removal via denitrification and are important sinks for excess sediment-bound phosphorus (P). However, the capacity for wetlands to provide bioremediation of nutrient pollution has been limited by historical losses of 50% of wetland areas in the U.S. (Dahl 2011). On North Carolina’s low-lying Albemarle-Pamlico peninsula, historic wetlands losses have been particularly dramatic because of ditching and drainage to reap the benefits of poorly-drained, but exceptionally fertile cropland (Hearns 1910, Copeland et al. 1983). Despite a nation-wide hiatus (Dahl 2011), wetland losses caused by development in this region have continued in recent decades (Carpenter and Dubbs 2012).
Losses of natural wetlands have made constructed public and private impounded wetlands especially important for North American waterfowl populations (ducks, geese and swans), which have recovered from historic lows of the 1930s (North American Waterfowl Management Plan Committee 2012). Overwintering waterfowl flocks are often concentrated in managed moist-soil impoundment habitats which provide a high density of preferred food items and attract a corresponding high density of waterfowl. Moist-soil management, is described as “an art,” as optimal practices vary considerably based on biological, climatic and physio-geographic context, but the key is managing hydrology to effectively support palatable wetland vegetation for waterfowl (Strader et al. 2005). Typically this involves a spring drawdown in which impoundments are dewatered passively by removing flash-board rises and/or actively by pumping.

Impoundment drawdown sends a pulse of water and nutrients downstream, the magnitude of which is based on water depth and the burden of nutrient concentrations. Since high bird densities are associated with elevated surface water nutrients (Gould and Fletcher 1978, Manny et al. 1994, Post et al. 1998, Olson et al. 2005), the potential exists for heavy nutrient loading from impoundments that attract large flocks of waterfowl. An investigation into the potential water quality impacts of waterfowl at a refuge in New Mexico concluded that waterfowl impoundments have the potential to export both N and P based on observed increases in concentrations from inflow to outflow (Brandvold et al. 1976). The Brandvold et al. (1976) study, however, lacked
nutrient budgets and has not been emulated in other regions. Thus, the export of
nutrients from heavily-used bird habitats to downstream lakes and estuaries has not
been well-explored.

Nutrient export from managed wetlands may be especially important in the
context of Lake Mattamuskeet, which drains large areas of public and private
impoundments and has suffered from recent declines in water quality and submerged
aquatic vegetation (SAV) associated with phytoplankton blooms (North Carolina
Department of Health Environment and Natural Resources 2013, U.S. Fish and Wildlife
Service 2015b). The loss of macrophyte coverage is a serious concern for Mattamuskeet
National Wildlife Refuge, which was established in 1934 to maintain and promote
wetland habitats for migratory bird populations and specifically wintering waterfowl in
and around the Lake. The Refuge attracts a peak of roughly 200,000 to 300,000 waterfowl
and is of critical importance to Atlantic Flyway populations, which depend on the
Lake’s declining beds of SAV.

The purpose of this paper is to quantify nutrient loading to Lake Mattamuskeet
by the surrounding managed impoundment wetlands. Since surface P concentrations
were relatively low, we mainly focus our analysis on the relative importance of N
loading. We compare N sources in the watershed, i.e. Agricultural runoff/leaching, wet
deposition, direct transport by bird flocks (Post et al. 1998) to generate a preliminary N
budget for Lake Mattamuskeet. Finally, we estimate the potential to mitigate
impoundment N export through modification of hydrologic management prescriptions for increased denitrification and/or decreased mass loading.

5.2 Methods

5.2.1 Site Description

Lake Mattamuskeet, the centerpiece of Mattamuskeet National Wildlife Refuge, is a natural lake system located on the Albemarle-Pamlico Peninsula on the eastern coast of North Carolina (Fig. 21). Understanding and mitigating local water quality problems is complicated because of the lake’s large surface area (16,500 ha), extensive shoreline (116 km), shallow depth (average of 1 meter), and long history of hydrological alteration. In the mid-19th century the first canal from the lake to Pamlico Sound decreased the lake’s depth from 3 m to 1 m. Three additional drainage canals have since decreased the lake’s surface area from its original 48,000 hectares to its current size (Forrest 1999, Waters et al. 2009, 2010). Between 1915 and 1932, the lake bottom was drained three times and farmed twice (Forrest 1999, Waters et al. 2010). The north-south-oriented North Carolina Highway 94 divides Lake Mattamuskeet into eastern and western regions, which have different nutrient regimes (Fig. 21; Waters et al., 2010).
Figure 23: Landcover map of the Lake Mattamuskeet watershed in North Carolina, USA. Red dot marks field site at Marsh Impoundment 10 North.

Lake Mattamuskeet historically contained dense beds of submerged aquatic vegetation (SAV), but since the late 2000s, the western region has been dominated by phytoplankton and characterized by low clarity related to suspended sediments and high phytoplankton biomass (Waters et al. 2010). Recent monitoring by the U.S. Fish and Wildlife Service indicates that a corresponding decline in SAV and water-quality conditions is occurring in the eastern region of the lake (U.S. Fish and Wildlife Service 2015b). Lake Mattamuskeet is rated as hypereutrophic (North Carolina Department of Health Environment and Natural Resources 2013) by an index based on chlorophyll $a$, total organic nitrogen, total phosphorus, and water clarity. Increasing trends in
chlorophyll $a$, total nitrogen and total phosphorus, and frequent exceedances of the North Carolina water quality standard for chlorophyll $a$ have been detected on both sides of Lake Mattamuskeet (U.S. Fish and Wildlife Service 2015b). Evidence from a recent en vitro dosing experiment indicates that the lake phytoplankton growth is N-limited (Davis et al. 2016), a result supported by other findings of N-limitation shallow eutrophic lakes (Healey and Hendzel 1980, Hecky and Guildford 1984, Downing and McCauley 1992). However the high N:P ratio (44 by mass) of lake water indicates a potential for seasonal P limitation (Kolzau et al. 2014). Nevertheless, the prevailing wisdom is that harmful algal blooms at Lake Mattamuskeet may be caused or exacerbated by allochthonous N inputs from the watershed (U.S. Fish and Wildlife Service 2015b, Davis et al. 2016).

Our study site was Marsh Impoundment 10 North (MI10N) (35°32'08"N, 76°04'24"W), which lies at the east end of the refuge, is closed to hunting and has restricted public access (Fig. 23). Dominant plant species in MI10N include Phragmites australis, Eleocharis quadrangulata, Eleocharis parvula, Panicum dichotomiflorum, Bacopa monieri, Echinochloa walteri, Centella spp. and Alternanthera philoxeroides. MI10N soil is mapped as Weeksville loam (95%) and Engelhard loamy very fine sand (5%) (Soil Survey Staff 2015).

A network of canals, water-control structures and pumps allow Mattamuskeet National Wildlife Refuge staff to control impoundment water levels, which they do,
following an Annual Marsh/Water Management Plan prepared by the US Fish and Wildlife North Carolina Migratory Bird Field Office to meet population and habitat objectives for migratory waterfowl and shorebirds (John Stanton, personal communication). The 2014 protocol recommended two phases of water level management: 1) water level at MI10N should be drawn down to ‘ditch top,’ a level at which much of impoundment surface soil is exposed, by 1 June; 2) re-flooding should start 1 September with a goal of 30 cm of standing water by 1 December. Starting 12 June 2013 pumps were run for approximately 8 days to draw down 16 cm of standing water from MI10N (Fig. 24). Starting 9 May 2014 pumps were run for approximately 17 days to draw down 34 cm of standing water (Fig. 24).

![Water Level at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge as measured hourly by water level recorders during the months surrounding spring drawdown in 2013 and 2014. Gray shading highlights period of prescribed drawdown by Refuge management.](image)

**Figure 24:** Water level at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge as measured hourly by water level recorders during the months surrounding spring drawdown in 2013 and 2014. Gray shading highlights period of prescribed drawdown by Refuge management.
5.2.2 Submerged Aquatic Vegetation

Prior to 2003, SAV surveys at Lake Mattamuskeet consisted of sampling % cover at approximately 300 plots during July/August. Recent surveys (since 2013) follow a modified protocol in which 102 plots were sampled, with the number stratified based on water depth (six points located in 0-12 inches, 28 points located in 13-24 inches, 36 points located in 25-36 inches, 27 points in 37-48 inches, and five points in deeper than 48 inches). Data was collected at 38 plots on the west side of the lake along 3 transects and 64 plots on the east side of the lake along 4 transects in 2013, 2014, and 2015. Prediction maps of SAV coverage were created by using ordinary kriging methods in ArcGIS 10.3 and provide an estimate of the percent coverage of SAV on the lake bottom, categorized relative to management concerns. 25% or less coverage suggests a severe management concern, 25-50% coverage should suggest a decline is occurring and 50% or greater SAV coverage is indicative of a healthy SAV population (John Stanton, personal communication).

5.2.3 Watershed Land Cover Estimation

Prior to European settlement, much of the land surrounding Lake Mattamuskeet consisted of pocosin peatlands (Richardson 1983), but in the past century, vast areas of pocosins have been ditched and drained to facilitate agriculture and silviculture (Copeland et al. 1983). As a result, the hydrology on the Albemarle Peninsula is a complicated maze of canals, drainage ditches and pumps. We estimate land cover by
clipping the 2006 National Land Cover Database with a watershed boundary created by
the U.S. Fish and Wildlife Service (Fry et al. 2011), a modification of the existing
boundary in the National Hydrography dataset based on local landowner reports of
drainage patterns. We estimate winter impoundment cover by clipping an
impoundment shapefile created in 2012 by the North Carolina Wildlife Resources
Commission with the watershed boundary.

5.2.4 Water and Soil Analyses

We collected four replicate impoundment surface water samples monthly in
spring of 2013 and 2014. We measured Nitrate/Nitrite (NO$_x$) and ammonia/ammonium
(NH$_x$) using a Lachat Quickchem 8000 autoanalyzer and total Phosphorus using the
persulfate digestion-molybdate blue method (Wetzel and Likens 2000). We measured
total N using a Total Nitrogen Measuring Unit (Shimadzu) in 2014 only (this instrument
was not available in 2013). We found total N to be strongly correlated ($r^2 = 0.87$) with
NH$_x$ at MI10N during spring of 2014. We assume that a similar relationship between
NH$_x$ and total N existed in 2013 and we use a simple linear model to estimate total N
from measured NH$_x$ data in 2013. Mean residual error from the model is less than 10
percent.

We collected impoundment soil samples using a 2 cm diameter punch tube in
May and July 2013 and monthly from March to June, 2014. In the field we composited
and homogenized soils from the 0-5 and 10-15 cm depth intervals of three random
(replicates collected from each of 16 field plots. In the lab we weighed, oven-dried and re-weighed subsamples to estimate wet:dry ratios. We analyzed subsamples for total carbon and total N using a CE Instruments Flash Elemental Analyzer, and for total P using the molybdate blue method (Wetzel and Likens 2000) following nitric-perchloric digestion.

5.2.5 Nitrogen Source Estimation

5.2.5.1 Impoundments

We estimate the mass of N inputs to Lake Mattamuskeet from springtime impoundment drawdown by multiplying impoundment water volume by total N concentration of surface water. We measured MI10N water level hourly using an automated level logger (Odyssey) and multiplied water depth at start of drawdown to by impoundment area to calculate water volume. We used the mean surface water concentration from the measurement date closest to the timing of drawdown for our loading calculation. These key sampling dates were: 9 June 2013, three days before drawdown; and on 31 May 2014, roughly eight days after drawdown. We follow the same protocol to estimate P inputs.

To estimate impoundment contributions to the Lake Mattamuskeet nutrient budget, we extrapolated our observations at MI10N to all impoundment areas in the watershed. This generalization is probably inaccurate for the 1001 ha of private impoundments on agricultural land, which attract overwintering waterfowl, but have
different management objectives during the growing season. Despite the management differences, we include these areas in our estimate of ‘impoundment’ contributions for the Lake N budget because of a lack of any data from agricultural impoundments. We report high and low impoundment N loads to Lake Mattamuskeet as the greater value and lesser value, respectively, from 2013 and 2014. The mean we report is the arithmetic mean of these two values.

5.2.5.2 Bird Transport

The capacity for waterfowl to serve as nutrient vectors has been studied across a variety of aquatic habitats (Manny et al. 1994, Post et al. 1998, Olson et al. 2005, Chaichana et al. 2010). Waterfowl at Lake Mattamuskeet, particularly Snow Goose (*Chen caerulescens*) and Tundra Swan (*Cygnus columbianus*), feed in adjacent wetland or agricultural habitats, but return to the Lake to roost where their defecation represents an allochthonous source of N. While we lack high resolution quantitative data on the movement of all species of waterfowl, we observed a consistent influx of geese, Tundra Swan and ducks into the lake each day around dusk. To estimate the amount of N imported to Lake Mattamuskeet directly by bird movements, we follow the logic of Hahn et al.’s (2007) defecation model for quantification of allochthonous nutrient input into freshwater bodies by herbivorous waterbirds.

We estimate bird populations on the lake by using the mean counts of four aerial censuses collected by the U.S. Fish and Wildlife Service in winter months during the
study period (Table 7). We assume these populations are present at the lake for a season of 120 days (mid-November to mid-March), use Manny et al.’s (1994) defecation rate for Canada Goose (33 g goose\(^{-1}\) d\(^{-1}\)) and assume that this rate scales allometrically across species by body mass (Nagy et al. 1999). We use the following body masses: Tundra Swan – 6.75 kg (Limpert and Earnst 1994); Atlantic Canada Goose – 4.6 kg (Mowbray et al. 2002); Snow Goose – 2.6 kg (Mowbray et al. 2000); Mallard mass for ducks – 1.2 kg (Drilling et al. 2002). We assume waterfowl feces has an N content of 45 mg g\(^{-1}\) based on a published data review (Hahn et al. 2007).

Table 7: Waterfowl counts from aerial surveys conducted at Lake Mattamuskeet in North Carolina, U.S.A. by the U.S. Fish and Wildlife Service (Wires, 2015)

<table>
<thead>
<tr>
<th>Date</th>
<th>Tundra Swan</th>
<th>Ducks</th>
<th>Canada Goose</th>
<th>Snow Goose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/29/2012</td>
<td>22,991</td>
<td>167,774</td>
<td>2,309</td>
<td>5,200</td>
<td>198,274</td>
</tr>
<tr>
<td>1/10/2013</td>
<td>4,177</td>
<td>155,435</td>
<td>3,534</td>
<td>11,500</td>
<td>174,646</td>
</tr>
<tr>
<td>12/17/2013</td>
<td>8,771</td>
<td>196,809</td>
<td>7,395</td>
<td>11,000</td>
<td>223,975</td>
</tr>
<tr>
<td>1/24/2014</td>
<td>14,007</td>
<td>211,697</td>
<td>27,861</td>
<td>30,000</td>
<td>283,565</td>
</tr>
<tr>
<td>mean</td>
<td>12,487</td>
<td>182,929</td>
<td>10,275</td>
<td>14,425</td>
<td>220,115</td>
</tr>
</tbody>
</table>

We multiplied our estimate of fecal mass produced by feces N content and report a range of estimates for waterfowl inputs to Lake Mattamuskeet. Our maximum value assumes that 60% of all bird excreta ends up in the lake, with the rest remaining in adjacent foraging areas, as Post et al (1998) found in a study of Snow Geese. Imported feces fractions of 12 to 26% reported by (Hahn et al. 2007) are probably more reasonable
for our estimation of a mixed species assemblage and we use these values as for our minimum and mean, respectively.

5.2.5.3 Agricultural Leaching/runoff

N export for fertilized agricultural lands in North Carolina’s coastal plain varies widely based on how well-drained soils are and whether or not drainage is controlled with flash-board risers (Gilliam et al. 1978, Skaggs et al. 1980, Deal et al. 1986). Well-drained croplands have the potential to export large quantities of NO$_3$-N because they lack the subsurface anoxia to support denitrification. Poorly-drained agricultural soils, conversely, experience prolonged saturation, facilitating denitrification and exhibit much lower export of NO$_3$-N and total N. Drainage control simply reduces the volume of outflowing water thus reducing total N flux.

As a result of variable drainage, among other factors, empirical N export data from cropland on North Carolina’s Albemarle Peninsula range from 6.8 to 47.2 kg N ha$^{-1}$ y$^{-1}$ (Gilliam et al. 1978, Skaggs et al. 1980). The low-end measurements come from low-lying poorly-draining deep peat soils, but even the highest estimate is from Portsmouth soil, which is classified as ‘poorly drained’ (Soil Survey Staff 2015). Cultivated soils surrounding Lake Mattamuskeet represent a mosaic of soil series, but like Portsmouth, are poorly drained silty or sandy loams (Soil Survey Staff 2015). For our estimations of agricultural inputs to Lake Mattamuskeet, we use: 1) Skaggs et al.’s (1980) 3-year average for Albemarle Peninsula mineral and shallow organic soils of 15.6 kg N ha$^{-1}$ y$^{-1}$
as a low estimate; 2) Gilliam et al.’s (1978) average from three Albemarle Peninsula
cultivated Portsmouth soils of 30.8 kg N ha\(^{-1}\) y\(^{-1}\) as a high estimate; and 3) the arithmetic
mean of the previous two values as a mean estimate. To calculate the range of total loads
into Lake Mattamuskeet, we multiply each value by the area of agricultural land in the
watershed.

5.2.5.4 Atmospheric Deposition

Local rates of wet deposition of N are reported from Pocosin Lakes National
Wildlife Refuge (Ward 2009) and a private restored wetland (Ardón et al. 2010b),
approximately 25 km to the northwest and 40 km to north of Lake Mattamuskeet,
respectively. We prefer these locally-measured values to modeled estimates from the
National Atmospheric Deposition Network, which lacks measuring stations in
northeastern North Carolina. We report mean values from these two studies as
minimum and maximum wet deposition rates, respectively, and their arithmetic mean
as a mean value.

5.2.6 Nitrous Oxide Emission

We measured emissions of a nitrification-denitrification byproduct, nitrous oxide
(N\(_2\)O) (Firestone 1982), using a static chamber method monthly from March to June 2014.
We built chambers from 1 m sections of 55 cm diameter transparent TEDLAR plastic
sleeves with circular Plexiglas caps fringed with closed-cell foam to make an air-tight
seal. We affixed the cap with a 2 m sampling tube (1 mm inner diameter), a wide-bore
closeable exhaust vent and a thermocouple for measuring internal chamber temperature (T). To set up the static chamber apparatus for N₂O sampling we attached the cap to the sleeve using binder clips, inserted a plastic brace into the base of the sleeve to maintain a cylindrical chamber headspace, and carried it into the wetland hanging from a 2 m PVC pole. With the exhaust vent open we lowered the chamber to rest on the rebar base using the PVC pole. As the chamber ‘skirt’ submerged, air displaced by standing water was able to escape via the exhaust vent. We closed the vent before extracting the first headspace sample. We conducted chamber set up and sampling from a submerged cinder block platform to avoid disturbing sediments and driving ebullition into the chamber. Before extracting each sample, we flushed the sampling tube to mix this small volume (approximately 3 ml) with the larger headspace (approximately 160 L). We extracted one 60 ml headspace sample immediately following chamber set up and four additional samples at 5 to 8 minutes intervals for a total of 5 samples over an incubation of approximately 30 minutes. We recorded internal chamber T immediately following each extracted sample. We measured chamber height above water level to calculate cylindrical chamber volume. During sampling dates with low or no surface water (May and June 2014), use of the chamber described above was not possible because standing water is necessary to create a sealed headspace. Instead we used a permanent collar static chamber method, with a water-filled gutter and remote rod sampling system
(described in detail in Winton and Richardson, 2015). Because of smaller chamber volume (approximately 15 L), we extracted 4 headspace samples instead of 5.

We stored gas samples collected at the field site in labeled Mylar bags for transport back to the Duke University Wetland Center laboratory. We analyzed samples for N₂O within one week of collection using a Varian 450 gas chromatograph equipped with an electron capture detector. We ran all samples in duplicate with the mean value used for gas flux calculations unless duplicate values differed by >10%, in which case the obviously outlying value was assumed to stem from analytical error and discarded. Flux was estimated by linear regression of sample concentration as a function of time elapsed. If a threshold r-squared value of 0.90 was not met, we removed one or two outlying points if it improved fit to > 0.90 (15% of incubations), otherwise such estimates were treated as failed incubations and excluded from subsequent analysis (3% of incubations).

We estimate the minimum detectable flux of 0.013 mg N₂O m⁻² h⁻¹.

5.2.7 Early Drawdown Simulation

We postulated that an earlier season impoundment drawdown may reduce the amount of N loadings going into Lake Mattamuskeet due to lower soluble N loads in impoundment surface water. Therefore we estimate a theoretical N export if drawdown were to have been completed on 1 May of 2013 and 2014, based on water level on that date and on total N concentrations, as measured on 9 May 2013 and 22 April 2014.
Otherwise we follow the same mass loading logic as described previously under ‘Impoundments.’

### 5.3 Results

We found that spring drawdown of MI10N exported relatively large amounts of N (14 and 22 kg N ha\(^{-1}\) in 2013 and 2014, respectively), but little P (<0.5 kg of P ha\(^{-1}\) both years) (Table 8). Our N loading rates from impoundment drawdown are remarkably similar to published annual N exports from Albemarle Peninsula agricultural fields, which range from 16 to 31 kg N ha\(^{-1}\) depending on soil drainage (Gilliam et al. 1978, Skaggs et al. 1980).
Table 8: Comparison of wetland size, drawdown volume, pre-drawdown surface water nutrients, nutrient export, and pre-drawdown peak bird use across 2 years at Mattamuskeet National Wildlife Refuge in North Carolina, USA and one year at Backus Lake in Michigan, USA (Kadlec, 1962)

<table>
<thead>
<tr>
<th>Wetland</th>
<th>Size</th>
<th>Drawdown</th>
<th>Surface water</th>
<th>Nutrient export</th>
<th>Peak Birds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ha</td>
<td>cm</td>
<td>m³</td>
<td>Total P</td>
<td>Total N</td>
</tr>
<tr>
<td>MI10N - 2014</td>
<td>88</td>
<td>34</td>
<td>299,200</td>
<td>0.121</td>
<td>6.49</td>
</tr>
<tr>
<td>MI10N - 2013</td>
<td>88</td>
<td>16</td>
<td>140,800</td>
<td>0.029</td>
<td>8.79</td>
</tr>
<tr>
<td>Backus Lake</td>
<td>142</td>
<td>55</td>
<td>781,000</td>
<td>0.023</td>
<td>0.05</td>
</tr>
</tbody>
</table>
While we observe that the magnitude of impoundment and agricultural field N sources is similar, we find that the form of N is different in each case. In contrast with agricultural runoff, which is typically high in NO₃ (Skaggs et al. 1980), we observed that MI10N surface waters had low concentrations of NO₃ (<0.06 mg L⁻¹ until after drawdown) and were instead dominated by high concentrations of NH₃ (Fig. 25).
Figure 25: Tukey boxplots comparing surface water ammonia/ammonium (NH₄), total nitrogen (TN), nitrate/nitrite (NOx) and total phosphorus (P) concentrations during the first half of two growing seasons at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, USA. Whiskers include points within 1.5 times the interquartile range; outliers are individual points. Letters indicate results of Tukey’s test of honest significant differences at α = 0.05. Differences in spring nutrient concentrations were not
statistically significant in all cases, as tested by ANOVA and Tukey’s Honest Significant Differences Test, but some true differences are likely obscured by low statistical power due to small sample sizes (n = 4). There were no significant differences among NO3 data. TN data for 2013 is modelled from NHx (see 5.2.4.1 Impoundments)

Spring season total N, NH3 and total P in MI10N surface water consistently showed a hump-shaped pattern, with the highest mean concentrations detected on 6 June, 2013 and 31 May, 2014, roughly coinciding with the drawdown target date on June 1 (Fig. 25). N dominated surface water with peak total N:P ratios of 303 and 53 (by mass) in 2013 and 2014, respectively (Table 8). Coupled nitrification-denitrification, as indicated by N2O emissions, was essentially undetectable in 2014 until after drawdown oxidized sediment surfaces (Fig. 26).
Figure 26: Tukey boxplots of nitrous oxide (N2O) emissions from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. Whiskers include points within 1.5 times the interquartile range; outliers are individual points. N2O emissions were almost entirely less than the minimum detectable threshold of 0.013 mg N2O m⁻² h⁻¹ until after spring drawdown oxidized surface soils around 23 May 2014.

Our analysis of earlier season simulated drawdowns indicates that drawdown on May 1 could have reduced N export by more than 70% in both years (Table 9). This analysis is based on observed hydrologic and nutrient data and considers only the avoided mass loss of dissolved N.

Table 9: Observed and simulated total nitrogen (TN) export from Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. Percent change refers to difference between simulated and observed N export for a given year.
Lake Mattamuskeet’s watershed has extensive cropland covering more than one-third of the land (Table 10) and we estimate that agricultural runoff is the largest source of N to the Lake (Table 11). Wet deposition and impoundment drawdown contribute roughly one-third and one-fifth of total N inputs, respectively. We find that fecal inputs (bird transport) are relatively minor (<2%) compared to these other sources despite the massive local population of overwintering waterfowl.

**Table 10: Land use of the watershed draining into Lake Mattamuskeet in North Carolina, U.S.A.**

<table>
<thead>
<tr>
<th>Land Use Type</th>
<th>Sub-Category</th>
<th>Area</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ha</td>
<td></td>
</tr>
<tr>
<td>Open Water</td>
<td>Lake Mattamuskeet</td>
<td>16500</td>
<td>-</td>
</tr>
<tr>
<td>Open Water</td>
<td>Non-lake</td>
<td>250</td>
<td>2.1</td>
</tr>
<tr>
<td>Development</td>
<td></td>
<td>604</td>
<td>5.0</td>
</tr>
<tr>
<td>Forest</td>
<td></td>
<td>522</td>
<td>4.3</td>
</tr>
<tr>
<td>Shrub/Scrub/Grass</td>
<td></td>
<td>150</td>
<td>1.2</td>
</tr>
<tr>
<td>Agriculture</td>
<td>Impounded</td>
<td>1001</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>Non-impounded</td>
<td>3464</td>
<td>28.6</td>
</tr>
<tr>
<td>Wetland</td>
<td>Impounded</td>
<td>1484</td>
<td>12.3</td>
</tr>
<tr>
<td></td>
<td>Non-impounded</td>
<td>4617</td>
<td>38.2</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>28592</td>
<td></td>
</tr>
<tr>
<td>Total (Excluding Lake)</td>
<td></td>
<td>12092</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 11: Comparison of the estimated relative importance of nitrogen sources to Lake Mattamuskeet in North Carolina, U.S.A. Reported percentage shares of total load are based on mean values

<table>
<thead>
<tr>
<th>Nitrogen Source</th>
<th>Loading Rate</th>
<th>Area Factor</th>
<th>Total Load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg N ha(^{-1}) yr(^{-1})</td>
<td>ha</td>
<td>Mg N yr(^{-1})</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Agricultural runoff</td>
<td>23.2</td>
<td>15.6</td>
<td>30.8</td>
</tr>
<tr>
<td>Wet deposition</td>
<td>5.0</td>
<td>4.2</td>
<td>5.8</td>
</tr>
<tr>
<td>Impoundments</td>
<td>18.1</td>
<td>14.1</td>
<td>22.1</td>
</tr>
<tr>
<td>Bird transport</td>
<td>0.2</td>
<td>0.1</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Our estimated N export from MI10N during drawdown is nearly two orders of magnitude greater than N export from an impoundment at Backus Lake in Michigan (Kadlec 1962), which supported two orders of magnitude fewer waterfowl (Table 8). Surprisingly, P export at MI10N and Backus Lake, however, was similar.

Surveys indicate that submerged aquatic vegetation (SAV) cover at Lake Mattamuskeet has declined over two timescales. SAV cover was consistently greater than 75% and 35% on the east and west sides respectively in the 1980s and 1990s, whereas recent surveys indicate that west side coverage is now consistently less than 15% and east side coverage is highly variable (Fig. 25A). The past three years of surveys indicate that SAV cover on the east side may now be in rapid decline (Fig. 25B).

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1 Gilliam et al. 1978; Skaggs et al. 1980  
2 Ward, 2009; Ardon et al. 2010
5.4 Discussion

5.4.1 Nitrogen vs Phosphorus

Since N is far more soluble than P, which is typically bound to sediments, it is not surprising that our analysis of nutrients exported by impoundment draw down show high N:P ratios of 50 and 300 in 2013 and 2014, respectively. Impoundment surface soils contain 370 mg P kg⁻¹, which is at the upper end of ‘medium’ for rice systems (International Rice Research Institute 1985), and have much lower N:P ratios (7.5) relative to surface water (Table 6), similar to other mineral soil wetlands on the North Carolina coastal plain (Richardson et al. 1988). It is possible that significant amounts of...
suspended sediments are also exported with impoundment surface water, which could occur via resuspension from wind, bioturbation, or the pumping process. Because high winds interfered with our ability to sample trace gas emissions, we specifically targeted sampling effort on calm days, and thus our surface water sampling may be biased against times of high sediment resuspension. Given the possibility that we are greatly underestimating P export via suspended sediments, we focus further analysis and discussion on the export of soluble N, which we feel is much better constrained.

5.4.2 Drawdown Timing

While agricultural lands often export high loads of NO$_x$-N because efficient drainage prevents the anoxic soil conditions necessary for removal via denitrification (Skaggs et al. 1980), in MI10N most N was exported in the form of NH$_x$, indicating a low capacity for nitrification. Low NO$_x$ concentrations (Fig. 23) and undetectable N$_2$O emissions prior to drawdown (Fig. 24), further indicate low nitrification and unfavorable conditions for N-removal via denitrification. Thus strategies to mitigate N loading from impoundments are antithetical to those for agricultural fields, which typically involve controlled drainage and use of buffering wetlands designed to provide an anoxic setting for denitrification (Gilliam et al. 1979, Cooper et al. 1987). For MI10N the problem is that soil remains wet for too long. An earlier seasonal drawdown (Table 9) would create an oxidized soil surface and hyporrheic zone, providing the potential for enhanced nitrification to complete the N cycle and stimulation of N removal.
An earlier drawdown would also have the potential to avoid N export because impoundment N concentrations would be lower. The observed drawdown timing appears to coincide with maximum surface water N concentrations, and thus maximum potential N export (Fig. 25). The hump-shaped pattern of surface water nutrients we observed in spring is likely the result of increased N mineralization relative to assimilation as wetland sediments warmed, followed by coupled nitrification-denitrification once surface sediments become oxidized by the drawdown (as indicated by the pattern of N₂O emissions; Fig. 26). These data suggest that if MI10N were drained earlier, before the acceleration of decomposition and N mineralization brought on by seasonal warming, it would load far less N.

Our early drawdown simulation suggests that a drawdown on 1 May could achieve more than 70% reductions in N export based on the avoided export of re-mineralized, concentrated NH₃. Early drawdown also has the potential to stimulate the permanent N removal process of denitrification. We caution, however, that our simple approach does not account for other potential feedbacks between water depth and biogeochemical processes. For example, decomposition of organic matter and mineralization could accelerate as water levels recede because of enhanced sunlight infiltration warming sediments. Since it takes several days of pumping to complete drawdown there is ample time for such processes and any feedbacks to play out. An
early drawdown strategy designed to reduce N export needs to be field-tested in a controlled experimental setting.

In addition to the potential for unforeseen feedbacks, early drawdown also poses other management drawbacks. For example, increased pumping cost is one likely drawback of earlier drawdown, though its severity will vary depending on idiosyncratic patterns of seasonal rainfall. Early drawdown in 2014 would have required a minor increase (<10%) in water volume pumped, but in 2013 the volume to pump would have had to nearly double—late drawdown in 2013 allowed evapotranspiration and passive drainage to do nearly half of the work. Another drawback of early drawdown may be enhanced emissions of the greenhouse gas, nitrous oxide, produced as a by-product of denitrification. However, a full accounting of the radiative impacts of early drawdown would need to include carbon emissions from diesel fuel burned by pumps, as well as impoundment methane emission and carbon storage dynamics (beyond the scope of this investigation).

The irony at Lake Mattamuskeet is that the drawdown of refuge impoundments, which is timed to optimize habitat quality in impoundments, also appears to maximize N export to the lake from these units. Heavy N loading is known to have deleterious effects on the habitat quality of shallow lakes and estuaries by stimulating phytoplankton (Hecky and Kilham 1988, Kolzau et al. 2014) at the expense of SAV (Davis and Brinson 1980). SAV at Lake Mattamuskeet is in “severe decline” (U.S. Fish
and Wildlife Service 2015b), as comprehensive surveys by the U.S. Fish and Wildlife Service demonstrate. Mean cover was roughly 70% in the 1990s; surveys from 2013/2014 reveal that SAV currently covers a mean of 30% of the lake bottom, with the healthier east side trending toward the more degraded west side (Fig. 27). Thus, some of the waterfowl resources gained within the 2485 ha of impoundments in the Lake Mattamuskeet watershed may be offset by losses in the waterfowl carrying capacity of the lake because of N-driven phytoplankton blooms and SAV loss exacerbated by impoundment drawdown. Therefore, current impoundment management prescriptions may have more mixed effects on landscape-level waterfowl resources than has been previously realized.

5.4.3 Role of Waterfowl

Despite our finding that waterfowl flocks transport relatively minor amounts of N directly to Lake Mattamuskeet, birds may still play an indirect role in downstream N loading via impoundment drawdown. The high population of overwintering waterfowl at MI10N (Table 12) probably inflates the pool of nutrients available for export. In addition to the potential to import N from adjacent agricultural lands (Post et al. 1998), birds can also contribute to mineralization of autochthonous N via the digestion and excretion of consumed plants and prey. Thus without directly moving any N, waterfowl may enhance export from impoundments by making N more soluble and mobile during drawdown.
Table 12: Waterfowl counts from ground surveys conducted at Marsh Impoundment 10 North at Mattamuskeet National Wildlife Refuge in North Carolina, U.S.A. by the U.S. Fish and Wildlife Service (Wires, 2015)

<table>
<thead>
<tr>
<th>Date</th>
<th>Tundra Swan</th>
<th>Ducks</th>
<th>Canada Goose</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/20/2012</td>
<td>770</td>
<td>3,142</td>
<td>60</td>
<td>3,972</td>
</tr>
<tr>
<td>12/6/2012</td>
<td>920</td>
<td>219</td>
<td>13</td>
<td>1,152</td>
</tr>
<tr>
<td>12/14/2012</td>
<td>294</td>
<td>345</td>
<td>0</td>
<td>639</td>
</tr>
<tr>
<td>12/28/2012</td>
<td>2231</td>
<td>508</td>
<td>320</td>
<td>3,059</td>
</tr>
<tr>
<td>1/8/2013</td>
<td>5,036</td>
<td>5,484</td>
<td>335</td>
<td>10,855</td>
</tr>
<tr>
<td>11/22/2013</td>
<td>710</td>
<td>815</td>
<td>215</td>
<td>1,740</td>
</tr>
<tr>
<td>12/2/2013</td>
<td>840</td>
<td>1,290</td>
<td>540</td>
<td>2,670</td>
</tr>
<tr>
<td>12/12/2013</td>
<td>1,111</td>
<td>280</td>
<td>405</td>
<td>1,796</td>
</tr>
<tr>
<td>12/19/2013</td>
<td>1,800</td>
<td>1,652</td>
<td>100</td>
<td>3,552</td>
</tr>
<tr>
<td>1/9/2014</td>
<td>6,450</td>
<td>25,529</td>
<td>95</td>
<td>32,074</td>
</tr>
<tr>
<td>Mean</td>
<td>2,016</td>
<td>3,926</td>
<td>208</td>
<td>6,150</td>
</tr>
</tbody>
</table>

While impoundment drawdown does not always export large amounts of N, as in the case of Backus Lake (Kadlec 1962), we may expect to find high N export in regions where waterfowl seasonally concentrate, as we have documented in eastern North Carolina. Similar effects may be occurring unrecognized in other important waterfowl wintering and migration areas along the Atlantic Coast as well as the Gulf Coast and the Mississippi Alluvial Valley. Hydrologic draw down N export across 36,000 ha of managed freshwater moist-soil impoundments at National Wildlife Refuges across the southeastern U.S. (Appendix A) could represent a widespread and hitherto unrecognized contributor of aquatic eutrophication.

Fertilizer runoff from agricultural lands is correctly recognized as the primary driver of anthropogenic eutrophication of lakes in many settings (Bennett et al. 1999, Qin
et al. 2007), but if we ignore potential N contributions from wildlife habitats, particularly those with dense bird populations and over which we have the capacity to exert hydrologic control, we may be overlooking a potent tool for nutrient mitigation. Since impoundments typically have a single outflow used once annually for drawdown, they could be considered a pollution point-source—one that our research suggests could be relatively easy to mitigate through existing management infrastructure.

5.4.4 Implications for Lake Mattamuskeet

Our data from MI10N indicate that Mattamuskeet National Wildlife Refuge moist-soil impoundments have the potential to be important exporters of N via seasonal hydrologic drawdown, comparable on a per-area basis to local fertilized agricultural lands. In most watersheds, impoundments represent a minor component of land cover and thus cannot contribute significantly to downstream loading even with a high export per ha. At Lake Mattamuskeet, however, some 2,500 ha of impoundments are packed into a 12,000 ha watershed. The large impoundment area combined with a high loading rate leads us to conclude that impoundments may collectively weigh significantly on Lake Mattamuskeet’s N budget.

We caution, however, that our extrapolation to the 1001 ha of privately owned impoundments on agricultural land is uncertain. These lands are managed for both agriculture and waterfowl, which makes them fundamentally different from moist-soil impoundments in a few crucial ways: 1) vegetation cover is corn or soy bean rather than
aquatic vegetation; 2) fertilizers are applied; and 3) timing of hydrologic drawdowns may be much earlier in the season. As a result, the timing and mechanism of nutrient loading may be much different in private impoundments compared to moist-soil units under refuge management. Nevertheless, if we use agricultural runoff values (Gilliam et al. 1978, Skaggs et al. 1980) instead of moist-soil impoundment values for agricultural impoundments, our study suggests the magnitude of N loading would change little (Table 11).

Our N budget indicates that agricultural fields are the most important source of anthropogenic N to Lake Mattamuskeet, but that the contribution from impoundments is not insignificant. This finding may be useful for managers of the Lake, because mitigation of agricultural sources of pollution can be expensive (Butt and Brown 2000, Rabotyagov et al. 2010), whereas our analysis suggests that impoundment N loading could be significantly reduced by early draw down—a relatively minor, low-cost alteration to current impoundment management.

6. Conclusions
6.1 Summary of research findings

The goal of this dissertation is to improve scientific understanding of how wetland restoration practices and waterfowl habitat management affect critical wetland biogeochemical processes related to greenhouse gas emissions and nutrient cycling. Via field experiments I tested how management factors, influenced responses of
biogeochemical process indicators, such as trace gas emissions, surface and pore water chemistry, soil properties and vegetation dynamics. I described relationships between restoration and management prescriptions and the biogeochemical functioning of wetlands and suggest how alterations in management might yield more desirable wetland biogeochemical performance via avoided emissions of greenhouse gases or nitrogen export.

To evaluate how organic matter amendments influenced the carbon balance of a restored wetland, I measured trace gas emissions across an experimental organic matter gradient (Chapter 2). I found that methane emissions were relatively low compared to reference wetlands and were not enhanced by added organic matter. Added organic matter decomposed aerobically during dry periods leading to greater carbon dioxide efflux from plots that received the heaviest amendments.

To improve understanding of optimal methods for measuring trace gas emissions from wetlands I compared the efficacy of two different methods for measuring emissions of methane (Chapter 3). I found that my remotely sampled water-filled-gutter chamber design, was far more resilient to soil-disturbance-induced measurement errors compared to an often-used rubber-gasket seal static chamber design. This design was superior in avoiding the purge of soil pore space methane into chamber headspace during setup and sampling, and it was far more likely to generate robust linear regressions and reliably methane flux data. This chamber successfully
obviates the need to construct costly boardwalks for reliable wetland methane emissions measurement.

Extensive research effort has been invested into the interplay between emergent wetland vegetation and methane emissions (Laanbroek 2010), but little is known about how aquatic herbivores impact dynamics of wetland biogeochemistry. Through a 2-year waterfowl exclosure experiment, I found that herbivory of belowground roots and rhizomes retarded spring emergence of hydrophytes, which starved soils of oxygen (Chapter 4). This lead to huge methane emissions from grazed plots, compared to densely vegetated plots protected from herbivory by exclosures. Herbivore effects on plant transport of oxygen also impacted the nitrogen cycle, as grazed soils were unable to nitrify and thus emitted undetectable levels of nitrous oxide. Thus, waterfowl herbivory fundamentally altered the methane and nitrogen cycling of our study wetland by disrupting plant-mediated transport of oxygen to soils.

Herbivore disruption of the nitrogen cycle at my waterfowl impoundment field site also contributed to heavy export of dissolved nitrogen during prescribed hydrologic drawdowns (Chapter 5). I found that the waterfowl impoundment exported as much nitrogen to downstream water bodies as adjacent fertilized agricultural fields and argue that such ecosystems, which are widespread, may constitute a significant unrecognized source of nitrogen to lakes and estuaries surrounded by impoundments with high densities of waterfowl. My hydrology and nutrient data indicate that by altering the
timing of prescribed drawdown, nitrogen export could be reduced significantly, as was shown by my analysis in which N loading was reduced by more than 70 percent.

6.2 Integration and implications of research findings

The overarching objective of this dissertation research is to address knowledge gaps in the scientific understanding of how wetland restoration practices and waterfowl management affect critical wetland biogeochemical processes related to greenhouse gas emission and nutrient cycling. This research advances the state of ecological understanding of the interplay between emergent vegetation and biogeochemical cycling in wetlands and contributes to a scholarly debate about the importance of restored wetlands as sources of greenhouse gases. In addition, my work provides practical guidance on how to best meet greenhouse and nutrient pollution mitigation goals in specific wetland restoration and management contexts.

The greenhouse gas implications of wetland restoration have been hotly debated in scientific publications (Bridgham et al. 2006, 2014, Mitsch et al. 2013, Neubauer 2014). This dissertation research advances this debate by providing evidence demonstrating that the practice of adding organic matter to restored wetlands does not exacerbate the undesirable emission of methane (Chapter 2). This research also provides guidance for restoration practitioners, suggesting that additions of organic matter above a moderate threshold will lead to increased emissions of carbon dioxide without improving other desired wetland functional responses (Bailey et al. 2007, Bruland and Richardson 2009).
In addition, my work testing the effectiveness of static chamber design (Chapter 3) will allow for future investigators of greenhouse gas emissions from wetlands to generate reliable data without having to invest in costly boardwalk construction.

The role of emergent vegetation in the biogeochemical functioning of wetlands is complex because hydrophytes exert influence via at least three mechanisms (Laanbroek 2010). Emergent vegetation: 1) contributes a carbon source to wetland sediments in the form of detritus and exudates (Ding *et al.* 2005); 2) transports oxygen into wetland sediments via aerenchyma and radial oxygen loss (Armstrong 1964); and 3) provides an efflux pathway for the ventilation of accumulated interstitial gases, especially methane (Dacey and Klug 1979). Research investigating impact of herbivory on these various plant effects on wetland biogeochemistry has been sparse. Chapter 4 of this dissertation demonstrates for the first time that herbivorous waterfowl fundamentally alter methane and nitrogen cycling in wetlands by disrupting plant-mediated oxygen transport. This finding fills an important knowledge gap because most investigations into wetland biogeochemical function typically ignore or overlook the impact of large-bodied fauna and my results indicate that their impacts can be potent.

My research on the effects of waterfowl on impoundment greenhouse gas emissions may be particularly useful for wildlife management organizations which control the hydrology and waterfowl populations for vast areas of wetlands (Strader *et al.* 2005), and which are also under pressure to meet greenhouse gas emission reduction
targets (Fish and Wildlife Service 2010). I found that prescribed hydrologic manipulations of impoundments to support germination of waterfowl-favored plants (Strader et al. 2005) also have the potential to exert strong controls over the emission of methane (Chapter 4) and the export of nitrogen (Chapter 5). My finding of the potential for waterfowl impoundments to export heavy loads of nitrogen may have important practical impact for the management of these habitats since aquatic eutrophication is such a pervasive problem (Smith 2003), including at the lake into which lake my field site drains (U.S. Fish and Wildlife Service 2015b).

My intended outcome is for this dissertation research to not only provide the foundation for practical wetland management solutions, but for it also to inspire additional needed research investigating the carbon balance of restored wetlands and the role of herbivores in wetland biogeochemical functioning. Like any research endeavor, this dissertation, in its search for answers, also demands the asking of many additional questions. Massive wetland restoration projects continue on the heels of development in the United Stated as part of the policy of ‘no net loss,’ and yet the implications of restoration practice on greenhouse gas budgets remains uncertain and debated in the scientific literature. In addition, the biogeochemical impacts of many herbivore species, inhabiting a wide diversity wetland habitats, remain completely unknown. As the world population swells with additional billions of human inhabitants over the coming decades, demands on shared wetland resources will intensify, and it
will become increasingly important to understand how best to maximize the value they provide. My hope is that other researchers will be inspired by this dissertation to join me in meeting these environmental challenges.
Appendix A

Table 13: Estimate of the extent of impoundment habitats similar to those at Mattamuskeet National Wildlife Refuge. Freshwater moist soil and/or emergent marsh waterfowl impoundment areas within National Wildlife Refuges (NWR1) and under state management in Waterfowl/Wildlife Management Areas (WMA) of the Southeastern U.S. region, including Alabama2, Arkansas3, Delaware4, Florida5, Georgia6, Kentucky7, Louisiana8, Maryland9, Mississippi10, North Carolina11, South Carolina12, Tennessee13, Virginia14. The total acreage reported here represents a fraction of total suitable waterfowl habitat as it excludes flooded agricultural lands, brackish impoundments, forested wetlands, ponds, reservoirs, lake margins and unmanaged natural marshes, as well as all privately owned wetlands.

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References


Angel, R., P. Claus, and R. Conrad. 2011. Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. The ISME journal 0:1–16.


Flebbe, P. A. 1982. Biogeochemistry of carbon, nitrogen, and phosphorus in the aquatic subsystem of selected Okefenokee Swamp sites. University of Georgia, Athens, Georgia, USA.


North Carolina Department of Health Environment and Natural Resources. 2013. Lake & Reservoir Assessments Tar-Pamlico River Basin. Raleigh, NC.


production, and methanogenic pathways in peatlands across an ombrotrophic–minerotrophic gradient. Soil Biology and Biochemistry 54:36–47.


Biography

R. Scott Winton born NC March 1985 in Durham, North Carolina. After transferring out of Jordan High School at the start of 11th grade, he graduated from Carolina Friends High School in 2003. Scott received an A.B. with honours in Geology-Biology from Brown University in Providence, Rhode Island in 2007. His honor thesis completed under the supervision of Prof. Timothy D. Herbert was titled: “A record of sea surface temperatures and productivity at the Peru Margin for the last 2,000 years.”

After three years abroad working at the University of Edinburgh School of Geoscience in Scotland; The Wilderness Society in Sydney, Australia; La Hesperia Biological Station in Pichincha, Ecuador, Scott returned to Durham to pursue a Ph.D. in the Duke University Wetland Center under the supervision of Prof. Curt Richardson. He was given honorable mention twice by the National Science Foundation Graduate Research Fellowship Program and awarded research grants from the Carolina Bird Club and Duke University Graduate School. In 2015, Scott designed and taught, as instructor of record, an undergraduate seminar, Biodiversity Issues and Field Methods, thanks to an Anne T. and Robert M. Bass Instructional Fellowship.

Scott is well-known on Duke’s campus for his interest in birds and advocacy for conservation. As representative of the Environmental Science and Policy program on the Duke University Graduate and Professional Student Council Scott wrote and passed resolutions advocating for the conservation of old growth forest on campus and for bird-
friendly building design. The latter led university administration to retrofit the Fitzpatrick Center and Scott was interviewed by local TV and print news. He was also featured in a video on Duke Today and stories in *Duke Environment* and *Duke Research*. Along with several other Nicholas School students he has worked to develop a research program investigating patterns of bird-window collisions.

Publications:

