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**WATER QUALITY IMPACTS OF NC'S LARGEST WETLANDS MITIGATION  
BANK**

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**ABSTRACT:** Wetlands provide important ecosystem services such as nutrient retention, flood abatement and habitat for biodiversity. Half of global wetland area has been lost and much of what remains is severely degraded. Wetland mitigation seeks to ameliorate this problem, by restoring former wetlands that have been drained back to their more natural ecosystem state. While the end result of wetland mitigation is clear and much research has been conducted evaluating the success of mitigation projects, there is still a lack of a clear understanding of the trajectory of restoring biogeochemical function in large-scale riverine wetlands. This project examined the trajectory of recovery of biogeochemical function of Timberlake Farm, the largest wetland mitigation project in North Carolina, as it undergoes hydrologic and biologic (re-vegetation) restoration. The main objectives of this research were: 1) to construct a water budget and monitor dissolved inputs and outputs of nitrogen and phosphorus prior to and following the re-inundation of the site in February of 2007; 2) examine the relationship between N and P cycling and key environmental driver(s) across the Timberlake hydrologic gradient; and 3) compare N and P cycling among the restored Timberlake site to an upstream agricultural field (pre-restoration state) and a reference wetland (post-restoration goal). This study found that the site is acting to remove dissolved N while being a source of dissolved P to downstream ecosystems. Hydrologic (frequency of flooding), environmental (redox), and geochemical (soil Al and Fe) characteristics of the Timberlake site as well as the current agricultural and two reference sites are important in determining denitrification potential and P sorption capacity. While converting this former agricultural field into a wetland has provided water quality benefits by removing 50-80% of the N inputs, an equal amount of N is being released to the atmosphere in the form of a potent greenhouse gas (nitrous oxide). Flooding soils that have received fertilizer also leads to release of P previously adsorbed to oxygenated sediments. Research over the first year of hydrologic restoration at Timberlake indicate that it is important to consider all costs and benefits of wetland restoration in order to better manage these ecosystems. This research has been conducted in partnership with the NC EEP and the NC State Water Quality Group. Data generated from WRRI funding provided critical preliminary data allowing the project team to secure funding from the Department of Energy, the National Science Foundation, the Lindbergh Foundation and the National Center for Airborne Laser Mapping to continue this research for at least the next three years.

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## SUMMARY AND CONCLUSIONS:

*Approach:* With the help (and additional financial support) of the NC Ecosystem Enhancement Program and the Great Dismal Swamp Mitigation Bank, LLC the Timberlake wetlands restoration project, a 400ha agricultural to wetland conversion project near Columbia, NC in February 2007 was fully instrumented with a variety of environmental sensors and dataloggers. WRRRI support was used to supplement the Timberlake work through instrumenting field sites at two reference wetland sites and an agricultural field site in May 2007. Since May 2007 research associated with this WRRRI funding has:

### *Objectives:*

I. Determined whether the Timberlake Farm restoration site is a net sink or source of nitrogen (N) and phosphorus (P) to downstream ecosystems in first year of hydrologic reconnection.

II. Examined the relationship between N and P cycling and key environmental driver(s) across the Timberlake hydrologic gradient.

III. Compared N and P cycling between the restored Timberlake site to an upstream agricultural field (pre-restoration state) and a reference wetland (post-restoration goal).

### *Findings:*

Project results thus far indicate that the site has been functioning primarily to reduce N exports (retaining 48-84% of N inputs), while serving as a source of dissolved P (releasing approximately 100 lb of P over the first year). P dynamics appear to be controlled by both hydrologic (frequency of flooding) and geochemical (concentrations of Al and Fe) characteristics of the site. High and highly variable emission rates of nitrous oxide (N<sub>2</sub>O), methane and carbon dioxide are being emitted from the restored site. Year 1 estimates suggest that 20-40% of the measured nitrogen “retention” from dissolved inputs is being released to the atmosphere as N<sub>2</sub>O, a potent greenhouse gas. The highly variable rates of denitrification suggest that more than one environmental variable is driving these transformations. Ongoing work at the site seeks to develop modeling approaches to determine the relative contribution of environmental drivers on denitrification and emissions of carbon dioxide and methane. Patterns observed across the different sites spanning a land use gradient (from active farming to pristine wetlands) will be used to test these models.

Two manuscripts arising from WRRRI funded work will be submitted in the fall of 2008. One manuscript will focus on the patterns and mechanisms driving P retention and loss from the Timberlake restoration site over the first year of restoration. Another manuscript will combine the water and nutrient budget to examine changes in water quality over the first year since restoration. Future publications will examine environmental drivers of denitrification and emissions of methane and carbon dioxide.

## RECOMMENDATIONS:

To date, this intensive study of NC's largest wetlands mitigation bank has found that:

- 1) The Timberlake restored wetland is acting to reduce dissolved N exports and increase dissolved P export relative to inputs.

These data suggest that it is important to consider both N and P dynamics when implementing wetland restoration projects. The majority of wetland restoration projects are focused on increasing N retention by promoting denitrification. Unfortunately, sites that are good for denitrification in former agricultural fields can also leach P due to desorption of legacy fertilizer applications.

- 2) Hydrologic factors (frequency of inundation) are important determinants of both denitrification and P-sorption (major processes for long-term storage of N and P respectively) within Timberlake and across different sites.

This result reinforces previous finding in other wetland restoration projects on the importance of restoring the hydrology of former wetland ecosystems in order to restore the function. But results from Timberlake also suggest that other factors, like availability of labile organic matter in the case of denitrification and concentrations of Al and Fe in the case of P sorption, are also important in restoring the function of these ecosystems. Future restoration and mitigation site selection should consider how soil characteristics and other environmental drivers (soil temperature, redox) will influence the time frame necessary to restore the full function of wetland ecosystems in former agriculture fields.

- 3) Agriculture to wetland conversion can lead to significant removal of water column nitrogen. Yet this positive finding is tempered by empirical data that suggest that a great deal of N is leaving the agricultural system as the potent greenhouse gas N<sub>2</sub>O.

While the fluxes of N<sub>2</sub>O are highly variable in space & time across the restored wetland, initial scaled up estimate suggests that N<sub>2</sub>O release in the first year of restoration is equivalent to 333 ± 100 tons of CO<sub>2</sub>. This amount of carbon release is sufficient to significantly offset the C sequestration potential of the site from the incorporation of soil organic matter through the site.

- 4) It is important to consider all possible tradeoffs (both local and global) when designing and implementing wetland restoration efforts in order maximize the function of these ecosystems.
- 5) Understanding patterns and processes in wetland restoration projects is expensive and time consuming. Collaborations between academic, government, and private investor groups provide unique opportunities to provide much needed information to maximize money spent on restoration efforts.

# Water quality impacts of NC's largest wetlands mitigation bank

## 1. Introduction

### 1.1 Background

#### Statement of Critical Water Resources Problem:

**High levels of nutrients and sediment are loaded to coastal ecosystems:** Globally, nutrient and sediment loading of terrestrial, freshwater and coastal ecosystems is occurring as a result of human waste disposal and agriculture. Humans have roughly doubled the annual supply of reactive nitrogen (N), while phosphorus (P) has increased by approximately 75% of pre-industrial levels (Vitousek et al. 1997, Bennett et al. 2001). Increased nutrient loading to coastal waters and estuaries leads to harmful algal blooms and regional hypoxia (Turner and Rabalais 1994, Green et al. 2004). Because natural wetlands have a high and long-term capacity to retain nitrogen and phosphorus from surface and subsurface runoff (Johnston 1991, Zedler 2003), it has been suggested that wetlands can serve a major role in ameliorating nutrient loading downstream (Verhoeven et al. 2006). Wetlands have the ability to retain N through microbial uptake and by transforming reactive nitrogen into inert gaseous forms (N<sub>2</sub>) through denitrification (Weller et al. 1994). Phosphorus retention in wetlands occurs mostly through sedimentation, mineral adsorption and plant uptake (Richardson 1985). However, much of this retention services have been lost because half of global wetland area has been lost and much of the remaining wetlands have been degraded (Zedler and Kercher 2005).

**Mitigation seeks to reverse these degradations, but are there hidden costs?** Concern over the loss of ecosystem services provided by wetlands has resulted in the initiation of major investments in wetland and river restoration throughout the US. Wetland and river restoration has emerged as a lucrative industry, with NC being in the vanguard of compensatory mitigation based on restoration practices. While more than one third of all river, and most wetland restoration projects are implemented to “manage and improve water quality”, most are rarely evaluated to determine if this goal is achieved (Zedler 2000, Bernhardt et al. 2005). The restoration of former agricultural areas, with high nutrient content in surface soils as a legacy of fertilizer use, presents the potential for both water quality improvement and degradation, even as new habitat for wildlife is created. There is evidence to suggest that flooding conditions could enhance N removal from surface waters while increasing P loading to surface waters if P is desorbed from soil minerals (Van Dijk et al. 2004). This research examined controls on N and P retention within Timberlake farm, the largest restoration project in North Carolina, as it undergoes hydrologic and biological (~revegetation) restoration. Understanding these dynamics at the patch level and landscape scale is critical to developing a predictive framework for water quality implications of riverine wetland restoration in former agricultural lands.

## **Related Research and Justification:**

*Preliminary research: Sediment N and P:* Past heavy fertilizer use during agricultural practices created high N and P in the sediments of Timberlake (Fig. 1). Nitrogen is highly variable through out the Timberlake Farm. Despite this, concentrations of  $\text{NO}_3$  measured recently in drainage waters is quite low ( $9.9 \pm 8.8 \mu\text{g NO}_3\text{-N L}^{-1}$ ) suggesting high rates of denitrification through out the site. Total dissolved nitrogen concentrations are very high ( $1.39 \pm 0.49 \text{ mg N L}^{-1}$ ). Phosphorus is higher in the higher elevation sites and lower in near the stream channel, suggesting that P might be lost through desorption processes. Concentrations of P are high across surface waters on the site ( $23.5 \pm 17.8 \mu\text{g PO}_4\text{-P L}^{-1}$ ).

*Ecosystem services provided by wetlands:* Wetlands contribute up to 40% of the earth's renewable ecosystem services, even though they cover only 15% of the earth's surface (Costanza et al. 1997). It is still difficult to predict how much of a wetland must be restored to regain various ecosystem services, or how much each ecosystem service would increase after drained fields are restored (Zedler and Kercher 2005). It is also difficult to determine how to maximize various ecosystem services simultaneously. Biodiversity tends to be higher where nutrient supply is low. However, maximum nutrient removal requires abundant nutrient supply (eutrophic conditions), which usually leads to dominance by single plant species (Zedler 2000). For example, if the main goal of the Timberlake Restoration project was to serve as a nutrient sink, cutting the hydrologic connections to downstream would maximize nutrient retention. But this would inhibit the potential of the site to provide habitat for migrating fish. There is the possibility that restoring the hydrologic connections of the site will provide nursing habitat for river herring.

*The Timberlake Restoration project will double the total acres of restored wetlands in NC in a single site.* As of 2003, the NC EEP reported 945.4 acres of wetlands that are in the process of being restored, enhanced or preserved (EEP 2005). While it is clear that there are economies of scale associated with the size of wetland restoration projects (i.e. larger projects have lower cost per acre, (EEP 2005), it is unclear what the ecosystem benefits are from large scale restoration projects. Comparing the restoration trajectory of this

large-scale project to smaller scale projects in North Carolina will provide important insights into the biogeochemical benefits or costs of large-scale restoration. Ultimately, this research will provide insights into a scientific basis to help make decisions regarding whether it is more efficient to restore a smaller number of large-scale sites versus a larger number of smaller-scale sites. Locally, there are significant questions about how ecosystem function in wetlands varies with location relative to streams and rivers. Currently the EEP, makes a distinction for compensatory mitigation between “wetlands” and “riverine wetlands,” and in fact this distinction has substantial financial implications: restored riverine wetlands are worth approximately twice that of non-riverine wetlands. However, the process basis for this distinction is poorly defined, and the EEP lacks the science to quantify the additional benefits provided by riverine wetlands. This research, having strong spatial, temporal, and hydrological components, will allow managers to understand how proximity to fluctuating rivers and streams impacts biogeochemical function, and thus potentially help the EEP more precisely define the distinction between riverine and non-riverine wetlands.

*Possible costs associated with large-scale restoration:* The creation of a large wetland ecosystem with anoxic sediments on formerly cultivated (and heavily fertilized) lands may have unforeseen consequences for trace gas emissions of methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) and release of bound phosphorus from sediments to downstream ecosystems. Jennifer Morse, a Ph.D. student at Duke University, is currently investigating this complementary aspect of the restoration project as the focus of her dissertation. Numerous studies have shown that wetting of former agricultural fields associated with wetland restoration can lead to the release of bound phosphorus (D'Angelo and Reddy 1994, Phillips 2001, Pant et al. 2002, Aldous et al. 2005). The flooding regime imposed on restoration projects will influence many physical and biotic properties that determine P mobility and availability. Soil pH and redox potential will affect the forms of metals that will bind or release P (Richardson 1985, Schlesinger 1997), and biologically mediated processes such as organic matter decomposition which might release P (Koerselman and Meuleman 1996).

## **1.2 STUDY OBJECTIVES**

### **2. STUDY DESIGN**

Once N and P enter the restored site through surface water or precipitation they can either be exported, stored long-term in biomass or soil organic matter (SOM), or, in the case of N, lost as N<sub>2</sub>O and N<sub>2</sub> through denitrification. Restoration of wetland and stream topography leads to increasing water residence time, and thus, it increases the opportunities for nutrient assimilation and transformation within the site. Ultimately, transforming a former agricultural field into a large, shallow, riverine wetland should shift the site from a net source to a net sink of N and P. While the direction of change is apparent, the rate of change and the magnitude of the impact are difficult to predict. Research at the site attempts to characterize the rate and magnitude of this change, with the ultimate goal being to understand how to accelerate the recovery of biogeochemical function in future restored coastal plain wetlands, as well as to constrain the realistic potential of restoration for biogeochemical function.

Hydrology was expected to be a major driver of the fate of N and P in Timberlake soils and sediments, and will explicitly examine denitrification rates and P-sorption capacity across the hydrologic gradients present on the site. These two processes are considered the most important long-term processes of retention (for P) and removal (for N) of these two nutrients (Richardson 1985, Walbridge and Struthers 1993, Peterson et al. 2001), (Bernhardt et al. 2003). Both the rates themselves and their relationships to environmental drivers

will be compared between Timberlake and other two sites (Palmetto Peartree Preserve, Preservation Area, and upstream farm; see Research sites below). At the patch scale the availability of O<sub>2</sub> and labile C should control rates of denitrification and P sorption in Timberlake and the natural wetlands in Palmetto-Peartree and the Preservation Area. The hydrologic setting of any landscape patch within the Timberlake landscape should strongly control the supply of both O<sub>2</sub> and C. Sites that are always wet should have low O<sub>2</sub> in the sediments, accumulate organic matter and because these soils are very N rich, should have very high rates of denitrification. In these low O<sub>2</sub> settings, there will be low P-sorption and release of iron-bound P due to anoxia, and thus more soluble P available for biological assimilation and hydrologic loss. Therefore, very wet landscapes patches will be most retentive of N, but least retentive of P. Patches that are only wet by precipitation should have high O<sub>2</sub>, most N in the easily leached form (nitrate, NO<sub>3</sub>) and most P bound to aluminum and iron aggregates. Thus these dry patches will be highly susceptible to hydrologic losses of N, but should be fairly retentive of P. The vast majority of the sites will alternate between these two conditions, due to a fluctuating water table, and thus both processes determining the fate of N and P will vary seasonally depending on hydrology and biological activity.

### OBJECTIVES:

- I. Determine whether the Timberlake Farm restoration site is a net sink or source of N and P to downstream ecosystems (both annually and seasonally)
- II. Examine the relationship between N and P cycling and key environmental driver(s) across the Timberlake hydrologic gradient.
- III. Compare N and P cycling between the restored Timberlake site to an upstream agricultural field (pre-restoration state) and a reference wetland (post-restoration goal).

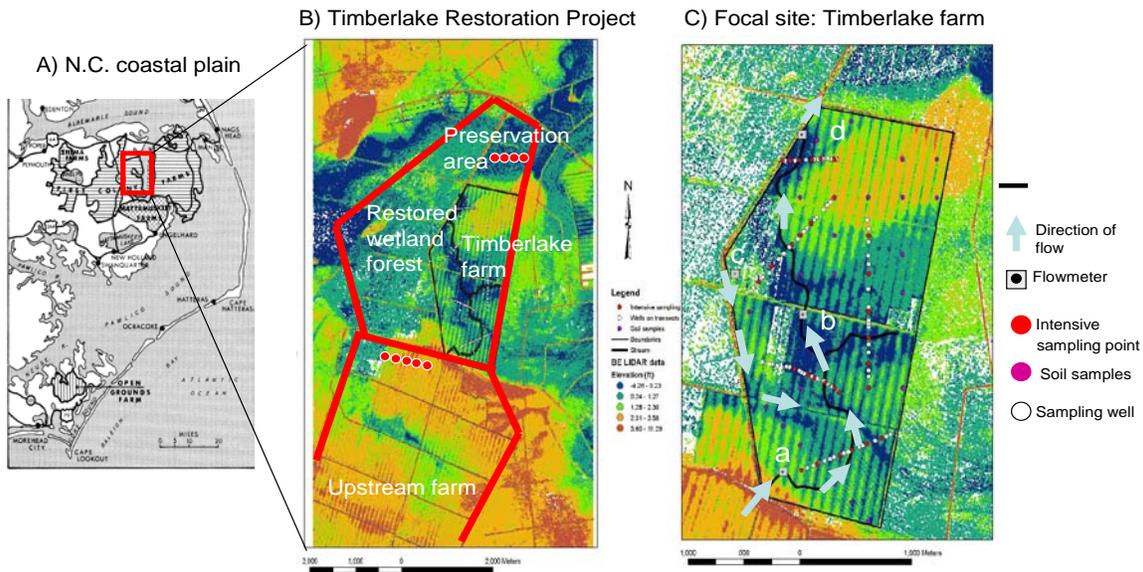
## **2.1 STUDY SITES**

*Restoration project:* Timberlake Restoration Project, Tyrrell County, NC, is a 4,000<sup>+</sup> acre compensatory mitigation site comprising former agricultural lands to be restored to a mixture of wetlands and riverine wetlands, as well as formerly logged and unimpacted swamp forests that will soon be hydrologically connected to the restored areas (Fig. 2). This land is owned by the Great Dismal Swamp Mitigation Bank, LLC (GDSMB) and it was purchased in the early 1990's by a group of private investors for the purpose of expanding their mitigation bank holdings for both their own mitigation needs as well as for generating profits through sales to other developers. Mitigation banking is now a substantial industry in environmental science and engineering, and has been suggested to be the dominant mechanism for future environmental restoration in the US.

The Timberlake Restoration Project, represents one of the largest privately owned mitigation banks on the East coast, as well as the single largest restoration project in North Carolina. Much of the forested section of the property has already been approved for wetland mitigation credits, i.e., the credits have been released by the Mitigation Bank Review Team (MBRT) but not sold. In the final phase of the project, the 1,000 acre former agricultural field (referred to as Timberlake Farm) is being converted into riverine wetland (with ditches filled, canals plugged, and 750,000 live saplings planted). Removal of a downstream gate-pump

system in February 2007 was the final step in the restoration process by reinstating the wind tide driven hydrologic regime.

Research efforts throughout 2007 were concentrated on the Timberlake Farm (Fig. 2C). Soils on the site include Hyde loam, Ponzer muck, Roper muck, Belhaven muck, Weeksville silt loam, and Pungo mucks: all of which are classified as rarely flooded, very poorly drained hydric soils (USDA SSURGO Database 2005; Tyrrell County Soil Survey, 1924). However, deforestation, drainage, and tillage associated with agriculture have caused much of the original peat and muck to be oxidized over time, such that the soils on the property may now be borderline mineral soils. The Timberlake Farm has been heavily instrumented by GDSMB in order to facilitate and encourage independent scientific research. Currently 140 groundwater wells are installed across the 1000 acre agricultural field. Forty wells are aligned along a grid for even sampling of the entire Timberlake Farm. The remaining 100 wells are installed across 5 broad transects (20 wells each) that span the full hydrologic gradient of the site. Each of these transects is centered on the lowest elevation point of the landscape, with 20 wells extending out laterally from these points to the highest elevation point in the landscape. Thus the central wells in each transect are expected to be in permanently flooded sediments, while the outermost wells on all transects are expected to have surface water only during rare events. Seventy of the 140 wells contain datalogging water level recorders, allowing us to have a highly refined model of ground and surface water heights throughout the site. Flow monitoring devices (either acoustic doppler flowmeters within canal or pressure transducers in a flume) and ISCO autosamplers have been installed at constricted flow locations on the site to continuously monitor flow onto and off the agricultural field and to provide triggered sampling during important hydrologic events (Fig. 2).



**Fig. 2.** Research site location. Panels B) and C) represent digital elevation models (LIDAR data). Red points indicate proposed location of sampling transect in upstream farm. Letter in panel C) denote locations of flowmeters and/or a flume with a pressure transducer.

*Wetland reference sites:* Pristine reference sites in the same basin are very hard to find, due to the extent of draining for agricultural activities. For comparison with the Timberlake farm, this project located two minimally impacted reference sites that share many characteristics.

*Reference 1:* Preservation Area within the Great Dismal Swamp Mitigation Bank: Five sampling points were established in the northern section of the Great Dismal Swamp Mitigation Bank. This is an area that was never put into agriculture and contains a 40+ year old stand of cedars and pines. This site had been monitored as part of the Great Dismal Swamp monitoring program, providing us with long-term data for water level.

*Reference 2:* Palmetto-Peartree Preserve is a relatively unimpacted riverine wetland site, similar to Timberlake farm, in the Palmetto Peartree Preserve, managed by the Conservation Fund. Palmetto-Peartree Preserve is a 10,000 acre endangered species mitigation bank for the red-cockaded woodpecker (RCW). Protection of RCW at the site offsets habitat lost in road construction. The preserve also provides habitat to other wildlife species, including bald eagle, peregrine falcon, and red wolf. With the assistance and advice of William Vaughn (Preserve manager) five sampling points were installed in a stand of pristine pocosin forest.

*Current agricultural site:* The agricultural upstream farm abuts the southern edge of Timberlake Farm. This project was granted permission by the landowners (the Mormon Church, Salt Lake City) and the farm manager (Ken Cherry) to sample on this property within an actively cultivated corn-soybean rotation field. In summer 2007 five sampling points were installed along an elevation gradient as above (5 wells and lysimeters; Fig. 2B). The agricultural reference site is critical to: 1) provide information on how the Timberlake Farm functioned under agricultural management (past condition); and 2) provide information on sources of fertilizer derived N and P to Timberlake (inputs). In Fall 2007 a diesel pump station was installed in the southern end of Timberlake Farm to divert water from the upstream farm during high precipitation. This means that during times of heavy rainfall, Timberlake Farm receives high inputs of water that are expected to contain high levels of inorganic N and P. Future work at the site will document the ability of the restored site to transform and retain these large pulses of inorganic nutrients.

## **2.2 SAMPLING METHODOLOGY**

### **Objective I. Mass Balance Ecosystem Budget Approach:**

Water and nutrient budgets were constructed to compare the inputs to and outputs from the former agricultural field at Timberlake Farm. Water budget: the site is hydrologically constrained with flow entering through two canals, and exiting through a single wide culvert (see Fig. 2). All three of these control points are instrumented with an acoustic Doppler flowmeter to continuously monitor water depth, velocity and direction. Based on extensive soil sampling and soil solution sampling the clay horizon below the surface soil layer is assumed to represent an effective hydrologic boundary at a depth of 30-40 cm. Thus the hydrologic system includes all surface soils and surface water above the clay lens. At all three control points, and within the center of the Timberlake Farm in a constriction point, ISCO autosamplers are installed to collect surface water. Weekly surface water samples are collected from all four locations. These data, together with continuous flow data have been used to construct a mass balance for inorganic and total dissolved N and for PO<sub>4</sub> for the entire Timberlake Farm area. In addition, high frequency samples have been collected over the course of storm hydrographs for at least one major rain event each season in order to examine hydrologic controls on nutrient concentrations.

### **Objective II. Environmental Drivers of Denitrification and P-sorption across the hydrologic gradient in Timberlake Farm.**

**a.) Soil pore water:** In 2005, 40 continuously recording groundwater wells were installed across the former agricultural fields in Timberlake Farm. In July/August of 2006 five additional sampling transects were set up along hydrologic flowpaths in the active floodplain of the restored riverine wetland with a total of 100 monitoring wells (30 with loggers for water level and temperature, a total of 70 wells with loggers throughout the site; Fig. 2). Funding from WRI supplemented existing infrastructure already at the site to create a more complete field data collection network. In association with the 30 wells with water table depth recording equipment along the 5 sampling transects (Fig. 2C), piezometers and lysimeters ( $15 \pm 5$  cm depth), redox probes ( $15 \pm 5$  cm depth), soil moisture probes and soil thermocouples were installed. These intensive sampling points span the hydrologic gradient across the entire Timberlake Farm providing a unique view of the environmental drivers of denitrification and P sorption. Funding from WRI supported the collection of bi-monthly water samples from soil lysimeters to be analyzed for  $\text{NO}_3$ ,  $\text{NH}_4$ , DON,  $\text{PO}_4$  and DOC.

**Table 1.** Hypothesized relationships within and across sites for key environmental drivers and processes measured at all intensive sampling points.

	Timberlake hydrologic gradient <b>Wet vs dry</b>	Palmetto-Peartree (wetland) vs Timberlake (TL) vs Farm <b>Among sites</b>	<b>Methods</b>
<b>Environmental drivers</b>			
Redox potential	>	PP>TL>Farm	Redox probes
Soil solution N	<	Farm>TL> PP	Lysimeters to sample
Soil solution P	<	Farm>TL> PP	Lysimeters to sample
Soil C content	>	PP>TL>Farm	CHN Analyzer
C mineralization	>	PP>TL>Farm	Flask incubations (19) Extracted from soil cores
Root biomass	<	PP>TL>Farm	
Soil temperature	<	Farm>TL> PP	Temperature loggers
Soil moisture	>	PP>TL>Farm	Soil moisture probes
Soil pH	=	PP>TL=Farm	pH meters
<b>Retention Processes</b>			
P-sorption capacity	<	PP>TL=Farm	P-isotherms (26) Enzyme assay method
Denitrification rates	>	TL>PP>Farm	(27, 28)

**b) Soil N and P pools:** At each intensive sampling point within each transects (n=33, Fig. 2C) 5 soil cores (10 cm diameter, 20 cm deep) were collected. Samples were separated into the top 5 cm and <5-20 cm depth and all cores from a point were homogenized to get two integrated samples (0-5cm and 5-20cm) from the area surrounding each monitoring well. Soil N and P pools were characterized using standard methods.

**c) Environmental drivers:** At each intensive sampling points (n=33, Fig. 2) spatial and temporal variability for a range of environmental drivers of N and P retention were characterized using standard methods (Tables 1 and 2).

**d) Rates of Denitrification and P-sorption:** Functional response measurements were focused on estimating denitrification rates and P adsorption because these are the most important processes for permanent, or long-term removal of N and P (Richardson 1985, Walbridge and Struthers 1993, Peterson et al. 2001, Bernhardt et al. 2003).

**d.1) P adsorption:** Sub-samples from the two homogenized cores at each location were used to create sediment P adsorption isotherms using methods from (Axt and Walbridge 1999). Each core was homogenized and then air dried. 2.0 g dry weight (dwe) of fresh soil (n=2) per sample was equilibrated in 50-ml centrifuge tubes with 25 ml of 0.01 M CaCl solutions containing  $130\text{mg P L}^{-1}$  added as  $\text{KH}_2\text{PO}_4$ . Samples were shaken mechanically for 24 hours and then centrifuged for 20 min at 0.314 g. Orthophosphate concentrations in the clear supernatants were analyzed. Sorption data from  $130\text{ mg P L}^{-1}$  was used to estimate a P sorption index (PSI) to compare it across the hydrologic gradient (Axt and Walbridge 1999). The remaining soil was used for elemental analysis of C and N, for nitric-perchloric digestion and subsequent analysis of total P, and oxalate extractable Fe and Al.

**d.2) Denitrification potential:** Sub-samples from the same soil cores used were used to determine denitrification potential using the denitrification enzyme assay method (Tiedje et al. 1989, Groffman et al. 1999), which provides conditions ideal for maximum potential denitrification rates and uses acetylene to block the conversion of  $\text{N}_2\text{O}$  to  $\text{N}_2$ . Gas samples were analyzed for  $\text{N}_2\text{O}$  on a Shimadzu GC-17A equipped with an electron capture detector. Potential denitrification rates were compared across the hydrologic gradient in Timberlake Farm to determine relationships between denitrification rates and environmental drivers.

**Objective III.** Compare N and P cycling between the restored Timberlake site to an upstream agricultural field (pre-restoration state) and two reference wetlands (post-restoration goal)

**Setting endpoints:** The same nutrient pools (Table 1) and environmental drivers (Table 2) measured at TL-Farm were also measured in current and former riverine wetlands in the same drainage basin with contrasting land-use history: currently farmed lands (upstream, agricultural site), and lands without ditches and minimal logging history (Palmetto-Peartree Preserve and Timberlake Preservation Area). Comparing the Timberlake Farm with natural riverine wetlands and currently farmed lands will provide realistic ranges at each end of the land-use history spectrum. In order to compare the more intensive sampling (n= 33 sampling points) from Timberlake Farm, to the sampling in ANWR and the upstream farm (n=5 in each site), a subset of sampling points from TL-FARM have been selected to match the elevation of sampling points in the other two sites. After controlling for elevation, the same environmental drivers were examined as correlates with denitrification and P-adsorption potential among the three sites.

### 3. MAJOR RESULTS

#### 3.1 Objective I: Mass Balance Ecosystem Budget

Q1: Is Timberlake Farm a net sink or a net source for nitrogen and phosphorus?

*H<sub>1</sub>*: On an annual basis, the former agricultural field will serve as a net N sink due to high denitrification rates associated with high soil NO<sub>3</sub><sup>-</sup> and available labile carbon from algal communities in the stream channel. As the vegetation develops, plants will provide more carbon sources and through uptake will serve as a long-term sink for N from upstream sources.

In the first year since re-flooding (which occurred on February 27, 2007), the Timberlake site has retained 48% of the surface water inputs of dissolved inorganic nitrogen, and 83% of the total inputs (including rainfall, Fig. 3). DIN concentrations were fairly low in both sites during the first seven months of monitoring and started increasing after October 2007. Only during two months (October and November 2007) were surface water inputs of N higher than rainfall inputs (Fig. 3b), illustrating the importance of measuring rainfall N inputs when building nutrient budgets for wetland restoration sites. The DIN is dominated by NH<sub>4</sub>, with usually less than 15 % of the DIN load occurring as NO<sub>3</sub>. This pattern might change in the next year as the upstream farm will start pumping their excess water on the Timberlake site. It should be noted that the retention estimates of the site are conservative, given that 2007 was year of exceptional drought in the state of NC. The exact fate of the N being “retained” is unclear. High rates of denitrification and high concentrations of dissolved organic nitrogen have both been measured at the site. These results suggest that both losses to the atmosphere and transformations to organic forms are important pathways in removing DIN from surface water in addition to assimilation of inorganic N into plant biomass and soil organic matter.

*H<sub>2</sub>*: On an annual basis, the site will be a net source of P after inundation due to mobilization of P bound in mineral forms as the soils become anoxic. As the vegetation develops, plant uptake will serve as a long-term sink for P from upstream sources

Phosphorus concentrations tended to be higher in the first seven months of monitoring, with marked declines in concentrations in both sites in the fall and winter (Fig. 4). As expected, after the initial flooding in February 2007, there was an increase in P concentration leaving the site (Fig 4a). The site has been a source of P in the first year (Fig. 4b). Increased P loads have been reported in other places where former agricultural fields have been flooded. The main drivers of P sorption in these acidic soils tend to be Al and Fe. The ability of both of these metals to sorb on to P, in particular Fe, depend on the redox potential of the soils. When soils become anoxic Fe bound P tends to be released. Aluminum is less sensitive to changes in redox, but by increasing the ability of non-crystalline forms of Al to bind to organic matter and sorb P, flooding can lead to increases in P sorption. Previous research has shown that soils in these southeastern wetlands tend to be more susceptible to changes in Al than in Fe (Richardson 1985). Similar to the increase in P concentration after initial flooding, subsequent storms were found to increase P concentrations of water leaving the site after rainfall events. Given the prolonged drought during the past year, it is difficult to predict how higher rainfall events will affect P leaving the site. Continued storm sampling will enable relationships between hydrologic conditions and P export to be developed.

### **3.2 Objective II: Environmental Drivers of Denitrification and P-sorption across the hydrologic gradient in Timberlake Farm.**

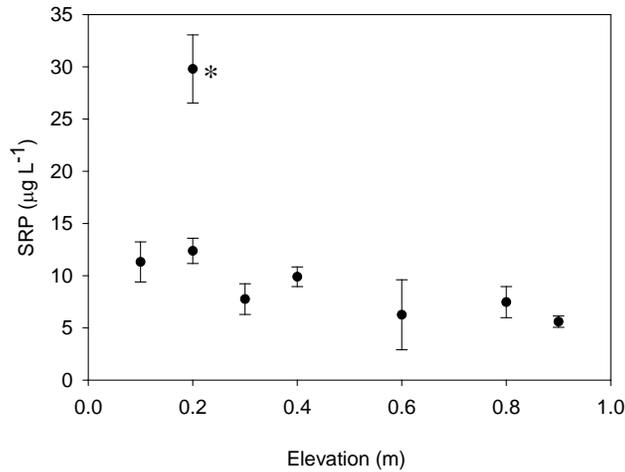
Q2 How do N and P pools vary across the hydrologic gradient within Timberlake Farm?

*H<sub>3</sub>*: As the frequency of inundation increases, the proportion of P in highly soluble pools ( $\text{PO}_4^{3-}$ ) will increase.

Shallow (15cm depth) piezometers and lysimeters from long-term sampling stations (n=33) were sampled bimonthly for one year. Elevation was used as a proxy for flooding frequency and redox conditions, with the lowest elevation points continuously submerged, while the higher elevation points are wet only during storm events. Soil solution concentrations of P (Fig. 5) were similar to those observed in surface waters (Fig. 4). Concentrations of P were inversely correlated with elevation (Fig. 5), supporting the

hypothesis that mobilization of P is controlled by hydrology through changes in redox. Future analysis will further examine P concentrations in relation to measured soil redox potential. In order to examine the potential mechanisms driving this pattern P sorption analyses were conducted (see  $H_6$ ).

*Fig. 5. Annual means of soluble reactive P ( $\mu\text{g L}^{-1}$ ) across an elevation gradient at the Timberlake restoration site. The point labeled with an \* denotes a higher average caused by one sampling date which had much higher P ( $130 \mu\text{g L}^{-1}$ ) than the other dates.*



$H_4$ : As the frequency of inundation increases, there will be lower concentrations of inorganic N (predominantly as  $\text{NH}_4^+$ ) in solution and higher total nitrogen content of the sediments.

Elevation was used as a proxy for frequency of inundation to examine landscape patterns in soil solution  $\text{NH}_4$ ,  $\text{NO}_3$  and TDN. Higher concentrations of N were measured in soil solution (Fig. 6) than in surface waters (Fig. 3). Similar to surface water,  $\text{NH}_4$  was the largest component of total dissolved nitrogen (70-90% of TDN was in the form of  $\text{NH}_4$ ). There was also a trend towards decreasing  $\text{NH}_4^+$  and TDN with increasing elevation. These data agree with the prediction that as frequency of inundation increases, there are lower concentrations of  $\text{NH}_4^+$ . Higher flooding frequencies lead to anoxic conditions which inhibit nitrification, which would otherwise convert  $\text{NH}_4$  to  $\text{NO}_3$ .

Q3 What environmental drivers control denitrification rates, greenhouse gas emissions, and P sorption capacity across the Timberlake Farm hydrologic gradient?

$H_5$ : The supply of labile carbon and of  $\text{NO}_3$  will control denitrification rates. C and N inputs will increase with the frequency of inundation (bringing surface water inputs).

Trace gas emissions have been measured across the Timberlake Restoration Site (TL) every two months since January 2007 along two intensive transects (n=33 sampling points). Figure 7 shows N<sub>2</sub>O and CH<sub>4</sub> emissions as CO<sub>2</sub>-equivalents, for more direct comparison. Emissions of N<sub>2</sub>O were most important during the two warmest months, while CH<sub>4</sub> fluxes were lower overall, occasionally negative, and extremely variable. The conversion of N<sub>2</sub>O and CH<sub>4</sub> to CO<sub>2</sub> equivalents was based on factors (310 for N<sub>2</sub>O and 21 for CH<sub>4</sub> over 100 years) published in the latest IPCC Report (Forster et al. 2007).

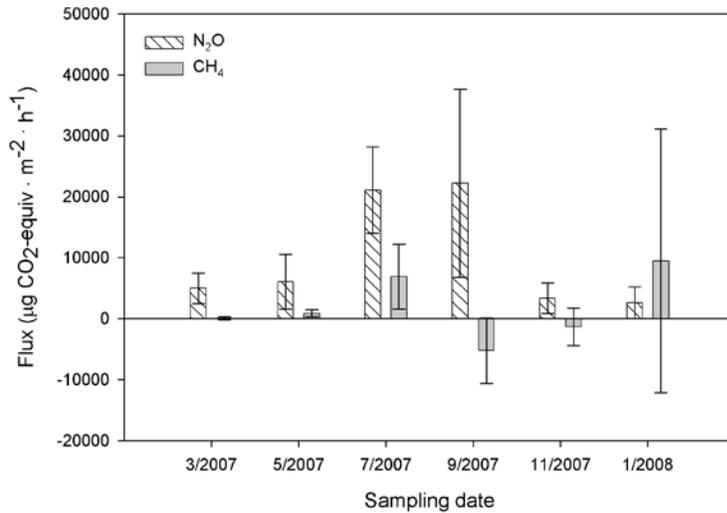


Figure 7. Mean N<sub>2</sub>O and CH<sub>4</sub> emissions (in CO<sub>2</sub> equivalents) for each sampling date in the Timberlake Restoration Site. Error bars represent the standard error.

Annual estimates of N<sub>2</sub>O and CH<sub>4</sub> emissions for the year (based on six sampling events) following hydrologic restoration in February 2007 are shown in Table 2. The annual emissions were estimated using the mean (µg · m<sup>-2</sup> · h<sup>-1</sup>) of all sampling points and dates for TL, converted to a yearly basis (24 h · 365 days) and to kg · ha<sup>-1</sup> from µg · m<sup>-2</sup>; results were then extrapolated to the 400 ha extent of the site. Annual estimates for reference sites and for CO<sub>2</sub> from all the sites will be available after May 2008 because sampling at the reference sites and measuring CO<sub>2</sub> began in July 2007.

Table 2. Annual N<sub>2</sub>O and CH<sub>4</sub> emissions estimate for Timberlake Restoration Site (3/07-4/08)

	Mean	Mean +/- SE
kg N <sub>2</sub> O · y <sup>-1</sup>	1169	827 - 1513
kg N <sub>2</sub> O · y <sup>-1</sup> (CO <sub>2</sub> -equiv)	375249	265,467 - 485,673
kg CH <sub>4</sub> · y <sup>-1</sup>	3194	-3503 - 9890
kg CH <sub>4</sub> · y <sup>-1</sup> (CO <sub>2</sub> -equiv)	67074	-73,563 - 207,690

Preliminary data analysis has focused on the relationships between environmental variables and gas fluxes by sampling point, using least squares multiple regression with air temperature and soil moisture as explanatory variables. Air temperature and soil moisture had a statistically significant relationship with CH<sub>4</sub> flux in two of 33 sampling points in the restored wetland, while

these values and N<sub>2</sub>O flux exhibited significant relationships in four different sampling points. As data collection continues, stepwise multiple regression analyses will be performed using additional environmental factors including redox potential, soil temperature, and water level to predict trace gas flux.

*H<sub>6</sub>*: The P sorption capacity of the sediments will be positively correlated with sediment redox potential, which will in turn decline with the frequency of inundation.

Again elevation was used as an indicator of redox conditions. Contrary to the initial hypothesis, there was not a strong relationship between elevation and single-point isotherm (Fig. 8a). There was, however, a strong relationship between the concentrations of oxalate extractable Al and Fe and single point isotherm expressed as the percentage of original P added that was adsorbed (Fig. 8b). This agrees with previous studies in similar wetlands which have shown that non-crystalline forms of Al and Fe are the main sites for P sorption (Richardson 1985, Axt and Walbridge 1999). Other relationships between soil organic matter, nitrogen content, and pH did not explain as much of the variation in P sorption. Aluminum concentrations explain more of the variation ( $r^2=0.68$ ) than Fe ( $r^2=0.58$ ), which suggests that the site can function as more long-term P sink than if Fe was binding most of the P.

### **3.3 Objective III. Compare N and P cycling between the restored Timberlake site to a pre-restoration site and two reference wetlands**

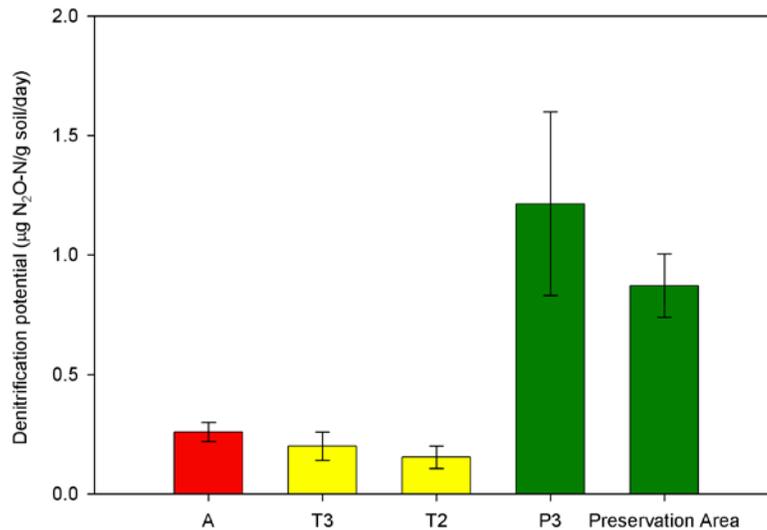
Q4 How different are rates of denitrification and P sorption capacity between Timberlake and nearby agricultural and protected wetland ecosystems?

*H<sub>7</sub>*: Denitrification rates will be highest in the restored wetland, intermediate in the Palmetto-Peartree site, and lowest in the actively drained agricultural field.

Relatively low denitrification potentials were estimated across all sites in June 2007 (Figure 9). Both transects within the restored site were not significantly different from the agricultural field while both forested wetlands had significantly higher denitrification potential. This ecosystem function requires anaerobic conditions in soil, in addition to sufficient supplies of NO<sub>3</sub><sup>-</sup> and labile carbon. These results

suggest that the dry conditions in soils, especially in the restored site and in the agricultural field, were limiting denitrification potential there. As the area begins to recover from the drought, denitrifying microbial activity is expected to increase across the sites.

Figure 9. Denitrification potentials across the Timberlake Restored Site (T3, T2) and the reference sites: agricultural field (A), Palmetto Peartree Preserve (P3), and TL Preservation Area. Bars represent mean and standard error.



*H<sub>8</sub>*: Phosphorus sorption capacity will be similar between the Timberlake and agricultural sites, low organic matter in these sediments will leave only mineral sorption of P. Sediments from the Palmetto-Peartree Preserve and the Preservation Area sites should have the highest P sorption capacity

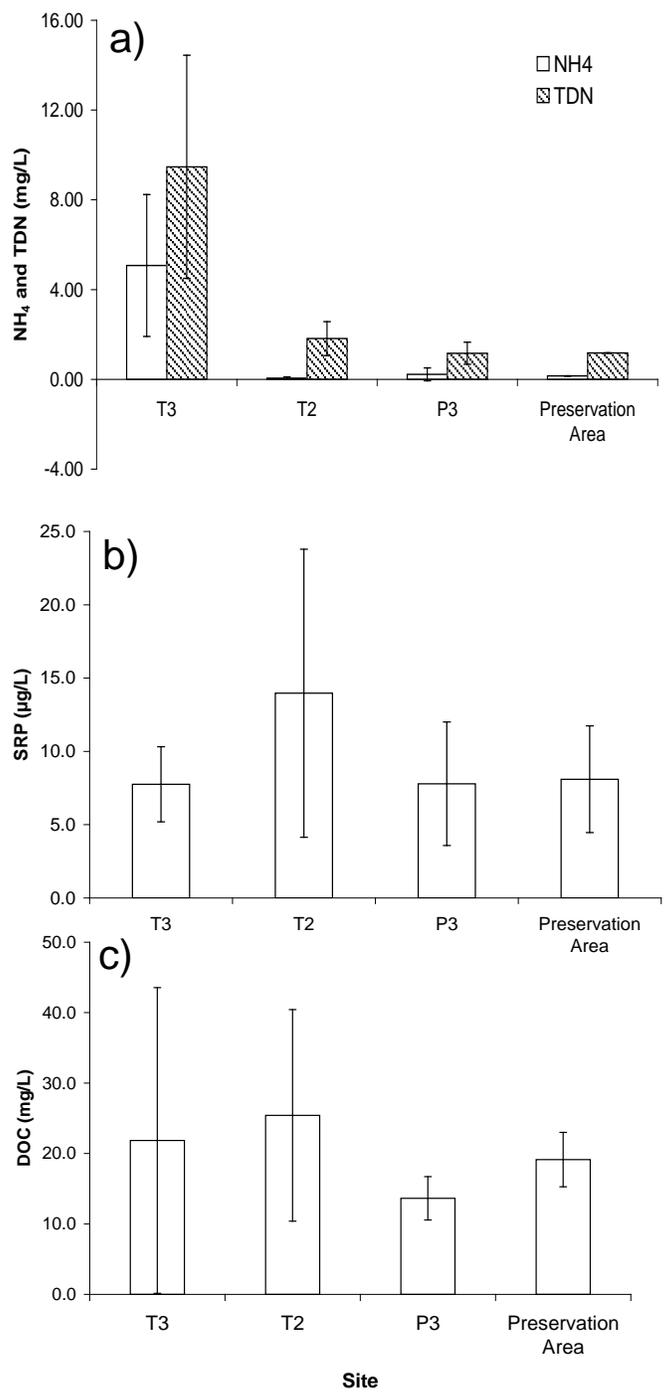
There was higher P sorption capacity in the Timberlake site than in Palmetto Peartree Preserve and the Agricultural Field (Fig. 10) with the preservation area having the highest P sorption capacity (Fig. 10). Across these 4 different sites, oxalate extractable Al was the best predictor ( $r^2 = 0.53$ ) for sorption capacity (Fig. 10). When both Fe and Al were considered together the predictive power of the relationship declined ( $r^2 = 0.23$ ). This suggests that Al is a better predictor of P sorption capacity than Fe across a land-use gradient. This information has management applications, because it can allow prioritization of sites for subsequent wetland restoration that have high Al content in the soil, as they will be the best sites to reduce P loading of surface waters.

Q5 How do the answers to Q2 and Q3 differ between Timberlake and nearby agricultural and protected wetland ecosystems?

*H<sub>9</sub>*: Because the availability of N and P is much higher at Timberlake than at Palmetto Peartree, higher concentrations of N and P should be measured in soil solution for Timberlake sites than for the two reference wetlands.

Because of the ongoing drought, it was often impossible to collect soil solution samples from all four sites, with no solution collected on most dates from lysimeters within the agricultural field. In January 2008 samples from Timberlake and the two reference sites were compared, with higher concentrations of NH<sub>4</sub>, TDN, SRP and DOC measured at the Timberlake site than in the reference sites (Fig. 11).

Fig. 11. Concentrations of a)  $NH_4$  and TDN; b) soluble reactive phosphorus; and c) dissolved organic carbon, in soil solution from 15cm depth from 4 sites collected on January 2008. T2 and T3 are transects within Timberlake restoration project, P3 is Palmetto Peartree preserve, and Preservation Area.



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## 5. Appendix

### 5.1 Public Presentations of this research (12 presentations)

Results from this project have been (or will be) presented in the following forums:

- **\*\* Invited talk: Ardón, M., Morse, J.L., M.W. Doyle and E.S. Bernhardt.** 2008. Biogeochemical consequences of North Carolina's largest wetland mitigation project. U.S. Geological Survey, Raleigh, NC
- **Ardón M., S. Montanari, J.L. Morse, M.W. Doyle and E.S. Bernhardt.** 2008. *Phosphorus retention in a large-scale wetland restoration project under a changing climate.* North American Benthological Society Meeting, Salt Lake City, UT
- **Morse, J.L., M. Ardón, and E.S. Bernhardt.** 2008. *Predicting the fate of nitrogen and the impact on water quality and greenhouse gas emissions from sea level rise and wetland restoration.* Lindbergh Foundation Annual Meeting, Atlanta, GA.
- **Morse, J.L., M. Ardón, and E.S. Bernhardt.** 2008. *Farm fields to wetlands: biogeochemical consequences of re-flooding in coastal plain agricultural lands.* Oosting Memorial Lecture Graduate Student Symposium, Durham, NC.
- **Morse, J.L., M. Ardón, M. Doyle, G. Poole, and E.S. Bernhardt.** 2008. *Scaling up gas flux measurements to annual and landscape scales.* NSF Riparian Zones Workshop, Indianapolis, IN.
- **Morse, J.L., M. Ardón, M. Doyle, and E.S. Bernhardt.** 2007. *Farm fields to wetlands: biogeochemical consequences of re-flooding in coastal plain agricultural lands.* North American Benthological Society Meeting, Columbia, SC.
- **Ardón, M., J.L. Morse, E.S. Bernhardt, and M.W. Doyle.** 2007. *Biogeochemical consequences of hydrological restoration of a 400-ha former agricultural field.* Ecological Society of America Meeting, San Jose, CA
- **\*\* Invited talk: Ardón, M.** 2007. *Organic matter processing and P cycling in tropical streams and temperate riverine wetlands.* University of North Carolina, Chapel Hill, NC.
- **\*\* Invited talk: Ardón, M., Morse, J.L., E.S. Bernhardt, and M.W. Doyle.** 2007. *How do we quantify trade-offs between various wetland ecosystem costs and benefits?* 10<sup>th</sup> International Symposium on Wetland Biogeochemistry: Frontiers in Biogeochemistry. Annapolis, MD.
- **Ardón, M.** 2007. *Organic matter processing and P cycling in tropical streams and temperate riverine wetlands.* Program in Ecology, Duke University, NC.
- **Morse, J.L., M. Ardón, E.S. Bernhardt, M.W. Doyle, and G.C. Poole.** 2007. *Predicting Patterns of and Limits to Denitrification Following Conversion of Former Agricultural Lands to Forested Wetlands.* 10<sup>th</sup> International Symposium on Wetland Biogeochemistry: Frontiers in Biogeochemistry. Annapolis, MD.
- **Morse, J.L., E.S. Bernhardt and G.C Poole.** 2006. *Linking hydrologic and edaphic parameters to denitrification and N<sub>2</sub>O emissions in a coastal plain riverine wetland.* NSF Denitrification RCN Workshop: Denitrification Modeling Across Terrestrial, Freshwater and Marine Systems, Millbrook, NY.

## **5.2 Additional Funding Sources to continue this research**

- Department of Energy, National Institute of Climate Change Research Coastal Research Program, Farm fields to wetlands: biogeochemical consequences and climate feedbacks due to sea level rise in coastal plain agricultural landscapes (\$375,000) Bernhardt (PI)
- National Science Foundation, Minority Postdoctoral Fellowship, Nutrient cycling in coastal wetland ecosystems under an accelerating hydrologic cycle (\$123,000) Ardón
- Lindbergh Foundation Research Grant (\$10,580) Morse
- National Center for Airborne Laser Mapping graduate student seed money award (LIDAR survey of research site, est. worth \$20-30K) Morse