FARM FIELDS TO WETLANDS:
BIOGEOCHEMICAL CONSEQUENCES
OF RE-FLOODING IN COASTAL PLAIN AGRICULTURAL LANDS

by

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Dissertation submitted in partial fulfillment of
the requirements for the degree of
Doctor of Philosophy in the University Program in Ecology
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ABSTRACT

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Abstract

Whether through sea level rise, farmland abandonment, or wetland restoration, agricultural soils in coastal areas will be inundated at increasing rates, renewing connections to sensitive surface waters and raising critical questions related to environmental tradeoffs. Wetland restoration in particular is often implemented not only to promote wildlife habitat, but also to improve water quality through nutrient removal, especially in agricultural catchments. The microbial process of denitrification is the central mechanism of nitrogen removal in wetlands and flooded soils, and can be seen as a potential environmental benefit of flooding agricultural lands. While denitrification undoubtedly can remove nitrogen from soil and surface water, higher soil moisture or flooding in wetland soils can also increase the production of greenhouse gases, specifically nitrous oxide and methane, representing a potential environmental tradeoff. Understanding the likely benefits of denitrification and the likely greenhouse gas costs of wetland restoration could help inform environmental policies concerning wetland restoration.

Determining whether restored wetlands are larger sources of greenhouse gases compared to contrasting land use types (agriculture and forested wetlands) was the first goal of this dissertation (Chapter 2). We measured gas fluxes from soil and water to the atmosphere, and related environmental variables, in four sites over two years to

estimate fluxes of the three major greenhouse gases. We found that carbon dioxide was the major contributor to the radiative balance across all sites, but that in the agricultural site and one of the forested wetland reference sites, nitrous oxide was the second most important contributor. Many studies have shown that methane is more important that nitrous oxide in most freshwater wetlands, as we found in the other forested wetland reference site and in flooded parts of the restored wetland. Overall, we did not find higher greenhouse gas fluxes in the restored wetland compared to agricultural soils or forested wetlands.

The controls over nitrous oxide are especially complex, because it can be produced by two complementary processes, nitrification and denitrification, which generally occur under different conditions in the environment. In Chapter 3, we determined the soil and environmental factors that best predicted nitrous oxide fluxes for a subset of our data encompassing gas fluxes measured in November 2007. We found that soil temperature and soil carbon dioxide flux, along with ammonium availability and denitrification potential, were good predictors of nitrous oxide (R^2_{adj} =0.81). Although the nitrous oxide model did not perform as well when applied to data from another sampling period, we expect to further develop our modeling efforts to include possible non-linear temperature effects and a larger range of environmental conditions.

In Chapter 4, we present results of a stable isotope tracer experiment to determine the relative contribution of nitrification and denitrification to nitrous oxide

fluxes in these different land use types, and to determine the response of these processes to changing soil moisture. We added two forms of nitrogen-15 to intact soil cores to distinguish nitrification from denitrification, and subjected the cores to drainage or to a simulated rain event. We found that across the range of soil moisture, the fraction of nitrous oxide produced by denitrification did not change, but within each soil type there was a response to the simulated rain. In mineral soils, the nitrous oxide fraction increased with increasing soil moisture, with the highest mole fraction [$N_2O/(N_2+N_2O)$] in the agricultural soils, while in the organic soils there was no change or even a decrease. The fraction of nitrous oxide derived from coupled nitrification-denitrification increased with increasing soil moisture, and was much higher than that from denitrification alone in the more organic soils. This suggests that, in these saturated acid-organic soils, nitrification plays an important and underappreciated role in contributing to nitrous oxide fluxes from freshwater wetlands. The results from the laboratory experiment were consistent with patterns we saw in the field and help explain the differential contribution of nitrification and denitrification to nitrous oxide fluxes in different land use types in coastal plain wetlands of North Carolina.

Overall, we found that both nitrification and denitrification contribute to nitrous oxide fluxes in coastal plain wetlands in North Carolina, and that nitrification is an especially important source in acid-organic soils under both field-moist and saturated conditions. Although freshwater wetlands, with an average nitrous oxide mole fraction

of 0.08, are generally seen as being insignificant sources of nitrous oxide, our study sites ranged from 0.10 to 0.30, placing them closer to agricultural fields (0.38; Schlesinger 2009). Although the ecosystems in our study produced more nitrous oxide than expected for freshwater wetlands, we found no significant tradeoff between the local water quality benefits conferred by denitrification and the global greenhouse gas costs in the restored wetland. These results suggest that, from a nitrogen perspective, wetland restoration in coastal agricultural lands has a net environmental benefit.

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1. Introduction

1.1 Wetlands, agriculture, and restoration

Since European settlement (1780's to 1980's), forty percent of wetlands in the South Atlantic region of the United States were converted into other land uses, primarily for forestry and drained agriculture (Dahl 1990). Currently, many of these actively drained farms are being abandoned due to economic barriers, such as high fuel prices for drainage pumps, or are being restored to wetlands through economic incentives like mitigation banking. Slowly rising sea levels linked to climate change are also bringing significant coastal inundation to the region. Whether through sea level rise, farmland abandonment, or wetland restoration, the inundation of heavily fertilized soils is coupled with renewed connections to sensitive surface waters, raising critical questions related to environmental tradeoffs. Likely benefits of re-flooding former wetlands include the creation of wildlife habitats and increased storage of carbon (C) in soils and vegetation. Removal of nitrogen (N) from soils or surface waters by microbial denitrification (DNF), which transforms nitrate (NO_3^-; a major pollutant) to inert dinitrogen gas (N_2), presents another significant potential benefit of re-flooding agricultural lands.

While DNF, along with other soil microbial processes in wetland soils, can be said to improve water quality, it can be a major natural source of greenhouse gases (GHGs) to the atmosphere; this is an important tradeoff that must be considered when

wetland restoration and DNF are promoted as means of improving surface and ground water quality. Emissions of the three major greenhouse gases [carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4)] have been well documented in many natural and human-dominated wetland environments, and are known to be strongly influenced by hydrology, soil properties, and nutrient availability. However, the rates, patterns, and determinants of the three main biological mechanisms by which these GHGs are produced have rarely been studied systematically and jointly, especially in restored wetlands. Understanding the controls over microbial GHG fluxes in wetlands is important to global change science. This knowledge may also help inform management practices for restoring agricultural wetlands, particularly as the creation and restoration of riparian wetlands and streams for water quality improvement and compensatory mitigation continues to expand.

1.2 Biogeochemistry of greenhouse gases

Trace gases emitted by soil microbial processes are a major part of biogenic greenhouse gas fluxes to the atmosphere, representing 30% of annual CO_2 and CH_4 fluxes and 70% of annual N_2O fluxes (Schlesinger 1997; Mosier 1998); Table 1). Biogenic trace gas fluxes occur predominantly from waterlogged, seasonally saturated, or ephemerally wet soils, as typically found in wetland, riparian, and stream ecosystems (Conrad 1995). These landscape locations, and their associated trace gas emissions, are particularly sensitive to hydrologic alterations and nutrient loading that result from land

Table 1: Comparison of biogenic trace gases in the atmosphere, their concentrations and trends, sources and sinks.

	CO_2	CH_4	N_2O
Atmospheric concentration (1, 2)	374.9 ppmv	1.730 ppmv	0.3170 ppmv
Current annual increase (1, 2)	1.7-2.4 ppmv	0.007 ppmv	0.0008 ppmv
Global warming potential (CO_2 equiv.) for 100 y (1)	1	25	298
Atmospheric residence time (3)	5 years	12 years	120 years
Major sources (3, 5)	Ocean degassing Soil respiration Plant respiration Industrial emissions Biomass burning	Methanogenesis Biomass burning Fossil fuels Landfills Agriculture Live/senescing plants	Nitrification and denitrification
Major sinks (3)	Ocean uptake Photosynthesis Carbonate rocks	Reactions with OH Soil methanotrophy	Stratospheric photolysis
Soils as % of annual flux to the atmosphere (3, 4)	30	30	70
Range of soil redox potentials	> 800 to –300mV	+50 to –250 mV	+120 to +250 mV

1: Forster et. al. (2007); 2: Keeling and Whorf 2005; 3: Schlesinger (1997); 4: Mosier (1998); 5: Keppler et al. 2006

use change and climate change (Schlesinger et al. 2006; Verhoeven et al. 2006), but riparian zones in particular have not been explicitly included in greenhouse gas inventories [e.g., (Groffman et al. 2000)].

The three major biogenic trace gases have global significance in terms of warming potential and chemical reactions in the atmosphere. The characteristics of CO_2, N_2O, and CH_4 in the atmosphere are summarized in Table 1. Carbon dioxide is far more abundant in the atmosphere than N_2O or CH_4, but the mean residence times of CH_4 and N_2O are much longer than CO_2, which contributes to their greater global warming potential (25 and 298 times the warming potential of CO_2, respectively (Forster et al. 2007). Soil microbial processes feature prominently amid the major sources of biogenic

trace gases, through soil respiration, methanogenesis, and microbial nitrification (NF) and DNF. The sink mechanisms for CO_2, N_2O, and CH_4 are quite different as well, with CO_2 being subject to more rapid removal from the atmosphere; CH_4 in the atmosphere is depleted following reactions with OH radicals, while the only mechanism for N_2O destruction is photolysis in the stratosphere (Schlesinger 1997). Both CH_4 and N_2O also affect atmospheric chemistry through involvement in reactions that lead to the depletion of stratospheric ozone (Mosier 1998).

Emissions of CO_2, N_2O, and CH_4 have been well documented in many natural and human-dominated wetland environments, such as riparian forests, rice paddies, and constructed treatment wetlands (Groffman et al. 2000; Mander et al. 2005; Mitsch et al. 2005; Forster et al. 2007). However, soil respiration, DNF, and methanogenesis in wetlands have rarely been studied systematically and jointly outside of treatment wetlands (but see (Yu et al. 2006). Fluxes of these trace gases depend on physical factors (e.g., temperature, soil bulk density, soil texture), hydrologic factors (e.g., water table depth and variability, water-filled pore space, water source), chemical factors [e.g., pH, redox potential, O_2, NO_3^-, dissolved organic C (DOC)], and biological factors (e.g., microbial communities, quality and quantity of soil organic matter, and plant nutrient demands; Conrad 1995; Mosier 1998). An examination of the effects of different factors on rates, patterns, and variability of these soil processes is warranted to understand how trace gas fluxes vary accordingly and to determine tradeoffs between their emissions.

1.2.1 Overview of microbial processes

Carbon dioxide is the byproduct of respiration in soils. The energy in organic carbon compounds is released by oxidation, and CO_2 is formed; the magnitude of the total energy released depends on the terminal electron acceptor. Oxygen is the most energetically favorable terminal electron acceptor, yielding 501 kJ $\cdot$ mol^{-1} from the oxidation of organic carbon (Hedin et al. 1998). The oxidation of organic matter is the energy source for certain heterotrophic anaerobic metabolisms as well, but these occur through multiple steps catalyzed by various microbial pathways, including fermentation, with much lower energy yields (Megonigal et al. 2004).

Microbial processes that produce N_2O are also complex. Under aerobic conditions, the nitrifying bacteria and archaea oxidize NH_4^+ to NO_2^- (e.g., *Nitrosomonas* spp.) and NO_2^- to NO_3^- (e.g., *Nitrobacter* spp.), with some N_2O produced as an intermediate byproduct (Paul and Clark 1996). Denitrifying microbes, mostly heterotrophic bacteria such as *Pseudomonas* spp. and *Alcaligenes* spp., are facultative aerobes that use NO_3^- as a terminal electron acceptor in the absence of O_2. The complete reduction of NO_3^- to N_2 occurs under anoxic conditions with low substrate availability (low NO_3^- and DOC), while N_2O is released as an intermediate precursor to N_2 (Knowles 1982). DNF has been shown to occur under aerobic conditions in the laboratory (Lloyd et al. 1987). The temporal lag between N_2O production and N_2O reduction to N_2 has been attributed to the *de novo* synthesis of DNF enzymes (Firestone et al. 1980; Knowles 1982).

Under the redox conceptual framework, the most favorable terminal electron acceptor after O_2 is NO_3^-, releasing 476 $kJ \cdot mol^{-1}$ (Stumm and Morgan 1996).

Methanogenesis describes two distinct pathways of CH_4 production that occur within the domain Archaea and are the least energetically favorable of the anaerobic metabolic processes: 1) acetate-splitting (e.g., *Methanosarcina* spp.; energy yield 93 $kJ \cdot mol^{-1}$) and by CO_2 reduction (e.g., *Methanococcus* spp., energy yield 66 $kJ \cdot mol^{-1}$). Net CH_4 flux from soils to the atmosphere can be attenuated by the consumption of CH_4 by methanotrophs in oxic environments (e.g., *Methylobacter* spp. and *Methylosinus* spp.; members of the γ- and α-Proteobacteria, respectively; Conrad 1995).

1.2.2 Controls on soil microbial processes

1.2.2.1 Temperature

Soil respiration, NF, DNF, and methanogenesis are all biological processes, and as such are stimulated by increasing temperatures. In temperate soils, the meaningful temperature range for microbial activity is generally between 5°C and 35°C, and can persist throughout the year in the Southeast United States (Megonigal et al. 1996). For example, Q_{10} values (average increases in rates per 10°C increase in temperature) can range from 2 to 4 for soil respiration (Fierer et al. 2003), DNF (Knowles 1982), and methanogenesis (Macdonald et al. 1998), indicating an exponential increase in emission rates with increasing temperatures. In mesocosm studies, hysteresis effects in CO_2 and CH_4 emissions have been shown in response to warming experiments (Updegraff et al.

1998). In field studies, CH_4 emission rates have been shown to be less sensitive to lower temperatures (4-9°C), with a strong response above 10°C (Wilson et al. 1989). The acetate-splitting pathway of methanogenesis tends to dominate the process at lower temperatures, with the proportion of CO_2-reducing methanogenesis increasing with temperature (Conrad 1995). Since surface soils are exposed to more varying temperatures than subsurface soils, microbial communities in surface soils are generally less sensitive to temperature changes than communities in subsurface soils (Fierer et al. 2003). Owing to the high specific heat of water, hydric soils and other saturated environments are likely to experience smaller diurnal temperature changes than drier soils.

1.2.2.2 Hydrologic factors

Under very dry conditions, low soil moisture inhibits microbial activities: emissions of CO_2, N_2O, and CH_4 are lower than under moist conditions (Conrad 1995). Rapid re-wetting of dry soils can cause a flush of CO_2 and N_2O, and can stimulate denitrification (Brumme et al. 1999; Venterink et al. 2002; Fierer and Schimel 2003). Methanogenesis can follow drying and re-wetting in floodplain wetlands, although the time lag in methane production can vary from days to months, depending on the season (Boon et al. 1997). Moist soils are favorable for decomposition of organic matter: CO_2 emissions are highest just below saturated levels. Under flooded conditions, available O_2

is rapidly depleted and its resupply is diffusion-limited; less energetically efficient anaerobic metabolic processes occur, leading to lower emission rates of CO_2 (Craft 2001).

Hydrologic factors are critical in controlling DNF and methanogenesis: it is primarily as a result of saturated soil pore spaces (whether from precipitation, surface runoff, overbank flooding, or groundwater) that anoxia develops. Many soil incubation studies as well as plot-scale field measurements have shown that indices relating to soil moisture are often the best predictors of DNF activity [e.g., (Davidson and Swank 1986; Pinay et al. 1993; Weier et al. 1993; Burt et al. 2002)]. Nitrous oxide emissions have been widely shown to be higher at less than fully saturated conditions, with N_2O:N_2 decreasing with increasing water filled pore space and lower O_2 availability [e.g., (Weier et al. 1993; Thomas et al. 1994; Ullah et al. 2005)]. Mean water table elevation can be a good predictor of dominant N-transforming processes, for example ammonification (0-10cm below the surface), NF (-10 to –30cm), and DNF (below –30cm) showed distinct patterns along a European climatic gradient (Hefting et al. 2004). Soil texture and bulk density are related to hydrology through the physical constraints they impose on water movement and gas diffusion, and have been shown to be good predictors of DNF in riparian soils, especially at coarser scales (Groffman et al. 1992; Brumme et al. 1999).

Methane emissions are also closely linked to flooded conditions, given that methanogenesis is restricted to anoxic zones, while CH_4 consumption occurs in oxidized surface soils (Whalen 2005). Where CH_4 production is significant at depth, much of it can

be consumed by aerobic methanotrophs (Conrad 1995). For example, CH_4 fluxes from peatlands with standing water can be an order of magnitude higher than in similar zones where the water table is 15cm below the surface (Macdonald et al. 1998). Other studies have also shown that water level must be near the surface for CH_4 emissions to be significant, but other factors are also frequently important (Fiedler and Sommer 2000; Updegraff et al. 2001).

1.2.2.3 Chemical factors

Supplies of electron donors and receivers can be used as predictors of soil microbial processes, including DNF and soil respiration by applying a thermodynamic approach (Hedin et al. 1998; Sobczak et al. 1998). In many cases, the availability of organic matter as an electron donor is the primary factor that controls rates of soil respiration, DNF, and methanogenesis (Megonigal et al. 2004). The quality of available organic matter, measured for example as C mineralization or water-extractable C, is the strongest control on CO_2 and CH_4 emissions in wetlands, particularly once anaerobic conditions have been established (Bridgham and Richardson 1992; Fiedler and Sommer 2000; Whalen 2005).

Nitrogen removal in riparian zones is tightly linked to supplies of both NO_3^- and soil organic matter (Addy et al. 1999; Groffman et al. 2002; Sabater et al. 2003), which are required for DNF. Carbon mineralization rates and concentrations of low molecular weight organic acids, indicators of C quality, have been shown to predict DNF well in

riparian zones (Seitzinger 1994; Baker and Vervier 2004; Hill and Cardaci 2004). In a laboratory study, high NO_3^- supplies resulted in higher N_2O:N_2 ratios, while increasing DOC supplies led to the production of N_2 rather than N_2O (Weier et al. 1993). It is important to note that, in the presence of O_2, NF can supply NO_3^- for DNF, so that NO_3^- concentrations in porewater or in water overlying sediments may not be strong predictors of DNF (Seitzinger 1994). Interactions between the vertical distribution of organic C in the soil profile and hydrologic flowpaths transporting NO_3^- result in hot spots of DNF (Hill et al. 2000; McClain et al. 2003); where availability of labile DOC is low, DNF rates will be lower (McCarty and Bremner 1992).

Low pH tends to lower the rates of microbial processes, with the exception of organisms that are adapted to acidic environments, such as wetland methanogens (Paul and Clark 1996; Whalen 2005). Denitrification has been found to occur at pH 4-11 under laboratory conditions, with optimum conditions around pH 7-8 (Thomas et al. 1994). In two forested ecosystems, base saturation was strongly correlated to pH and was a good predictor of NF parameters (Davidson and Swank 1986). Field results for CH_4 emissions show interactions between plant communities, microbial communities, and pH, with no clear predictor emerging for methanogenesis (Bridgham and Richardson 1992; Whalen 2005).

Soil redox potential is another edaphic parameter that has been studied as a controller or correlate of anaerobic microbial processes, although it is the competition for

electron donors by microbes that establishes soil redox potential (Megonigal et al. 2004). Across a soil redox gradient in a forested wetland, redox potential (Eh) was shown to be significantly correlated with O_2 concentrations. Typical Eh values for oxidized soils are +400 to +700 mV, while seasonally flooded soils have widely varying values – encompassing the oxidized range down to –300 mV (Patrick et al. 1996). Methanogenesis is thought to occur primarily at very low redox potentials, given its unfavorable thermodynamic status, but significant CH_4 production has been measured at +300mV (Yu et al. 2006). In the same study, N_2O production was highest at +250mV, which seems contrary to thermodynamic theory, yet the authors explained this apparent anomalous pattern using evidence of microsites of anoxia within oxic soil horizons for the production of CH_4 at high redox potentials (Yu et al. 2006). Another study has shown redox potentials over -75mV to be inversely correlated with CH_4 fluxes in wetlands (Fiedler and Sommer 2000). In a laboratory study, maxiumum CH_4 emissions were measured at different redox potentials depending on the soil type, ranging from –220 to –150 mV, while N_2O production was highest at +120 to +250 mV. This study suggests that holding soil redox potential between –150 and +120 mV would minimize production of both greenhouse gases (Yu et al. 2001).

1.2.2.4 Biological factors

Plant dynamics, microbial community composition, and animal activities such as bioturbation are also clearly important to trace gas emissions from soils. The

contribution of aboveground plant litter and root biomass to the pool of mineralizable C plays a key role in regulating soil microbial processes, including DNF and CH_4 production (Baker and Vervier 2004; Liikanen et al. 2005; Whalen 2005). The role of vascular plant aerenchyma as a conduit for CH_4 from belowground has been widely reported; this mechanism also bypasses the layer of methanotrophic organisms that might diminish CH_4 fluxes to the atmosphere (Whalen 2005). Bioturbation by soil fauna can be an important factor that introduces O_2 to anoxic soils, altering biogeochemical processes (Paul and Clark 1996; Megonigal et al. 2004).

The interaction of physical, chemical, and biological parameters that can control trace gas emissions from microbial processes is complex; that microbial communities differ in composition and in DNF function and respond differently to environmental variability is not surprising. Two fields with different land use histories showed significantly different DNF activities (DNF rates, DNF enzyme activities, N_2O:N_2 ratios); these differences were attributed to community composition and diversity (Cavigelli and Robertson 2000; Cavigelli and Robertson 2001). Relating microbial processes to community structure represents a new frontier at the micro-scale in ecosystem ecology, and is due largely to the wider availability of molecular tools.

1.2.3 Variability across ecosystem types

Upland forested ecosystems can be net sinks for CH_4 due to methanotrophic activities in surface soils (Whalen 2005). Riparian forests, and other seasonally wet

environments, can be sources of both CH_4 (15-900 mg $CH_4 \cdot m^{-2} \cdot d^{-1}$) and N_2O (e.g., 4.8-43.9 mg $N_2O \cdot m^{-2} \cdot d^{-1}$; (Brumme et al. 1999), but rates and patterns of emission vary widely in space and time, due to the complex factors that govern their production (Matson and Vitousek 1990; Fiedler and Sommer 2000; Groffman et al. 2000; Yu et al. 2006).

In agricultural settings, substantial fractions of fertilizer N additions are subject to nitrification and denitrification, which have been shown to contribute equally to N_2O fluxes from soils, up to 156 mg $N_2O \cdot m^{-2} \cdot d^{-1}$ (Panek et al. 2000). Agricultural fields have been determined to represent the largest source of N_2O from anthropogenic activities (International Panel on Climate Change 2001). In addition, N-fertilization and irrigation in grasslands can inhibit the consumption of CH_4 in surface soils and enhance N_2O production (Mosier et al. 1991; Panek et al. 2000). Rice paddies are a major source of trace gases from soils: CH_4 production can reach over 500 mg $CH_4 \cdot m^{-2} \cdot d^{-1}$ (Neue and Sass 1994).

Treatment and constructed wetlands provide another eutrophic perspective on trace gases: emission rates for both CH_4 and N_2O ranged over 3 orders of magnitude in a study in Estonia (Mander et al. 2005). These highly variable rates were still much lower than rates reported for agricultural fields, including rice paddies, leading the authors to suggest that wetland restoration should not result in major increases in greenhouse gases worldwide (Mander et al. 2005). However, other authors have raised concerns that

wetland restoration in agricultural lands could have negative consequences in terms of GHG emissions [e.g. (Verhoeven et al. 2006)].

In trace gas studies, research methods and resulting flux measurements vary widely across spatial and temporal scales, experimental conditions, and reporting conventions: from incubations in test tubes, to laboratory mesocosms, to in situ rates from surface soils and deep groundwater. Additionally, there is much variability and uncertainty in extrapolating field-scale, short-term measurements to annual, regional and global scales, especially in the face of regional land use change and global climate change (Groffman et al. 2000b). As a result, mass-balance, empirical, or simulation models have been developed for field-scale to global scale estimations of GHG fluxes. These can include relationships of soil parameters (soil NO_3^-, respiration, and soil physical properties; (Del Grosso et al. 2000), bulk density, or mapped soil type to predict ecosystem N_2O or CH_4 emissions from soils (Groffman et al. 1992; Fiedler and Sommer 2000). Refining large-scale models and incorporating more accurate accounting procedures that include more realistic variety of mechanisms for trace gas emissions may be of importance for global climate change models (Groffman et al. 2000; Skiba and Smith 2000).

1.3 Research questions

My dissertation focuses on quantifying GHG fluxes and determining their environmental controls in coastal freshwater wetlands under different land uses. The specific questions that are addressed in the research are:

1. What are the rates and spatial patterns of CO_2, N_2O, and CH_4 emissions from agricultural, restored, and forested wetlands and how do they vary throughout the year? (Chapter 2)
2. How do environmental factors influence N_2O fluxes and N cycling, and can we develop predictive models for these processes? (Chapter 3)
3. How do the microbial sources of N_2O (nitrification and denitrification) and partitioning of gaseous products (N_2O vs. N_2) vary across land uses and hydrological setting? (Chapter 4)

This research may contribute to our understanding of the contribution of agricultural and restored wetlands to GHG fluxes, and could inform global climate change scenarios and policies for wetland restoration.

2. Greenhouse gas fluxes in coastal plain wetlands under contrasting land uses

2.1 Introduction

The extent of wetlands worldwide has been diminished by about half through human activities such as clearing, filling, ditching, and drainage (Millennium Ecosystem Assessment 2005). In the US, the majority of wetland conversions occurred from the 1780's through 1980, primarily driven by agricultural expansion (Dahl 1990). In North Carolina, 13% of the state's wetlands were converted to agriculture from 1970-1980, particularly in the coastal plain (Dahl 1990; Heimlich et al. 1998). About 27% of active US farmland occurs on former wetland soils (Heimlich et al. 1998; USDA 1999), and this pattern is also seen in many regions of the world (Zedler 2003).

The loss of both inland and coastal wetlands has impaired wetland ecosystem services, including wildlife habitat, protection from flooding, carbon (C) storage, and water quality benefits (Zedler and Kercher 2005). Drainage of wetlands has caused large C losses from soil and lower rates of C storage, contributing to global climate change (Bridgham et al. 2006). On regional and local scales, decreased wetland area and the resulting decreased capacity to retain nutrients in watersheds, along with increased fertilizer use and agricultural intensity, contribute to water pollution, including coastal zone eutrophication and hypoxia (Turner and Rabalais 1994; Boesch et al. 2001; Rönnberg and Bonsdorff 2004; Mitsch et al. 2005; Turner et al. 2008).

In recognition of wetland ecosystem services, a 1989 federal policy known as "no net loss" promoted compensatory mitigation to offset future wetland losses through wetland restoration or creation (National Research Council 2001). Wetland restoration efforts are intended to replace habitat and improve water quality (Mitsch 2005; Verhoeven et al. 2006). Marginally productive agricultural areas in former wetlands are often candidates for restoration because of their landscape position, residual organic soils, and poorly drained status (Heimlich et al. 1998; Zedler 2003; Neely 2008).

Wetland restoration practices generally aim to restore ecosystem functions by re-establishing wetland hydrology and vegetation (Zedler and Kercher 2005) – e.g., recontouring, filling ditches, reconnecting wetland areas to surface waters, and planting obligate and facultative wetland species (Needham 2006). Quantification of ecosystem function resulting from restoration is rarely required or attempted; proxies such as survival of planted trees and mean growing season water table depth (WTD) generally suffice for evaluating wetland restoration (e.g., US Army Corps of Engineers 1997).While wetland restoration ecology aims to predict restoration outcomes and trajectories for multiple ecosystem services (Zedler and Callaway 1999; Zedler 2000), in restoration practice, specific functional goals are rarely set, and the possibility that some ecosystem services may be promoted at the expense of others is seldom addressed (Jackson et al. 2005; Zedler and Kercher 2005). In fact, the same conditions that promote nutrient removal from polluted waters may suppress biodiversity or increase greenhouse gas

(GHG) emissions (Verhoeven et al. 2006; Wilcock et al. 2008). These potential tradeoffs need to be identified and evaluated, so that they can be incorporated into environmental policies, if warranted.

Nutrient removal by wetlands from surface and subsurface waters through sediment deposition, organic matter accumulation, adsorption to particles, and biological uptake is well documented (Richardson 1985; Seitzinger 1988; Johnston 1991). For nitrogen (N), microbial denitrification (DNF) is the main mechanism of permanent removal, as it converts nitrate (NO_3^-) to inert dinitrogen gas (N_2). Because it is an anaerobic heterotrophic process, DNF may be stimulated during transient wet events, such as rainstorms (Poe et al. 2003), or when wetlands are restored by re-flooding—if sufficient NO_3^- and labile C are available. It follows that when agricultural wetlands are restored, the cessation of fertilizer inputs and facilitated drainage, coupled with enhanced DNF, could result in lower aqueous N export (Verhoeven et al. 2006; Orr et al. 2007).

Promoting enhanced DNF is not an unequivocal environmental gain (Schlesinger et al. 2006). Under incomplete anoxia or high NO_3^- availability, DNF can produce nitrous oxide [N_2O; (Davidson et al. 2000)] – a stratospheric ozone-depleting gas with 298 times the global warming potential of carbon dioxide [CO_2; (Forster et al. 2007)]. Agricultural lands, through N fertilization and soil emissions, are the largest source of N_2O to the atmosphere (Mosier et al. 1998). Although undisturbed wetlands have not been shown

to be major sources of N_2O globally (Bridgham et al. 2006), agricultural N inputs or legacy N in abandoned agricultural soils, along with re-flooding, could enhance DNF and promote N_2O emissions in agricultural restored wetlands [RW; (Verhoeven et al. 2006)].

Furthermore, the same conditions that promote DNF may also increase methane (CH_4) production, the most radiatively important GHG after CO_2. Emissions from wetlands represent 15-40% of global CH_4 fluxes (Bridgham et al. 2006; Forster et al. 2007). Methane (global warming potential = 25) is produced by methanogenic microbes in highly reduced soils or in anoxic microsites and is consumed by methanotrophic microbes in oxic environments. Flooded soils tend to have lower rates of soil respiration, and thus lower CO_2 emissions from wetland soils could partially offset the increased production of N_2O and CH_4 trace gases (Whiting and Chanton 2001). Because agricultural RWs are influenced by many factors that vary by land use (e.g., hydrologic variability, soil texture, pH, microbial and plant community composition), they are likely to differ from pre-restoration land uses and from comparable less disturbed forested wetlands in terms of GHG fluxes.

Our study was designed to evaluate the effect of restoration on multiple ecosystem services in a 440ha former agricultural restored wetland in coastal North Carolina. One major objective was to quantify GHG fluxes in the RW and compare them to GHG fluxes from nearby agricultural fields and natural wetlands. We measured GHG

fluxes (CO_2, N_2O, and CH_4) following restoration across the RW, an adjacent active agricultural field (Ag), and in two forested wetlands (FW). We sampled gas fluxes from soil and water to the atmosphere bimonthly from July 2007 to June 2009, to capture seasonal variability, as temperature and moisture are likely to control enzyme-mediated activities such as GHG production and consumption.

We hypothesized that hydrologic variability, temperature, and N availability would be the main drivers of GHG fluxes within and across sites, such that i) under dry conditions, GHG fluxes would be relatively low but would be dominated by CO_2; ii) under intermittently flooded conditions, nutrients would cycle more rapidly, thus producing more CO_2 and N_2O; and iii) under permanently flooded conditions, CH_4 would be the dominant GHG. Therefore, we expected to find that GHG fluxes would be iv) low in the Ag site, except perhaps following fertilization when N_2O would be high, v) that the RW would have the highest fluxes of N_2O given legacy fertilizer and reflooded conditions, and vi) that FWs would have the highest fluxes of CH_4, based on their relatively undisturbed hydrology, organic soils, and reducing environments.

2.2 Methods

2.2.1 Geographic setting and history

Our study sites are located in the Albemarle-Pamlico Peninsula, in the Outer Coastal Plain of NC (Figure 1). There is little topographic relief, with more than 54% of the 5000 km^2 peninsula under 1m elevation (Poulter and Halpin 2008). The Albemarle-

Pamlico Peninsula is bounded by the Albemarle, Croatan, and Pamlico sounds. The climate is classified as humid-subtropical, with mean annual precipitation of 1330 mm·yr^{-1} and temperature of 16.6°C (State Climate Office, Raleigh, NC). Hydrology in this low-relief basin is driven by precipitation, evapotranspiration, and wind tides (Richardson and McCarthy 1994).

The region was historically dominated by pocosin wetlands with deep Histosol soils, pine forests, and an understory of evergreen shrub-scrub vegetation (Richardson 2003), as well as swamp forests along blackwater creeks. Much of the landscape was logged in the 19th and 20th centuries; large-scale conversion to agriculture occurred in the 1970-80s with the construction of large canals and drainage systems (Carter 1975). By 1979, only 9% of historical pocosins remained in the NC coastal plain (Richardson 1983). Much of the Albemarle-Pamlico Peninsula is currently in agriculture (corn-soybean row-crops), of which 80% requires active drainage to maintain arable fields (Neely 2008).

2.2.2 Site descriptions

The primary study location is a large compensatory mitigation site (1704ha; 35°54′22″ N, 76°09′25″E; Figure 1), known as the Timberlake Restoration Project (TLRP) which is owned by Great Dismal Swamp Mitigation Bank, LLC. TLRP drains to the Little Alligator River, which flows into the Alligator River and Albemarle Sound. The elevation in TLRP ranges from -0.4 m to 5.1 m above sea level. The TLRP property historically was the headwaters for coastal blackwater streams that flow into the

Alligator River, with pocosin vegetation in higher elevation areas (Needham 2006). Swamp forests in the site were cleared, drained, and converted to agriculture in the 1970's, while some areas remained forested. The TLRP property currently contains drained shrub-scrub wetlands, restored and selectively timbered forested wetlands, and former agricultural fields undergoing stream and wetland restoration areas (Needham 2006). The former corn and soybean farmland within TLRP (440ha), last harvested in 2004, is the restored wetland (RW) that is the focus of our study.

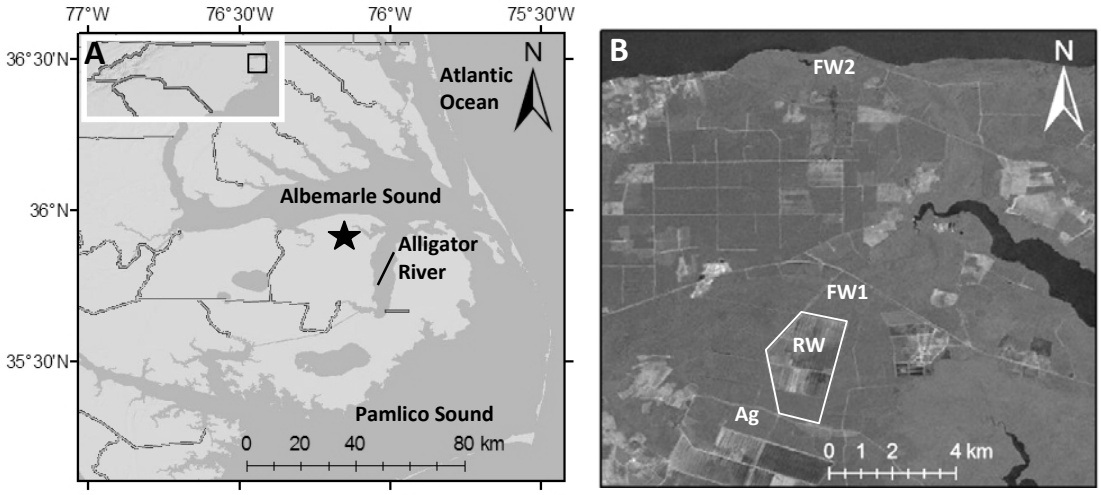


Figure 1: Geographic location of study: A) overview and B) detailed view Timberlake preservation wetland (FW1), and Palmetto Peartree Preserve (FW2). Adapted from Ardón et al. (submitted)

Restoration of the TLRP agricultural area aimed to re-establish a dynamic hydrologic regime and native vegetation, by regrading the land surface, plugging drainage canals, removing the drainage pump, and planting 750,000 live saplings from eight species of obligate and facultative wetland trees: *Taxodium distichum, Nyssa sylvatica* var. *biflora, Nyssa aquatica, Fraxinus pennsylvanica, Salix nigra, Chamaecyparis*

thyoides, Quercus nigra, Quercus michauxii, Quercus phellos, and *Quercus falcate var. pagodafolia* (Needham 2006). Hydrologic reconnection of TLRP to the upstream restored forest and downstream waters was completed in 2007, reinstating the precipitation- and wind tide-driven hydrologic regime. The two dominant soil series in the RW are very poorly drained hydric soils: Ponzer muck (loamy, mixed, dysic, thermic Terric Haplosaprist) and Hyde loam [fine-silty, mixed, active, thermic Typic Umbraquult (USDA 2009)].

The Ag site, cropped in a corn and soybean rotation since the mid-1970s, is immediately adjacent to RW and is drained by a system of ditches and pumps, part of which discharges into RW. It is mainly comprised of Weeksville silt loam soils [coarse-silty, mixed, semiactive, thermic Typic Umbraquult (USDA 2009)]. One of the reference sites, FW1 was established in a minimally impacted forested wetland portion of TLRP, dominated by a mixed hardwood forest [oak-gum-cypress (Needham 2006)]. The soils in FW1 are mapped as Dorovan muck [dysic, thermic Typic Haplosaprist (USDA 2009)]. The other reference FW2 is located 8km away in the Palmetto Peartree Preserve, in a swamp hardwood stand of cypress and tupelo. Soils in FW2 are mapped as Belhaven muck [loamy, mixed, dysic, thermic Terric Haplosaprist (USDA 2009)].

2.2.3 Sampling locations

Just prior to hydrologic restoration in 2007, we established one transect within each of the two main soil types in RW, with 12 permanent sampling locations in the

Hyde loam series and 21 in the Ponzer muck series, to capture the expected extent of the flooding gradient. We set up fewer sampling locations (n=5) within each of the three reference sites. At each sampling location, we excavated a soil profile to 50cm depth, measuring the thickness of the surface organic horizon (where present) and the depth to the relatively impermeable mineral horizon below (except in the Ag site, where we were only able to auger to 15cm due to soil compaction). We defined our system boundaries as the surface horizon above the mineral confining layer; in all sites, depth to this mineral horizon was at least 15cm and was well over 50cm in FW sites.

2.2.4 Environmental variables

Each sampling location was instrumented with 5 platinum-tipped redox electrodes (Vepraskas and Faulkner 2001). To monitor near-surface and surface waters, we installed slotted PVC monitoring wells to just above the clay layer (or to 45cm depth where the clay layer was deeper than 45cm), and programmed a water level recorder (Levelogger Gold or Silver, Solinst Instruments) in each well to record pressure and temperature every 15min. The slots extended to 10cm above the ground surface, allowing the Leveloggers to register the pressure of overlying water even when the water table was above the ground surface. Well positions and elevations were professionally surveyed by R. Sanderson in 2008.

At each sampling location, we collected soil samples from 0-15cm to determine soil characteristics, including organic carbon content, pH, soil texture, and bulk density.

We used a 5cm diameter soil sampler with a slide hammer attachment (AMS Instruments) for bulk density (BD) sampling without significant compaction in Ag and RW sites. Compaction was a problem in FW1 and FW2; to collect intact samples, we sharpened 10cm diameter PVC cylinders, pushed them into the soil, and carefully dug around them to remove them. Soil organic C content was determined with a Carlo Erba Elemental Analyzer. Soil pH was measured on replicate 3g samples in 5mL 0.01M $CaCl_2$, which is preferable to water when soils have high C content (Hendershot et al. 1993).

2.2.5 Gas flux measurements

We applied the static chamber approach (Livingston and Hutchinson 1995) to measure soil-atmosphere and water-atmosphere gas fluxes, and therefore positioned the enclosures to avoid large plants. As a result, our CO_2 flux measurements do not encompass photosynthetic uptake and should be interpreted as soil respiration. At each sampling location, we installed a soil collar (15cm tall x 20cm diameter; SDR-35 PVC pipe) to a depth of approximately 5cm in the soil. Chamber tops were built from opaque 20cm molded PVC caps with gas-tight rubber gaskets by adding a 1/4" Swagelok brass sampling port with rubber septum, vent tube (5mm i.d.; 6cm long), internal fan (7cfm; 5V DC; Jameco Electronics Inc.) to each cap [adapted from (Livingston and Hutchinson 1995; McLain et al. 2002)]. Incubations consisted of placing the chamber top tightly on the soil collar after clipping any vegetation taller than 10cm found inside the collar.

Gas samples were collected bimonthly from July 2007 – June 2009 (n=11 sampling dates, excluding November 2008), with all sites and sampling points visited during three consecutive sampling days. We collected 10mL headspace samples with a glass syringe immediately following cap placement and after each of two intervals of 30-40min. Gas samples were collected in triplicate and injected into 9mL pre-evacuated glass vials (Teledyne Tekmar). Air temperature, barometric pressure, and soil temperature at 5cm were recorded at the beginning of each sampling interval. Chamber height, water depth, and redox potentials were recorded once for each location on each sampling date. Redox potentials were measured using a voltmeter and calomel reference electrode (Fisher Scientific), and values were corrected by adding 241mV to correct to the standard hydrogen electrode. The median value of the five redox probes was used in subsequent data analyses.

When surface water was above 10cm, we used a similar approach, except that a floating PVC collar imbedded in a polystyrene platform was used to capture water-air gas exchange. For floating collar samples, water samples for determination of dissolved gas concentrations were collected in 120ml pre-evacuated glass bottles during each incubation, and headspace equilibration techniques were used to extract a 10ml gas sample for GC analysis (Hudson 2004). Water temperature and volume of the water sample were also measured.

Gas vials were analyzed for CO_2, N_2O, and CH_4 concentrations injected by a Tekmar 7050 Headspace Autosampler to a Shimadzu 17A gas chromatograph with ECD and FID detectors, retrofitted with 6-port valves and a methanizer in series to allow the determination of the three gases from the same sample. Ultra-high purity N_2 was used as the carrier gas and P5 mixture served as the make-up gas for the ECD. A Nafion tube (Perma Pure LLC) and counter-current medical breathing air were used to remove water vapor from the sample stream. Samples were analyzed as soon as feasible after collection, always within two weeks (sample holding time was confirmed to be at least two weeks during lab testing). Peak areas of samples and known standards were determined with GCsolution software version 2.3 and exported to a Microsoft Access database for data storage. Gas concentrations in vials were calculated from linear regression ($R^2 > 0.95$) of concentrations of certified primary standards (Airgas) against peak areas; concentrations of field samples were obtained by averaging values from duplicate samples analyzed in the same analytical batch, unless the relative percent difference exceeded 20%, in which case the maximum value was used (assuming that a vial had leaked since field sampling).

Under ideal conditions, gases accumulate (or are consumed) linearly over time during static chamber incubations, and the slope of the concentration vs. time is used to estimate the flux. Static chambers are sensitive to disturbance and chamber effects; gas fluxes can be underestimated due to chamber effects if high concentrations in the

chamber limit the diffusion of gases from soil to atmosphere (Livingston and Hutchinson 1995). Therefore, we excluded incubations with elevated initial concentrations (attributed to disturbance). From replicate determinations of known standards for each gas, we estimated the minimum detectable concentration difference for each sampling date [MDCD (Yates et al. 2006; Matson et al. 2009)]. To estimate gas flux, the slope of the concentration vs. time line was used when $R^2 > 0.90$. When the accumulation rate was non-linear, and the change in concentration during the first interval was greater than MDCD, we used the rate during the first interval; when a longer incubation was needed to exceed MDCD, we used the rate calculated from the initial to the final sample. Incubations in which the concentration increased significantly then decreased significantly, or vice versa, were excluded from the data set as failed incubations (see Table 1), while incubations during which there was no detectable concentration change were set to zero. Gas concentrations ($g \cdot cm^{-3}$) were multiplied by chamber height to report flux rates by surface area.

To estimate water-atmosphere gas exchange, we determined the concentration of gases dissolved in the water samples (Hudson 2004), the gas transfer velocity using water temperature and the Bunsen coefficient for each gas (Fogg and Sangster 2003), and the change in gas concentrations in the chamber volume during the first time interval of the incubations (Conrad and Seiler 1988; MacIntyre et al. 1995).

2.2.6 Data analysis

Field and analytical data files were assimilated and manipulated within a relational database in Microsoft Access. Data files were extracted for flux calculations and statistical analyses using the R programming language (R Development Core Team 2009) and its `RODBC` package (Ripley and Lapsley 2009). Due to our study's unbalanced sampling design (n=5 at each reference site vs. n=33 in RW) and to determine differences due to hydrology in the restored wetland, we created three post-hoc groupings of sampling points in RW, based on 1) fewest missing values for GHG fluxes; and 2) daily mean water table depth (WTD), such that n=5 for each group: RW-dry (WTD < -20cm), RW-int (mean WTD between -15 and -5cm), and RW-wet (mean WTD > 10cm).

For statistical comparisons, performing parametric tests such as ANOVA to detect group differences was not appropriate because results were not normally distributed. Instead of relying on a theoretical normal probability distribution, we used a resampling approach that essentially exchanges the group labels (Ag, RW-dry, RW-int, RW-wet, FW1, and FW2) attached to observations and generates a distribution of test statistics by Monte Carlo sampling (Good 2000). Specifically, we used permutation one-way tests with adjusted p-values for multiple comparisons between groups [`oneway_test` and Nemenyi-Damico-Wolfe-Dunn test, R package `coin` (Torsten et al. 2008)]. This method was used to determine whether gas fluxes and soil properties differed between groups. Tests on pH data were conducted on hydrogen ion concentrations, before transforming results back to pH notation. We converted N_2O and

CH_4 to CO_2-equivalents by multiplying by 298 and 25, respectively, based on the global warming potential for the 100-y time horizon in the IPCC's Fourth Assessment Report (Forster et al. 2007). To estimate cumulative annual gas fluxes, we estimated the areas under each curve (minimum, mean, and maximum) over the two-year period using the trapezoidal rule [`trap.rule`, R package `Hmisc` (Harrell 2009)].

2.3 Results

2.3.1 Environmental variables as drivers of GHG fluxes

During the two years of this study, data from air temperature loggers did not show obvious differences among sites (Figure 2A). Daily mean air temperature during the study was 16.0°C, with an overall range of -14 to 41°C. Air temperature patterns showed three even phases which are typical of the region's seasonality: warming (spring; mean daily temperature rising from lows to ~20°C), hot (summer; temperatures consistently above 20°C), and cooling (fall/winter; mean daily temperature dropping from ~20°C to annual lows). Seasonal patterns in water levels differing in magnitude were seen in three sites (not Ag; Figure 2B): lower water levels in mid- to late-growing season, rising during October through May, and dropping again during the growing season. This pattern did not closely track air temperature trends, especially during the warming season when temperatures and water levels both tended to rise.

The reference sites and RW exhibited marked overall differences in water table depth and variation. The daily mean WTD was within 10cm of the surface less than 1%

of the time in the actively drained Ag field, 4% in FW1 points and 11% of the time in FW2 points (Figure 2B). The 15cm depth of wells in Ag points was too shallow to record many valid measurements of WTD, as active drainage maintained low water tables for agricultural purposes (Figure 2B). The RW was much wetter overall, with WTD within 10cm 58% of the time; surface water was recorded 27% of the time. Within the RW, water table was within 10cm of the surface 5% of the time in the dry subgroup (RW-dry), 24% of the time in the intermittent group (RW-int), and 82% of the time in RW-wet.

We continued to examine differences between groups based on the three reference sites and the three hydrologic subgroups in RW, beginning with soil properties (Table 2). Bulk density was inversely proportional to patterns in water levels, SOC, and soil N (TN), with Ag, RW-dry, and RW-int sites having the lowest water levels, SOC, and TN, and the highest BD. The three wettest sites (RW-wet, FW1, and FW2) were different from only the Ag site in terms of BD ($p < 0.039$), SOC ($p < 0.078$), and TN ($p < 0.10$). Soils in RW-wet were lower in SOC (32.2%) compared to FW2 (47.2%; $p=0.067$). Soil C:N ratios were higher in the RW sites (22.5-28.7) compared to the reference sites (17.2-19.5; $p < 0.07$).

The Ag and FW2 sites had higher pH than RW-wet, FW1, and RW-int (5.53 and 4.78 vs. 3.75 and 4.29; $p < 0.096$). Redox potential measurements showed three clear groupings: Ag soils were generally oxic (367 mV), the three sites with intermediate

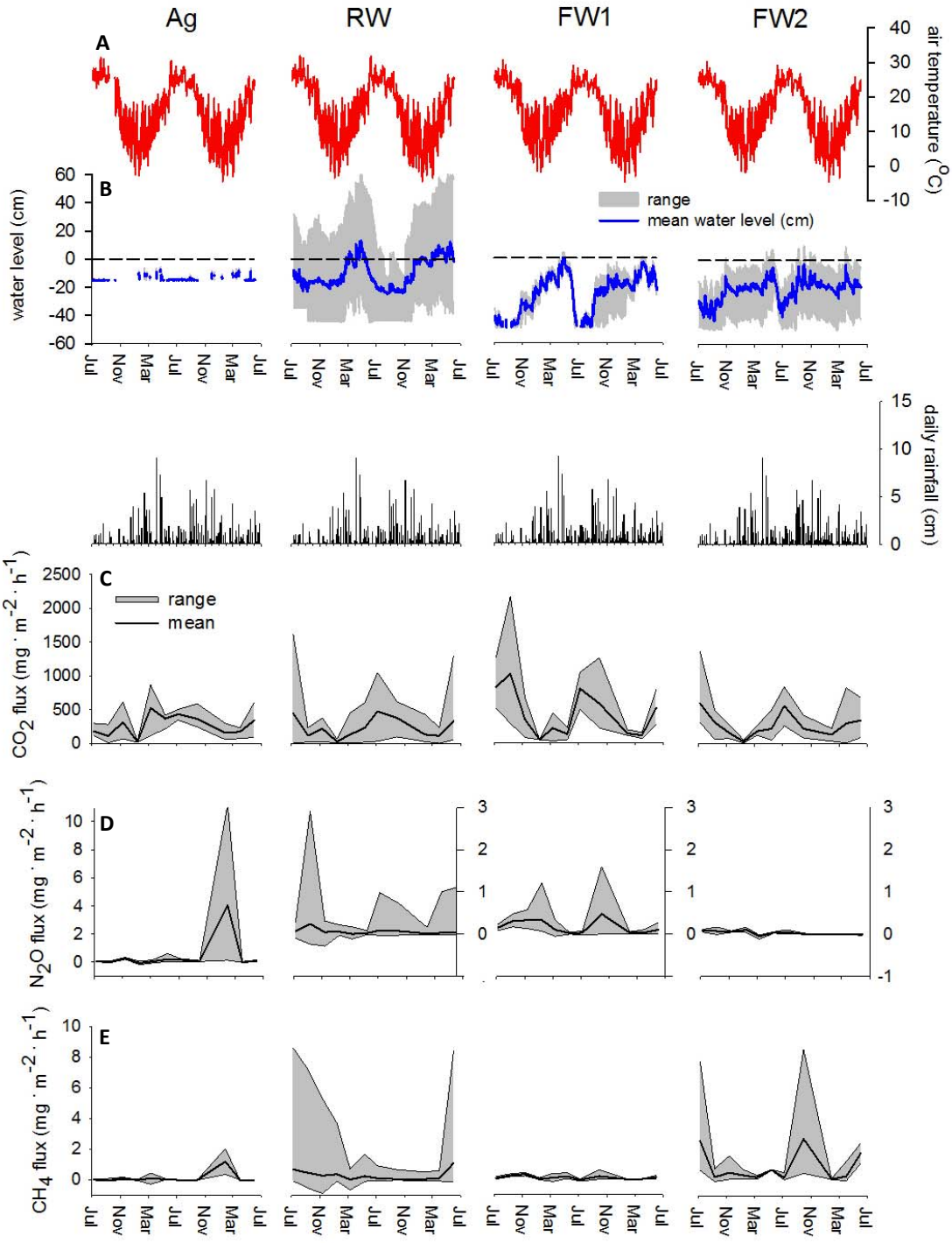


Figure 2: Environmental variables and GHG fluxes (July 2007 – June 2009). A) air temperature; B) water level (daily mean and range); and GHG fluxes (mean and range; C: CO_2; D: N_2O; E: CH_4)

wetness were similar (5.27 - 89.3 mV at RW-int, FW1, and RW-dry), and the two wettest sites had reducing environments (-100 and -180 at RW-wet and FW2, respectively). A simple multiple linear regression approach to explain GHG flux based on environmental variables across all observations was not fruitful; an analysis of variables controlling N_2O fluxes for one sampling period is presented in Chapter 3.

2.3.2 Greenhouse gas fluxes—temporal and spatial patterns

Strong seasonality of CO_2 flux from soil was observed in the FW and RW sites, with highest rates during the warmest months (maximum at FW1 in September 2007; 2161 mg $CO_2\,m^{-2}\cdot h^{-1}$) and very low rates during the coldest months (< 20 mg $CO_2\cdot m^{-2}\cdot h^{-1}$; Table 2); this pattern was not clear in the Ag soils, where soil respiration was lower overall and did not appear to follow temperature trends (Figure 2D). We found similar but weaker seasonal patterns for N_2O, with higher fluxes during warmer months in RW and FW1; however, the magnitude of warm season increases in these sites was dwarfed by very high fluxes measured in the Ag points in January 2009 (11.2 mg $N_2O\cdot m^{-2}\cdot h^{-1}$; Table 2; Figure 2E). Conversely, N_2O fluxes in FW2 were uniformly low throughout the study period. Methane fluxes were occasionally high and highly variable over time in RW and FW2, with the highest fluxes measured during warm months (8.50 and 8.58 mg $CH_4\cdot m^{-2}\cdot h^{-1}$; Figure 2E).Yet this pattern was not consistent across all sites, nor in all warm months; for example, the largest CH_4 fluxes in the Ag site were measured in January 2009, while CH_4 fluxes in FW1 were consistently low (Figure 2F).

Table 2: General soil characteristics (0-15cm depth).
Means (± standard error) were compared for six groups (including the 3 RW subgroups); different letters indicate differences at p < 0.10.

group	n	BD ($g\ cm^{-3}$)	Soil Organic C (%)	Soil Total N (%)	C : N	pH	Redox Potential n	Redox Potential (mV)
Ag	5	1.31 ± 0.02a	3.8 ± 0.6c	0.19 ± 0.04b	19.5 ± 0.5b	5.53a	55	367 ± 30.4a
RW (all)	33	0.70 ± 0.06	17.5 ± 2.4	0.67 ± 0.09	25.0 ± 0.7	3.84	363	-1.61 ± 14.8
RW-dry	5	0.98 ± 0.09ab	8.7 ± 2.3bc	0.37 ± 0.08ab	22.5 ± 1.3ab	4.40ab	55	89.3 ± 36.6b
RW-int	5	0.87 ± 0.14ab	11.8 ± 4.7bc	0.46 ± 0.16ab	23.5 ± 1.6ab	4.29b	55	5.27 ± 34.4b
RW-wet	5	0.34 ± 0.06bc	32.2 ± 4.8b	1.11 ± 0.14a	28.7 ± 1.5a	3.75b	55	-100 ± 36.6c
FW1	5	0.17 ± 0.04bc	39.4 ± 3.4ab	2.27 ± 0.32a	17.2 ± 0.2b	4.03b	55	12.1 ± 19.7b
FW2	5	0.06 ± 0.01c	47.2 ± 0.6a	2.57 ± 0.03a	18.4 ± 0.2b	4.78a	55	-180 ± 19.8c

Table 3: Summary statistics for GHG gas fluxes
Results are means ± SE, median, minimum, and maximum for n=11 sampling dates). For each gas, we report the percentage of incubations that were excluded (ND) and the percentage of valid incubations that were linear ($r^2 \geq 0.9$).

		CO_2 ($mg \cdot m^{-2} \cdot h^{-1}$)						N_2O ($\mu g \cdot m^{-2} \cdot h^{-1}$)						CH_4 ($\mu g \cdot m^{-2} \cdot h^{-1}$)					
group	n	% ND	% linear	mean ± s.e.	med	min	max	% ND	%linear	mean ± s.e.	med	min	max	% ND	% linear	mean ± s.e.	med	min	max
Ag	55	9.0	58	290 ± 28.8	246	10.8	865	20	47	458 ± 271	104	-173	11200	27	24	75.9 ± 53.0	0.0	-285	2020
RW (all)	363	17	65	263 ± 14.5	173	4.3	1614	15	34	56.5 ± 12.6	12.3	-286	2909	27	42	304 ± 68.1	20.1	-891	8580
RW-dry	55	11	69	335 ± 36.8	294	8.5	1100	9.0	38	56.7 ± 21.2	24.2	-120	999	20	36	10.2 ± 9.36	0.0	-96.6	236.0
RW- int	55	13	64	317 ± 36.2	350	10.7	1290	14	31	65.4 ± 25.3	21.5	-122	1070	14	44	74.5 ± 44.3	0.0	-538	1720
RW-wet	55	27	69	150 ± 20.5	95.8	4.3	560	11	36	33.6 ± 18.2	12.2	-286	2910	36	64	1100 ± 366	31.3	-660	8450
FW1	55	11	60	472 ± 64.4	325	20.0	2160	13	44	178 ± 43.9	77.9	-49.7	1600	20	42	88.3 ± 24.8	22.3	-165	608
FW2	55	14	56	301 ± 39.8	231	11.0	1370	14	27	29.6 ± 7.84	13.7	-117	166	27	36	939 ± 285	282	-92.7	8500

Pronounced spatial variability of all three gases was shown to occur within sites on a given sampling event. Spatial variability was lower for soil CO_2 compared to N_2O and CH_4, as seen by similar rises and drops in maximum, mean, and minimum CO_2 fluxes on the same sampling date (Figure 2D).Within RW and FW sites, spikes in the maximum N_2O and CH_4 fluxes were not similarly seen in the mean and minimum rates on the same measurement day (Figure 2D-E). Within the Ag site, spatial variability was very high on a single day in January 2009, when the range of measured N_2O fluxes was over 10 times larger than the range of values measured there during the rest of the study (Figure 2E).

2.3.3 Greenhouse gas fluxes—group comparisons

Statistical comparisons between groups were made using RW hydrologic subgroups and the three reference sites. Mean soil CO_2 fluxes were not dissimilar across the three land use types, ranging from 290 mg $CO_2 \cdot m^{-2} \cdot h^{-1}$ in Ag to 472 mg $CO_2 \cdot m^{-2} \cdot h^{-1}$ in FW1 (Table 3; Figure 3A); but rates were significantly lower in RW-wet (254 mg $CO_2 \cdot m^{-2} \cdot h^{-1}$; $p < 0.046$) compared to all other sites. Mean N_2O fluxes were very low across the three RW groups and FW2 (0.030 to 0.065 mg $N_2O \cdot m^{-2} \cdot h^{-1}$; $p < 0.027$) ; while the mean N_2O flux in the Ag site was large with a high degree of uncertainty (0.46 ± 0.27 mg $N_2O \cdot m^{-2} \cdot h^{-1}$).

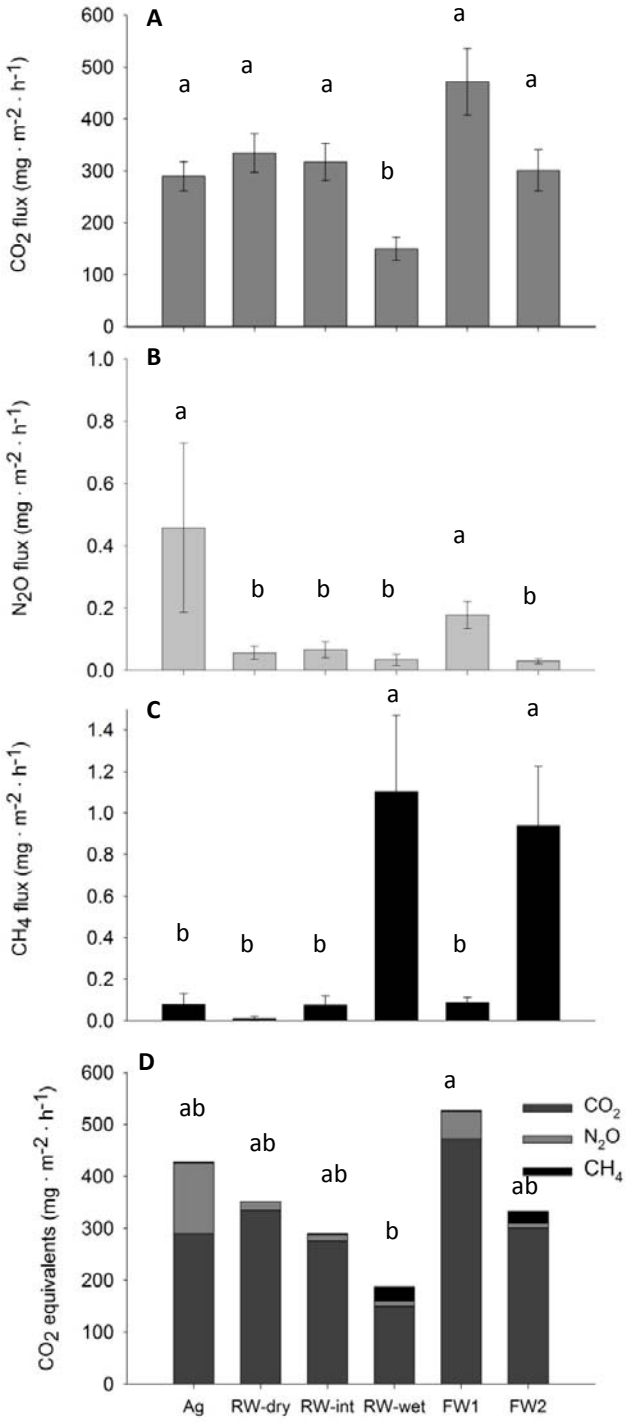


Figure 3: Mean GHG fluxes (± SE) and CO_2 equivalents by site. Agricultural field (Ag), restored wetland (RW) with 3 hydrologic classes, and two forested wetland sites (FW1, FW2)

Mean CH_4 fluxes differed across the sites, with significantly higher fluxes at the two wettest sites (FW2 and RW-wet) compared to the other sites (0.94 and 0.27 mg $CH_4 \cdot m^{-2} \cdot h^{-1}$, respectively; $p < 0.012$). Although negative fluxes of N_2O and CH_4 were measured on occasion in all sites during the course of the study, zero median CH_4 fluxes in Ag, RW-dry, and RW-int suggest the importance of CH_4 consumption in those sites (Table 3).

After converting mean N_2O and CH_4 fluxes to CO_2-equivalents, the three GHG components were summed for each group, allowing us to compare their cumulative contributions to global radiative balance [(Bridgham et al. 2006); Figure 3D]. For all groups, the main component of GHG fluxes was CO_2, with N_2O the next most important contributor, except in FW2 and RW-wet, in which CH_4 was the second largest source. The FW1 site had the highest radiative balance overall (527 mg CO_2-eq $\cdot m^{-2} \cdot h^{-1}$) but was only statistically distinguishable from RW-wet ($p=0.037$), which had the lowest radiative balance (Figure 3D). Because we did not specifically measure CH_4 ebullition, which has been shown to be up to 90% of total CH_4 flux in flooded environments (MacIntyre et al. 1995), we recalculated the CH_4 contribution of RW-wet and FW2, only the two sites in which ebullition would have been significant, assuming our measured CH_4 fluxes represented 10% of total CH_4 flux. Under this scenario, there was no difference in overall radiative balance among the six sites ($p > 0.1$, data not shown).

2.3.4 Greenhouse gas fluxes—cumulative annual fluxes

By integrating the curves of minimum, mean, and maximum fluxes in Figures 2C-E over all sampling dates, we estimated three values of annual fluxes for each gas at each of the six sites (Table 4). For CO_2, the smallest mean annual flux was estimated in RW-wet, with a mean of 13000 kg $CO_2 \cdot ha^{-1} \cdot y^{-1}$. The RW overall had high variability [relative percent difference (RPD) 318%], but each RW hydrologic subgroup had RPD comparable to that of the other sites (117-124% vs. 97-151% in reference sites; Table 4). Mean cumulative soil CO_2 flux was slightly higher in the Ag and FW2 sites (24600 and 28000 kg $CO_2 \cdot ha^{-1} \cdot y^{-1}$), but estimates had a smaller range and were better constrained (RPD of 97% and 151%, respectively). FW1 had the highest mean annual flux (42700 kg $CO_2 \cdot ha^{-1} \cdot y^{-1}$) with a comparatively low RPD of 123%.

Mean annual N_2O flux estimates in Ag and FW1 were 14 and 5 times higher than the smallest mean annual flux at FW2. Although N_2O results at FW1 and Ag sites had

Table 4: Estimated cumulative annual GHG fluxes from July 2007 - June 2009 by calculating the areas under each curve, along with relative percent difference [RPD = (max-min)/mean x 100%]

	CO_2 kg · ha^{-1} · y^{-1}				N_2O kg · ha^{-1} · y^{-1}				CH_4 kg · ha^{-1} · y^{-1}			
group	**min**	**mean**	**max**	**RPD**	**min**	**mean**	**max**	**RPD**	**min**	**mean**	**max**	**RPD**
Ag	11600	24600	35600	97	3.0	40.3	106	256	-0.75	10.7	22.3	215
RW	2220	24100	78800	318	-7.6	5.9	68.9	1300	-21.2	29.6	332	1190
RW-dry	14700	31000	52800	123	0.34	4.9	17.1	356	-3.3	0.83	6.62	1200
RW-int	14000	27800	48400	124	-1.4	7.7	21.4	296	-8.5	7.19	35.1	606
RW-wet	3850	13000	19000	117	-2.6	2.4	9.9	521	38.4	122	259	181
FW1	20000	42700	7270	123	3.2	13.9	32.5	211	-0.7	7.5	20.6	284
FW2	11000	28000	53500	151	-0.2	2.8	5.7	211	24	75.7	197	228

higher uncertainty relative to CO_2 estimates (RPD 211-255%), all three RW groups had low mean annual N_2O fluxes (2.4 - 4.9 kg $N_2O \cdot ha^{-1} \cdot y^{-1}$) and higher uncertainty (RPD = 296-521%; Table 4). For CH_4 mean annual fluxes, uncertainty around estimates was high for all sites, with RPD of 216-284% for the reference sites and 181-1200% for RW. The highest CH_4 mean annual fluxes were estimated at RW-wet and FW2 (122 and 75.7 kg $CH_4 \cdot ha^{-1} \cdot y^{-1}$), while values from the other sites were less than half as large (7.5 - 29.6 kg $CH_4 \cdot ha^{-1} \cdot y^{-1}$; Table 4).

2.4 Discussion

2.4.1 Land use and environmental variables as drivers of GHG fluxes

Despite high temporal and spatial variability of gas fluxes within sites, we found some significant differences between sites, which we can partially attribute to land use and environmental variables. Not surprisingly, soil respiration was lowest in the most consistently flooded site, with the lowest redox potential and lowest quality organic matter (RW-wet). We had expected that soil respiration in both FW sites would be lower than in the Ag site, as is typically the case (Schlesinger 1997), but we found no differences; a drought during this study period could have had an equalizing role by suppressing soil respiration in the drier Ag soils and increasing soil respiration in the reference wetlands.

We had expected that N_2O fluxes would be highest in sites with intermittently flooded hydrology and high N availability. The soil C:N ratio has been shown to be a

relatively good predictor of DNF rates, with low C:N enhancing DNF rates and high C:N limiting DNF [e.g., (Hume et al. 2002; Hunt et al. 2007)]. Based on mean redox potentials of the bulk soil, thermodynamic theory would predict DNF (optimal at 200mV) to be favored at FW1, RW-int, and RW-dry (5.3 to 89 mV). We found the highest fluxes in Ag soils and FW1; in FW1, mean redox potential was intermediate and N availability was high (based on low soil C:N). We expected to find higher N_2O fluxes in the RW, but N availability there was actually lower compared to other sites.

In this study, we report that N_2O can be a more important contributor to the total GHG flux than CH_4 in restored former agricultural wetlands, forested wetlands with unsaturated surface soils, as well as in agricultural soils. CH_4 was more important than N_2O in the two sites with the highest water tables, as has been found in most wetland studies (Bridgham et al. 2006). CH_4 is likely to be most dominant in nutrient-poor and highly reduced wetlands, such as peatlands (Bridgham and Richardson 1992), while in N-rich, less reducing environments, N_2O can be a larger component of GHG balance than CH_4, especially over longer time horizons due to the longer residence time of N_2O in the atmosphere (Zou et al. 2005; Forster et al. 2007).

Despite their high warming potential, both trace gases were minor contributors compared to CO_2 at all sites. Flooded soils, where CH_4 fluxes were highest, had lower soil respiration, providing an important offset such that differences in total GHG emissions (CO_2, N_2O, and CH_4) were not significantly different between the contrasting

land uses. Within the RW, we found the lowest total GHG flux in the wettest portions of the restored wetland (Figure 3D).

2.4.2 Spatial and temporal variability

Beyond estimating the radiative balance, measuring CO_2, N_2O, and CH_4 jointly has advantages over only measuring one or both trace gases. Because soil respiration is a process that reflects overall biological activity in soils, and because CO_2 is not appreciably consumed in soils, its fluxes can be relatively conservative tracers during an incubation. N_2O and CH_4 are both produced and consumed by a variety of specialized microbes within soils, thus they represent the net effect of many interacting physical variables and biological processes. Therefore, the magnitude and direction of N_2O and CH_4 fluxes is generally more difficult to measure and less predictable than for CO_2. As expected, we found much higher spatial and temporal variability within sites for N_2O and CH_4 fluxes (relative percent difference of 211-1300% and 181-1200%, respectively; Table 4), compared to soil CO_2 flux (relative percent difference: 97-151%).

Seasonal patterns were more pronounced for soil respiration than for N_2O and CH_4 fluxes across all sites: CO_2 fluxes generally were higher during warmer months, peaking in July/September for the FW and RW sites, mirroring air temperature changes. N_2O and CH_4 fluxes were likewise generally higher when temperatures were warmer, but were also driven by site-specific variability. Within the RW, the timing of the highest N_2O fluxes was different for each group, occurring in April 2009 in RW-dry, in

Table 5: Means of GHG fluxes in selected freshwater wetlands and other ecosystems

Source	Ecosystem	Ag.	Drained	Restored	CO_2	N_2O	CH_4
					($kg \cdot ha^{-1} \cdot y^{-1}$)		
Matson et al. 2009	Boreal forests, Canada					0	-0.6
Zona et al. 2009	Boreal peatland, tundra		x				87.6
Mosier et al. 1986	Temperate croplands, barley/corn, CO	x				68.8	
Oorts et al. 2007	Temperate croplands, corn/soy, France	x			14600	1.7	
Gleason et al. 2009	Temperate croplands, ND	x	x		57000	3.3	283
Rolston et al. 1978	Temperate croplands, ryegrass, CA	x				946	
Weier et al. 1996	Temperate croplands, sugarcane, Australia	x				275	
This study	Temperate cropland, corn/soy, NC	x	x		24600	40.3	10.7
	Temperate forested WL, NC				35400	8.35	41.6
	Temperate restored agricultural WL, NC		x	x	24100	5.9	29.6
Bridgham et al. 2006	Freshwater WL, N. America						1150
Mander et al. 2008	Temperate constructed WL, Estonia	x			17400	15.5	106
Sovik and Klove 2007	Temperate constructed WL, Norway	x				314	4620
Stadmark and Leonardson 2005	Temperate constructed WL, Sweden	x				0	3190
Pulliam 1993	Temperate forested WL, GA						228
Brumme et al. 1999	Temperate forested WL, Germany					11.5	
Butterbach-Bahl et al. 2002	Temperate forests, Germany					5.8	
Liikanen et al. 2009	Temperate marshes, Baltic Sea					0.1	757
Dinsmore et al. 2009	Temperate peat bog, Scotland					0	1.4
Freeman et al. 1993	Temperate peat monolith, Wales	x	x		4080	2.3	1180
Hendriks et al. 2007	Temperate peatlands, Netherlands	x	x	x	31800	0	417
Brumme et al. 1999	Temperate pine forest, WI					1.6	
Hefting et al. 2003	Temperate riparian forest, Netherlands	x				31.4	
Altor and Mitsch 2006	Temperate riparian marshes, OH			x			840
Hernandez and Mitsch 2006	Temperate riparian marshes, OH			x		0.6	
Altor and Mitsch 2008	Temperate riparian mesocosms, OH			x	97000		140
Bridgham and Richardson 1992	Temperate peatlands, NC				56200		
Gleason et al. 2009	Temperate wet meadow, ND	x	x	x	59700	2.5	212
Xiong et al. 2007	Subtropical rice paddies, China	x	x			19	5560
Zou et al. 2005	Subtropical rice paddies, China	x	x			25.8	515
Yu et al. 2008	Subtropical forested WL, LA				22000	85.6	1242
Alford et al. 1997	Subtropical forested WL, LA						534
Liu et al. 2008	Subtropical plantations, China	x			35600	9.2	3450
Bartlett et al. 1988	Tropical forested WL, Amazon						701
Livingston et al. 1988	Tropical forests, Amazon					1.8	
Brumme et al. 1999	Tropical wet forest, Brazil					6.8	
Brumme et al. 1999	Tropical wet forest, Costa Rica					9.3	

September 2007 in RW-int, and November 2008 in RW-wet, but not clearly associated with hydrologic status. For CH_4 within RW, the seasonal pattern was more evident, with relatively higher fluxes during warmer months, and the highest fluxes in RW-dry and RW-int occurring in June 2009– a warm period with wetter soils than in prior summers.

Our conclusions about N_2O flux from the Ag site would have been very different without the January 2009 samples. Because temperatures were around 8°C during sampling, it is unlikely that this was a freeze-thaw effect, as has been documented elsewhere in winter [e.g., (Papen and Butterbach-Bahl 1999)]. This N_2O pulse was not seen in the other sites on this date, supporting this conclusion. Based on our communications with the farm manager, we know that the Ag site is generally fertilized in early winter (December/January) and early spring (March). Our water chemistry sampling (Ardón et al, submitted) shows mean NO_3-N concentrations of 1.68 $mg \cdot L^{-1}$ in January 2009, while May-November 2008 concentrations were 0.052 mg NO_3-$N \cdot L^{-1}$. Other periods of high NO_3^- concentrations in Ag drainage water did occur during our study but were not reflected in N_2O fluxes, showing the unpredictability of trace gas fluxes.

For CH_4, we had expected that continuously flooded sites would have the highest fluxes; according to our soil redox measurements, methanogenesis [optimal at -400mV (Megonigal et al. 2004)] would be likely to dominate in FW2 and RW-wet (mean of -180mV and -100mV, respectively). We did find that CH_4 fluxes were highest in these two wettest sites. In the Ag site, the highest CH_4 flux was also measured during January

2009, which could be the result of wetter soils or reduced CH_4 consumption as a result of higher N availability: NH_4^+ can suppress CH_4 consumption by CH_4 oxidizers, while NO_3^- can raise the redox potential of soils (Le Mer and Roger 2001; Liu and Greaver 2009).

2.4.3 GHG fluxes in context

When comparing our results to some published studies of GHG fluxes, ranging from northern peatlands and constructed wetlands to tropical forests and agricultural fields, our mean rates for CO_2, N_2O and CH_4 fluxes fall within the range of reported literature values (Table 5). Our CH_4 flux rates (mean of 10.7-41.6 $kg \cdot ha^{-1} \cdot y^{-1}$) are low in this context, and especially for wetlands, compared to the table's overall mean of 1026 $kg \cdot ha^{-1} \cdot y^{-1}$. The highest annual estimate of CH_4 flux in our study was in RW-wet, with 259 $kg \cdot ha^{-1} \cdot y^{-1}$, still far below the mean CH_4 flux in Table 5. Clearly CH_4 fluxes can be quite variable spatially and by ecosystem, with values from individual studies spanning 3 orders of magnitude. Literature values for N_2O fluxes from natural wetlands and other undisturbed ecosystems were relatively low (0-16 $kg \cdot ha^{-1} \cdot y^{-1}$, except for a Louisiana swamp with 85.6 kg $N_2O \cdot ha^{-1} \cdot y^{-1}$) while elevated values were reported in constructed wetlands, rice paddies, and especially in agricultural watersheds (up to 946 $kg \cdot ha^{-1} \cdot y^{-1}$; Table 5).

We can also view our N_2O flux estimates in the context of other N fluxes we have measured in TLRP. We have found that atmospheric N inputs to TLRP are 5.6 $kg\ N \cdot ha^{-1} \cdot y^{-1}$; Ardón et al. submitted), approximately equal to mean estimated N_2O-N flux across

RW (5.9 kg N $\cdot ha^{-1} \cdot y^{-1}$). However, if we assume a typical N_2O mole fraction [N_2O-N/(N_2O-N+N_2-N)] reported for freshwater wetlands and agricultural fields (0.08 - 0.37; Schlesinger 2009), our estimated N-gas fluxes exceed atmospheric N inputs by about 3 - 10 times. Assuming our estimates of N-gas flux and atmospheric N deposition are reasonably accurate, this would imply that 1) there is excess N available in RW soils (despite lower pools in RW compared to the other sites), either from legacy N fertilizers or N mineralization; or 2) N fixation is contributing substantially to the N budget of TLRP. Our surface water monitoring results show that NO_3-N inputs to the site in surface water (mean of 24 kg N $\cdot y^{-1}$) are balanced or exceeded by NO_3-N outputs in surface water (mean of 34 kg N $\cdot y^{-1}$; Ardón et al. submitted), so DNF of surface water NO_3 is not likely to be the source of N-gas emissions.

2.4.4 Implications for wetland restoration

The large extent (440ha) and hydrologic variability of the RW are not typical of conventional RWs, which tend to be much smaller in area. The extent of the RW, along with its hydrologic regime (driven by precipitation and evapotranspiration) and low relief, allowed us to identify patterns of GHG fluxes within the RW that were associated with hydrological variables. Such relationships could help us understand the consequences of restoration for the many similar low-lying forested wetlands and peatlands in the South Atlantic region were converted to drained agriculture (Carter

1975; Dahl 1990), including in coastal NC and SC, and lands surrounding the Chesapeake Bay.

Low pH is characteristic of organic soils, and this acidity in TLRP persisted despite 30y of agriculture which included liming among other management practices (R. Needham, pers. comm.). Although we expected to find large pools of legacy N two years after the last crop harvest, based on fertilization history, we found lower soil N content in RW compared to FW sites. If soil N pools and N_2O fluxes in RW were enhanced by fertilizer-derived N, it is possible that these pools were depleted and gas fluxes subsided within the initial two years following abandonment.

Contemporary elevated N inputs to RW are therefore external to the system. Based on other work at TLRP (Ardón et al. submitted), inputs of N to RW from surface water, rain, and drainage from the Ag site are equal parts NH_4-N, NO_3-N, and dissolved organic N. The RW appears to retain 70% of total N inputs—retaining 98% of NO_3-N and 25% of NH_4-N, while acting as a net source of dissolved organic N. The dominance of NH_4-N over NO_3-N suggests that DNF rates are high and that nitrification, an aerobic, chemolithotrophic microbial process that converts NH_4^+ to NO_3^- could be limited by high acidity or by high organic C (Davidsson and Stahl 2000). Other work at TLRP has found that soil DNF potential is relatively high throughout the site (Chapter 3), which indicates that nitrification could be a major determinant of DNF here. The role of nitrification in controlling DNF rates in RWs is not frequently discussed when considering the

importance of DNF to wetland restoration and ecosystem services [e.g., (Schlesinger et al. 2006; Verhoeven et al. 2006)], yet it is likely to be important in acidic wetlands.

Over the two years of our study, when considering the net radiative balance of the three GHGs, we found that CO_2 was the largest component (66% to 100% of total GHGs) and did not differ between land uses in our study. Restoring wetland hydrology to TLRP has led to significant NO_3 retention or removal (Ardon et al. submitted) but does not appear to have significantly increased trace gas emissions [*contra* (Verhoeven et al. 2006)]. This conclusion has been reached in a few other studies in constructed wetlands (Mander et al. 2005) and restored prairie wetlands (Gleason et al. 2009). The current study, by presenting data for acidic coastal freshwater wetlands in a humid-subtropical climate, provides a regional and biogeochemical perspective that has not been presented to date.

3. Can we forecast nitrous oxide fluxes in coastal plain wetlands from environmental variables and soil processes?

3.1 Introduction

Water quality in many agricultural watersheds and receiving waters worldwide is severely degraded as a result of excess nitrogen (N) fertilizer usage (Galloway et al. 2004) and the decreased extent of wetlands that historically reduced the amount of N in downstream waters (Mitsch and Day 2006). Nitrogen pollution in watersheds has a multitude of detrimental effects, including human health hazards, algal blooms in waterways, and declines in fisheries and biodiversity (Carpenter et al. 1998; Townsend et al. 2003). Increasingly, wetland restoration is proposed to remedy the problem of N pollution in watersheds, because wetlands can serve as major sinks for N through plant uptake, sedimentation, and microbial N transformations (Zedler 2003).

Microbial denitrification (DNF) is the main mechanism of permanent N removal, because it converts nitrate (NO_3^-) in soil and water to inert atmospheric dinitrogen (N_2) gas (Knowles 1982). Microbial nitrification (NF) is an important controller of denitrification, through its production of NO_3^- from ammonium (NH_4^+). Nitrification and DNF can jointly provide a water quality benefit; however, both processes can produce nitrous oxide (N_2O), a trace gas that is harmful to the stratospheric ozone layer and contributes to the heat-trapping capacity of the atmosphere (Bremner 1997; Forster et al. 2007). Understanding whether N_2O fluxes from NF and DNF represent a substantial

drawback compared to the water quality benefits of these processes requires an integrative assessment of their complex biological and environmental controls (Schlesinger et al. 2006).

The microbial sources of N_2O have long been known, but N_2O fluxes from ecosystems are difficult to predict because they represent the net effect of multiple, interacting, and highly variable processes influenced by a variety of environmental factors. Davidson (1991) provides a useful framework for considering how environmental factors affect the flux of N_2O: 1) by controlling the rates of NF and DNF; 2) by affecting the proportion of N_2O produced by each process; and 3) by controlling the flux of N_2O from soil and water to the atmosphere (Davidson 1991). The spatial and temporal hierarchies at which controlling factors operate, from the organismal level to ecosystem scale, and from hourly to interannual scales, can provide important insights as well (Groffman 1991; Brumme et al. 1999).

Rate-controlling factors include temperature, the availability of substrates [bioavailable carbon (C), NH_4^+ and NO_3^-], and the presence of oxygen (O_2)–although they can have opposite effects due to the nature of each process. Nitrification tends to be favored in environments with high O_2, excess NH_4^+, and low C availability, because it is performed by aerobic chemolithoautotrophic microbes that oxidize NH_4^+ to NO_3^- and fix C (Davidson 1991; Paul and Clark 1996). Conversely, DNF is favored under low O_2, high

C, high NO_3^- (Firestone et al. 1980), because it is a heterotrophic metabolic pathway that uses organic C as the electron donor and NO_3^- as the electron acceptor (Knowles 1982).

Some environmental factors, such as soil pH, can influence both process rates and the proportion of N_2O that is produced. Nitrification and DNF enzyme activities can be inhibited at low pH, although there is evidence that some communities of nitrifiers and denitrifiers are not inhibited by conditions in acidic soils (Weier and Gilliam 1986; Weier and Gilliam 1986; De Boer et al. 1991; Simek and Cooper 2002). The proportion of NF and DNF resulting in N_2O has been found to be higher at low pH (Stevens et al. 1998; Morkved et al. 2007). Soil O_2 also can regulate the proportion of end products of both NF and DNF. Higher proportions of N_2O are produced by NF at intermediate O_2 availability or 40-60% water filled pore space (WFPS), and by DNF under low O_2 to incomplete anoxia or 60-80% WFPS (Davidson et al. 2000; Schindlbacher et al. 2004). A third factor that can affect the proportion of N_2O produced from DNF is the ratio of electron donors to acceptors ($C:NO_3^-$): high $C:NO_3^-$ yields more N_2, while low $C:NO_3^-$ results in more N_2O (Firestone et al. 1980). The proportion of N_2O produced by NF and DNF is not likely to be a fixed ratio, because it is influenced by multiple factors that vary spatially and temporally.

The third type of factor influencing N_2O fluxes is related to physical parameters that control diffusion and mass flow of gases from water and soil to the atmosphere (Davidson 1991). These can include soil properties such as particle size, bulk density,

drainage class, and hydrologic variables such as water table depth, soil moisture, and precipitation. Although the soil parameters control fluxes of gases through soil pore spaces, they are generally measured at coarser scales and do not vary temporally (Groffman 1991). Hydrologic parameters respond to weather events and climate trends, thus can vary on rapid time scales (minutes to hours) as well as seasonally, with freeze/thaw cycles and changes in evapotranspiration. Parameters that limit the flux of N_2O from soil and water also encourage the consumption of N_2O by denitrifiers, because it is reduced to N_2 in the final step of DNF.

These environmental factors interact at multiple spatial and temporal scales, and together influence NF and DNF rates and N_2O fluxes. In a wetland ecosystem, some factors may exert a greater influence on net N_2O fluxes; identifying such emergent factors that regulate N_2O fluxes could have great utility for ecosystem management. In particular, the scale of temporal and spatial variability associated with important predictors could inform monitoring and modeling efforts, even if such predictors are likely to vary by ecosystem or regionally (Groffman et al. 2000). In restored wetlands influenced by agricultural nutrient loading, many conditions exist that might promote high N_2O fluxes: high N and C availability, low pH, high or variable soil moisture, and poorly drained soils. Alternatively, N_2O fluxes could be low in such systems if pH and O_2 availability inhibit NF rates, and thus the supply of NO_3^- for DNF. Or if soil properties and hydrology impede transport of water and substrates through soils, N

cycling would be slower, thus limiting N_2O fluxes. If factors akin to state variables (such as pH, bulk density, or soil C:N) were found to be better predictors of N_2O fluxes than more variable hydrologic or chemical parameters such as soil moisture or NO_3^- pools, such information could be used to guide restoration practices, monitoring efforts, and models of N_2O fluxes.

3.1.1 Research approach and hypotheses

During a multi-year study of gas fluxes from agricultural, restored, and forested coastal plain wetlands, we found some significant differences in soil properties and hydrologic patterns between land use types, and that N_2O fluxes tended to be higher in drier sites with higher N availability (Chapter 2). With the goal of relating N_2O fluxes to environmental variables and soil processes in a statistical framework across land use types, we measured a variety of potential predictors during the summer and fall of 2007. To develop a predictive model for N_2O fluxes, we used a space-for-time substitution, incorporating observations across all the sampling points for a given sampling period. Additionally, we wished to determine whether variables determined from soil cores or variables measured in the field contemporaneously-with gas sampling were more useful for predictive purposes. In particular, we aimed to find out whether widely-used laboratory assays for NF and DNF were related to field N_2O fluxes. Identifying relationships between N_2O flux and slowly changing variables, such as soil properties, vs. rapidly changing variables, such as soil NO_3^- pools or water table depth, would

provide insights into functional relationships between processes and could be useful for monitoring and modeling N_2O fluxes.

We hypothesized that hydrologic variables, such as water table depth, would be the main drivers and have a bell-shaped relationship with N_2O fluxes (Davidson 1991), with highest rates at intermediate soil moisture due to contributions from both NF and DNF. We expected that high soil NO_3^- content would lead to high DNF and N_2O production, while high soil C content would more likely lead to complete DNF, resulting in N_2. We also expected to find that soil redox potential would integrate hydrologic and metabolic conditions, and be the best predictor of the dominant metabolism, such that NF would occur above 250 mV and DNF at +250 to100mv, with high N_2O fluxes occurring around 200-250mV.

3.2 Methods

3.2.1 Field sampling

This multi-year study of GHG emissions following wetland restoration focused on a 440ha restored wetland (RW) and three reference sites [one agricultural field (Ag), and two forested wetlands (FW1 and FW2)] in the Albemarle-Pamlico Peninsula in the coastal plain of North Carolina. The sites are described extensively in Chapter 2. We collected soil samples (0-15cm) in June 2007 and in October 2007 from all 48 sampling points across the four sites, and measured N_2O fluxes and related environmental variables in July and November 2007. The methods and sampling approach for

measuring and calculating gas fluxes are also described extensively in Chapter 2. At each sampling location (n=48), we installed one EC-5 soil moisture sensor (Decagon Devices, Pullman, WA) to measure volumetric water content and five platinum-tipped redox electrodes (Vepraskas and Faulkner 2001) to measure soil redox potential with a voltmeter and calomel reference electrode (Fisher Scientific).

This approach resulted in four groupings of predictor variables, based on the method of data collection and the timescales of variability: 1) soil properties that change on annual to longer time scales; 2) soil solute pools that vary on more rapid time scales; 3) soil biogeochemical processes, and 4) contemporaneously measured field-based variables (hereafter field-based variables), such as hydrologic data, porewater chemistry, soil temperature, and other gas fluxes. Because summer and fall 2007 were an abnormally dry period, the water table in many sampling points was below the depth of our monitoring wells and piezometers; we therefore had to exclude hydrologic variables related to water table depth and porewater chemistry altogether due to the number of missing observations.

3.2.2 Laboratory analyses

Soil properties (pH, bulk density, %C, %N, and C:N) were determined using standard methods described in Chapter 2. Soil chemical variables were measured on subsamples of fresh soil sieved with a 2-mm mesh. We extracted inorganic N from duplicate 2.5g soil samples with 25mL 2M KCl, and analyzed the extracts for NH_4^+ and

NO_3^- on a Lachat QuickChem 8000 automated system using the phenate method for NH_4^+ and the hydrazine reduction method for NO_2^-+ NO_3^- (Lachat Instruments, Milwaukee, WI). We adapted a procedure by McDowell et al. (McDowell et al. 2006) to determine the bioavailability of water-extractable dissolved organic C (DOC). Briefly, we extracted duplicate 1g (dry weight equivalent) subsamples of fresh soil with 30ml of nanopure water for 15minutes on a shaker table, and then centrifuged the samples at 3400 rpm for 10 minutes. We filtered 10mL of supernatant through a Whatman GF/F filter for analysis of initial concentrations of DOC and total dissolved N (TDN) by Shimadzu TOC-V total carbon analyzer with a TNM-1 nitrogen module (Shimadzu Scientific Instruments, Columbia, Maryland, USA).We incubated 10ml of filtered supernatant with 100µL of nutrient solution (NH_4NO_3 and KH_2PO_4) and 50µLof inoculum (unfiltered supernatant pooled from five samples from different sites) for 7 days in amber glass vials in the dark. After 7 days, we filtered the samples and determined DOC contents. We calculated the percent of bioavailable DOC (BDOC) based on the difference between initial and final DOC concentrations.

Soil biogeochemical processes were likewise determined on fresh soils and included DNF potential, net nitrification (NF), and active microbial biomass. Denitrification potential was measured as DNF enzyme activity [DEA; (Tiedje et al. 1989; Groffman et al. 1999)]. In this anoxic assay, 5g of soil in a slurry with excess NO_3^- and labile C were incubated with acetylene, which blocks the activity of the nitrous oxide

reductase enzyme, allowing the denitrification rate to be estimated through the accumulation of headspace N_2O. Net NF was estimated using a nitrapyrin-inhibition assay (Kemp and Dodds 2002), in which parallel 5g soil samples (with and without nitrapyrin) were incubated in centrifuge tubes for 7 days in the dark on a shaker table at 150rpm (aerated daily); accumulation of NH_4^+ due to blocked NF was measured on the Lachat QuickChem 8000. A protocol for estimating active microbial biomass by substrate-induced respiration (SIR) using autolyzed yeast as the substrate was modified from West and Sparling 1986 and Bradford et al. 2008. Additionally, we adapted methods commonly used to determine C-mineralization rates (e.g., Fierer and Schimel 2002) to determine the cumulative and maximum rate of CH_4, N_2O, and CO_2 production, incubating replicate 5g soil subsamples in gastight amber glass vials for 7-days under parallel field-moist (ambient atmosphere; CH_4.ox, N_2O.ox, CO_2.ox) and anoxic conditions (N_2 atmosphere: CH_4.anox, N_2O.anox, CO_2.anox). Concentrations of CO_2, N_2O, and CH_4 were measured on a Shimadzu 17A gas chromatograph with ECD and FID detectors, retrofitted with 6-port valves and a methanizer in series with the FID to allow the determination of the three gases from the same sample.

3.2.3 Data analysis and modeling

We chose to use results from October 2007 soil sampling and November 2007 gas sampling for model development because ancillary information on soil and environmental variables was most complete for this dataset, and to use data from June

and July 2007 for model validation. To put this subset of gas analyses into context with our previous work on biogeochemical differences by land use in these sites (Chapter 2), we tested group differences in DNF potential (as DEA) using permutation one-way tests with adjusted p-values for multiple comparisons between groups [oneway_test and Nemenyi-Damico-Wolfe-Dunn test, R package coin (Torsten et al. 2008)].

We compiled soil variables from October 2007 samples and environmental variables from November 2007 from 48 sampling points into a dataset without missing values for predictor variables for as many observations as possible. Thus we excluded hydrologic variables based on water level and variables related to porewater chemistry, and achieved a dataset of 20 observations (out of a possible 48) that had no missing values for all 25 potential predictors in the N_2O model. We used principal components analysis [PCA; `princomp`; R package `stats`; (Venables and Ripley 2002)] to identify correlations between predictor variables and to see how observations clustered together according to land use in parameter space.

We applied multiple linear regressions with laboratory-based and field-based factors to develop models for N_2O fluxes, and included only soil variables to develop models for DNF potential. The assays for net NF proved to be uninformative, with very low values across most observations, so those results were not modeled but were included in the subsequent data analysis as potential predictors of N_2O fluxes and DNF potential. To avoid overfitting the model, and to identify the best predictors of N_2O flux

in each group of variables, we built submodels for each of the five variable groups (soil properties, soil chemistry, soil biological processes, hydrology, and other measurements).

We used a stepwise method based on Akaike's Information Criterion (AIC) with forward and backward selection [`stepAIC`; R package `MASS`; (Venables and Ripley 2002)] to identify the combination of variables within each submodel that best fit the N_2O flux data. We then combined the five reduced submodels into one model and repeated the stepwise AIC model selection procedure to identify the models that best fit the N_2O flux data. Final model comparisons were based on AIC_c to account for small sample size (McQuarrie and Tsai 1998). We repeated this multiple regression process to develop a model for DNF potential using soil characteristics, soil chemistry, and soil process variables. Again, we built models for each group of variables, used stepwise AIC to identify the best submodels, and then combined the submodels to identify the two models that best fit the DNF potential data over all groups of variables.

After building models for N_2O flux, we tested their performance with data from October 2007 soils and November 2007 field measurements that was not used to build the models (n=12). Although they were spatial models, with no temporal component, we wished to test their performance with input data from another season, so we used data from June/July 2007. We were not able to test the DNF potential models with October/November 2007 data, because the whole dataset was used to build the models;

data from June/July 2007 were used to test the second DNF potential model only, because the soil incubation assays were not performed in June 2007.

3.3 Results

3.3.1 N_2O flux patterns by land use type

Previous work examined patterns in gas fluxes by land use type and found higher mean N_2O fluxes in the Ag site and FW1 over the two years of the study (Chapter 1); this pattern held true again in November 2007 (Figure 4). When comparing N_2O fluxes from July and November 2007 to the rest of the study period, we can see that the range of N_2O fluxes in July and November 2007 was lower than the long-term mean in the Ag site, similar to the long-term mean for the RW sites, and higher than the long-term mean in FW2 (Figure 4). In the Ag and FW1 sites, November 2007 N_2O fluxes were much higher than fluxes in July 2007, and in RW-wet, mean N_2O fluxes in July 2007 were negative (Figure 4). DNF potential only differed between Ag and FW2 sites. Neither N_2O fluxes nor DNF potential showed a clear relationship with redox potential, and N_2O flux patterns did not match patterns in DNF potential across sites. When N_2O fluxes from the entire study period were plotted against redox potential and against available paired DNF potential results, no simple relationship emerged (Figure 5).

3.3.2 Distributions of and correlations between predictor variables

Prior to building multiple regression models, we examined the distributions of predictor variables through box-and-whisker plots, and the correlations of variables

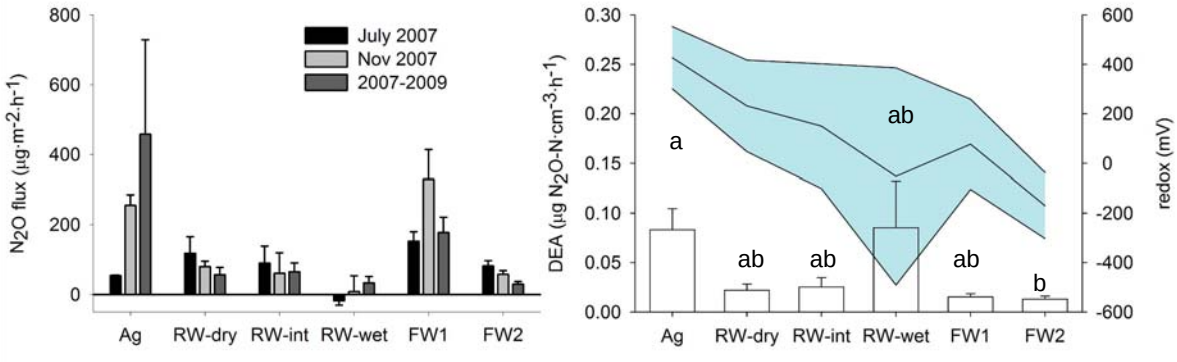


Figure 4: N_2O fluxes and denitrification potential (DEA) by site in fall 2007. A) N_2O flux for July 2007, November 2007 and the entire study period; B) DEA for October 2007 soils is shown against 95% confidence interval of redox potential (right y-axis) in November 2007. Data (means ± SE) are displayed by site, with RW results for 5 dry, 5 intermittently flooded, and 5 wet points. Different letters indicate differences in DEA between sites at $p < 0.10$.

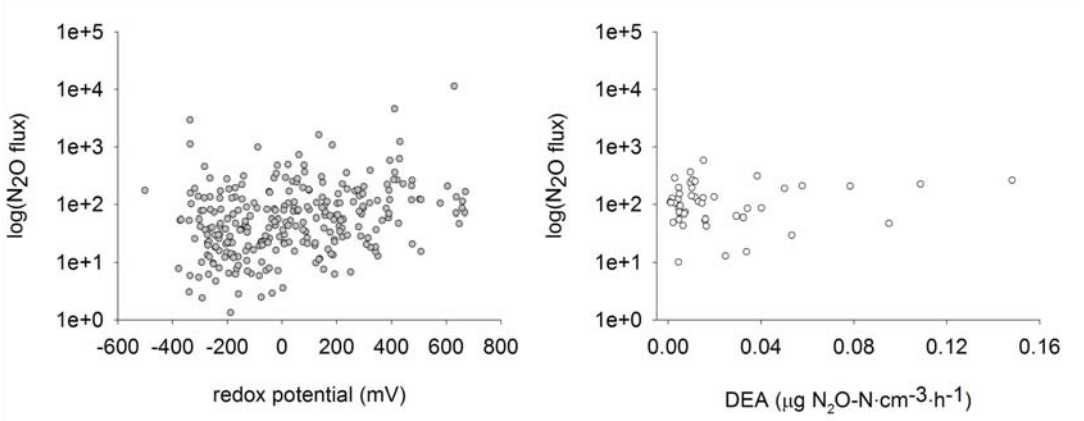
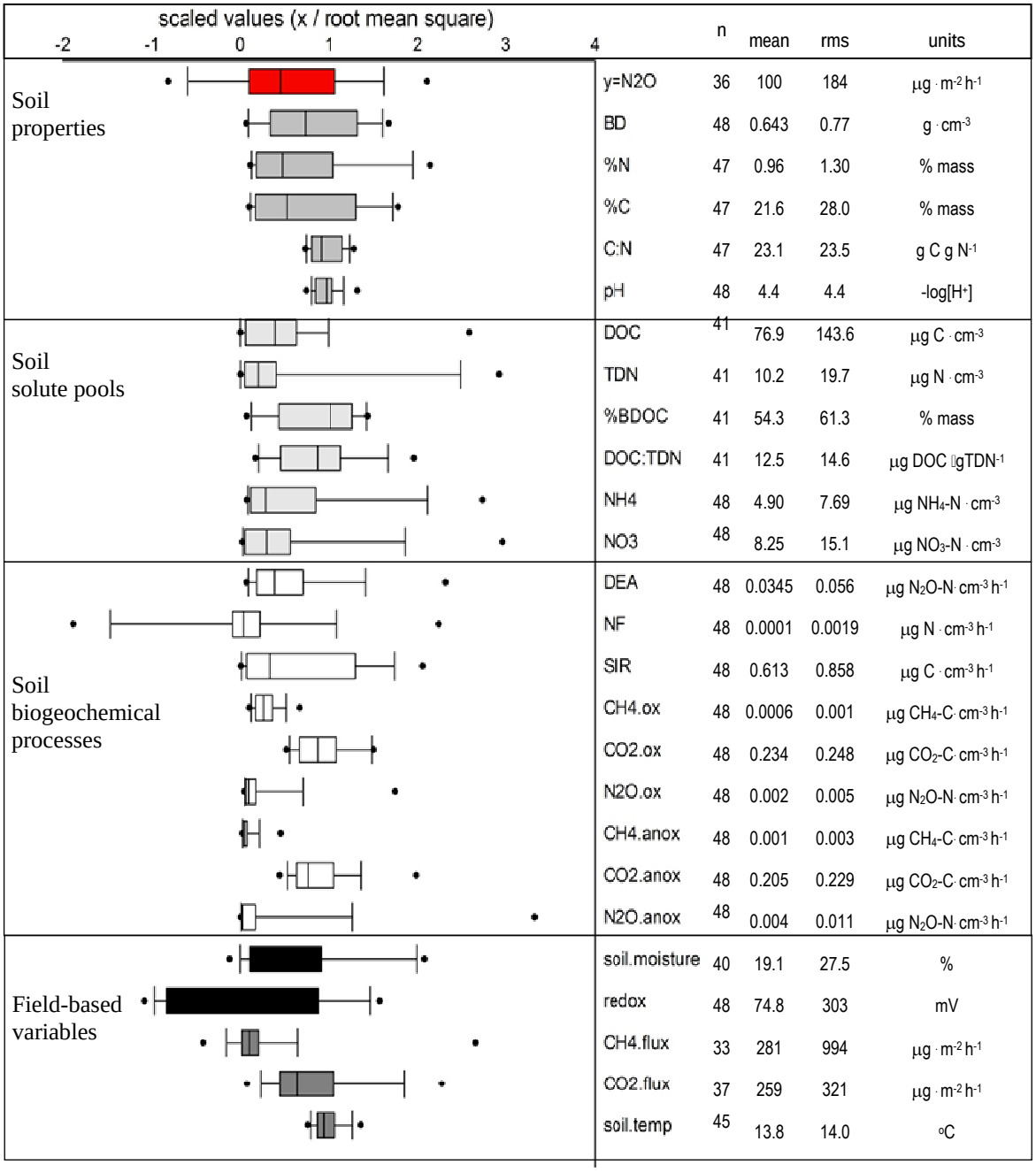


Figure 5: Log-transformed N_2O fluxes vs. redox potential (July 2007-June 2009) and denitrification potential (DEA; June and October 2007)



	n	mean	rms	units
y=N2O	36	100	184	µg · m^{-2} h^{-1}
BD	48	0.643	0.77	g · cm^{-3}
%N	47	0.96	1.30	% mass
%C	47	21.6	28.0	% mass
C:N	47	23.1	23.5	g C g N^{-1}
pH	48	4.4	4.4	-log[H$^+$]
DOC	41	76.9	143.6	µg C · cm^{-3}
TDN	41	10.2	19.7	µg N · cm^{-3}
%BDOC	41	54.3	61.3	% mass
DOC:TDN	41	12.5	14.6	µg DOC gTDN^{-1}
NH4	48	4.90	7.69	µg NH_4-N · cm^{-3}
NO3	48	8.25	15.1	µg NO_3-N · cm^{-3}
DEA	48	0.0345	0.056	µg N_2O-N· cm^{-3} h^{-1}
NF	48	0.0001	0.0019	µg N · cm^{-3} h^{-1}
SIR	48	0.613	0.858	µg C · cm^{-3} h^{-1}
CH4.ox	48	0.0006	0.001	µg CH_4-C· cm^{-3} h^{-1}
CO2.ox	48	0.234	0.248	µg CO_2-C· cm^{-3} h^{-1}
N2O.ox	48	0.002	0.005	µg N_2O-N· cm^{-3} h^{-1}
CH4.anox	48	0.001	0.003	µg CH_4-C· cm^{-3} h^{-1}
CO2.anox	48	0.205	0.229	µg CO_2-C· cm^{-3} h^{-1}
N2O.anox	48	0.004	0.011	µg N_2O-N· cm^{-3} h^{-1}
soil.moisture	40	19.1	27.5	%
redox	48	74.8	303	mV
CH4.flux	33	281	994	µg · m^{-2} h^{-1}
CO2.flux	37	259	321	µg · m^{-2} h^{-1}
soil.temp	45	13.8	14.0	°C

Figure 6: Boxplots for candidate variables (k=25) for N_2O multiple linear regression. Boxes represent 25-75% of results, error bars extend to 10% and 90% percentiles, and points correspond to individual values outside those boundaries. Variables are grouped by type. The companion table lists the number of observations, mean, root mean square, and units for each variable.

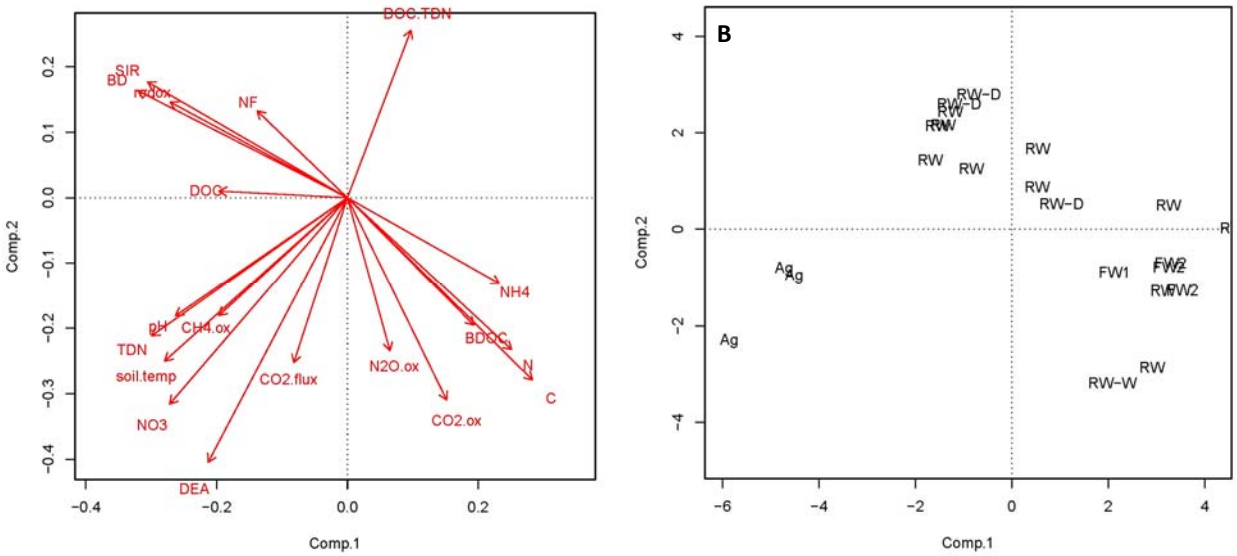


Figure 7: Biplot of principal components analysis for 19 predictor variables. The two principal axes displayed account for 43% and 15% of the variability. Points in A) correspond to variables in Figure 6, while points in B) are labeled according to land use type (Ag, FW1, FW2 for reference sites; RW-D and RW-W for 5 driest and 5 wettest among RW points)

through PCA (Figures 6 and 7). Figure 6 shows the relative distribution of values in 25 candidate predictor variables by creating box-and-whisker plots of values scaled by the root mean square of each variable. The highest variability is seen in variables related to soil biogeochemical processes, with intermediate variability found in soil solute and field-based variables, while the smallest range is seen in soil properties. Through PCA, we found that many predictor variables were correlated [SIR, BD, redox, DOC, and NF at one end of axis 1, with NH_4^+, %BDOC, %C, %N, N_2O.ox and CO_2.ox (gases measured during ambient incubations) at the opposite end], while the orthogonal axis grouped DOC:TDN against NO_3^-, DNF potential, CO_2 flux, pH, TDN, soil temperature, and

CH_4.ox from ambient incubations (Figure 7A). The first two components of the PCA explained 43% and 15% of the variability. Additionally, PCA revealed three distinct clusters by land use, such that Ag sites were separated from RW/RW-dry sites and from FW1/FW2/RW-wet/RW sites, although the separation between RW and FW was not complete (Figure 7B).

3.3.3 Multiple regression models for N_2O flux

Stepwise multiple regression with automated variable selection by AIC yielded submodels for N_2O fluxes for each group of candidate variables (Table 6). The model based on soil properties included pH, %N, and %C and the soil solute submodel with KCl-extractable NO_3^+ and NH_4^+ explained a similar proportion of the variance in N_2O flux (R^2_{adj} = 0.44 and 0.43, respectively). The biogeochemical process submodel included DNF potential (DEA) alone as the best available predictor, explaining 21% of the variance (p=0.044; Table 6). The best model from field-based contemporaneously measured variables found that soil CO_2 flux and soil temperature explained 61% of the variance in N_2O fluxes.

The four submodels were combined into one model with nine parameters, which was refined again using stepwise AIC variable selection. This process yielded the final

N_2O model based on four parameters: two field-based variables (soil CO_2 flux and soil temperature), one soil solute pool (NH_4^+), and one biogeochemical process variable (DNF potential as DEA), with R^2_{adj} of 0.81. The AIC values were corrected to AIC_c to account for small sample size (McQuarrie and Tsai 1998), and the model with the smallest AIC_c was deemed to best fit the data out of the competing models. The Δ_i values in Table 6 represent the difference between the lowest AIC_c value and the AIC_c values of competing models. The w_i columns represent the relative weight of each model given the competing models. The overall best model for N_2O flux had a Δ_i of over 10, which means that there was very strong support for this model, and its weight was over 0.99. We then compared the submodels to one another, to determine whether environmental or soil variables were more useful in predicting N_2O fluxes. The model with soil CO_2 flux and soil temperature had a weight of 0.98, vastly outperforming the other submodels when the combined best model was removed from the comparison. Among the remaining submodels, the soil solute model was the best fit to the N_2O data, contributing two-thirds of the weight, while the soil properties submodel accounted for most of the remainder (Table 6).

3.3.4 Multiple regression models for denitrification potential

The DNF potential modeling effort identified submodels for each group of soil variables, with the soil properties model and the soil solute pools model fitting the data equally well (R^2_{adj} = 0.61 and 0.62, respectively). The soil properties model included %N,

Table 6: Model selection results for N_2O fluxes and DNF potential

				Reduced submodels						
N_2O models (n=20)	**AIC- selected variables**	**R^2_{adj}**	**K**	**AIC**	**AIC_c**	**Δ_i^{sub}**	**w_i^{sub}**	**Δ_i^{sub1}**	**w_i^{sub1}**	
Overall N_2O model	CO_2.flux + soil.temp + NH_4 - DEA	0.81	4	157.0	159.7					
Submodels										**Rejected variables**
Field -based	CO_2.flux + soil.temp	0.61	2	169.3	169.3	0	0.98			CH_4.flux, redox, soil moisture
Soil solutes	NH_4 + NO_3	0.43	2	176.9	177.6	8.2	0.016	0	0.66	DOC, TDN, %BDOC, DOC:TDN
Soil properties	pH - %N + %C	0.44	3	177.6	179.1	9.8	0.007	1.5	0.31	BD, C:N
Biogeochemical processes	DEA	0.21	1	183.8	184.1	14.7	0.001	6.5	0.03	NF, SIR, CH_4.ox, CO_2.ox, N_2O.ox, CH_4.anox, CO_2.anox, N_2O.anox
DEA models (n=39)										
Overall DEA model	CH_4.ox - pH+ pH^2	0.68	3	-311.0	-310.3	0	0.97			
Submodels										**Rejected variables**
Soil properties	pH^2- pH - %N	0.61	3	-303.4	-302.7	7.61	0.022	0	0.74	BD, %C, C:N
Soil solutes	TDN - DOC + NO_3 + DOC:TDN + $(NH_4)^{-1}$	0.62	5	-302.4	-300.6	9.69	0.007	2.08	0.26	%BDOC
Biogeochemical processes	CH_4.ox	0.31	1	-281.6	-281.4	28.8	0.00	21.2	0	NF, SIR CO_2.ox, N_2O.ox, CH_4.anox, CO_2.anox, N_2O.anox

Table 7: Estimated coefficients and SEs, and relative importance (RI) for the two best models of N_2O flux and DNF potential. Multiple R^2 is shown to assess fit to the data.

N_2O					DNF potential				
Variable type	**variable**	**est. coef**	**SE**	**RI**	**Variable type**	**variable**	**est. coef**	**SE**	**RI**
	(Int)	-515	99.2			(Int)	0.295	0.0699	
Field-based	CO_2.flux	0.311	0.071	0.36	Soil property	pH	-0.138	0.0289	0.21
Field-based	soil.temp	38.3	7.62	0.28	Soil property	pH^2	0.0161	3.00E-03	0.28
Solute pool	NH_4	17.5	4.11	0.12	Biogeochemical	CH_4.ox	55.2	15.2	0.21
Biogeochemical	DEA	-1052	424	0.09					R^2 = 0.70
				R^2 = 0.85					
	(Int)	-178	80.4			(Int)	0.385	0.0733	
Field-based	CO_2.flux	0.370	0.09	0.44	Soil property	%N	-7.86E-03	3.65E-03	0.07
Field-based	soil.temp	13.6	6.17	0.22	Soil property	pH	-0.164	0.0308	0.25
				R^2 = 0.66	Soil property	pH^2	0.0188	3.19E-03	0.32
									R^2 = 0.64

pH, and $(pH)^2$ while the soil solute model was based on TDN, DOC, NO_3^-, DOC:TDN, and $(NH_4^-)^{-1}$ (Table 6). With pH there was both a linear and quadratic relationship to DNF potential, while NH_4^+ had an inverse linear relationship. Model selection for biogeochemical process variables identified CH_4 production under oxic, field-moist conditions (CH_4.ox) as the best biogeochemical predictor of DNF potential ($R^2_{adj} = 0.31$). The three submodels were combined into an overall model with nine parameters, from which the best predictors of DNF potential were identified by stepwise AIC model selection: CH_4.ox, pH, and $(pH)^2$. This overall model fit the DNF potential results with an adjusted R^2 of 0.68; compared to the candidate submodels, it was the best fit to the data, with a weight of 0.97 (Table 6). When comparing the three submodels to one another, the soil properties model was determined to have the best fit, with a weight of 0.74, while the soil solute model contributed the remainder of the weight. While the biogeochemical variable CH_4.ox was selected in the final model, alone it was a very poor predictor of DNF potential (Table 6).

3.3.5 Model fits and model validation

For each of the top two models for N_2O flux and DNF potential, we tabulated the estimated coefficients (and their standard errors) in Table 7, along with relative importance, or proportion of the variance accounted for by each coefficient, and multiple R^2 values. The best N_2O model accounted for 85% of the variance in N_2O flux, nearly half of which was due to the influence of soil CO_2 flux in the model; soil temperature was the

next most important variable, while together NH_4^+ and DNF potential contributed 21% to the explained variance. In second best N_2O model, soil CO_2 flux was twice as important as soil temperature in explaining N_2O flux (relative importance = 0.44 and 0.22, respectively). Soil temperature, CO_2 flux, and NH_4^+ were positively related to N_2O flux, while DEA had a negative relationship. The fits of the two best models to the DNF potential data were more similar, each explaining 70% and 64% of the variance in DNF potential, respectively (Table 7). In the best model of DNF potential, pH and its square accounted for 49% of the variance, with CH_4.ox contributing 21% to the model. In the next best DNF potential model, pH again accounted for the bulk of the variance (57%), with %N representing only 7%. The pH and $(pH)^2$ coefficients were similar in both DNF potential models, with a U-shaped relationship and a minimum around pH=4.2 (plot not shown). In the DNF potential models, DNF potential increased with increasing CH_4.ox in the first case, while DNF potential had a negative relationship to soil %N (Table 7)

Having identified two models with the best fit to N_2O flux data, we plotted observed against fitted values for each model (Figure 8A) to see how the models captured the range of values. Both models appeared to perform well at intermediate and high values, but did not correctly predict negative flux values. When the N_2O models were validated with data from the same time period but not used in model building, they had similar fits (model 1 R^2 = 0.67 and model 2 R^2= 0.62; Figure 8B). Using data

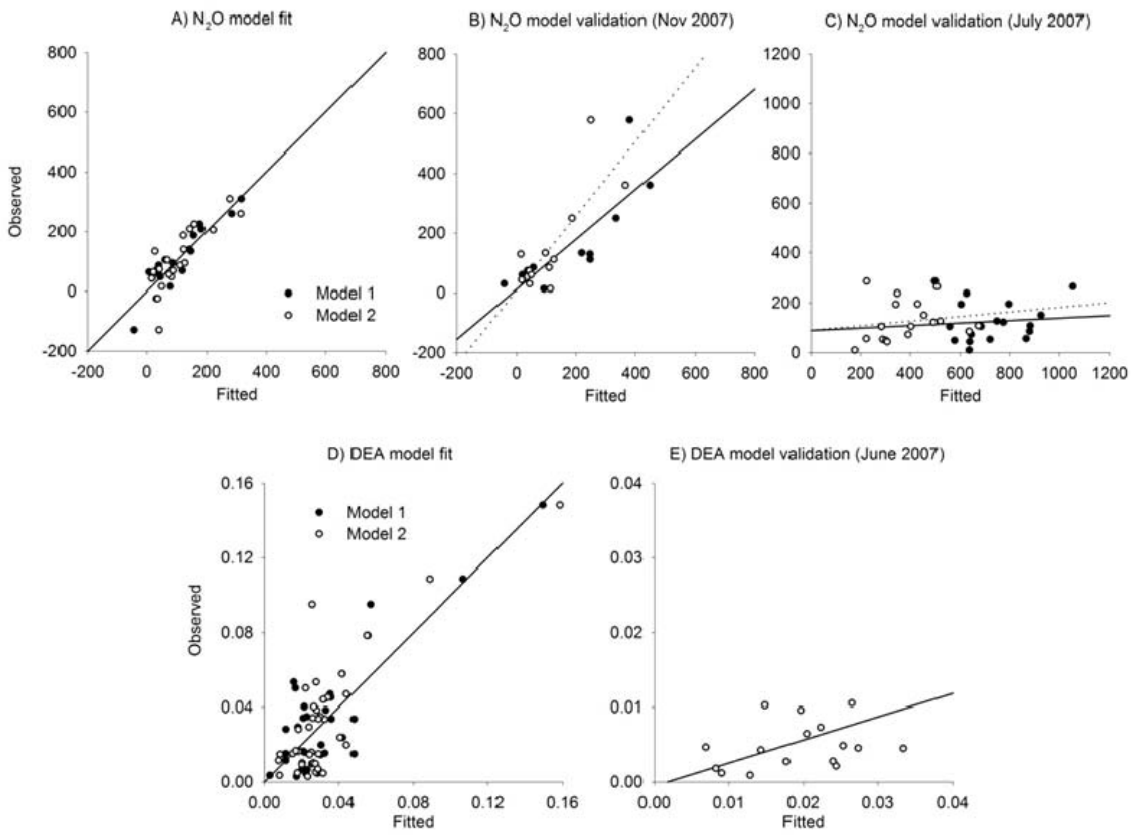


Figure 8: Model fits to observed data and validation of N_2O and DEA models. A) Fitted vs. observed N_2O flux for modeled dataset (model 1 R^2=0.85 and model 2 R^2=0.66); Validation of N_2O models with B) data from Oct/Nov 2007 not used in model building (model 1 slope=0.84 and R^2=0.67; model 2 slope=1.24 and R^2=0.62); and C) data from June/July 2007 (model 1 slope=0.05 and R^2<0.01; model 2 slope=0.09 and R^2=0.03); D) fitted vs. observed DEA for modeled dataset (model 1 slope and R^2=0.71 and model 2 slope and R^2=0.64); E) Validation of DEA model 2 with soils data from June 2007 (slope=0.31 and R^2=0.44).

from June and July 2007, the two N_2O models performed poorly, vastly overpredicting N_2O fluxes (Figure 8C). The fit of the two best models of DNF potential was assessed in Figure 8D and found to be similar for both models, without major outliers in the data. Model validation for models of DNF potential was limited to testing the second model

with data from June 2007, because the October soils dataset was used in model building. The fit of the second model to the June 2007 dataset was better than the fit of the N_2O flux models, with a slope of 0.31 and R^2 of 0.44 (Figure 8E).

3.4 Discussion

We first looked for patterns in N_2O fluxes, net nitrification (NF), and denitrification (DNF) potential (as measured by DEA) across land uses for measurements made in Fall 2007 (Figure 4). We found lower N_2O fluxes in the restored wetland (RW), regardless of hydrologic status (represented by redox potential), compared to either the adjacent agricultural field (Ag) or forested wetlands (FW). Although we found higher DEA in the Ag site compared to FW2, there were no other differences between land uses. The variability of both DNF potential and redox potential were highest within RW-wet. We conclude that DNF potential as measured by DEA was not a good single indicator of N_2O flux patterns across land uses.

Initially, we hypothesized that hydrologic variables would be primary controls on N_2O fluxes, with highest rates at intermediate soil moisture due to contributions from both NF and DNF. However, we did not find a simple relationship between N_2O fluxes and redox potential across the entire study period. We found no simple relationship between N_2O fluxes and either of its two biological sources (Figure 5; NF results not shown). This was not surprising, given that multiple levels of controls can interact to

influence both NF and DNF rates, product ratios, and transport to the atmosphere (Davidson 1991).

The principal components analysis of 19 variables showed that observations were grouped quite distinctly by land use type, with observations from the Ag site aligning with variables that could be associated with soil fertility (e.g., pH, NO_3-, soil respiration) while observations from the FW and RW-wet sites aligned with variables that were associated with high organic matter (soil C and N content, NH_4^+, and BDOC). The third grouping of variables, along which the drier RW sites were aligned, was directly opposite the organic matter gradient, and included variables such as substrate inducible respiration, dissolved organic C, redox potential, and bulk density. These variables could be related by high mineral content, such that particle surface area was greater, supporting larger microbial biomass per volume of soil sampled, while still having better drainage than soils high in organic matter, enabling higher redox potentials (Figures 7A and B).

To identify the most important processes and environmental factors in determining N_2O fluxes and DNF, we used a multiple regression approach that included both N_2O sources and environmental variables. If DNF were the main contributor to N_2O fluxes, as is likely to be true in most wetlands based on O_2 availability and water-filled pore space (Davidson et al. 2000; Ciarlo et al. 2007), the proportion of N_2O produced by DNF would likely be regulated in part by environmental factors, not just

by DNF rate. We found that the best models for N_2O fluxes depended primarily on environmental variables (CO_2 flux and soil temperature), explaining 64-66% of the variance, with some additional contribution to the top N_2O model by NH_4^+ availability and DEA (21% additional variance explained; Table 7).

The tight relationship between N_2O flux and CO_2 flux has been recently demonstrated in a synthesis of gas exchange studies in eight ecosystems throughout the world, which found a slope of 0.66 (r^2=0.79) in rice paddies, 0.19 (r^2=0.66) in temperate forests, and 0.16 (r^2=0.79) in dry croplands (Xu et al. 2008). In our dataset, in a model with soil CO_2 flux alone, the slope was 0.41 (r^2=0.64, excluding one negative N_2O flux measurement), placing these acid organic soils closer to rice paddies than temperate forests or croplands. Some models of N-gas products from DNF, such as DAYCENT, include CO_2 respiration as an index of C availability (Del Grosso et al. 2000). Emissions of N_2O have been shown to have great temperature sensitivity under excess N availability, with lower temperature responses in N-limited forests (Schindlbacher et al. 2004; Barnard et al. 2005; Grant and Pattey 2008). The correlation of N_2O fluxes with soil CO_2 fluxes and temperature shows that these environmental variables can be useful for predictive purposes because they integrate physical factors, such as diffusivity and solubility, with biological factors such as heterotrophic respiration and enzyme activities that increase soil C and N availability. Our N_2O models did not appear to predict N_2O consumption well, although only one observation of N_2O flux was negative (Figure 8A).

The role of N availability, NF, and DNF in regulating N_2O fluxes was less intuitive. We expected that high soil NO_3^- content would lead to high DNF and N_2O production, while high soil C would promote the production of N_2 over N_2O. Although NH_4^+ and NO_3^+ together helped explain 43% of the variation in N_2O flux in the soil chemistry submodel, the final model included NH_4^+ rather than NO_3^- as an important predictor, perhaps because NO_3^- was correlated with soil temperature and soil CO_2 flux (Table 7, Figure 7).

Since we found a positive relationship between NH_4^+ and N_2O flux in the first N_2O model, one explanation could be that N_2O fluxes arise from NF, driven by high NH_4^+ availability and relatively high soil moisture in these wetlands. However, we have no evidence for direct NF emissions of N_2O. While we do not have directly measured DNF rates either, DNF potential has a positive linear relationship with N_2O flux in the biogeochemical submodel (Table 6, r^2=0.21). But when considered with other variables in the first N_2O model, the relationship is more complex: with increasing soil CO_2 flux, increasing temperature, and increasing NH_4^+, higher DNF potential results in lower N_2O flux. Perhaps high NH_4^+ with high DEA promotes more complete DNF and lower N_2O flux, while high NH_4^+ with low DEA means that excess N is available, leading to incomplete DNF and higher N_2O flux, which is consistent with the Davidson (1991) "leaky pipe" model of N_2O flux. Although the relationship was not straightforward nor the most informative, we found that soil N availability and DNF potential did explain

21% of the variation in N_2O fluxes (Table 6). However, assays for net NF did not contribute to our models of N_2O flux.

Although we expected that soil redox potential would integrate hydrologic and metabolic conditions, and be the best predictor of N_2O flux, we found no such relationship: redox potential alone was not related to N_2O flux, and it did not contribute to the overall N_2O flux model. Perhaps this was because point measurements of redox potential in the soil do not reflect conditions in microsites within the soil that might contribute to N_2O production, or because this was an abnormally dry period. Other factors that surprisingly were not selected for the final models were variables related to C availability, soil pH, and active microbial biomass; however they were likely correlated with model parameters. Carbon quality and availability were likely strongly related to CO_2 flux, while soil pH was correlated with NH_4^+ (Figure 7).

With respect to DNF potential, we found that pH was the most important variable in predicting DEA in the two top models. When plotting DNF potential as a function of pH in these models, we found that the curve was U-shaped with DNF potential minimized at pH 4.2 (not shown). Many other studies have found that acidity tends to inhibit DNF in laboratory settings; however, in acid soils, it appears that microbial communities can be adapted to low pH and denitrify even at low pH (Weier and Gilliam 1986; Simek and Cooper 2002). Despite the strength of the correlation between pH and DEA, the differences among land use types we found in DNF potential

(Figure 4) were not the same as land use differences we saw in soil pH (Chapter 2, Table 2). The influence of ongoing liming in the Ag site, along with waning effects of past liming in RW, could explain the wide range in soil pH across land use types (3.75 in RW-wet to 5.53 in Ag; Chapter 2, Table 2).

In the overall DNF potential model, the rate of CH_4 production under ambient moisture and oxygen availability contributed 21% to the explained variation in DEA (Table 7). We interpret this as an indicator of the soil's water-holding capacity and prevalence of anoxic microsites during the incubation, which would be positively related to DNF potential. In the second DNF potential model, soil N content was negatively related to DEA; in the PCA, we found that soil extractable NO_3 was strongly correlated with DEA, while soil N, C and % bioavailable DOC were orthogonal to DEA (Figure 7A). Overall, however, the fact that pH and %N, as slow-changing soil properties, were important in predicting DNF potential ($R^2_{adj} = 0.64$) was interesting: this suggests that DEA may be a robust parameter that likewise does not change very rapidly. The assay was indeed developed to facilitate cross-system comparisons and to give a broader view of DNF, given that actual DNF rates are difficult to measure and are highly variable (Groffman 1987; Groffman et al. 2006).

The model validation we performed helps us address the question of the broader applicability of these models, and relates to the multiple levels of control over N_2O flux and DNF potential. When applied to data from the same time period that was not used

to build the models, both N_2O flux models fit the data reasonably well (model 1 R^2=0.67 and model 2 R^2=0.62, Figure 8). However, when these spatial models were applied to data from June/July 2007, both models overpredicted N_2O flux, with poor fit to observed values (Figure 8C). The range of N_2O fluxes encompassed in July 2007 was lower overall and included more observations of negative N_2O flux (Figure 4), and temperatures were higher in July than November. The most likely explanations for poor model performance for July N_2O fluxes are: 1) non-linear responses to hot temperatures; and 2) autotrophic respiration by plants during the growing season, which would increase CO_2 flux but not affect N_2O production, leading to overprediction of N_2O flux. These models may be applicable outside the growing season, when autotrophic respiration is not a major factor. Other explanations could be that the influence of drier conditions was not captured by model parameters; the influence of vegetation during the growing season, competing for available N; or greater importance of other unmeasured variables.

The DNF potential model with pH and %N was also tested with data from June/July 2007, and it performed much better than the N_2O models, explaining 44% of the variation in DEA. This gives support to the idea that DNF potential is a parameter that integrates soil properties, microbial processes, and environmental variables over longer periods than measurements of soil N pools or N cycling rates. On the other hand, these qualities make DEA assays a relatively blunt tool for assessing responses to

seasonal changes or weather events, and suggest that DNF potential is only partially useful for predicting sensitive and rapidly changing variables such as field N_2O fluxes.

This study found that contemporaneously measured environmental variables were the best predictors of N_2O fluxes for a given sampling event, and that soil N availability and DNF potential can provide additional predictive ability for such models. Although there is much support in the literature for hydrologic controls over N_2O fluxes, we did not find evidence for this during summer and fall 2007, which was an abnormally dry period. We expect to expand our models to the two-year dataset of gas fluxes and environmental variables using multilevel non-linear modeling approaches. Another question remains the contribution of NF and DNF to N_2O production. Our analyses in this study could not distinguish between NF and DNF as sources of N_2O in these wetlands. This approach was not intended to measure the production of N_2 from DNF—thus we cannot identify the factors controlling $N_2O/(N_2+N_2O)$, which is critical in addressing the relative environmental benefits and tradeoffs of wetland restoration in agricultural landscapes.

4. Nitrous oxide and N_2 emissions from nitrification and denitrification in coastal plain wetlands under contrasting land uses

4.1 Introduction

Wetland restoration, especially in eutrophic conditions with high nitrogen (N) availability such as in agricultural watersheds, has the potential to remove significant quantities of nitrogen (N) from surface waters and soil (Zedler 2003), Ardón et al. in review), with microbial denitrification (DNF) being the major permanent N removal mechanism. However, DNF and nitrification (NF), a related microbial process in the N cycle, can produce nitrous oxide (N_2O) as a byproduct, which is a potent greenhouse gas (Forster et al. 2007). Emissions of N_2O by microbial processes represent 70% of annual N_2O fluxes to the atmosphere (Schlesinger 1997; Mosier 1998), and are primarily generated in waterlogged or periodically saturated soils, as typically found in wetland and stream ecosystems, and through agricultural practices (Conrad 1995; Panek et al. 2000). If NF and DNF convert a substantial portion of their inputs to N_2O rather than N_2, this could offset some of the local or regional water-quality benefits of DNF, by creating a global cost (Schlesinger et al. 2006; Verhoeven et al. 2006).

The biology of nitrifying and denitrifying microbes is fundamental to understanding their roles in biogeochemical cycling. Nitrifiers are dominantly chemolithoautotrophic bacteria and archaea, deriving energy for carbon (C) fixation by chemically oxidizing NH_4^+ to NO_2^- and NO_2^- to NO_3^-, with some N_2O produced as an

intermediate by-product (Paul and Clark 1996). They are held to grow slowly and to be poor competitors for NH_4^+ with respect to heterotrophic microbes (Megonigal et al. 2004). Denitrifying bacteria are mostly heterotrophic facultative aerobes that use NO_3^- as a terminal electron acceptor in the absence of O_2. The complete reduction of NO_3^- to N_2 occurs under anoxic conditions with low substrate availability (low NO_3^- and DOC), while N_2O is released as an intermediate precursor to N_2 (Knowles 1982).

Measuring DNF rates and products can be difficult: DNF is highly variable spatially and temporally, and fluxes of its major product, N_2, are undetectable under most circumstances against the atmospheric background of 78% N_2, and the proportion of N_2O and N_2 is highly variable as well (Seitzinger et al. 2006; Groffman et al. 2009). Measuring N_2O is relatively easier, but attributing its production to NF or DNF is not straightforward, because the multiple conditions that control NF and DNF rates and products are not clearly separate (Arah 1997; Stevens et al. 1997). While NF and DNF are influenced by soil pH and the availability of C and N, the primary determinant of their rates and end-products is held to be the availability of oxygen (O_2), or related parameters affecting O_2 such as soil moisture or drainage status (Weier et al. 1993; Davidson et al. 2000; De Boer and Kowalchuk 2001; Simek and Cooper 2002).

Field- and ecosystem-scale factors, including vegetation, climate, and land use history, also can be important in regulating NF and DNF and their gaseous products (Matson and Vitousek 1990; Groffman 1991; Bergsma et al. 2002). Literature values

suggest N_2O:N_2 ratios ranging from 1% in streams to 40% in fertilized and irrigated farm fields, although many denitrification models assume a 1% ratio (Mulholland et al. 2004; Panek et al. 2000). Schlesinger (2009) recently published a summary of N_2O mole fractions [$N_2O/(N_2O+N_2)$] from DNF, identifying mean values (± standard error, SE) for agricultural soils (0.375±0.035), soils with natural or recovering vegetation (0.492±0.066), and freshwater wetlands/flooded soils (0.082±0.024). Because restored wetlands in agricultural catchments share attributes of agricultural soils and have recovering vegetation, the mole fraction of N_2O from NF and DNF in such systems could potentially be higher than the average freshwater wetland. Furthermore, while the review paper suggests that wetlands have relatively low contributions of N_2O compared to total DNF, this estimate does not include potential additional contributions of N_2O from NF, which should increase with increasing soil moisture (Schlesinger 2009).

In this study, we sought to determine 1) rates and patterns of NF and DNF; 2) their relative contributions to N_2O emission rates; and 3) the mole fraction of N_2O produced by NF and DNF, across three land use types and under differing soil moisture conditions. In a ^{15}N stable isotope tracer experiment, we determined the mole fraction of N_2O in intact cores, under both wet and dry conditions in a restored wetland, an adjacent active agricultural field, and a nearby forested wetland in the Coastal Plain of North Carolina.

We expected that soil moisture would be a first-order control on NF and DNF: drier conditions would have low N_2O emissions from NF, increasing with increasing soil moisture and reaching a maximum at 70-80% water-filled pore space (WFPS), above which DNF would tend to go to completion, with negligible N_2O production (Davidson et al. 2000). Where soil NO_3^- concentrations are high, DNF-derived N_2O production would be relatively stronger; with high available C, DNF would tend to produce N_2. Increasing C availability may increase N mineralization rates, leading to higher availability of NH_4^+ and higher NF and DNF rates (Schlesinger 1997). Based on published pH effects on NF and DNF rates and end-products, we expected that more acidic soils would have higher N_2O emissions.

4.2 Methods

4.2.1 Field sampling

For this experiment, we focused on four sites within the three land use types that were described in detail in Chapter 1: an active agricultural field (Ag), a forested wetland (FW1 in Chapters1 and 2; hereafter FW), and two soil types within a restored wetland [surface mineral soil: Hyde loam (R-min); surface organic soil: Ponzer muck (R-org)].

We collected intact soil cores (in 30cm aluminum sleeves; 20cm soil with 10cm headspace; 5cm diameter) using a soil sampler with slide hammer (AMS Samplers, American Falls, ID). For the ^{15}N tracer experiment, we collected soil cores for three

replicates at each of two moisture levels and two N treatments ($^{15}NO_3^-$, $^{15}NH_4^+$) at each site, and 6 additional cores per site were collected for physico-chemical analyses and biological assays. We determined bulk density (BD), %C, %N, KCl-extractable NH_4 and NO_3, soil pH, net nitrification, denitrification enzyme activity (DEA), and bioavailable dissolved organic C (BDOC) following methods detailed in Chapters 1 and 2. To estimate percent water-filled pore space, volumetric water content (derived from BD and gravimetric water content) was divided by total porosity (Linn and Doran 1984). To estimate total porosity, soil particle density of 2.65g $\cdot cm^{-3}$ is conventionally assumed for mineral soils; since this did not apply for organic soils, we adjusted soil particle density for soil organic content based on soil %C and a regression method developed by (Rühlmann et al. 2006). Assays for net NF and BDOC proved uninformative; results are not presented.

4.2.2 Laboratory experiment:

We prepared tracer solutions of 99 atom% $K^{15}NO_3$ and 98 atom% $(^{15}NH_4)_2SO_4$ to reach a target N enrichment of 10% of soil inorganic N (DIN) pools. Two moisture treatments were applied to examine the effects of soil moisture on NF and DNF: we stretched the range of field moisture by draining the cores for 48h (D) and simulating a 1 cm rain event (simulated rain: SR). Half of the cores (SR) received 20mL of dilute tracer solution and the other half (D) was allowed to drain and evaporate for 48h before adding 2mL of concentrated ^{15}N tracer solution, with both moisture treatments receiving

the same quantity of ^{15}N. Because the Ag and R-min soils contained twice as much DIN as the R-org and FW soils by mass, they received twice the mass of ^{15}N tracer but the same volume of water according to moisture treatment. Solutions were injected through the core length using a modified copper tube and syringe. Cores were sealed with Teflon tape and plastic caps equipped with brass compression fittings and septa for gas sampling; the assembly had proved to be gas-tight during a pilot study. Soil cores were maintained upright in a dark growth chamber at 20ºC for 48h after tracer additions.

Pre-evacuated (<30mTorr; (Hamilton and Ostrom 2007) 12mL glass Exetainers (Labco, UK) were used to collect headspace gas samples after 48h of incubation for $^{15}N_2O$ and $^{15}N_2$ analysis by IRMS at UC Davis Stable Isotope Laboratory (Davis, CA). Vials were filled with 13mL of headspace and immediately placed upside down in 50mL centrifuge tubes filled with He-purged DI water, to limit N_2 exchange with the atmosphere. Samples were shipped by ground service and analyzed within 7 days of collection. Following the incubations, duplicate 10g subsamples of incubated soils were shaken with 2M KCl to extract DIN. To determine $^{15}NH_4$ and $^{15}NO_3$ in each soil sample, the ^{15}N diffusion method (Brooks et al. 1989; Herman et al. 1995) was applied to trap DIN on acidified filter disks for analysis of ^{15}N by IRMS at UC Davis Stable Isotope Lab.

4.2.3 Calculations and data analysis

To calculate the flux of N_2O and N_2 from the ^{15}N-labeled soil, we applied the "non-equilibrium" equations developed by Stevens et al. (1997) and elaborated by

(Bergsma et al. 1999; Bergsma et al. 2001). From the volume and concentration of ^{15}N tracer solution added to each soil core, we calculated the percent of ^{15}N recovered in soil as $^{15}NH_4$-N and NO_3-N and in the headspace as $^{15}N_2O$ and $^{15}N_2$. We used analysis of variance (R statistical software, package `stats`) to determine site differences for soil characteristics (with Tukey's HSD for post-hoc comparisons), and to determine the effects and interactions of site, moisture treatment, and ^{15}N source on the mole fraction of $^{15}N_2O$. We used simple linear regression (SigmaPlot 11) to quantify the effect of soil moisture on N-gases derived from $^{15}NH_4$ vs. $^{15}NO_3$.

4.3 Results

We found differences between sites in soil characteristics that could influence NF and DNF rates and end-products, with differences primarily between the two mineral surface soils compared to the two organic surface soils (Table 8). Bulk density and NO_3-N availability were higher in Ag and R-min soils, while soil %C, %N, and NH_4-N were 3-10x higher in the organic soils R-org and FW. Differences between means of soil pH between sites were determined on H^+ ion concentrations; only FW was found to be significantly lower in pH (3.13) compared to the other sites (3.75-4.86; Table 8). Denitrification enzyme activity did not differ significantly between sites (Table 8).

To assess NF and DNF during the 48h incubations, we examined the recovery of added $^{15}NO_3$ and $^{15}NH_4$ in the various soil and gaseous pools (Figure 9). We found

Table 8: Soil physical and chemical properties by site for ^{15}N experiment
Mean values ± SE are shown, except for soil pH for which the range of values is given. Different letters indicate significant differences at $p < 0.1$

Site	BD ($g \cdot cm^{-3}$)	%C	%N	C:N	NO_3 ($mg\ N \cdot m^{-3}$)	NH_4 ($mg\ N \cdot m^{-3}$)	pH (range)	DEA ($mg\ N \cdot m^{-3} \cdot h^{-1}$)
Ag	1.24 ± 0.12^{a}	3.72 ± 0.15^{c}	0.19 ± 0.01^{c}	19.5 ± 0.3^{b}	167 ± 10.6^{a}	10.3 ± 1.73^{b}	4.86 (4.55-5.18)^{a}	1.68 ± 0.57^{a}
R-min	1.02 ± 0.05^{ab}	4.97 ± 0.09^{c}	0.25 ± 0.01^{c}	19.8 ± 0.1^{b}	171 ± 34.9^{a}	17.5 ± 0.88^{b}	4.18 (3.97-4.43)^{a}	2.02 ± 0.60^{a}
R-org	0.89 ± 0.01^{b}	13.2 ± 1.7^{b}	0.52 ± 0.05^{b}	24.9 ± 1.1^{a}	77.8 ± 26.6^{b}	22.4 ± 2.8^{ab}	3.76 (3.59-3.95)^{a}	0.84 ± 0.13^{a}
FW	0.27 ± 0.03^{c}	34.8 ± 1.6^{a}	1.79 ± 0.10^{a}	19.5 ± 0.3^{b}	77.3 ± 19.0^{b}	31.9 ± 3.6^{a}	3.13 (3.07-3.17)^{b}	2.47 ± 0.55^{a}

greater recovery of $^{15}NO_3$ in saturated vs. drained treatments in all sites except FW, and the highest production of $^{15}NH_4$ was about 1% of $^{15}NO_3$ in FW, but less than 0.5% in other sites (Figure 9A). The drained mineral soils had less than 0.5% total N-gas production, increasing to 1% in Ag and 2% in R-min under SR conditions (Figure 9B). The organic soils had more $^{15}NO_3$ tracer recovered as $^{15}N_2$ than as $^{15}N_2O$, with 1-2% N_2 in the drained treatment, while 21% and 68% of the tracer were recovered as $^{15}N_2$ in the SR treatment in R-org and FW, respectively (Figure 9B). Recovery of $^{15}N_2O$ was less than 0.5% N_2O in both organic drained soils, increasing to 2% with SR in FW (Figure 9B). The $^{15}N_2O$ mole fraction of added $^{15}NO_3$ increased in all sites with higher soil moisture, from less than 0.05 to above 0.1 in R-min and 0.35 in Ag, except in FW where the mole fraction decreased from 0.12 to 0.02 (Figure 9C).

Following $^{15}NH_4$ enrichment, all sites and moisture treatments showed evidence of NF as seen by the recovery of $^{15}NO_3$ (Figure 9D). In the mineral soils, we found no change in $^{15}NH_4$ recovery but greater $^{15}NO_3$ production under SR vs. drained conditions

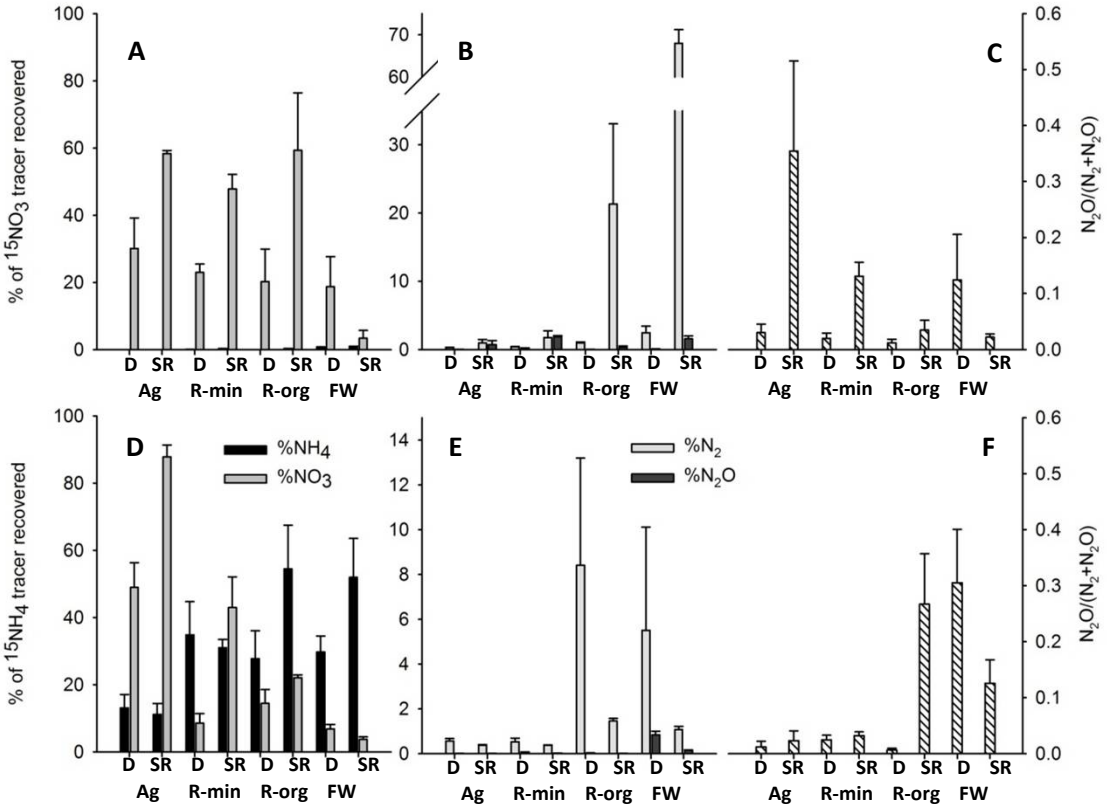


Figure 9: Recovery of ^{15}N tracers (mean ± SE), in extractable and gaseous forms by site and moisture treatment (D=drained and SR=simulated rain). A) %$^{15}NO_3$ recovered in soil extracts; B) %$^{15}NO_3$ recovered in $^{15}N_2$ and $^{15}N_2O$; C) mole fraction of N_2O from $^{15}NO_3$; D) %$^{15}NH_4$ recovered in soil extracts, E) %$^{15}NH_4$ recovered in $^{15}N_2$ and $^{15}N_2O$; F) mole fraction of N_2O from $^{15}NH_4$.

(Figure 9D). In the organic soils, a greater proportion of the added $^{15}NH_4$ was recovered under SR (52-54%) than in drained soils (28-30%). Patterns of $^{15}NO_3$ production were different among the two organic soils, increasing in R-org and decreasing in FW with increasing soil moisture (Figure 9D). N-gas production from $^{15}NH_4$ was dominated by $^{15}N_2$ across all sites and moisture treatments, but was especially low in the mineral soils, representing less than 1% of added $^{15}NH_4$ (Figure 9E). In both organic soils, both $^{15}N_2$

Table 9: Analysis of variance summary for $N_2O/(N_2O+N_2)$ by site, rain, and N source

Source	df	SS	MS	F value	P
Site	3	0.0552	0.0184	1.84	0.160
Moisture	1	0.0390	0.0390	3.90	0.0567*
N source	1	0.00087	0.0087	0.0866	0.770
site : moisture	3	0.176	0.0586	5.86	0.00261*
site : N source	3	0.197	0.0657	6.57	0.00138*
moisture : N source	1	0.0123	0.0123	1.23	0.275
site : moisture : N source	3	0.116	0.0386	3.86	0.0182*
Residuals	32	0.320	0.0100		

Sources marked (*) are significant at $p < 0.1$

and $^{15}N_2O$ production in drained treatments was greater than in SR treatments (Figure 9E). The mole fraction of $^{15}N_2O$ was below 0.05 in both mineral soils across moisture treatments; in R-org, the $^{15}N_2O$ mole fraction was low in drained conditions and increased to 0.28 with the SR treatment, while the opposite response to soil moisture was seen in FW, with higher $^{15}N_2O$ mole fractions in drained versus SR conditions (0.3 vs. 0.12; Figure 9F).

The experiment was designed as a three-way analysis of variance, with results of the analysis of $^{15}N_2O$ mole fractions summarized in Table 9. As the description of Figures 9C and 9F implied, there were significant interactions between the three factors (site x moisture treatment x N source; p=0.018) and significant two-way interactions between site and moisture (p=0.0026) and between site and N source (p=0.0014; Table 9). Only the moisture treatment as a single factor had a significant effect on the $^{15}N_2O$ mole fraction (p=0.057); the effects of N source and site alone were not significant (Table 9).

With this experiment, we wished to quantify the role of soil moisture (as %WFPS) in regulating N_2O and N_2 production across all sites. Figure 10Aand 10C show that the fluxes of both N_2 and N_2O from the $^{15}NO_3$ tracer increased with increasing WFPS within each site, with N_2 dominating N-gas fluxes across the range of WFPS. The effect of the moisture treatments (as shown by the lines for each site) differed by site (see also

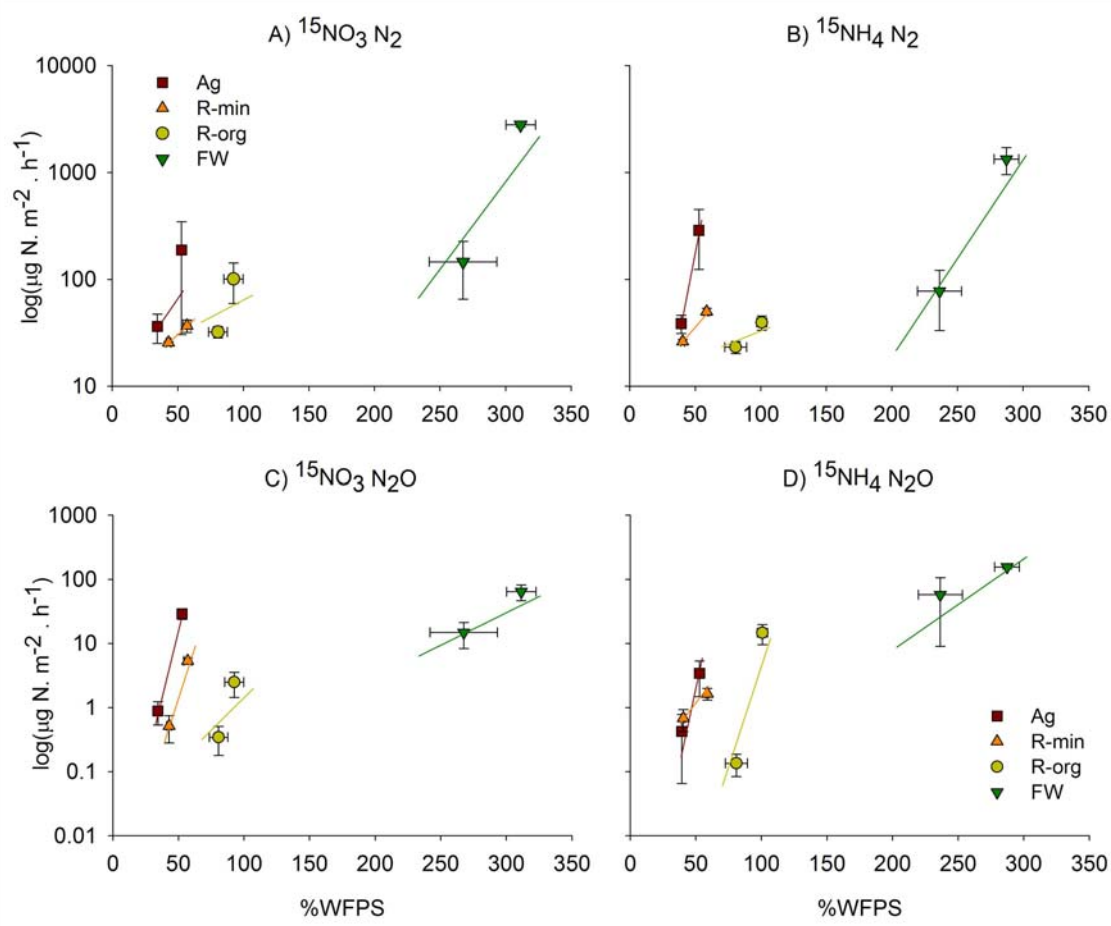


Figure 10: $^{15}N_2$ and $^{15}N_2O$ by site as a function of water-filled pore space. Top panels: log N_2 from A) $^{15}NO_3$ and B) $^{15}NH_4$; bottom panels: log N_2O from C) $^{15}NO_3$ and D) $^{15}NH_4$. Each point is the mean of 3 cores per treatment (drained vs. simulated rain), with error bars (SE) for N-gas and WFPS.

Table 8). Fluxes of N_2 from the $^{15}NH_4$ tracer increased similarly to N_2 in the $^{15}NO_3$ treatment (Figure 10B). For the mineral soils and RW-org, we can see that differences in N_2 and N_2O fluxes within sites between the two moisture treatments were greater than differences between sites in the same moisture treatment, with higher N_2 and N_2O fluxes under SR conditions; for FW, fluxes of N_2 and N_2O were generally higher than other sites under either drained or SR conditions (Figures 10A-D).

When directly comparing N-gas fluxes from $^{15}NO_3$ and from $^{15}NH_4$, we see that about 1.5 times as much N_2 is produced from the $^{15}NH_4$ than $^{15}NO_3$ under SR conditions in the mineral soils, while about twice as much N_2 is produced from the $^{15}NO_3$ tracer than from $^{15}NH_4$ under both drained and SR conditions in the organic soils (Figure 11A). The differences between the tracers were much more pronounced for N_2O: $^{15}NH_4$-derived N_2O was 2.5-6 times greater than $^{15}NO_3$-derived N_2O in the wetter organic soils

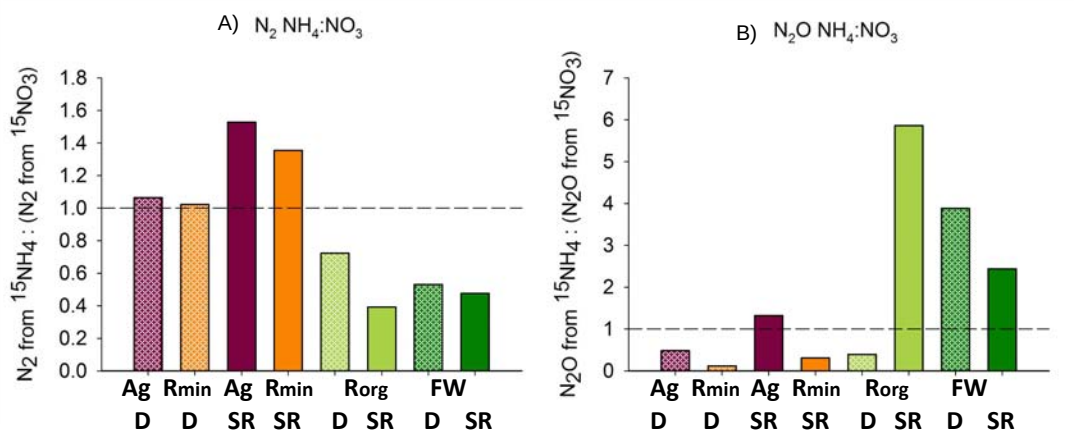


Figure 11: Ratio of N-gases from $^{15}NH_4$ versus $^{15}NO_3$ by site and treatment A) N_2 from $^{15}NH_4$: N_2 from $^{15}NO_3$ and B) N_2O from $^{15}NH_4$: N_2 from $^{15}NO_3$, with sites and treatments ordered from driest to wettest.

and about 1.4 times greater in the SR treatment in the Ag soils (Figure 11B). In the drained mineral soils, the SR treatment in the R-min soils, and the drained treatment in the R-org soils, $^{15}NO_3$-derived N_2O was more than twice as high as N_2O flux from $^{15}NH_4$ (Figure 11B).

4.4 Discussion

This experiment allowed us to determine the relative production of the denitrification (DNF) end-products by following $^{15}NO_3$ through to $^{15}N_2$ and $^{15}N_2O$ in different soils under two moisture treatments. As expected, DNF-derived N_2 and N_2O increased with increasing soil moisture (expressed as % water-filled pore space; WFPS) within each site, because DNF is an anaerobic process that is favored when O_2 concentrations decrease under more saturated conditions. Based on extensive evidence from the literature, we expected the mole fraction of N_2O [$N_2O/(N_2+ N_2O)$] to decrease, with DNF going to completion under saturating conditions (Firestone et al. 1979; Davidson et al. 2000). However, we found that the N_2O mole fraction from DNF increased in the simulated rain (SR) treatment in the mineral soils (Ag and R-min) and in the restored wetland organic soils (R-org; Figure 9C). The N_2O mole fraction from DNF decreased with increasing soil moisture only in the reference forested wetland site (FW; Figure 9C). This increased production of N_2O from DNF with increasing soil moisture was unexpected; one explanation might be that a greater volume of the drier, more mineral soils was rendered anoxic enough to undergo DNF, but not anoxic enough

throughout the soil volume to undergo complete DNF to N_2, as likely occurred in FW soils in the SR treatment.

Through this experimental approach, we also were able to examine the products of coupled nitrification-denitrification (NF-DNF) by adding $^{15}NH_4$ to soils. By comparing trends in gaseous products from $^{15}NH_4$ additions to results from $^{15}NO_3$ additions, we could infer the contribution of NF to N_2O production. We found that coupled NF-DNF was clearly important in these soils, occurring across the range of soil moisture in the experiment (Figure 10B and 10D). Dinitrogen derived from NF-DNF increased with increasing WFPS, within each site as expected, with similar yields compared to $^{15}NO_3$-derived N_2, suggesting an efficient connection between NF and DNF in these soils (Figure 11A). It is important to note that although NF is an aerobic process and DNF is primarily anaerobic, these two processes can coexist in the bulk soil, which includes both oxic and anoxic microsites even under saturated conditions.

Despite the apparent tight connection between NF and DNF products, we found that $^{15}NH_4$-derived N_2O departed from expectations based on $^{15}NO_3$-derived N_2O (Figure 11B). We found that in mineral soils under both drained and SR conditions, and in R-org under drained conditions, N_2O produced from $^{15}NH_4$ was equal to or lower than N_2O produced from DNF, meaning that less N_2O was produced by NF-DNF than by DNF alone. One explanation for this result could be that demand for NH_4 by soil microbes in these soils was higher than their demand for NO_3^-, leaving relatively more $15NO_3$ to be

denitrified than $^{15}NH_4$ available to be nitrified. The high mole fraction of N_2O from DNF in the Ag soils contributes to our understanding of high N_2O fluxes measured during two years of field study in that site (Chapter 2).

In the wetter organic soils, (R-org soils under SR conditions and in FW in both treatments), there appears to be more N_2O produced from coupled NF-DNF than from DNF alone (Figure 11B), suggesting N_2O production from NF in addition to what was produced from DNF. This large contribution of NF to N_2O declined with increasing saturation, from a ratio of about 6 in R-org SR, down to 2.5 in FW-SR. This could be consistent with theory—that N_2O from NF increases with increasing WFPS (Davidson et al. 2000), while DNF could be going further toward completion as well, converting N_2O to N_2, as seen in Figure 11B.

Soils with the highest calculated WFPS (R-org and FW) had the highest NH_4 availability and lowest pH, two factors which have also been found to be correlated with high N_2O mole fractions (Weier and Gilliam 1986; Weier and Gilliam 1986; De Boer and Kowalchuk 2001). In our other work, we have found a correlation between NH_4 availability and field N_2O fluxes (Chapter 3), a strong influence of pH on DNF potential (Chapter 3), and higher N_2O fluxes from the FW site over two years of study (see Chapter 2). In these acidic organic soils high in NH_4^+, NF is more sensitive than DNF in response to hydrologic variation, and is probably a more important driver of N_2O flux than DNF.

This study shows that land use history is an important control of N cycling in these sites which were all once acidic organic wetlands. Through clearing of forests, drainage, fertilization, liming, tillage, and oxidation of soil organic matter, the now-mineral surface soils in the more agriculturally influenced sites (Ag and R-min) have different N cycling patterns compared to the still-organic surface soils in R-org and FW. These land use practices altered the physical and chemical conditions in the soil, likely leading to distinct microbial communities in each site, as has been found elsewhere (Hartman et al. 2008). The acid organic soils clearly support communities of acid-tolerant nitrifiers at pH ranges that have been shown to inhibit NF in the laboratory.

Since most models of N_2O production focus on DNF as the primary mechanism for its production, having been developed especially for in circum-neutral soils and for agricultural applications (e.g., Del Grosso et al. 2005; Groffman et al. 2009), they might accurately predict the higher N_2O mole fraction of Ag soils at the intermediate WFPS we achieved in our experiment. Yet in the acidic, high-NH_4 organic soils, the N_2O flux is driven also by NF, with N_2O mole fractions around 0.28-0.30, putting them closer to agricultural soils (0.38) and upland soils (0.49) than to freshwater wetlands (0.08), even under saturated conditions. As wetlands are increasingly being restored to promote water quality, the role of pH and hydrology in controlling N_2O fluxes needs to be better incorporated into our understanding.

5. Conclusions

In our two-year monitoring study of greenhouse gas emissions from four coastal plain wetland ecosystems, we found that while CO_2 was the dominant GHG across the land use types in our study, in soils with high N availability and lower water tables (Ag site and FW1), N_2O was a larger contributor to the radiative balance than CH_4. Methane was a larger source in sites that were consistently flooded (RW-wet and FW2). We did not find that the restored wetland was a significantly higher source of GHG compared to agricultural soils or natural wetland.

For a subset of our data, we examined the influence of soil properties, soil chemistry, soil microbial processes, hydrology, and other environmental variables on N_2O fluxes and denitrification (DNF) potential. We found that contemporaneously measured environmental variables (CO_2 flux and soil temperature) along with NH_4 availability and DNF potential together were strong predictors of N_2O fluxes in November 2007 (r^2=0.85). The factors that best explained the variability in DNF potential were pH and either CH_4 flux or %N in soil. The predictive ability of the best model for N_2O flux was not very good when applied to a dataset from July 2007; suggesting non-linear temperature effects, or a the contribution of plant during the growing season. The model for DNF potential performed better when applied to soil data from June 2007, suggesting that DNF potential results are more stable over time and less responsive to changing environmental conditions.

We conducted a stable isotope tracer experiment using ^{15}N to determine whether nitrification (NF) or DNF was the main source of N_2O in four soil types, and whether the mole fraction of N_2O was sensitive to alterations in soil moisture. We found that, within each soil type, N_2O mole fraction from DNF changed with increasing moisture: in the two mineral soils (Ag and R-min), the N_2O mole fraction increased, while in the organic soils, it increased in R-org and decreased in FW1 with increasing moisture. We found higher production of N_2O from NF-DNF than from DNF in the wetter organic soils, which suggests that NF can still be an important contributor to N_2O fluxes under saturated conditions.

These patterns of higher N_2O mole fraction from NF and DNF were consistent with results in the previous two chapters, and suggest that the large measured N_2O fluxes in the Ag site come from DNF, while NF is an additional contributor to high N_2O fluxes in the acid organic soils of FW. The importance of NF-derived N_2O in acidic wetland environments needs to be incorporated into models of N cycling and should be considered in wetland restoration practices.

Overall, we found that both nitrification and denitrification contribute to nitrous oxide fluxes in coastal plain wetlands in North Carolina, and that nitrification is an especially important source in acid-organic soils under both field-moist and saturated conditions. Although freshwater wetlands, with an average N_2O mole fraction of 0.08, are generally seen as being insignificant sources of N_2O, our study sites ranged from 0.10

to 0.30, placing them closer to agricultural fields (0.38; Schlesinger 2009). Although the ecosystems in our study produced more N_2O than expected for freshwater wetlands, we found no significant tradeoff between the local water quality benefits conferred by denitrification and the global greenhouse gas costs in the restored wetland. These results suggest that, from a N perspective, wetland restoration in coastal agricultural lands has a net environmental benefit.

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Biography

Jennifer L. Morse was born in March 1975 in Varese, Italy. She lived in Varese and Reggio Emilia, Italy, and Antibes, France, before moving to Virginia in 1986. She attended Thomas Jefferson High School for Science and Technology, graduating in 1993, before attending the College of William and Mary in Virginia. Jen received a B.S. *cum laude* from the College of William and Mary in 1997 with a double major in Biology and Environmental Science.

In 2002, Jen completed a M.S. degree in Biology with a concentration in Environmental Science and Policy at George Mason University. Her thesis was titled, "Sediment deposition and nutrient availability in two tidal freshwater marshes on the Mattaponi River, Virginia," and was conducted under the guidance of Dr. Mark R. Walbridge and Dr. J. Patrick Megonigal. After her M.S. studies, Jen continued to work in ecology research, as a lab technician and manager in Dr. Margaret Palmer's stream ecology lab at the University of Maryland, College Park.

Jen arrived at Duke University in 2004 to pursue a Ph.D. in Ecology with Dr. Emily Bernhardt and Dr. Curt Richardson. To support her graduate research, she has won several fellowships and grants, including a three-year U.S. EPA STAR Graduate Fellowship (2005), a research grant from the Lindbergh Foundation (2008), and the Katherine Goodman Stern Dissertation Fellowship (2009). She was chosen as an Oosting Memorial Graduate Student Speaker at Duke University and as a participant in the

Preparing Future Faculty program in 2008. Along with Sean Berthrong and Elizabeth Sudduth, Jen developed and taught a course in Ecosystem Restoration as adjunct faculty in the Environmental Science Department at Elon University in January 2009.

Jen is a member in several professional societies, including the Ecological Society of America, the Society of Wetland Scientists, the North American Benthological Society, and the American Geophysical Union. She has been especially active in the Biogeosciences section of the Ecological Society of America, serving as the web master from 2005 to 2010. Jen has been a participant in several workshops sponsored by the National Science Foundation, including the Denitrification Research Coordination Network workshop on modeling denitrification, which resulted in a publication.

Jen lives in Durham with Colie Hoffman, her partner of five years. They would like to be legally married in the United States someday soon.

Publications

Ardón, M., **J.L. Morse**, M.W. Doyle, and E.S. Bernhardt. The water quality consequences of restoring wetland hydrology to a large agricultural watershed in the southeastern coastal plain. (Ecosystems, in review)

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