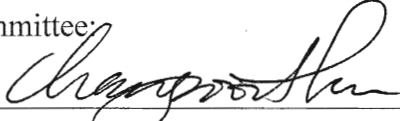
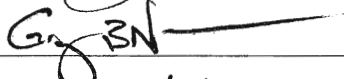
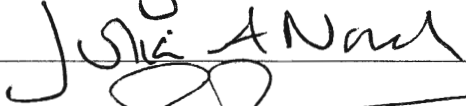


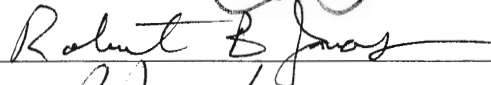
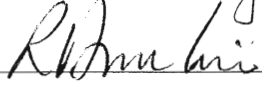
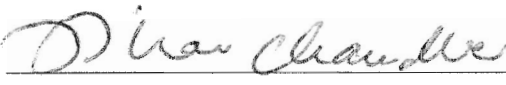


THE EFFECTS OF HYDROLOGIC CONNECTIVITY, AGE-RELATED SOIL  
PROPERTIES, AND MICROTOPOGRAPHY ON NITROGEN DYNAMICS IN  
CREATED WETLANDS OF THE VIRGINIA PIEDMONT

by

Kristin Wolf  
A Dissertation  
Submitted to the  
Graduate Faculty  
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Doctor of Philosophy  
Environmental Science and Public Policy

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The Effects of Hydrologic Connectivity, Age-Related Soil Properties, and  
Microtopography on Nitrogen Dynamics in Created Wetlands of the Virginia Piedmont

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## Abstract

### THE EFFECTS OF HYDROLOGIC CONNECTIVITY, AGE-RELATED SOIL PROPERTIES, AND MICROTOPOGRAPHY ON NITROGEN DYNAMICS IN CREATED WETLANDS OF THE VIRGINIA PIEDMONT

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George Mason University, 2010

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Wetlands are unique ecosystems that provide the essential ecosystem service of excess nutrient retention, processing, and removal from the landscape. In the early 1990s the U.S. adopted a “no net loss” policy towards wetlands in order protect these remaining wetland resources from the impacts of development. Under this policy the practice of wetland mitigation banking became the preferred method of compensating for “unavoidable” structural and functional impacts to natural wetlands. Created and restored wetlands, however, do not necessarily develop the same structural or functional capacity of their natural counterparts in the required five to ten year monitoring period. This study investigated four created non-tidal freshwater wetlands of varying ages and two natural non-tidal freshwater reference wetlands in the northern Virginia Piedmont. The purpose of this study was to identify how the design features of hydrologic connectivity and microtopography influence nitrogen cycling in created wetlands and investigate whether



created wetland soils develop with age in order to support nitrogen processing and removal comparable to that of natural wetlands. Ammonification, nitrification, and net nitrogen mineralization (ammonification + nitrification) were determined using *in situ* incubation of modified ion exchange resin cores and denitrification potential was determined with denitrification enzyme activity. Principal component analyses were conducted on hydrologic and soil variables to identify hydrologic connectivity, soil moisture, and soil condition indices. Total nitrogen sedimentation and ammonification rates increased with the hydrologic connectivity index. Nitrification and denitrification potential increased with soil moisture, soil condition, and microtopographic indices. Net nitrogen mineralization also increased with soil condition index. Nitrogen flux rates demonstrated age-related patterns, with younger created wetlands having lower rates of ammonification, nitrification, nitrogen mineralization, and denitrification potential than older created wetlands, which had flux rates similar to natural reference wetlands. Results demonstrated a clear, but variable age-related trajectory of coupled soil and nitrogen cycling development in created wetlands that trend toward natural wetlands. Findings of this study support the incorporation of hydrologic connectivity and microtopography into the design and regulatory evaluation of created wetlands, as well the monitoring of soil development indicators in these wetlands, in order to improve their ecosystem service of water quality improvement through the functional development of nitrogen cycling.

## **Chapter 1: The effects of hydrologic connectivity on nitrogen fluxes in created and natural wetlands of the Virginia Piedmont**

### **Abstract**

Natural wetlands are often connected to the surrounding hydrologic system through groundwater and/or overbank flow, but created and restored wetlands may only have a limited and static surface water connection. This lack of dynamic hydrologic connectivity may depress coupled nitrogen (N) fluxes, as the N cycle requires fluctuating aerobic and anaerobic conditions for nitrification and denitrification, respectively. Furthermore, the natural flood pulses that hydrologically connected wetlands experience are an important source of N to the wetland. This study investigated the effects of hydrologic connectivity on N cycling in created and natural freshwater wetlands of varying hydrology in the Piedmont physiographic province of Virginia. A principal component analysis of hydrologic variables was used to identify a hydrologic connectivity index (HC) as a measure of sedimentation and surficial hydrology dynamic. Average ( $\pm$  one standard error) flux rates were  $1.27 \pm 0.77$  g-N  $m^{-2}$   $yr^{-1}$  for total nitrogen sedimentation,  $15.11 \pm 2.23$   $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$  for ammonification,  $4.26 \pm 1.07$   $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$  for nitrification,  $19.36 \pm 2.52$   $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$  for net N mineralization, and  $119.15 \pm 13.73$   $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$  for denitrification potential. Nitrogen sedimentation and ammonification fluxes increased with the index of HC. In plots experiencing greater

HC, greater inputs of allochthonous N subsidized enhanced rates of ammonification. Nitrification fluxes and denitrification potentials were not directly correlated with the HC index, but did increase with an index of soil moisture. Nitrification flux and denitrification potential were coupled in these wetlands. Findings of this study support incorporation of hydrologic connectivity into the design of created and restored wetlands. Hydrologic connectivity could also be considered as a regulatory requirement of mitigation banking to ensure the establishment of a sustainable hydrologic regime that supports N cycling and retention.

## **1. Introduction**

Hydrology is the essential determinant of ecosystem structure and function, including biogeochemical cycles, in wetland systems (Mitsch and Gosselink 2000). A wetland's connection to the surrounding hydrologic system influences the timing, amplitude, duration, and frequency of flooding; the aerobic/anaerobic condition of the soil; and the importation, transformation, and sequestration/removal of nutrients (Tockner et al. 1999, Craft and Casey 2000, Hein et al. 1999, Noe and Hupp 2005) – all of which create the biogeochemical character of the wetland. The ability of a wetland to sequester, transform, and remove excess nitrogen (N) that would otherwise contribute to eutrophication in downstream systems provides a watershed with the vital ecosystem service of water quality improvement (Verhoeven et al. 2006, Mitsch and Gosselink 2003). Indeed, wetland creation and restoration has often been proposed as a management

option to remove excess nutrients at the landscape scale (Lowrance et al. 1997, Haycock et al. 1997, Mitsch and Day 2006). Improvement of water quality as an ecosystem service is particularly important for wetlands in the Chesapeake Bay watershed (Lowrance et al. 1997), where N pollution is a primary cause of its impaired status (Chesapeake Bay Foundation 2010). If wetlands are isolated from the surrounding hydroscape, they cannot effectively intercept and filter incoming water and the opportunity for improved water quality and flood protection in the wetland's local watershed is missed.

Nitrogen cycles, in particular, are heavily controlled by hydrologic variables, as fluctuating redox conditions are required for N to cycle completely from its organic to inorganic form through N mineralization (comprised of ammonification and nitrification processes) and for its removal as  $N_2$  gas by denitrification (Reddy and Patrick 1984, Hefting et al. 2004). While ammonification can occur under aerobic and anaerobic conditions (it is expedited under the former; Ponnampereuma 1972, Brinson et al. 1981), nitrification and denitrification require aerobic and anaerobic conditions, respectively (Ponnampereuma 1972). A fluctuating hydroperiod, and subsequent fluctuation in redox potential, favors nitrification during dry drawdown periods, producing nitrate ( $NO_3^-$ ) substrate that can later be denitrified to  $N_2$  during wet flooded periods (Reddy and Patrick 1984). Hydrology also influences more recently discovered N processes whose contribution to N cycling is currently less clear, such as anaerobic ammonium oxidation (anammox) (Matheson et al. 2003) and dissimilatory nitrate reduction to ammonium (DNRA) (An and Gardner 2002). Furthermore, the flood-pulse dynamic that characterizes hydrologically connected wetlands provides an important source of

allochthonous material that may stimulate the N cycle by contributing additional mineralizable organic N substrate (Junk et al. 1989, Odum et al. 1995, Nahlik and Mitsch 2008),  $\text{NO}_3^-$  for denitrification (Pinay and Naiman 2002, Richardson et al. 2004), and carbon to enhance the metabolism of heterotrophic bacteria (Robertson et al. 1999).

Despite the essential value of hydrologic connectivity in transporting energy and materials and providing essential ecosystem services, this characteristic is often overlooked when ecosystems are anthropogenically altered (Pringle 2003). This is often a design flaw in the creation and restoration of wetland systems, notably in cases where the mitigation wetland is not the hydrologic equivalent of the replaced natural wetland at the landscape scale (Bedford 1996). Many created wetlands incorporate a surface-driven, epiperched “perching design” that relies on the compaction of clayey substrate to retain water at or near the surface for a required number of days during the growing season (Whittecar and Daniels 1999). The result is a wetland that is disconnected from groundwater exchange, has limited surface water exchange, has muted temporal variability in water stage, and is generally far wetter than natural wetlands (Brooks et al. 2005, Hoeltje and Cole 2007). The N filtering capacity of a wetland is largely determined by hydrologic factors that regulate the flow path and residence time of mobile N species like  $\text{NO}_3^-$  (Groffman et al. 1998; Fink and Mitsch 2007). Maintaining groundwater exchange increases residence time (Cook and Hauer 2007) and the potential for N to come in contact with the biologically active soil surface that can process N (Ponnamperuma 1972, Pinay and Naiman 2002). The lack of hydrologic connectivity in natural wetlands has been shown to limit primary productivity in depressional wetlands

(Cook and Hauer 2007) and nutrient accumulation associated with sedimentation in riparian wetlands (Noe and Hupp 2005). The lack of hydrologic connectivity and flood pulses in created and restored wetlands has been shown to depress denitrification (Hunter and Faulkner 2001, Hernandez and Mitsch 2007). The closed material cycles of hydrologically disconnected wetlands, created or natural, thus lowers N input and the N sequestration/removal potential of the wetland in general (Hopkinson 1992). While the restoration of hydrologic connectivity may be problematic in intensely developed landscapes where invasive species, toxins, and excess nutrients may be transported downstream (Jackson and Pringle 2010), wetlands are generally beneficial systems to reconnect to the landscape due to their filtration capability.

Although there has been a long precedence of N-related scientific research in natural ecosystems, there is less information on N cycling in created wetlands. Under the 1990 Memoranda of Agreement between the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency, wetland mitigation must compensate for the loss of acreage and function of natural wetlands (US EPA 1990). Wetland mitigation can result in a net loss of wetland function when improperly carried out (Erwin 1991, Kentula et al. 1992, Zedler 1996, Hoeltje and Cole 2007). Although N cycling is an essential function of natural wetland systems, criteria for mitigation success typically only include the presence of surface water during the growing season, indication of soil anoxia, and hydrophytic wetland vegetation (NRC 2001). While mimicking the hydrologic structure or connectivity of natural wetlands does not ensure that N processes will function as they

do in natural systems, it creates an environment that can facilitate the development of this important nutrient function.

This study investigated the impact of hydrology in general and hydrologic connectivity in particular, on N flux rates in created and natural wetlands in the Piedmont physiographic province. The created wetlands represent a range of hydrogeomorphic settings and hydrologic-connectivity designs to surrounding hydroscapes. The study focused on the following research questions:

- (1) What are the hydrologic characteristics and range of hydrologic connectivity of nontidal, freshwater created and natural wetlands of the Virginia Piedmont?
- (2) How do these wetlands cycle N, including N sedimentation, ammonification, nitrification, and denitrification potential flux rates?
- (3) How does the hydrologic character of these wetlands influence N flux rates?

## **2. Site Descriptions**

### *2.1 General setting*

All study sites were non-tidal freshwater wetlands located in the northern Virginia Piedmont physiographic province (mean annual precipitation 40 cm, mean temperature min 6°C / max 19°C) (Figure 1). The mitigation wetland banks were constructed on old farm fields and cattle pasturelands and were created to mitigate for various local construction projects that impacted a mixture of bottomland forested floodplain,

shrub/scrub, and emergent wetlands and open water ponds. The sites represent the typical range in hydrologic regime found in created and natural wetlands. All of the created wetlands were under 11 years old and had predominantly herbaceous cover, with some open water aquatic areas, shrub-scrub areas, and/or young stands of trees. The created wetlands are intended to mature into a mixture of bottomland forested, shrub/scrub, and emergent wetlands and open water areas that they were meant to replace. The natural wetlands include bottomland riparian forested wetlands and open herbaceous wetlands.

## *2.2 Created wetlands*

Loudoun County Mitigation Bank (LC) is a 32-acre wetland and upland buffer complex, constructed by Wetland Studies and Solutions, Inc. (WSSI) in the summer of 2006 in Loudoun County, Virginia (Latitude 39°1'58.98" N Longitude 77°36'26.10" W). LC contains disked areas that were tilled with a disk roller during construction and undisked areas that were designed as adjacent paired plots for another study (Ahn and Peralta 2009). The site is enclosed within a berm on the floodplain of Big Branch Creek and Goose Creek. The wetland contains two contiguous areas (cell 1 and cell 2) that are separated by a central berm. Cell 2 receives flow from an unnamed tributary of Goose Creek through a head race attached to a cross vane structure with flow impacts regulated by an Agridrain structure and gate valve. LC also receives surface water runoff from an upland housing development and forested buffer, as well as minor groundwater inputs



from toe-slope intercept seepage. Vegetation is currently dominated by herbaceous plants with small, container-grown, planted woody vegetation interspersed throughout.

Clifton Farm (CF) is a 2.14-acre mitigation wetland, constructed by Angler Environmental in 2005 in Fauquier County, Virginia (Latitude 38°46'38.75" N Longitude 77°47'40.61" W). The site receives groundwater from a small upland reservoir and surface water runoff, but has no stream connection. Vegetation is herbaceous with planted, woody vegetation throughout.

Bull Run Mitigation Bank (BR) is a 50-acre wetland and upland buffer complex, constructed by WSSI in 2002 in Prince William County, Virginia (Latitude 38°51'12.74" N Longitude 77°32'58.52" W). The site receives water from Bull Run from an AgriDrain structure that routes water via a central ditch through the wetland , as well as overbank flow from Bull Run (average of one overflow event per year; WSSI 2004), which sharply bends around the corner of the site. The wetland receives limited surface water runoff from uplands and negligible groundwater. Vegetation is predominantly herbaceous, with small, planted woody vegetation throughout.

North Fork Wetlands Bank (NF) is a 125-acre wetland, constructed by WSSI in 1999 in Prince William County, Virginia (Latitude 38°49'31.53" N Longitude 77°40'9.17" W). With the exception of minor contributions from toe-slope intercept seepage, the site is disconnected from the groundwater by an underlying clay liner and is hydrologically divided into four areas: main pod area – fed by upland surface water runoff and a tributary of the North Fork of Broad Run that is controlled by an artificial dam; overbank flow area – fed by overbank flow from the tributary; vernal pool area – located in the

southwest quadrant of the wetland and fed solely by precipitation; and depressional wetland area – fed by surface runoff from the adjacent Boy Scout campgrounds.

Vegetation is mostly herbaceous, interspersed with young tree saplings and shrubs in projected forested areas, patches of shrub/scrub and emergent vegetation, and aquatic plants in the open water area.

The wetlands designed by WSSI (LC, BR, and NF) contain at least a 0.3 m low permeability subsoil layer covered with 0.2 m of commercially available topsoil. This design creates a perched, surface-driven water table close to the soil surface and limits groundwater exchange in the wetland (Ahn and Peralta 2009).

### *2.3 Natural wetlands*

Manassas National Battlefield Park (BFP), established in 1940, is a 5,000 acre site with areas of natural wetland coverage located in Prince William County, Virginia (Latitude 38°49'24.98" N Longitude 77°30'28.30" W). An area of herbaceous wetland within a matrix of forested floodplain was selected for study and comparison to the created wetlands. The site is connected to Bull Run by a culvert on its eastern end and also receives groundwater and upland surface water runoff. Vegetation is mostly herbaceous with a few mature trees interspersed throughout.

Banshee Reeks Nature Preserve (BSR), established 1999, is a 725-acre site with areas of natural seep and riparian wetlands located in Loudoun County, Virginia (Latitude 39°1'16.44" N Longitude 77°35'49.10" W). The studied floodplain riparian

wetlands receive water from groundwater springs, surface water runoff, and occasional overbank flooding from Goose Creek. Vegetation is mature bottomland forest with little understory.

### **3. Methods**

#### *3.1 Sampling design*

Study plots at the four created and two natural wetlands were selected so that typical hydrology, vegetation, and any experimental manipulation (disking) of the wetland site was represented. At LC three disked plots (LC BB from cell 1 and LC DD and LC EE from cell 2), paired with three undisked plots (LC B from cell 1 and LC D and LC E from cell 2) were selected for a site total of six plots. At NF two plots in the main pod area (NF 40 and NF 41) and two plots in the vernal pool area (NF 14 and NF 15) were selected. At CF two plots, one on either side of a central berm (CF 3 and CF 4) were selected. At BR four plots arranged perpendicularly to the overbank flowpath were selected (BR 3, BR 4, BR 5, and BR 6; plot numbers increasing with distance from the corner of the site that receives overbank flow), with the central ditch separating the distance between BR 5 and BR 6. At BFP two plots in an open, herbaceous area were selected (BFP 1 and BFP 3), and at BSR two plots in a forested riparian area were selected (BSR 4 and BSR 5). There were a total of 16 plots in 4 created wetlands and 4

plots in 2 natural wetlands – 20 plots in all. Soil sampling in the wetlands occurred over a two-day period the second week of every month from July 2008 to July 2009.

### 3.2 N mineralization

Soil net N mineralization was measured *in situ* using a modification of the DiStefano and Gholz (1986) resin core technique for use in wetlands that was developed by Noe (in press). The method incubates in place a soil core with six ion-exchange resin bead bags, three placed above and three below the soil core, to quantify inorganic N loading to the soil core (two outer resin bags) and production from inside the intact soil core (two inner resin bags + soil core) in hydrologically dynamic wetlands soils. The two middle bags were used as a quality control check to ensure that inner and outer bags were not saturated with ions and incapable of trapping nutrients. A preliminary study has shown that the outer and inner bags are sufficient for removing all dissolved inorganic N from water entering and leaving the modified resin core in wetland soils (Noe, in press).

Study plots were sampled by randomly placing a 1-m<sup>2</sup> quadrat at the beginning of the study that divided the sampling area into 100 10-cm<sup>2</sup> cells. The surficial soil (0-5 cm) of two adjacent 10 cm<sup>2</sup> cells were randomly sampled each month each with beveled, thin-walled, PVC core tubes, 7.8 cm in diameter and 11 cm in length, that were driven into the ground until the soil surface was 3 cm below the top of the tube. Cores were then removed, and the excess 3 cm of soil on the bottom of the core was scraped out, resulting in a soil core depth of 5 cm and a soil volume of 251.3 cm<sup>3</sup> in the middle of the core tube.

The first core was processed as an initial core and placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within 48 hours to yield initial 2M KCl extractable soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentrations (Keeney and Nelson 1982). The initial core was also used for soil characterization and denitrification flux measurements (see below).

The second core was processed as a resin core by placing 3 resin bags each on the top and bottom of the extracted soil core. Each resin bag was constructed using an undyed nylon stocking (Kayser-Roth, Inc., Greensboro, North Carolina, USA) stretched over a 7.6 outer diameter, nitrile O-ring (Macro Rubber & Plastics Products, Inc., North Andover, Massachusetts, USA), filled with mixed-bed ion-exchange resin beads (Rexyn I-300, Fisher Scientific, Pittsburgh, Pennsylvania, USA), and closed with two stainless steel staples. The two outer and middle bags each contained 20 g of resin and the two inner bags contained 30 g of resin (necessitated by the higher rate of capture by inner bags in a pilot study). Each bag was sealed into the core along its outer edge with a silicone caulk free of anti-microbial agents (Silicone I rubber sealant, General Electric Co., Fairfield, Connecticut, USA) to prevent leakage and flow bypass around the resin. Wire supports were added over the outer bags on both the top and bottom of the core tube to keep the bags in place, and the resin core was inserted back into the core hole and made flush with the surrounding soil surface. Resin cores were then incubated *in situ* for approximately one month (range of 26 to 34 days) and then harvested, placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within one week. Monthly estimates of net ammonification and nitrification were calculated as

the sum of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively, in the soil core and two inner bags in the resin core minus the initial core. Net N mineralization was calculated as the sum of ammonification and nitrification relative to the dry mass of soil in the resin core. We present cumulative annual flux calculated as the sum of monthly incubations: (resin core soil  $\mu\text{mol}$  + 2 inner resin bags  $\mu\text{mol}$  – initial core soil  $\mu\text{mol}$ ) / ((total incubation days x (plot mean bulk density ( $\text{kg-dw cm}^{-3}$ ) x volume of soil core ( $251.3 \text{ cm}^3$ ))) for  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_3^- + \text{NH}_4^+$  (N mineralization) expressed as  $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$ .

### *3.3 Denitrification enzyme assay (DEA)*

Monthly denitrification potential was determined for each initial core using the denitrification enzyme assay (DEA) procedure (Smith and Tiedje 1979; Tiedje et al 1989; Groffman 1999). Assays were run in duplicate and consisted of a slurry of 25 g of homogenized field moist soil and a 25 mL solution of 1 mM glucose, 1 mM  $\text{KNO}_3$ , and 1  $\text{g L}^{-1}$  chloramphenicol mixed in a 125 ml Erlenmeyer flask. Flasks were sealed with rubber septa, made anaerobic by bubbling the slurry for 10 min followed by a 1 min headspace flush with  $\text{N}_2$  gas, and evacuated with a vacuum pump. Assays were brought to atmospheric pressure with  $\text{N}_2$  gas and injected with  $\text{H}_2\text{SO}_4$ -scrubbed acetylene (modified from Hyman and Arp 1979) to 10% of the volume of the flask headspace. Flasks were then incubated on a rotary shaker table with gas samples taken at 30 and 90 min. Gas samples were stored in freshly evacuated 2 mL glass vacutainer vials (Tyco Healthcare Group LP, Mansfield, MA, USA) until they could be analyzed for  $\text{N}_2\text{O}$  on a

Shimadzu 8A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with electron capture detection, generally within three days of sampling.

Denitrification enzyme activity was calculated as:  $M = C_g \cdot (V_g + V_l \cdot \beta)$  where,  $M$  = total amount of  $N_2O$  in water plus gas phase ( $\mu\text{g } N_2O\text{-N}$ ),  $C_g$  = concentration of  $N_2O$  in the gas phase ( $\mu\text{g } N_2O\text{-N/L}$ ),  $V_g$  = volume of the gas phase (L),  $V_l$  = volume of liquid phase (L), and  $\beta$  = Bunsen coefficient (0.544 @ 25°C) and expressed as  $\mu\text{mol } N_2O\text{-N kg-dw}^{-1} \text{ d}^{-1}$ .

### *3.4 Soil physicochemical properties*

#### *3.4.1 Laboratory analyses*

Gravimetric soil moisture (GSM) was determined for each initial core and resin core by removing a ~40 g dry-weight equivalent (dw-eq) subsample of homogenized soil, recording initial field-moist weight, and drying at 60°C until a constant weight was achieved. Bulk density (BD) was determined for each core by first weighing the entire field-moist core of soil, converting to dry weight based on GSM percentage, and dividing by the total volume of the soil in the core (251.3 cm<sup>3</sup>). Volumetric soil moisture (VSM) was calculated as  $BD \times (GSM / \text{density of water, assuming } 1.0 \text{ g-H}_2\text{O mL}^{-1})$ . Water-filled pore space (WFPS) was calculated as  $VSM / [1 - (BD/\text{quartz parent material density, assuming } 2.65 \text{ g cm}^{-3})]$ . Extractable  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was determined for each initial and resin soil core and resin bag, within 48 hours of collection, by adding 40 ml of 2 M KCl to a 4 g dw-eq subsample of soil or 2 g ww subsample of resin beads, agitating on a

shaker table for 1 h, and centrifuging (soil samples only) at 2500 rpm for 5 minutes. The supernatant was then filtered through a syringe with a 0.2 µm polyethersulfone filter tip (Pall Corporation, Port Washington, New York, USA) and analyzed on an Astoria 3020 series segmented flow autoanalyzer (Astoria-Pacific International, Clackamas, Oregon, USA). External reference standards and sample blanks (empty extraction vessel for soil blanks or extraction vessel with new resin for resin bead blanks) were included in each monthly run to validate results and to correct incubated resin and soil extractable concentrations, respectively.

#### *3.4.2 Field analyses*

Redox potential was measured each month by inserting a RE 300 ExStik<sup>®</sup> ORP meter (Extech Instruments Corporation, Waltham, Massachusetts, USA) to a depth of approximately 3 cm into the soil between the initial core and resin core sampling locations. Redox potential was recorded after drift was sufficiently stabilized (approximately 1 min). The depth of standing water, when present, was measured each month within the sampling quadrat. Ceramic sedimentation tiles (20 x 20 cm) were installed monthly at the same location that was adjacent to and representative of each plot, yet avoided any microtopographic or vegetative irregularities. Deposited sediment (excluding coarse woody debris and litter fall free of mineral sediment) was harvested from the tiles after each collection period, oven dried and weighed to determine monthly accumulation, and ground, subsampled, and analyzed for total C and N concentration on



an elemental analyzer (Perkin-Elmer 2400 Series II CHN/O Analyzer (Nelson and Sommers 1996, Waltham, Massachusetts). Sediment deposition rates were used to describe hydrologic connectivity and identify allochthonous sources of C and N. Cumulative annual sediment, C, and N deposition rates were calculated for each plot.

### *3.5 Statistical analysis*

The average and coefficient of variation (CV) of hydrologic variables for initial cores at each plot were included in the statistical analysis of the data in order to provide a measure of hydrologic spatial and temporal variation in the wetlands. Temporal variation in hydrology is an important characteristic of hydrologic connectivity, as it provides information on the occurrence, duration, and intensity of flooding in the wetland, as well as the changes in redox potential that follow flood events. All data were reviewed for normality and those variables failing tests for normality were transformed, usually by natural log transformation. A principal component analysis (PCA) was conducted on all hydrologic parameters to identify underlying multivariate gradients that may be influencing N fluxes. N sedimentation was included with the hydrologic variables because it represented an index of allochthonous N sediment inputs to the wetlands along with mass and C sedimentation rates. The final model was produced using a varimax rotated component matrix with eigenvalues  $> 1.4$ . A constrained ordination was selected for the analysis, as it yielded components with the highest explanatory power of N fluxes in the final regression analysis compared to an unconstrained PCA. Principal component

analysis scores were entered into a plot-wise hierarchical cluster analysis (HCA) that classified plots into five hydrologic groups (HGs). PCA component scores were then entered as independent variables into a multiple linear regression analysis to determine the influence of each component on cumulative annual N sedimentation,  $\text{NH}_4^+$  (ammonification) and  $\text{NO}_3^-$  (nitrification) production, N mineralization, and DEA. Pearson's product-moment correlation matrices were used for further investigation of univariate relationships among hydrologic variables and nutrient fluxes. All statistical tests were performed using SPSS version 15 (SPSS 2006), and tests were considered significant at  $\alpha = 0.05$ , unless otherwise noted.

## **4. Results**

### *4.1 Hydrology characterization*

The PCA resulted in a three component model that explained a cumulative 67.6% of total spatial and temporal variance in hydrologic data (Table 1). Component 1 explained 29.2% of total variance and consisted of variables that characterized an index of hydrologic connectivity (HC), including higher redox variability, deeper standing water and greater standing water variability, and greater sediment TC, TN, and mass accumulation. Component 2 explained 23.2% of total variance and consisted of variables that characterized spatial variation in soil moisture (SM), specifically, higher gravimetric and volumetric soil moisture and lower WFPS. The SM component also contained redox

potential, which showed positive loading on the component. Component 3 explained 15.2% of the total variance and consisted of variables that characterized soil moisture temporal variability (SMVAR), including volumetric soil moisture variability and WFPS variability.

A hierarchical cluster analysis of PCA scores by plot identified five hydrologic groups (HG1-5 ranked by increasing HC; Figure 2). HG1 included the natural wetland plots BSR 4 and BSR 5 and was characterized by the lowest HC, moderate SM, and high SMVAR. Compared to other hydrologic groups, HG1 received the least amount of sediment, had the lowest TC and TN sedimentation, and had soil with the highest redox potential and lowest redox variability (Table 2). HG2 included the created wetland plots BR 4, BR 5, BR 6, and NF 40 and was characterized by moderate HC and SM and low SMVAR. This group had the second lowest standing water, standing water variability, and BD (Table 2). HG3 included the created wetland plots LC D, LC EE, NF 14, NF 15, NF 41, and CF 4 and was characterized by moderate HC and SM and high SMVAR. This group had the highest TC and TN sedimentation (Table 2). HG4 included the created wetland plots LC B, LC DD, and LC E and was characterized by moderate HC and the lowest SM and SMVAR. This group had the deepest standing water, highest WFPS and BD, and lowest gravimetric soil moisture, volumetric soil moisture variability, and redox potential (Table 2). HG5 included both natural and created wetland plots, BFP 1, BFP 3, and BR 3, and was characterized by the highest HC, SM, and SMVAR. HG5 had the highest gravimetric soil moisture, volumetric soil moisture, standing water variability, lowest BD, and received far more sedimentation mass than other groups (Table 2). LC

BB and CF 3, both created plots, were identified by the HCA as individual outliers due to their high SMVAR scores and general dissimilarity with other groups, and thus, were not included in any hydrologic group.

#### *4.2 N flux rate characterization*

Average annual TN sedimentation was ( $1.27 \pm 0.77$  g-N  $m^{-2}$   $yr^{-1}$ ; mean  $\pm$  one S.E.) and was highest for HG5 ( $5.35 \pm 4.50$  g-N  $m^{-2}$   $yr^{-1}$ ) and lowest for HG1 ( $0.15 \pm 0.001$  g-N  $m^{-2}$   $yr^{-1}$ , Table 2). The average cumulative net annual ammonification rate was  $15.11 \pm 2.23$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$  (prorated daily mean based on number of incubation days) and was highest for HG2 ( $24.80 \pm 3.93$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ) and lowest for HG1 ( $-0.60 \pm 0.06$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ). Average net nitrification rate for the study wetlands was  $4.26 \pm 1.07$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$  and was highest for HG1 ( $8.10 \pm 0.60$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ) and lowest for HG4 ( $0.020 \pm 0.01$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ). Average net N mineralization rate was  $19.36 \pm 2.52$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$  and was highest for HG2 ( $30.51 \pm 4.25$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ) and lowest for HG1 ( $7.45 \pm 0.03$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ). Average DEA was  $119.15 \pm 13.73$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$  and was highest in HG5 ( $179.53 \pm 34.61$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ) and lowest in HG4 ( $59.47 \pm 5.08$   $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ ).

#### *4.3 The effects of hydrology on N flux rates*

Hydrologic connectivity index was a significant positive predictor of plot annual TN sedimentation (Multiple linear regression,  $n=20$ ,  $R^2=0.505$ ,  $P<0.001$ , Figure 3a), but

SM ( $P=0.407$ ) and SMVAR ( $P=0.957$ ) were not. This is not surprising since TN sedimentation was included in the PCA and had high loading on the HC component. Total N sedimentation also was positively correlated with TC sedimentation (Pearson product-moment correlation,  $n=20$ ,  $r=0.817$ ,  $P<0.001$ ), standing water variability ( $r=0.541$ ,  $P=0.014$ ), redox variability ( $r=0.513$ ,  $P=0.021$ ), average standing water ( $r=0.487$ ,  $P=0.029$ ), and ammonification ( $r=0.464$ ,  $P=0.039$ ). HC was also a significant positive predictor of ammonification ( $R^2=0.364$ ,  $P=0.005$ , Figure 3b), but SM ( $P=0.081$ ) and SMVAR ( $P=0.099$ ) were not. Ammonification was positively correlated with standing water variability ( $r=0.335$ ,  $P=0.008$ ), redox variability ( $r=0.419$ ,  $P=0.066$ ), sediment mass accumulation ( $r=0.417$ ,  $P=0.068$ ), average standing water ( $r=0.397$ ,  $P=0.083$ ), and N mineralization ( $r=0.872$ ,  $P<0.001$ ). Nitrification was not significantly predicted by HC ( $P=0.132$ ) or SMVAR ( $P=0.957$ ), but was predicted by SM at  $\alpha=0.10$  ( $R^2=0.186$ ,  $P=0.058$ , Figure 4a). Nitrification was negatively correlated with average WFPS ( $r=-0.520$ ,  $P=0.019$ ) and positively correlated with average gravimetric soil moisture ( $r=0.460$ ,  $P=0.041$ ), average redox ( $r=0.460$ ,  $P=0.041$ ), and, at  $\alpha=0.10$ , denitrification potential ( $r=0.434$ ,  $P=0.056$ ). N mineralization was not significantly predicted by HC ( $P=0.100$ ), SM ( $P=0.172$ ), or SMVAR ( $P=0.251$ ) and was significantly correlated only with ammonification ( $r=0.872$ ,  $P<0.001$ ).

Soil moisture index was a significant positive predictor of DEA ( $R^2=0.494$ ,  $P<0.001$ , Figure 4b), but not HC ( $P=0.472$ ) or SMVAR ( $P=0.892$ ). Denitrification enzyme assay was positively correlated with average gravimetric soil moisture ( $r=0.776$ ,  $P<0.001$ ), average volumetric soil moisture ( $r=0.504$ ,  $P=0.023$ ), ammonification

( $r=0.464$ ,  $P=0.039$ ), and, at  $\alpha=0.10$ , nitrification ( $r=0.434$ ,  $P=0.056$ ) and negatively correlated with average WFPS ( $r=-0.686$ ,  $P=0.001$ ).

## **5. Discussion**

### *5.1 Hydrology characterization*

Wetland hydrologic connectivity describes the degree to which a wetland is connected to the surrounding hydrologic system. Hopkinson (1992) uses the term to differentiate between hydrologically “open” systems, in which material and nutrient inputs largely exceed ecosystem standing stock and community need, and “closed” systems, in which the reverse is true. Craft and Casey (2000) investigated how hydrologic connectivity led to differences in sediment and nutrient deposition of open riparian wetlands versus closed, depressional wetlands, and Noe and Hupp (2005) demonstrated differences in sediment and nutrient deposition of hydrologically connected vs. disconnected riparian wetlands. Additionally, Richardson et al. (2004) found that hydrologic connectivity led to the delivery of river  $\text{NO}_3^-$  to C-rich floodplain sediments and N removal along the Upper Mississippi River.

While most studies attempt to characterize hydrologic connectivity on a categorical basis (hydrologically open vs. closed), this study attempted to quantify hydrologic connectivity as a continuous variable in order to differentiate wetlands and predict N fluxes. Hydrologic connectivity, as defined for our analysis, is a component derived from a PCA of all measured hydrologic variables and is specifically comprised of

those variables that indicate sedimentation and the surficial hydrologic dynamic of the wetland. The combination of these variables provided an index of HC through measures of sediment deposition mass and quality, standing water depth and standing water variability, and redox fluctuation (Table 1).

The separation of hydrologic variables into the PCA components hydrologic connectivity (HC), soil moisture (SM), and soil moisture variability (SMVAR) indicates independent gradients in the surface and sub-surface hydrology, as well as the temporal variability in sub-surface hydrology of the study wetlands, respectively (Table 1). As the study design is heavily weighted to the created wetland plots ( $n=16$ ) over the natural wetland plots ( $n=4$ ) among a total of six wetlands, these results emphasize the large hydrologic gradients found in created systems. The wide range of wetland hydrology represented in this study suggests that results can be generalized to many other created wetlands. Some created wetland plots, like LC B, LC DD, and LC E of HG4, demonstrate the epi-saturated, perching design common in created wetlands (Whittecar and Daniels 1999), where despite having the deepest average standing water depth, the plots also had the lowest average gravimetric and volumetric soil moisture and highest average WFPS, owing to the relatively high soil bulk density of these plots (HG4 mean:  $1.38 \pm 0.05 \text{ g cm}^{-3}$ , overall wetland mean:  $1.12 \pm 0.05 \text{ g cm}^{-3}$ , one-sample t-test,  $n=3$ ,  $P=0.001$ ). The high bulk density of these soils may in turn explain the lower redox potential of these plots by the limited oxygen availability in low porosity soil (Grabel and Seimer 1968; Powell and Bouchard 2010). This is in contrast to other created wetland plots like BR 3, and natural plots like BFP 1 and BFP 3 of HG5, where surface and sub-surface hydrology are closely

linked likely as a result of them having the lowest soil bulk density (HG5 also had highest HC, SM, and SMVAR scores). While the SMVAR component had the lowest explanatory power of hydrologic variance across wetlands, it added an important temporal variability measure that indicated soil moisture fluctuation on a monthly basis.

### *5.2 The effects of hydrology on N flux rates*

Total N sedimentation in this study increased with greater HC (Figure 3a). While TN sedimentation was a variable used to define the HC component, identifying the positive relationship between HC and TN sedimentation is useful in examining patterns between N inputs through sedimentation and other hydrologic variables within the collective HC index. It is also useful in validating HC as a measure of the hydrologic openness of a wetland (along with TC sedimentation and total mass sedimentation), as other HC variables are useful indicators for hydrologic pulsing (i.e. variability of standing water and redox potential). This relationship supports the idea that wetlands that are disconnected from surrounding hydrology receive less sediment and associated nutrients and diminished hydrologic pulsing to stimulate biogeochemical cycles (Hopkinson 1992, Richardson et al. 2004, Noe and Hupp 2005). For instance, HG1 plots received little to no flooding during the study, and thus, had the least TN sedimentation and lowest HC (Table 2), probably due to the deep incision of Goose Creek relative to these natural floodplain plots at Banshee Reeks Nature Preserve. These plots did receive considerable fresh leaf litter material deposited on the sedimentation tiles; however, this likely autochthonous material was not sampled or included as sediment accumulation. HG5, in contrast, received the highest average TN sedimentation as a result of the group having the highest



HC, in addition to the highest SM and SMVAR (Table 2). HG5 was mostly composed of natural wetland plots in the floodplain of Bull Run at Manassas National Battlefield Park. Large differences in the functioning of natural wetlands were demonstrated by the contrast in hydrology and N sedimentation inputs between these two natural wetland sites. Total N sedimentation fluxes were strongly correlated with TC sedimentation fluxes, but not sediment mass accumulation, indicating that the dominant source of N was associated with organic matter and not mineral sediment.

The range of annual TN sedimentation was  $0.6 - 36.0 \text{ g-N m}^{-2} \text{ yr}^{-1}$  for all study plots. LC plots had some filamentous algal growth during the last month of collection (July 2009), which resulted in elevated TN sedimentation averages. BR 3 and BFP 1 had particularly high TN sedimentation ( $36.0$  and  $12.3 \text{ g-N m}^{-2} \text{ yr}^{-1}$ , respectively), as they were the most hydrologically open wetland plots of the study. The range of annual TN sedimentation for the remaining 12 plots was  $0.6 - 4.9 \text{ g-N m}^{-2} \text{ yr}^{-1}$ , which is at the lower end of the range found for open floodplain systems ( $1.4 - 8.0 \text{ g-N m}^{-2} \text{ yr}^{-1}$ ; Craft and Casey 2000) and within the range found for closed depressional wetland systems ( $1.5 - 5.3 \text{ g-N m}^{-2} \text{ yr}^{-1}$ ; Craft and Casey 2000). The range of TN sedimentation for all plots in this study spanned the range found for hydrologically connected riparian wetlands ( $4.2 - 13.4 \text{ g-N m}^{-2} \text{ yr}^{-1}$ ) (with lower minimum values) to hydrologically disconnected riparian wetlands ( $3.5 - 4.8 \text{ g-N m}^{-2} \text{ yr}^{-1}$ ) (with higher maximum values) of Virginia and Maryland (Noe and Hupp 2005). The wetlands in this study represent a wide range of sedimentation regimes that when paired with associated hydrologic inputs indicate a gradient of hydrologic openness. Some wetland plots demonstrated negligible amounts of

sedimentation throughout the course of the study and can be considered hydrologically closed systems, while others had a steady source of monthly sediment deposition and can be considered open systems.

Ammonification rates also increased with greater HC in this study (Figure 3b). The relationship between HC and net  $\text{NH}_4^+$  production is dynamic: Although an increase in standing water and subsequent decrease in redox potential creates a reducing environment that favors the persistence of  $\text{NH}_4^+$  as the primary N mineralization product (Reddy and Patrick 1984), organic N is more efficiently mineralized under aerobic conditions (Ponnamperuma 1972, Brinson et al. 1981, Bridgham et al. 1998). Decreases in standing water during drawdown periods generally favor  $\text{NO}_3^-$  production; however, the increased rate of aerobic decomposition and  $\text{NH}_4^+$  production may have compensated for losses by nitrification. While constituent variables within the HC index may have varying effects on ammonification, their combined effect as HC supports greater rates of this N cycling process.

Ammonification was positively correlated with TN sedimentation. This supports the idea that wetlands that are connected to surrounding hydrology, notably in the form of a stream for this study, receive important nutrient input from stream bank overflow. Ammonium production increased with TN sedimentation likely as a result of the increased mineralizable N substrate and energy in the form of labile C. This allochthonous subsidy prevents the exhaustion of material and energy supplies that occurs with the continuous cycling of autochthonous matter in closed wetland ecosystems that favor immobilization over ammonification (Reddy and DeLaune 2008).

Ammonification was also positively correlated with N mineralization, as it was the primary contributor to net N mineralization. Ammonification was also positively correlated with denitrification potential, which may be due to the increase in inorganic N substrate availability (as more  $\text{NH}_4^+$  becomes available for nitrification) and/or due to the anaerobic redox conditions that support both of these processes.

HG1 had the lowest rate of ammonification, likely because the group did not have standing water or significant TN sedimentation for the duration of the study, and because it had the highest redox potential of all groups. Because there were sufficient soil oxygen levels for the oxidation of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  and little variability in the borderline aerobic redox level (mV= +291.45) to retain inorganic N in its reduced form, production and/or persistence of  $\text{NH}_4^+$  at these plots was limited. In contrast, HG2 had the highest rates of ammonification (and conversely lowest rates of nitrification), likely due to the fact that plots within it received more C and N deposition as mineralizable substrate, a variable hydroperiod to enhance organic decomposition, and thus, organic N mineralization, and adequate soil moisture to retain  $\text{NH}_4^+$  with little loss by conversion to  $\text{NO}_3^-$ .

Ammonification rate patterns were similar to those of Hefting et al. (2004) that found that higher water table levels (within -10 cm of the soil surface) favored ammonification and fully inhibited nitrification.

Nitrification was significantly predicted at  $\alpha=0.10$  by SM (Figure 4a). Nitrification increased with average GSM and redox and decreased with WFPS, probably as a result of the increased air-filled porosity of low WFPS soils that enabled oxygen transport into the soil matrix. Nitrification was also positively correlated with

denitrification potential, which is to be expected, as increases in  $\text{NO}_3^-$  substrate availability should support higher levels of denitrification. HG4 had the lowest nitrification rate, probably due to the markedly high bulk density and low redox potential, which excludes both water and oxygen from pore space, resulting in the highest WFPS. HG1 had the highest rate of nitrification, again, due to this group's high redox potential. Nitrification rates in this study were within range of those found in natural and restored saltwater marshes in North Carolina (Thompson et al. 1995). As saltwater marshes tend to be highly anoxic, and thus, nitrate deficient, this highlights the particularly low rate of nitrification in the created wetlands in this study. Percent nitrification (of net N mineralization) for wetlands, excluding BSR, was low, averaging 20.7% (range -17.3 to 54.0%) and was similar to bogs and acidic fens of northern Minnesota (Bridgham et al. 1998), owing to the wetter hydrologic regime of those study wetlands.

Nitrogen mineralization was not predicted by any of the PCA components or individual hydrologic variables. Nitrogen mineralization is calculated as the sum of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  production. Considering that nitrification and ammonification are each facilitated by dissimilar hydrologic conditions – dry aerobic and wet anaerobic regimes, respectively – this may explain the lack of correlation between net N mineralization and the hydrologic variables.

Nitrogen mineralization rates across HGs followed the same pattern as ammonification rates – highest for HG2 and lowest for HG1 – probably because ammonification comprised a much larger percentage of total N mineralization than nitrification in created wetlands (89%), which are heavily weighted in this study design.

Net N mineralization rates for this study were lower than those found in a restored riparian wetland in Maryland (Verhoeven et al. 2001), but similar to those found in natural temperate wetlands (Zhu and Ehrenfeld 1999, Fickbohm and Zhu 2006, Wassen and Olde Venterink 2006). Using the same *in situ* modified resin core technique, net N mineralization rates were similar to the range found in another natural floodplain wetland in the Virginia Piedmont (Noe, in press).

The SM component, which includes a positive association with soil redox, was a significant predictor of DEA (Figure 4b) and was positively correlated with nitrification at  $\alpha=0.10$ . Denitrification requires anaerobic conditions to reduce  $\text{NO}_3^-$  to  $\text{N}_2$ . Denitrification can only occur, however, so long as  $\text{NO}_3^-$  is available as a substrate. Considering the low proportion of ammonium that is nitrified in the created wetlands (nitrification was 11% of total N mineralization), it is likely that denitrifying microbes are limited by  $\text{NO}_3^-$  associated with low redox potential and low allochthonous N inputs in many of the created study wetlands. These results support those of Richardson et al. (2004), who found that denitrification rates significantly increase with the addition of  $\text{NO}_3^-$  and that hydrologic connectivity is a key component to delivering  $\text{NO}_3^-$  to floodplain wetlands. Study results also support similar studies where denitrification was positively correlated with redox potential (Verhoeven et al. 2001) and soil moisture (Schnabel et al. 1997, Hunter and Faulkner 2001, Groffman and Crawford 2003).

HG5 had the highest DEA rates, as well as the highest HC, SM, and SMVAR (Figure 2). The three plots that comprise this group were the most hydrologically dynamic of the study plots. BFP 1 and BFP 3 receive overbank flooding from Bull Run,

as well as runoff from a nearby semi-wooded slope and a berm that elevates a two-lane road above the wetland. These plots have some of the lowest bulk density values (BFP 1:  $0.70\pm 0.04 \text{ g cm}^{-3}$  and BFP 3:  $0.96\pm 0.05 \text{ g cm}^{-3}$ ) of the study plots, allowing water to percolate into the soil matrix and increase soil moisture. BR 3 is situated at the corner of the created wetland where Bull Run sharply bends around the perimeter of the wetland and the lower natural levee functions as a crevasse; consequently, the plot receives significantly higher sediment deposition and intensity of flooding, evidenced by the larger textured sands the plot received as sediment. The sand supplement to the plot paired with the lower bulk density of the soils ( $0.88\pm 0.05 \text{ g cm}^{-3}$ ) may aid in drainage at BR 3 and contribute to the higher variability of soil moisture and standing water depth found at this plot. The combination of soil and hydrologic variables at BFP 1, BFP 3, and BR 3 resulted in HG5 having some of the highest N fluxes in the study, including the highest DEA and TN sedimentation and second highest nitrification, ammonification, and N mineralization rates. This is probably the result of the high overbank and surface water flow transporting mineralizable substrate to the plots to enhance ammonification and fluctuating water levels that produce  $\text{NO}_3^-$  during dry periods and then denitrify  $\text{NO}_3^-$  during wet periods. Considering that denitrification potential was more than four times the rate of N mineralization, it is likely that there is an adequate microbial denitrifying community present in the study wetlands to denitrify any  $\text{NO}_3^-$  substrate that becomes available. The fact that HG4 had the lowest nitrification and DEA, HG5 had the second highest nitrification rate and highest DEA, and that nitrification and denitrification potential were found to be positively correlated suggests a close coupling of nitrification

and denitrification in these wetlands. Nitrate can become limited in closed wetland systems that do not receive a sufficient allochthonous source of organic N for ammonification or  $\text{NO}_3^-$  entering the wetland. In these closed systems, N cycling relies on an autochthonous organic N source as substrate for ammonification, which is the rate-limiting step in the N cycle. The increase in ammonification with greater HC indicates the importance of a hydrologically connected wetland design that can directly subsidize the system with allochthonous N and fluctuating hydrology to support enhanced  $\text{NH}_4^+$  production and subsequently facilitate enhanced nitrification and denitrification.

Denitrification enzyme activity rates for this study were higher than those found in restored, riverine wetlands in the mid-western U.S. (Orr et al. 2007), lower than those found in restored wetlands in Louisiana (Hunter and Faulkner 2001) and North Carolina (Bruland and Richardson 2005), and within range of those found in a created marsh receiving river water in Texas (Scott et al. 2008). Study DEA rates were within range of denitrification rates obtained using *in-situ* acetylene block technique for a created, riverine wetland in Ohio (Hernandez and Mitsch 2007). In comparison to denitrification rates of natural wetlands, DEA rates were lower than non-tidal riverine wetlands in the Virginia coastal plain (Pavel et al. 1996), mineral wetland soils in the central U.S. (D'Angelo and Reddy 1999), and European riverine wetlands (Hefting et al. 2004).

## **6. Conclusions**

Investigating the overall effects of hydrology on N fluxes in created and natural wetlands demonstrated that greater hydrologic connectivity can significantly increase TN

sedimentation and ammonification. The sediment received by hydrologically connected wetlands acts as a material and energy subsidy that can stimulate coupled N processing and removal. The fluctuating water level and redox potential associated with hydrologic connectivity facilitates N cycling by expediting the breakdown of organic N into  $\text{NH}_4^+$ , stimulating nitrification under aerobic conditions, and converting  $\text{NO}_3^-$  to  $\text{N}_2$  when conditions become anaerobic. If hydrologic variables remain static, certain N processes are enhanced, while other N processes are limited, and the N cycle as a whole will not function at its potential efficiency. For instance, if the wetland is created with an epiperched, perched design, the soil environment may become homogeneously anaerobic, and thus, unable to efficiently mineralize organic matter or produce  $\text{NO}_3^-$  as a denitrification substrate. Under these conditions, the wetland would be unable to intercept and permanently remove organic and inorganic N that may contribute to eutrophication in downstream systems, propagation of harmful invasive species, and losses in biodiversity. Overall, greater surface water connectivity increased N inputs, coupled N processes, and N removal in both created and natural wetlands. Designing a created wetland that is connected to the surrounding hydrologic system (beyond precipitation and overland surface flow) enables the wetland to experience the hydrologic pulses that characterize many natural systems and enables them to better mirror these systems in both structure and function. Incorporating hydrologic connectivity into the design of a created wetland, however, has certain tradeoffs associated with it, as there is an innate security in having a disconnected, clay-lined wetland that is essentially guaranteed to hold water. This “bathtub” hydrologic design typically ensures that the wetland will have sufficient



standing water for a certain percent of the growing season, as required by the Army Corps of Engineers guidelines for wetland creation and restoration. This highlights the disparity between the current regulations for wetland mitigation and the goal of creating a more natural hydrologic regime that supports N removal in created and restored wetlands. Furthermore, increased nutrient inputs could have the unintended impact of stimulating invasive species establishment and growth and decreasing biodiversity (Zedler and Kercher 2004), although this is not likely to be of great concern in newly created wetlands.

The results of this study indicate that enhancing N cycling in created and restored wetlands could be accomplished by siting and designing them in a way that incorporates sustainable wetland hydrology and establishes a suitable hydrologic connection to the surrounding watershed. Incorporating hydrologic connectivity into created and restored wetland design can maximize their important ecosystem services of water quality improvement and flood protection. As such, metrics of hydrologic connectivity could be included as part of the regulatory requirements for assessing hydrology in compensatory mitigation wetlands.

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Table 1. Hydrologic variables included in the three component PCA model for hydrologic variables. Component loading values for each hydrologic variable are provided in parentheses.

	<b>Component 1 (Hydrologic Connectivity)</b>	<b>Component 2 (Soil Moisture)</b>	<b>Component 3 (Soil Moisture Variation)</b>
CV redox potential (0.929)		Avg gravimetric soil moisture (0.931)	CV volumetric soil moisture (0.920)
CV standing water (0.798)		Avg volumetric soil moisture ( 0.764)	CV water-filled pore space (0.865)
Avg total nitrogen sedimentation (0.785)		Avg water-filled pore space (-0.738)	
Avg standing water (0.742)		Avg redox potential (0.552)	
Avg total carbon sedimentation (0.655)			
Avg sediment mass accumulation (0.565)			
% variance explained	29.3%	23.2 %	15.2%

Table 2. Hydrologic characteristics and nitrogen fluxes (mean  $\pm$  one standard error) for hydrologic groups (HG),  $n$  = number of plots.

	HG1 ( $n=2$ )	HG2 ( $n=4$ )	HG3 ( $n=6$ )	HG4 ( $n=3$ )	HG5 ( $n=3$ )
Gravimetric soil moisture (%)	60.20 $\pm$ 0.06	67.75 $\pm$ 0.03	47.17 $\pm$ 0.02	40.67 $\pm$ 0.02	76.00 $\pm$ 0.06
Volumetric soil moisture (%)	62.01 $\pm$ 0.03	63.10 $\pm$ 0.02	56.00 $\pm$ 0.01	57.33 $\pm$ 0.01	67.67 $\pm$ 0.02
CV Volumetric soil moisture	17.79 $\pm$ 1.86	9.81 $\pm$ 1.48	12.94 $\pm$ 1.20	7.83 $\pm$ 0.40	17.18 $\pm$ 2.53
Water-filled pore space (%)	98.50 $\pm$ 0.05	100.75 $\pm$ 0.04	111.67 $\pm$ 0.04	124.00 $\pm$ 0.07	99.67 $\pm$ 0.03
CV Water-filled pore space	16.48 $\pm$ 1.50	14.52 $\pm$ 1.23	18.45 $\pm$ 0.96	15.95 $\pm$ 1.63	18.54 $\pm$ 1.03
Standing water (cm)	0	2.27 $\pm$ 0.07	3.45 $\pm$ 0.94	5.53 $\pm$ 1.30	3.51 $\pm$ 1.36
CV Standing water	0	21.12 $\pm$ 8.58	111.83 $\pm$ 13.58	77.18 $\pm$ 8.18	137.79 $\pm$ 10.39
Annual sediment accumulation (g m <sup>-2</sup> )	208.00 $\pm$ 43.00	499.58 $\pm$ 206.10	283.0 $\pm$ 92.06	813.59 $\pm$ 293.74	4265.63 $\pm$ 3420.39
Total carbon sedimentation (g-C m <sup>-2</sup> mo <sup>-1</sup> )	1.06 $\pm$ 0.38	2.76 $\pm$ 1.06	4.27 $\pm$ 1.41	24.28 $\pm$ 5.53	22.74 $\pm$ 13.64
Total nitrogen sedimentation (g-N m <sup>-2</sup> mo <sup>-1</sup> )	0.15 $\pm$ 0.01	0.44 $\pm$ 0.51	0.40 $\pm$ 0.11	0.81 $\pm$ 0.24	5.35 $\pm$ 4.50
Redox potential (mV)	291.45 $\pm$ 0.25	250.76 $\pm$ 23.40	239.22 $\pm$ 10.38	160.92 $\pm$ 28.31	261.61 $\pm$ 7.28
CV Redox potential	22.17 $\pm$ 3.40	58.60 $\pm$ 6.36	64.54 $\pm$ 4.89	98.74 $\pm$ 15.07	73.03 $\pm$ 8.16
Bulk density (g-dw cm <sup>-3</sup> )	1.00 $\pm$ 0.02	0.91 $\pm$ 0.02	1.23 $\pm$ 0.04	1.38 $\pm$ 0.05	0.85 $\pm$ 0.09
Ammonification ( $\mu$ mol-N kg <sup>-1</sup> d <sup>-1</sup> )	-0.60 $\pm$ 0.06	24.80 $\pm$ 3.92	13.15 $\pm$ 3.79	19.80 $\pm$ 2.04	15.46 $\pm$ 2.43
Nitrification ( $\mu$ mol-N kg <sup>-1</sup> d <sup>-1</sup> )	8.10 $\pm$ 0.60	5.73 $\pm$ 1.61	4.65 $\pm$ 2.17	0.20 $\pm$ 1.09	6.80 $\pm$ 3.61
Nitrogen mineralization ( $\mu$ mol-N kg <sup>-1</sup> d <sup>-1</sup> )	7.45 $\pm$ 6.26	30.50 $\pm$ 4.25	17.77 $\pm$ 4.87	14.23 $\pm$ 3.21	26.63 $\pm$ 4.63
DEA ( $\mu$ mol-N kg <sup>-1</sup> d <sup>-1</sup> )	144.31 $\pm$ 19.34	175.03 $\pm$ 33.76	85.54 $\pm$ 12.51	59.47 $\pm$ 5.08	179.53 $\pm$ 34.61

## Figures Legend

Figure 1. Site map of wetland locations in the Virginia Piedmont, USA.

Figure 2. Scatter-plot of hydroconnectivity vs. soil moisture component scores per plot.

Symbols represent hydrologic group (HG) with empty symbols indicating natural wetlands. All HG2 symbols, denoted as “X”, are created wetlands.

Figure 3. Linear regression of hydroconnectivity component vs. (a) TN sedimentation

and (b) ammonification. Plot symbols indicate hydrologic group (HG) with empty symbols indicating natural wetlands. All HG2 symbols, denoted as “X”, are created wetlands.

Figure 4. Linear regression of soil moisture component vs. (a) nitrification and (b) DEA.

Plot symbols indicate hydrologic group (HG) with empty symbols indicating natural wetlands. All HG2 symbols, denoted as “X”, are created wetlands.

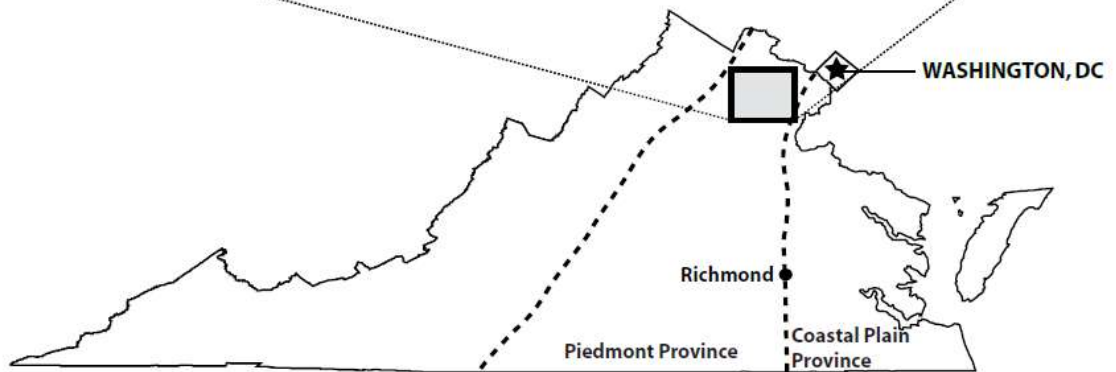
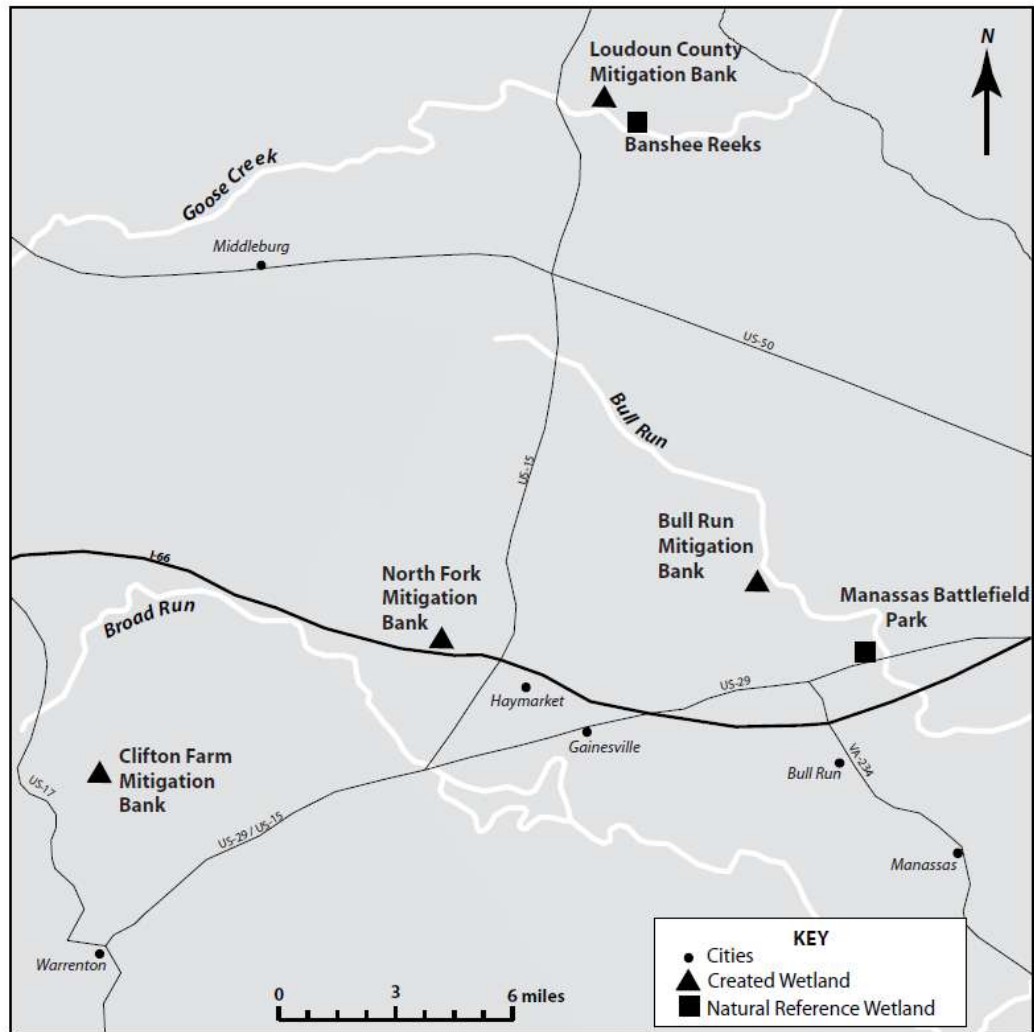


Figure 1.

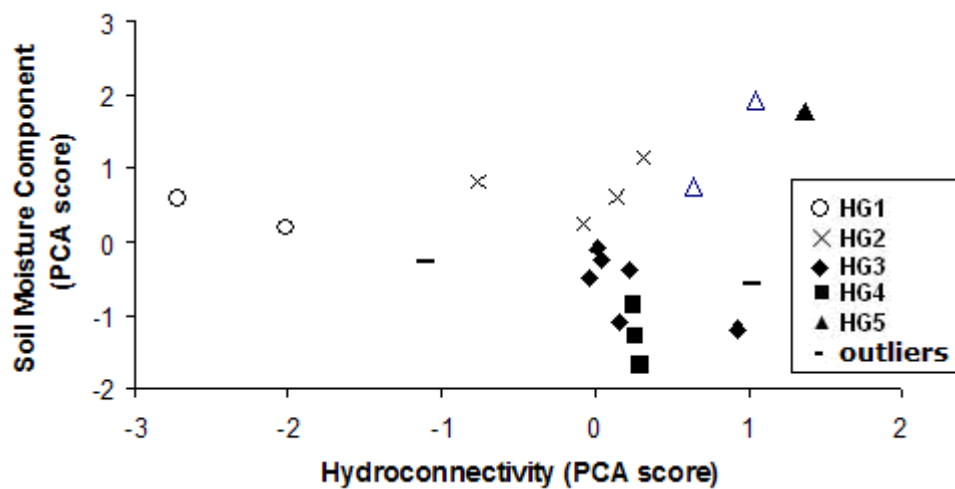


Figure 2.

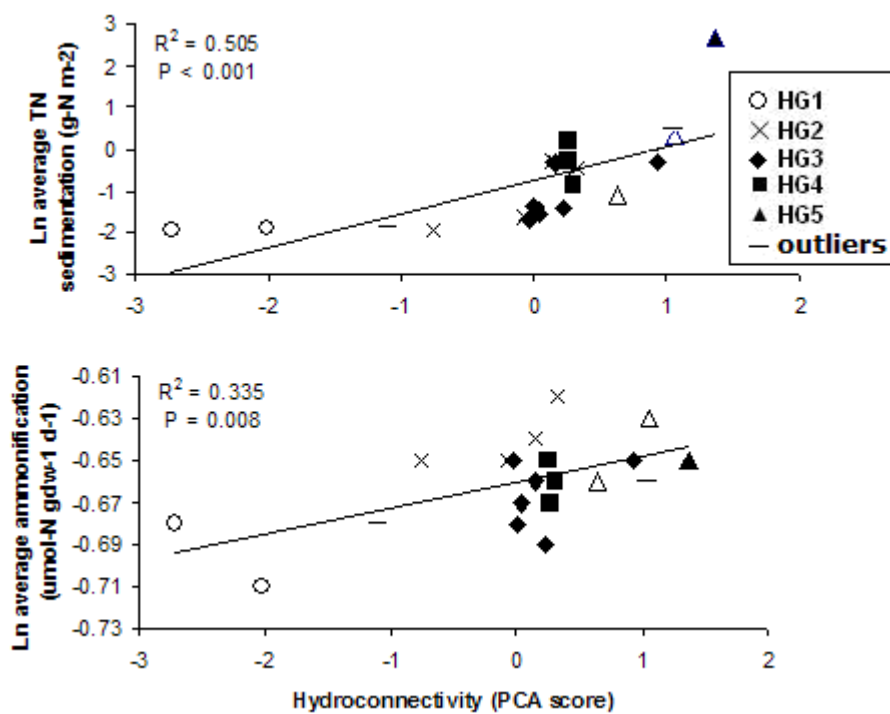


Figure 3

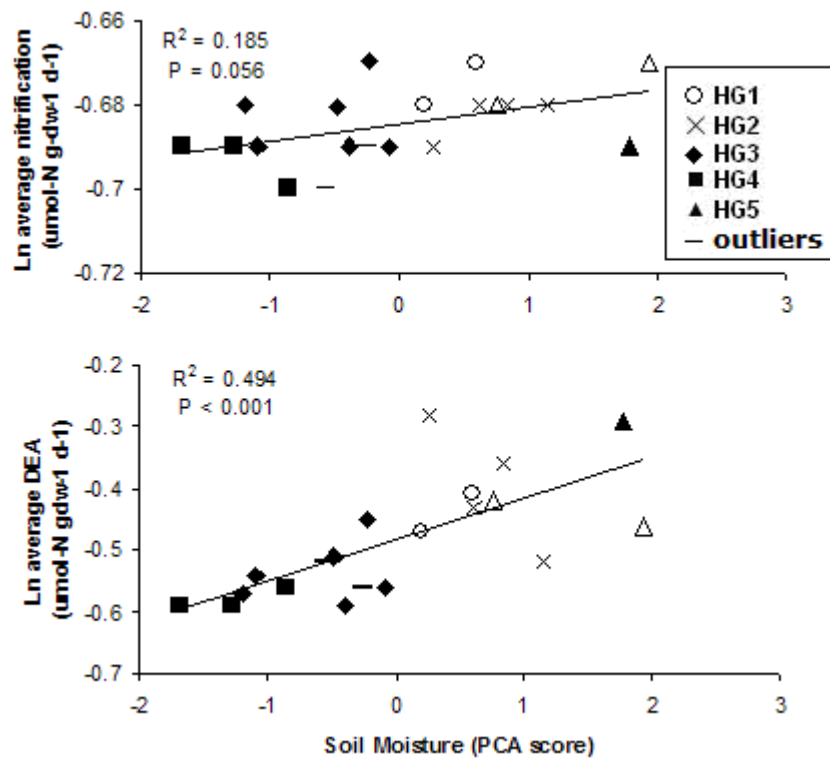


Figure 4.

## **Chapter 2: Development of soil properties and nitrogen cycling in created wetlands**

### **Abstract**

Mitigation wetlands are expected to compensate for the loss of structure and function of natural wetlands within 5 to 10 years of creation; however, the age-based trajectory of development in wetlands is unclear. This study investigates the development of coupled structural (soil properties) and functional (nitrogen cycling) attributes of created non-tidal freshwater wetlands of varying ages and natural reference wetlands to determine if created wetlands do in fact attain the important water quality ecosystem service of nitrogen (N) cycling over time. A principal component analysis was conducted to identify overall soil condition and soil texture indices. Soil condition index and its constituent variables, including gravimetric soil moisture, total organic carbon (TOC), and total nitrogen (TN) generally increased and bulk density decreased with age of the created wetland. Natural wetlands generally had higher overall soil condition, gravimetric soil moisture, TOC, and TN, as well as lower bulk density than created wetlands. Soil texture showed no significant relationship with age or between created and natural reference wetlands. Nitrogen flux rates also demonstrated age-related patterns, with younger created wetlands having lower rates of ammonification, nitrification, nitrogen mineralization, and denitrification potential than older created wetlands and natural



reference wetlands. Nitrification, mineralization, and denitrification potential each increased with greater soil condition index. Results show a clear age-related trajectory in coupled soil condition and N cycle development in created wetlands and demonstrate a close link between the development and maturation of soil properties and N cycling that is essential for water quality improvement. These findings can be used to enhance N processing in created wetlands and inform the regulatory evaluation of mitigation wetlands by identifying structural indicators of N processing performance.

## **1. Introduction**

Under Section 404 of the Clean Water Act and the 1990 Memorandum of Agreement between the Environmental Protection Agency and the Army Corps of Engineers, wetlands that are structurally or functionally impacted by development in the United States must be compensated for by the creation of additional wetlands or the restoration, enhancement, or preservation of existing ones (U.S. Environmental Protection Agency 1990). The practice of compensatory mitigation assumes that created and restored wetlands will replace losses in wetland structure and function within the 5 to 10 year monitoring period required by mitigation regulations (U.S. Army Corps of Engineers 2010). The developmental trajectory of mitigation wetlands, however, is highly variable (Simenstad and Thom 1996, Zedler and Callaway 1999, Morgan and Short 2002) and some wetlands never develop the structural or functional attributes of their natural counterparts (Erwin 1991, Kentula et al. 1992, Hoeltje and Cole 2007).

Created and restored wetlands have been shown to differ from natural wetlands in their hydrology (Confer and Niering 1992, Shaffer et al. 1999, Cole and Brooks 2002), vegetation (Confer and Niering 1992, Galatowitsch and van der Valk 1996, Campbell et al. 2002), and soil characteristics (Bishel-Machung 1996, Stolt et al. 2000, Verhoeven et al. 2001, Campbell et al. 2002, Bruland and Richardson 2005), and have been generally unsuccessful in meeting the performance criteria that have been legally mandated (National Research Council 2001).

Among the primary wetland components that are evaluated in mitigation projects, soils are often the least considered indicator of quality in created or restored wetlands (Shaffer and Ernst 1999, Ahn and Peralta 2009), despite their importance for successful ecosystem development. Soil properties such as organic matter can be an excellent indicator of soil quality (Bruland and Richardson 2006) as it is essential for many biogeochemical properties within the soil matrix. Organic matter should accumulate in wetlands with consecutive growing seasons, as vegetative seasonal dieback and litter fall contribute autogenic materials to the soil (Reddy and D'Angelo 1997). While organic matter has been shown to increase with age in created and restored wetlands (Ballentine and Schneider 2009), this is not always the case (Bishel-Machung 1996, Shaffer and Ernst 1999). The lack of organic carbon (C) accumulation, in particular, has been found as characteristic of created wetlands (Whittecar and Daniels 1999, Stolt et al. 2000, Hossler and Bouchard 2010), which has implications for vegetation (Stauffer and Brooks 1997), microbial communities (Duncan and Groffman 1994, Ahn and Peralta 2009), and C and nitrogen (N) cycling (Bruland et al. 2009, Sutton-Grier et al. 2009, Kayranli et al.

2010). In general, the age-based trajectory for soil development in created and restored wetlands is unclear.

Proper development of the structural attributes of wetland soil is critical for the development of more complex functional attributes of wetlands. For instance, the structural maturation of created wetland soils is important because it is the physical substrate for soil N cycling. The N cycle, in turn, provides the vital function of excess N removal from the landscape, which can prevent the eutrophication of downstream systems (Vitousek et al. 1997; Saunders and Kalff; 2001; Galloway et al. 2003). The development of soil properties in created and restored wetlands should demonstrate considerable influence over how these managed systems process N, especially with regard to soil quality and redox condition. The primary component processes of the N cycle include the conversion of organic N to ammonium ( $\text{NH}_4^+$ ) by ammonification, the transformation of  $\text{NH}_4^+$  to nitrate ( $\text{NO}_3^-$ ) by nitrification, and the conversion of  $\text{NO}_3^-$  to dinitrogen gas ( $\text{N}_2$ ) by denitrification. Each of these N processes requires an energy source in the form of labile C in addition to their respective N substrates (Reddy and Patrick 1984), which depend upon the availability of these elements in the soil. N processing also requires fluctuating redox conditions, specifically, aerobic conditions for expedited ammonification and nitrification, followed by anaerobic conditions for  $\text{NO}_3^-$  removal by denitrification (Ponnamperuma 1972, Reddy and Patrick 1984). The redox environment under which each of these processes occurs is again largely dependent on soil characteristics.

Considering the obvious link between soil properties and coupled N processes, the design, construction, and maturation of mitigation wetlands may impact these elements concurrently. For instance, heavy machinery used in the creation of wetlands can compact soils, reduce soil porosity (Stolt et al. 2000, Bruland and Richardson 2005) and associated redox potential, and inhibit effective N mineralization (ammonification + nitrification). Additionally, the altered texture of created wetland soils (Shaffer and Ernst 1999, Whittecar and Daniels 1999, Stolt et al. 2000, Campbell et al. 2002, Hossler and Bouchard 2010), paired with their higher bulk density (Bishel-Machung et al. 1996, Campbell et al. 2002, Hunter et al. 2008, Hossler and Bouchard 2010), affects N cycling by decreasing water-holding capacity and altering water and nutrient distribution throughout the wetland (Bruland and Richardson 2005). Furthermore, the incorporation of organic material into the soils of newly created wetlands can be age-related (Craft 1997), and the speed with and degree to which this occurs depends on autochthonous inputs from extant vegetation (Atkinson and Cairns 2001, Ballentine and Schneider 2009), allochthonous inputs from surface runoff and overbank flooding, temperature, hydrology, and other variables (Bowden 1987, Reddy and D'Angelo 1997, Kayranli et al. 2010). Despite the many connections that can be made between age, soil characteristics, and the coupled N cycle, their relationship within created and restored wetlands has yet to be determined. Understanding the timeline by which age-related soil properties develop and N processing functions mature has important wetland design and regulatory implications.

This study investigates the effects of age-related soil properties on N flux rates in created compared to natural non-tidal freshwater wetlands in the Piedmont physiographic province of Virginia. Soil properties and N fluxes were compared among two natural reference wetlands and four created wetlands, aged three, four, seven, and ten years, and the influence of soil properties on N fluxes was identified. The study focused on the following research questions:

- (1) How do soil properties in created wetlands develop over time?
- (2) How do N flux rates differ between created wetlands of different ages and between created and natural wetlands?
- (3) How do these age-related soil properties influence N flux rates in created and natural reference wetlands?

## **2. Site Descriptions**

### *2.1 General setting*

All study sites were non-tidal freshwater wetlands located in the northern Virginia Piedmont physiographic province (mean annual precipitation 40 cm, mean temperature min 6°C / max 19°C) (Figure 1). The mitigation wetland banks were created to mitigate for various local construction projects that impacted a mixture of bottomland forested floodplain, shrub/scrub, and emergent wetlands and open water ponds. All of the created

wetlands are under 11 years old and have predominantly herbaceous cover, with some open water aquatic areas, shrub-scrub areas, and/or young stands of trees. The created wetlands are intended to mature into a mixture of bottomland forested, shrub/scrub, and emergent wetlands and open water areas similar to those they were intended to replace. The natural wetlands include bottomland forested riparian wetlands and open herbaceous riparian wetlands.

## *2.2 Created wetlands*

Loudoun County Mitigation Bank (LC) is a 32-acre wetland and upland buffer complex, constructed by Wetland Studies and Solutions, Inc. (WSSI) in the summer of 2006 in Loudoun County, Virginia (39°1'58.98" N, 77°36'26.10" W). LC receives surface water runoff from an upland housing development and forested buffer, as well as minor groundwater inputs from toe-slope intercept seepage. Vegetation is currently dominated by herbaceous plants with small, container-grown, planted woody vegetation interspersed throughout.

Clifton Farm (CF) is a 2.14-acre mitigation wetland, constructed by Angler Environmental in 2005 in Fauquier County, Virginia (38°46'38.75" N, 77°47'40.61" W). The site receives groundwater from a small upland reservoir and surface water runoff, but has no stream connection. Vegetation is herbaceous with planted, woody vegetation throughout.

Bull Run Mitigation Bank (BR) is a 50-acre wetland and upland buffer complex, constructed by WSSI in 2002 in Prince William County, Virginia (38°51'12.74" N, 77°32'58.52" W). The site receives water from Bull Run from an AgriDrain structure that routes water via a central ditch through the wetland , as well as overbank flow from Bull Run (average of one overflow event per year (WSSI 2004)), which sharply bends around the corner of the site. The wetland receives limited surface water runoff from wetlands and negligible groundwater. Vegetation is predominantly herbaceous, with small, planted woody vegetation throughout.

North Fork Wetlands Bank (NF) is a 125-acre wetland, constructed by WSSI in 1999 in Prince William County, Virginia (38°49'31.53" N, 77°40'9.17" W). With the exception of minor contributions from toe-slope intercept seepage, the site is disconnected from the groundwater by an underlying clay liner and is hydrologically divided into four areas: main pod area – fed by upland surface water runoff and a tributary of the North Fork of Broad Run that is controlled by an artificial dam; overbank flow area – fed by overbank flow from the tributary; vernal pool area – located in the southwest quadrant of the wetland and fed solely by precipitation; and depressional wetland area – fed by surface runoff from the adjacent Boy Scout campgrounds. Vegetation is mostly herbaceous, interspersed with young tree saplings and shrubs in projected forested areas, patches of shrub/scrub and emergent vegetation, and aquatic plants in the open water area.

The wetlands designed by WSSI (LC, BR, and NF) contain at least a 0.3 m low permeability subsoil layer covered with the original topsoil from the site that was

supplemented with commercially available topsoil to a depth of 0.2 m. This design creates a perched, surface-driven water table close to the soil surface and limits groundwater exchange in the wetland (Ahn and Peralta 2009). These wetlands or portions thereof were also disked during construction in order to incorporate microtopography, which has been found to increase plant diversity and species richness (Moser et al. 2007), as well as moisture content, nutrient retention, and nutrient heterogeneity (Moser et al. 2009) in these study wetlands.

### *2.3 Natural wetlands*

Manassas National Battlefield Park (BFP), established in 1940, is a 5,000 acre site with areas of natural wetland coverage located in Prince William County, Virginia (38°49'24.98" N, 77°30'28.30" W). An area of herbaceous wetland within a matrix of forested floodplain was selected for study and comparison to the created wetlands. The site is connected to Bull Run by a culvert on its eastern end and also receives groundwater and upland surface water runoff. Vegetation is mostly herbaceous with a few mature trees interspersed throughout.

Banshee Reeks Nature Preserve (BSR), established 1999, is a 725-acre site with areas of seep and riparian wetlands located in Loudoun County, Virginia (39°1'16.44" N, 77°35'49.10" W). These floodplain riparian wetlands receive water from groundwater springs, surface water runoff, and occasional overbank flooding from Goose Creek. Vegetation is mature bottomland forest with little understory.



### 3. Methods

#### 3.1 Sampling design

Study plots at the four created and two natural wetlands were selected so that typical soils, hydrology, vegetation, and any experimental manipulation (disking) of the wetland site was represented. At the 3 yr old wetland (LC) three disked plots, paired with three undisked plots, were selected for a site total of six plots. At the 4 yr old wetland (CF) two plots, one on either side of a central berm, were selected. At the 7 yr old wetland (BR) four plots arranged perpendicularly to the overbank flowpath were selected. At the 10 yr old wetland (NF) two plots in the main pod area and two plots in the vernal pool area were selected. At BFP natural wetland two plots in an open, herbaceous area were selected, and at BSR natural wetland two plots in a forested riparian area were selected, for a total of 20 plots. Soil sampling in the wetlands occurred over a two-day period the second week of every month from July 2008 to July 2009.

#### 3.2 N mineralization

Soil net N mineralization was measured *in situ* using a modification of the DiStefano and Gholz (1986) resin core technique for use in wetlands that was developed by Noe (in press). The method incubates in place a soil core with six ion-exchange resin

bead bags, three placed above and three below the soil core, to quantify inorganic N loading to the soil core (two outer resin bags) and production from inside the intact soil core (two inner resin bags + soil core) in hydrologically dynamic wetlands soils. The two middle bags were used as a quality control check to ensure that inner and outer bags were not saturated with ions and incapable of trapping nutrients. A preliminary study has shown that the outer and inner bags are sufficient for removing all dissolved inorganic N from water entering and leaving the modified resin core in wetland soils (Noe, in press).

Study plots were sampled by randomly placing a 1-m<sup>2</sup> quadrat at the beginning of the study that divided the sampling area into 100 10-cm<sup>2</sup> cells. The surficial soil (0-5 cm) of two adjacent 10 cm<sup>2</sup> cells were randomly sampled each month each with a beveled, thin-walled, PVC core tube, 7.8 cm in diameter and 11 cm in length, that was driven into the ground until the soil surface was 3 cm below the top of the tube. Cores were then removed, and the excess 3 cm of soil on the bottom of the core was scraped out, resulting in a soil core depth of 5 cm and a soil volume of 251.3 cm<sup>3</sup> in the middle of the core tube. The first core was processed as an initial core and placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within 48 hours to yield initial 2M KCl extractable soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations (Keeney and Nelson 1982). The initial core was also used for soil characterization and denitrification flux measurements (see below). The second core was processed as a resin core by placing 3 resin bags each on the top and bottom of the extracted soil core. Each resin bag was constructed using an undyed nylon stocking (Kayser-Roth, Inc., Greensboro, North Carolina, USA) stretched over a 7.6 outer diameter, nitrile O-ring (Macro Rubber & Plastics Products, Inc., North

Andover, Massachusetts, USA), filled with mixed-bed, ion-exchange resin beads (Rexyn I-300, Fisher Scientific, Pittsburgh, Pennsylvania, USA), and closed with two stainless steel staples. The two outer and middle bags each contained 20 g of resin and the two inner bags contained 30 g of resin (necessitated by the higher rate of capture by inner bags in a pilot study). Each bag was sealed into the core along its outer edge with a silicone caulk free of anti-microbial agents (Silicone I rubber sealant, General Electric Co., Fairfield, Connecticut, USA) to prevent leakage and flow bypass around the resin. Wire supports were added over the outer bags on both the top and bottom of the core tube to keep the bags in place, and the resin core was inserted back into the core hole and made flush with the surrounding soil surface. Resin cores were then incubated *in situ* for approximately one month (range of 26 to 34 days) and then harvested, placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within one week. Monthly estimates of net ammonification and nitrification were calculated as the sum of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively, in the soil core and two inner bags in the resin core compared to the initial core. Net N mineralization was calculated as the sum of ammonification and nitrification relative to the dry mass of soil in the resin core. We present cumulative annual flux calculated as the sum of monthly incubations: (resin core soil  $\mu\text{mol}$  + 2 inner resin bags  $\mu\text{mol}$  – initial core soil  $\mu\text{mol}$ ) / ((total incubation days x (plot mean bulk density ( $\text{kg-dw cm}^{-3}$ ) x volume of soil core ( $251.3 \text{ cm}^3$ ))) for  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_3^- + \text{NH}_4^+$  (N mineralization) expressed as  $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$ .

### 3.3 Denitrification enzyme assay (DEA)

Monthly denitrification potential was determined for each initial core using the denitrification enzyme assay (DEA) procedure (Smith and Tiedje 1979; Tiejde et al 1989; Groffman 1999). Assays were run in duplicate and consisted of a slurry of 25 g of homogenized field-moist soil and a 25 mL solution of 1 mM glucose, 1 mM KNO<sub>3</sub>, and 1 g L<sup>-1</sup> chloramphenicol mixed in a 125 ml Erlenmeyer flask. Flasks were sealed with rubber septa, made anaerobic by bubbling the slurry for 10 min followed by a 1 min headspace flush with N<sub>2</sub> gas, and evacuated with a vacuum pump. Assays were brought to atmospheric pressure with N<sub>2</sub> gas and injected with H<sub>2</sub>SO<sub>4</sub> scrubbed acetylene (modified from Hyman and Arp 1979) to 10% of the volume of the flask headspace. Flasks were then incubated on a rotary shaker table with gas samples taken at 30 and 90 min. Gas samples were stored in freshly evacuated 2 mL glass vacutainer vials (Tyco Healthcare Group LP, Mansfield, MA, USA) until they could be analyzed for N<sub>2</sub>O on a Shimadzu 8A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with electron capture detection, generally within three days of sampling. DEA was calculated as:  $M = C_g * (V_g + V_l \times \beta)$  where, M = total amount of N<sub>2</sub>O in water plus gas phase ( $\mu\text{g N}_2\text{O-N}$ ),  $C_g$  = concentration of N<sub>2</sub>O in the gas phase ( $\mu\text{g N}_2\text{O-N/L}$ ),  $V_g$  = volume of the gas phase (L),  $V_l$  = volume of liquid phase (L), and  $\beta$  = Bunsen coefficient (0.544 @ 25°C) and expressed as  $\mu\text{mol-N kg-dw}^{-1} \text{d}^{-1}$ .

### *3.4 Soil physicochemical properties*

#### *3.4.1 Field analysis*

Redox potential was measured each month by inserting a RE 300 ExStik<sup>®</sup> ORP meter (Extech Instruments Corporation, Waltham, Massachusetts, USA) to a depth of approximately 3 cm into the soil between the initial core and resin core sampling locations. Redox potential was recorded after drift was sufficiently stabilized (approximately 1 min). Ceramic sedimentation tiles (20 x 20 cm) were installed monthly at an adjacent location that was representative of each plot, yet avoided any microtopographic or vegetative irregularities. Deposited sediment (excluding coarse woody debris and litter free of mineral sediment) was harvested from the tiles during each collection period for subsequent elemental analysis.

#### *3.4.2 Laboratory analysis*

Gravimetric soil moisture (GSM) was determined for each initial core and resin core by removing a ~40 g dry-weight equivalent (dw-eq) subsample of homogenized soil, recording initial field-moist weight, and drying at 60°C until a constant weight was achieved. Bulk density (BD) was determined for each core by first weighing the entire field-moist core, converting to dry weight based on GSM percentage, and dividing by the total volume of the soil in the core (251.3 cm<sup>3</sup>). Volumetric (VSM) was calculated as BD x (GSM / density of water, assuming 1.0 g-H<sub>2</sub>O mL<sup>-1</sup>). Water-filled pore space (WFPS) was calculated as  $VSM / [(1 - (BD / \text{quartz parent material density, assuming } 2.65 \text{ g cm}^{-3}))]$ .

Total soil C and N was determined by dry combustion of oven-dried, ground subsamples from each core on a 2400 Series II CHN/O elemental analyzer (Perkin-Elmer, Waltham, Massachusetts). Total soil organic carbon (TOC) was determined by HCl vapor digestion followed by dry combustion on an elemental analyzer. Sediment collected from sedimentation tiles was oven dried and weighed to determine monthly accumulation, and ground, subsampled, and analyzed for total C and N concentration on the elemental analyzer (Nelson and Sommers 1996). Cumulative monthly sediment, C, and N deposition rates were calculated for each plot. Soil texture analysis was conducted using a LISST-100X laser particle size analyzer (Sequoia Scientific, Inc., Bellevue, Washington, USA). A mixture of 0.02 g of well-mixed soil (combusted at 550°C for 4 hours to remove organics and then sieved to  $\leq 250 \mu\text{m}$ ), 10 mL sodium hexametaphosphate solution (50 g NaHMP/L), and 90 mL deionized water (final soil concentration of 200 mg/L) was placed in an ultrasonic bath for 5 minutes and then agitated on a shaker table at 100 rpm for 16 hours in order to breakup soil aggregates before being analyzed on the LISST-100X fitted with a stirring mixing chamber (Gee and Bauder 1986). The LISST-100X reports particle volume concentrations for 32 log-spaced size classes ranging in size from 1.2 to 250  $\mu\text{m}$ . Median particle size (D50) was interpolated from the cumulative size distribution of LISST output. The percent clay ( $< 2 \mu\text{m}$ ), silt (2 – 50  $\mu\text{m}$ ), and fine sands (50 – 250  $\mu\text{m}$ ) were determined from their appropriate LISST-100X output size classes (Gee and Bauder 1986, U.S. Department of Agriculture definition), corrected for the amount of soil that was retained on the 250- $\mu\text{m}$  sieve. Extractable  $\text{NO}_3^-$  and  $\text{NH}_4^+$  was determined for each initial and resin soil core and resin bag, within 48 hours of

collection, by adding 40 ml of 2 M KCl to a 4 g dw-eq subsample of soil or 2 g ww subsample of resin beads, agitating on a shaker table for 1 h, and centrifuging (soil samples only) at 2500 rpm for 5 minutes. The supernatant was then filtered through a syringe with a 0.2  $\mu\text{m}$  polyethersulfone filter tip (Pall Corporation, Port Washington, New York, USA) and analyzed on an Astoria 3020 series segmented flow autoanalyzer (Astoria-Pacific International, Clackamas, Oregon, USA). External reference standards and sample blanks (empty extraction vessel for soil blanks or extraction vessel with new resin for resin bead blanks) were included in each monthly run to validate results and to correct incubated resin and soil extractable concentrations, respectively.

### *3.5 Statistical analysis*

All data were reviewed for normality and those variables failing tests for normality were transformed, usually by natural log transformation. A principal component analysis (PCA) was conducted on all soil parameters to identify underlying multivariate components that may be influencing N fluxes. The final model was produced using a varimax rotated component matrix with an eigenvalue cutoff of 1.0. One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post-hoc test was used to determine significant mean differences in TOC, TN, bulk density, and gravimetric soil moisture variables, as well as soil condition and texture PCA components between wetlands. Principal component analysis scores were then entered as independent variables into a regression analysis to determine the influence of each

component on ammonification, nitrification, net N mineralization, and DEA. Pearson Product-moment correlation matrices were used for further investigation of univariate relationships with nutrient fluxes. All statistical tests were performed using SPSS version 15 (SPSS 2006), and tests were considered significant at  $\alpha = 0.05$ , unless otherwise noted.

## **4. Results**

### *4.1 Identification of soil condition and texture indices*

The PCA resulted in a two component model that explained a cumulative 83% of total variance in soil structural variables (Table 1). Component 1 explained 45.5% of total variance and consisted of variables that characterized soil condition index (SC), including higher TOC, TN, and gravimetric soil moisture and lower bulk density. Component 2 explained 37.5% of total variance and consisted of variables that characterized soil particle size and redox index, specifically, higher sand percentage and higher redox potential, larger soil particle diameter, and lower clay content. Soil texture classification was determined as silt loam for all wetland plots, with the exception of NF 15 and LC E, which were classified as sandy loam and silty clay loam, respectively.

### *4.2 How do soil properties in created wetlands develop over time?*



Natural wetlands and the 7 yr old wetland had the highest SC scores (one-way ANOVA,  $df = 5, 14$ ,  $P < 0.001$ , Figure 2a), followed by the 10 yr old wetland, and then the 3 and 4 yr old wetlands, which had significantly lower SC scores than the other wetlands. An expectedly similar pattern was apparent across wetland ages for soil variables that comprised the SC component; gravimetric soil moisture, TOC, and TN were highest for the 7 yr old and natural wetlands, followed by the 10 yr old wetland, and then the 3 and 4 yr old wetlands, which had the lowest values ( $P < 0.001$ , Figure 2b;  $P < 0.001$ , Figure 2d;  $P < 0.001$ , Figure 2e; respectively). Bulk density followed the opposite pattern in that 3 and 4 yr old wetlands had the highest bulk density, followed by the 10 yr old and BSR natural wetlands, and finally the 7 yr old and BFP natural wetlands, which had the lowest bulk density ( $P < 0.001$ , Figure 2c). The soil particle size and redox component scores and variables that comprised this component ( $P > 0.05$ , data not shown) showed no significant differences across wetland ages (Table 2 and Figure 2f,  $P = 0.46$ ).

#### *4.3 How do N flux rates differ between created wetlands of different ages and between created and natural wetlands?*

Soil net ammonification rates were generally greater in older created wetlands and one of the natural wetlands, but were also highly variable along the age trajectory to natural wetlands ( $P = 0.008$ , Figure 3a). Seven yr old and BFP natural wetlands had the highest ammonification rates, followed by 10, 3, and 4 yr old wetlands, and BSR natural wetland, which had the lowest ammonification rate (one-way ANOVA,  $df = 5, 14$ , Figure

3a). Net N mineralization, the sum of net ammonification and net nitrification, showed the same type of pattern as ammonification among the wetland sites ( $P=0.015$ , Figure 3c). The similarities and differences in pattern between ammonification and net N mineralization was due to the relatively low nitrification rates, which when added to ammonification changed the pattern for net N mineralization slightly and added greater variability.

Net nitrification rates had the clearest age-related trajectory (Figure 3b). Natural wetlands had the highest nitrification rates, followed by 7 and 10 yr old wetlands, and 3 and 4 yr old wetlands, which had the lowest nitrification rates ( $P=0.023$ , Figure 3b). Potential denitrification rates had a step pattern with age (Figure 3d). Three and 4 yr old wetlands had significantly lower DEA rates than 7 and 10 yr old and natural wetlands ( $P=0.009$ , Figure 3d).

#### *4.4 How do these age-related soil properties influence N flux rates in created and natural reference wetlands?*

Ammonification was not significantly influenced by SC (Linear regression,  $n=20$ ,  $R^2=0.154$ ,  $P=0.087$ , Figure 4a), but was positively correlated with TOC (Pearson product-moment correlation,  $n=20$ ,  $r=0.449$ ,  $P=0.047$ ) within the SC component. Ammonification was also positively correlated with TC sedimentation ( $r=0.860$ ,  $P<0.001$ ). The SC component was a significant positive predictor of nitrification (Figure 4b). Within the SC component, nitrification was positively correlated with TOC ( $r=0.532$ ,  $P=0.016$ ), TN ( $r=0.591$ ,  $P=0.006$ ), and gravimetric soil moisture ( $r=0.460$ ,

$P=0.041$ ), and negatively correlated with bulk density ( $r=-0.569$ ,  $P=0.009$ ). Nitrification was also positively correlated with redox potential ( $r=0.460$ ,  $P=0.041$ ), a variable within the particle size and redox component. SC was a significant positive predictor of N mineralization (Figure 4c). Within the SC component N mineralization was significantly positively correlated with TOC ( $r=0.549$ ,  $P=0.012$ ) and TN ( $r=0.481$ ,  $P=0.032$ ). SC was a significant positive predictor of DEA (Figure 4d). Within the SC component, DEA was positively correlated with TOC ( $r=0.771$ ,  $P<0.001$ ), TN ( $r=0.647$ ,  $P=0.002$ ), and gravimetric soil moisture ( $r=0.776$ ,  $P<0.001$ ), and negatively correlated with bulk density ( $r=-0.774$ ,  $P<0.001$ ). The soil particle size and redox component was not predictive of any of the N cycling rates ( $P>0.05$ ).

## **5. Discussion**

### *5.1 Soil condition and texture indices*

The separation of soil variables into the PCA components SC and soil particle size and redox indicates that soil quality (TOC and TN), moisture content, and bulk density vary independently of soil texture and redox characteristics in the study wetlands (Table 1). Variables within the SC gradient are highly interconnected. For instance, when higher amounts of organic matter, as indicated by TOC, are incorporated into the soil matrix, bulk density decreases and soil porosity increases, enabling more water to infiltrate pore space and gravimetric soil moisture to increase (Reddy and DeLaune

2008). Variables within the particle size and redox gradient are similarly interconnected, as higher percentages of sand increase average particle diameter, decrease the percentage of clay, and increase redox potential. While variables within the particle size and redox component should influence and be influenced by SC variables (i.e. higher clay content increases bulk density, higher bulk density decreases redox, etc.), particle size and redox index variables did not vary substantially between wetlands as SC variables did (Figure 2, Table 2). The fact that texture variables showed minimal differences between wetlands indicates that the textural composition of top soil that was added to created wetlands soils during construction was similar to that of the natural reference wetlands and was consistent among created wetlands, with aging having negligible effect on the texture of created wetland soils. These results differ from those studies that found differences in soil texture between wetlands of various ages or between created and natural wetlands (Zedler 1996, Shaffer and Ernst 1999, Whittecar and Daniels 1999, Stolt et al. 2000, Campbell et al. 2002, Hossler and Bouchard 2010).

### *5.2 Changes in created wetland soil properties over time and compared to natural reference wetlands*

A consistent pattern of SC and variables therein was apparent across wetland ages (Figure 2). Created wetlands that were 3 and 4 years old showed markedly less soil development, specifically less TOC, TN, gravimetric soil moisture, and higher bulk density than older created wetlands (7 and 10 years old) and natural wetlands. Newly

created wetlands have only a few growing seasons to accumulate autochthonous C and N from extant vegetation, and also less time for overflow events and surface water inputs to supply allochthonous organic matter and nutrients to the soil. The lack of organic matter paired with the compaction caused by the heavy equipment used during construction results in a higher soil bulk density that excludes water and air from the soil matrix. The combination of these factors results in an immature, dense soil that is deficient in organic material and has relatively little development. Results are consistent with studies that found that created wetlands had lower soil organic matter (Confer and Niering 1992, Bishel-Machung 1996, Shaffer and Ernst 1999, Campbell et al. 2002, Bruland and Richardson 2006) and organic C (Whittecar and Daniels 1999, Stolt et al. 2000, Hossler and Bouchard 2010) and higher bulk density (Bishel-Machung 1996, Campbell et al. 2002, Hunter et al. 2008, Hossler and Bouchard 2010) than natural reference wetlands.

Seven and 10 yr old created wetlands generally had values for SC and its constituent variables that were intermediate between the younger (3 and 4 yr old) created wetlands and natural wetlands (Figure 2). The 10 yr old wetland had significantly lower bulk density and higher TOC than 3 and 4 yr old wetlands, but lower TOC, TN, and overall SC score than natural wetlands. The 7 yr old wetland soils were most similar to natural wetland soils and were the same as one or both natural wetlands in terms of gravimetric soil moisture, bulk density, TOC, and overall SC, and differed only by having lower soil TN. The fact that the 7 yr old wetland soils were more similar to those of natural wetlands than the 10 yr old wetland soils may be the result of the higher TC and TN sedimentation rate in the 7 yr old wetland (Table 2), which may have

supplemented organic matter and nutrients to the created wetland soils. Additional organic material in the soil would decrease bulk density and increase gravimetric soil moisture, causing the 7 yr old wetland soil to better resemble the soil properties of the natural wetlands. The 7 yr old wetland contains a plot that has been highlighted in a related study for its exceptional hydrologic connectivity to the adjacent stream, the effects of which may also be seen in the soil characteristics of this particular wetland (Wolf et al., in review).

A trajectory of soil development was apparent with the older created wetlands having similar soil conditions as the natural wetlands. These created wetlands did not, however, demonstrate a fully linear trajectory of soil development, which suggests that while age can predict general patterns of soil development, local site variability (i.e site location, hydrologic connectivity, planted vegetation, and other design features) has considerable influence on soil development as well. These results are similar to studies demonstrating an age-related trajectory of soil development (Craft 1997, Ballentine and Schneider 2009) and are inconsistent with those studies that found that soil organic matter or other developmental indicators did not increase or trend towards that of natural wetlands with age (Bishel-Machung 1996, Shaffer and Ernst 1999, Campbell et al. 2002). Study results also indicated that created wetlands may (7 yr old wetland) or may not (10 yr old wetland) accumulate natural levels of organic C within 10 years in order to fulfill the energy requirements of the N cycle, lower bulk density, and hold moisture within the surficial soil matrix. Thus, the ability of a created wetland to exhibit the same surficial soil characteristics as natural wetlands is possible, but not guaranteed, supporting the

notion that the developmental trajectory of created wetlands is highly variable (Simenstad and Thom 1996, Zedler and Callaway 1999, Morgan and Short 2002).

### *5.3 The effects of age-related soil properties on N flux rates*

Ammonification was positively correlated with both soil TOC and TC sedimentation. The conversion of organic N to  $\text{NH}_4^+$  requires energy in the form of labile C; thus as more TOC accumulates, either by autochthonous sources within the wetland or allochthonous sources like sedimentation, the process of microbial ammonification can more easily fulfill its energy and organic N substrate requirements. Total organic C and TC sedimentation also explain differences in ammonification rates between wetlands; BFP and BSR natural wetland have the highest and lowest ammonification rates, respectively, and BFP natural wetland has almost twice the amount of soil TOC and more than 9 times the amount of TC sedimentation as BSR natural wetland. Banshee Reeks natural wetland plots were located along a highly incised floodplain of Goose Creek, which prevented any significant sediment deposition from occurring during the sampling year. This wetland also had little understory vegetation, in contrast to BFP plots that had thick tussocks of *Juncus effuses* that contributed to soil TOC in this wetland. It should be noted that 2 plots in LC had filamentous algal growth on the sedimentation tiles during the spring collection months. This may have resulted in an overestimation of C and N sedimentation rates for these plots; however, ammonification at LC remained low in spite of this issue. Overall, 3 and 4 yr old created wetlands had lower ammonification rates

than 7 and 10 yr old wetlands, which were similar to BFP natural wetland, but higher than rates for BSR natural wetlands (Figure 3). These results support Pinay et al. (1995) in the assumption that ammonification is stimulated by allochthonous import of organic material in sediments.

Nitrification increased with greater SC and was positively correlated with soil TN and TOC (Figure 4). This relationship is to be expected as microbial nitrification requires inorganic N substrate in the form of  $\text{NH}_4^+$  ( $\text{NH}_4^+$  production is positively correlated with TN, although not significantly) (Reddy and D'Angelo 1997). It also suggests that heterotrophic rather than chemoautotrophic nitrification may dominate in study wetlands and supports the assertion that this process can be of substantial significance (Paul and Clark 1996). Nitrification was also positively correlated with gravimetric soil moisture and redox potential and negatively correlated with bulk density. As bulk density decreases there is greater pore space in the soil matrix for air and water to infiltrate. As nitrification requires aerobic conditions, the process is facilitated by low density soils that not only have higher oxygen content, and thus redox potential, but also have greater gravimetric soil moisture. This would explain the counterintuitive positive relationship between nitrification and gravimetric soil moisture. Results are similar to Strauss et al. (2004) that found that oxygen penetration into the sediments of the Upper Mississippi regulated nitrification, as well as Neill (1995) and Zak and Grigal (1991) that found higher nitrification rates in a non-flooded prairie marsh and dried swamp forest soils of Minnesota, respectively. Nitrification rates across wetlands demonstrated an increase by age group, with 3 and 4 yr old wetlands having the lowest rates, 7 and 10 yr old wetlands



having intermediate rates, and natural wetlands having the highest rates (Figure 3). This pattern is likely the result of the SC gradient that exists, with natural wetlands exhibiting the highest SC and young created wetlands that exhibit the lowest SC. Zak et al. (1990) also found nitrification rates in old fields of Minnesota to increase with age, indicating that this trend is not limited to wetlands ecosystems and may be linked to successional processes.

Nitrogen mineralization increased with SC and was positively correlated with TOC and TN (Figure 4). These patterns are expectedly similar to its component processes of ammonification and nitrification and are likewise explained. Nitrogen mineralization follows a similar pattern across wetland ages as ammonification, because ammonification comprised a much larger percentage of total N mineralization (89%) in created wetlands and BFP natural wetland (70%). The high contribution of ammonification to net N mineralization in created and BFP natural wetland is similar to patterns found in constructed salt marsh soils with consistently low redox potentials (Langis et al. 1991). BSR natural wetland, in contrast, had received solely nitrification contributions as net N mineralization (200%), which were offset by the site's net negative ammonification rate (-100% of total N mineralization). Like ammonification patterns, 3 and 4 yr old created wetlands had lower N mineralization rates than 7 and 10 yr old wetlands, which were similar to BFP natural wetland, but higher than rates for BSR natural wetlands (Figure 3). Results are similar to Verhoeven et al. (1990) and Fickbohm and Zhu (2006) that found that N mineralization increased with soil organic matter in fens and bogs and temperate wetlands, respectively. Pinay et al. (1995) also attributed higher ammonification rates

partly to the soil organic matter content in finer textured soils; although this study found no relationship between N mineralization and texture.

Denitrification potential also increased with SC and was positively correlated with soil TOC, TN, and gravimetric soil moisture and negatively correlated with bulk density, much like that of nitrification (Figure 4). The heterotrophic bacteria responsible for denitrification require a C energy source, like the other component processes of the N cycle, as well as an N substrate in the form of  $\text{NO}_3^-$ . While denitrification requires anaerobic conditions, it is usually limited by the availability of  $\text{NO}_3^-$  substrate (Martin and Reddy 1996), which is produced under aerobic conditions; thus, fluctuating redox conditions may be optimal for supporting greater denitrification rates (Groffman and Tiedje 1988, Neill 1995). Nitrification and denitrification potential have been shown in a companion study to be coupled in the study wetlands (Wolf et al., in review), so while denitrification is facilitated by higher gravimetric soil moisture, it is also stimulated by lower bulk density soils, which may have higher organic matter and enough oxygen to produce  $\text{NO}_3^-$  substrate. Results are similar to studies that found that denitrification increased with organic matter or C (Reddy and D'Angelo 1997, Burt et al. 1999, Davidsson and Stahl 2000, Groffman and Crawford 2003, Sutton-Grier et al. 2009), soil moisture (Schnabel et al. 1997, Hunter and Faulkner 2001, Groffman and Crawford 2003) and decreasing bulk density (Burt et al. 1999). Soil texture, however, did not influence denitrification potential in this study as it did in other studies (Groffman and Tiedje 1989, Groffman et al. 1992, Pinay et al. 2000, Hefting et al. 2004).

## 6. Conclusions

A general but variable age-based trajectory of soil development was apparent in the created and natural wetlands of this study. Younger created wetlands (3 and 4 yrs old) showed significantly less soil development than older created wetlands (7 and 10 yrs old), while older created wetlands showed less (10 yr old) or similar (7 yr old) soil development to that of natural wetlands. The structural development of soils in the study wetlands over time translates to a similar pattern of N cycle function, with younger created wetlands generally demonstrating lower ammonification, nitrification, and potential denitrification rates than older created and natural wetlands. This indicates a close link between age-related soil properties, a structural attribute, and N cycling, a functional attribute, in the study wetlands.

While soil development and N cycling similar to that of natural wetlands is possible to achieve within ten years of creation, it is not guaranteed, indicating that environmental factors independent of time expectedly influence structural and functional development of wetland features. The function of N retention and removal is an essential ecosystem service that natural wetlands provide, and as such, should be a required function to be attained within the monitoring period of mitigation wetlands. While testing each component process of the N cycle is not efficient or cost effective, monitoring age-related soil properties, such as organic matter or organic C, which influence other important biogeochemical soil properties, may be an appropriate way to assess the functionality of created wetlands. Initial supplementation of organic C may be necessary

in some cases (Bruland et al. 2009, Sutton-Grier et al. 2009), until autogenic sources can contribute adequate nutrients to support a healthy microbial community with N cycling rates similar to natural wetlands. This study recommends that soil characteristics, like organic matter/C, be included as part of wetland mitigation monitoring requirements as an indicator of N cycling capacity to ensure that created wetlands function more like the natural wetlands they are meant to replace.

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Table 1. Two component PCA model for soil variables. Loading values in parentheses.

	<b>Component 1 (Soil condition)</b>	<b>Component 2 (Particle size &amp; redox)</b>
	Avg total organic carbon (.935)	Avg D50 (.970)
	Avg total nitrogen (.934)	Avg sand % (.934)
	Avg bulk density (-.924)	Avg clay % (-.841)
	Avg gravimetric soil moisture (.903)	Avg redox potential (.571)
% variance explained	45.5%	37.5%

Table 2. Soil texture, redox potential, and nutrient sedimentation by wetland age (mean  $\pm$  one standard error.)

	LC 3 yr old (n=6)	CF 4 yr old (n=2)	BR 7 yr old (n=4)	NF 10 yr old (n=4)	BFP Natural (n=2)	BSR Natural (n=2)
Particle size (D50)	15.06 $\pm$ 2.10	18.87 $\pm$ 0.70	19.94 $\pm$ 0.72	19.91 $\pm$ 3.10	19.12 $\pm$ 1.66	17.93 $\pm$ 1.58
Sand (%)	26.72 $\pm$ 2.61	30.40 $\pm$ 2.90	30.60 $\pm$ 0.44	31.60 $\pm$ 4.62	28.90 $\pm$ 0.80	25.85 $\pm$ 4.85
Clay (%)	15.10 $\pm$ 2.80	11.95 $\pm$ 0.65	9.60 $\pm$ 1.07	11.73 $\pm$ 2.18	9.30 $\pm$ 2.31	10.11 $\pm$ 0.20
Redox (mV)	175 $\pm$ 17	293 $\pm$ 51	267 $\pm$ 16	241 $\pm$ 16	258 $\pm$ 11	291 $\pm$ 1
Carbon sedimentation (g-C m <sup>-2</sup> mo <sup>-1</sup> )	18.21 $\pm$ 4.15	2.17 $\pm$ 0.57	14.54 $\pm$ 11.69	2.25 $\pm$ 0.20	9.39 $\pm$ 4.82	1.06 $\pm$ 0.38
Nitrogen sedimentation (g-N m <sup>-2</sup> mo <sup>-1</sup> )	0.92 $\pm$ 0.17	0.20 $\pm$ 0.04	3.97 $\pm$ 3.45	0.21 $\pm$ 0.02	0.86 $\pm$ 0.53	0.15 $\pm$ 0.001

## Figures Legend

Figure 1. Site map of wetland locations in the Virginia Piedmont, USA.

Figure 2. Boxplots showing average (a) soil condition (SC) component score, (b) gravimetric soil moisture, (c) bulk density (BD), (d) total organic carbon (TOC), (e) total nitrogen (TN), and (f) soil texture (TXT) component score for 3, 4, 7, and 10 year old created wetlands and BFP and BSR natural wetlands. *P*-values indicate results of Fisher's least significant difference (LSD) post-hoc test for one-way analysis of variance (ANOVA). Letters (a-d) represent significant differences between wetland plots for each variable with 'a' indicating the lowest values for that variable.

Figure 3. Average (a) ammonification, (b) nitrification, (c) N mineralization, and (d) denitrification potential (DEA) rate for 3, 4, 7, and 10 year old created wetlands and BFP and BSR natural wetlands. *P*-values indicate results of Fisher's least significant difference (LSD) post-hoc test for one-way analysis of variance (ANOVA). Letters (a-c) represent significant differences between wetland plots for each variable with 'a' indicating the lowest values for that variable.

Figure 4. Linear regression of soil condition component vs. (a) ammonification, (b) nitrification, (c) nitrogen mineralization, and (d) denitrification potential (DEA).

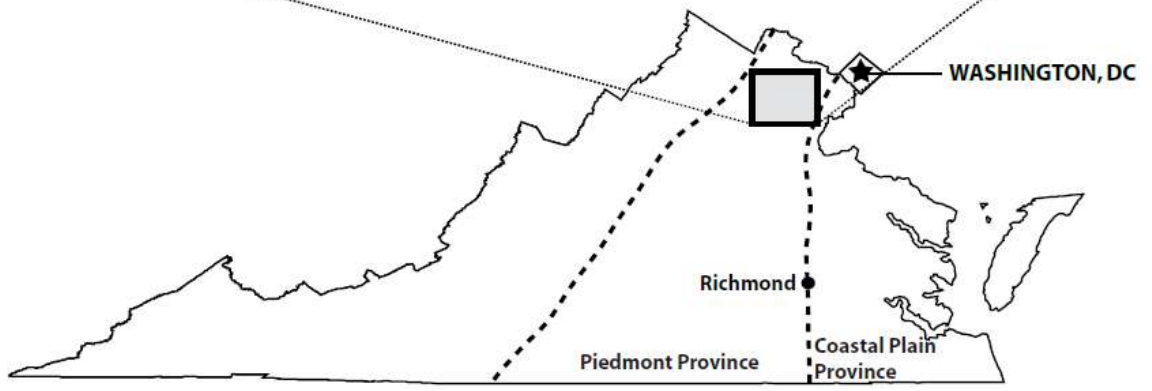
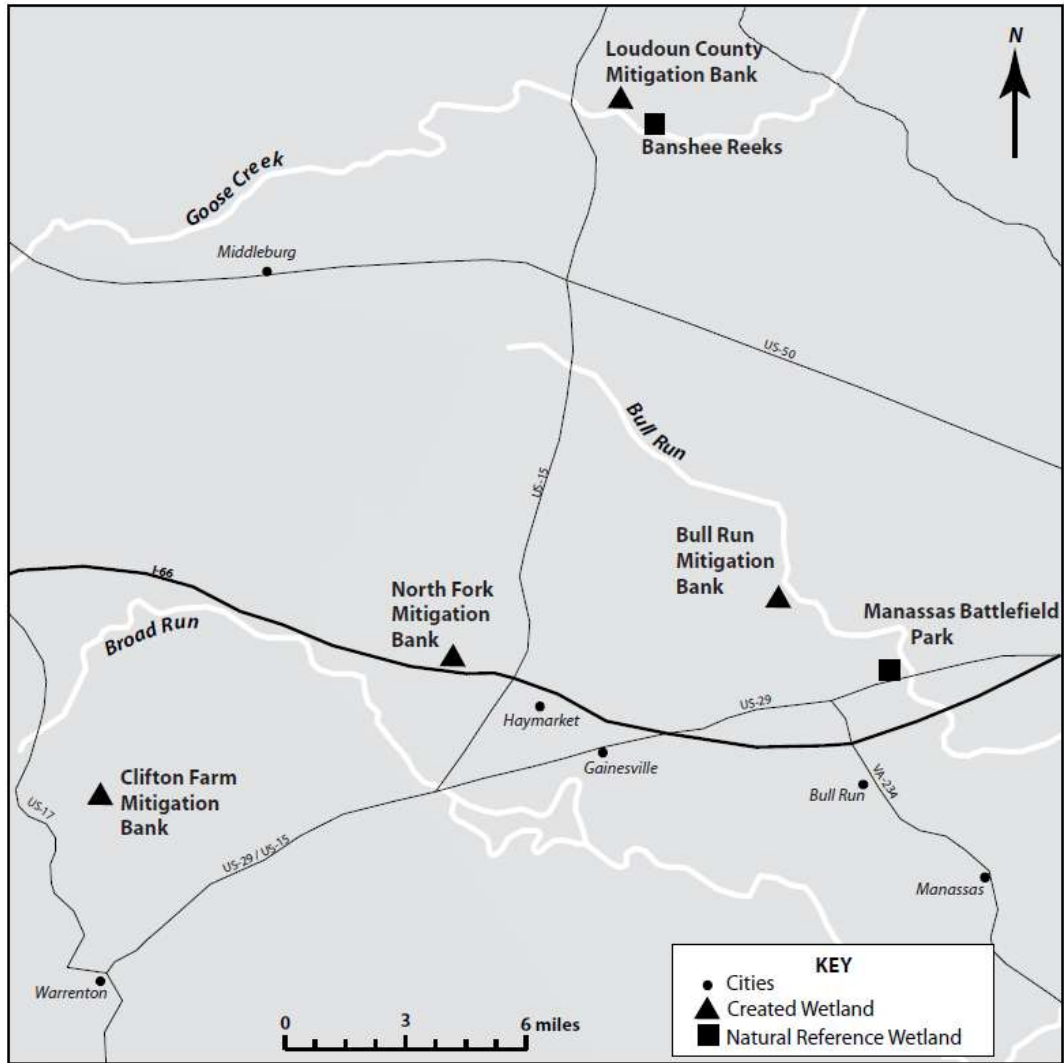


Figure 1

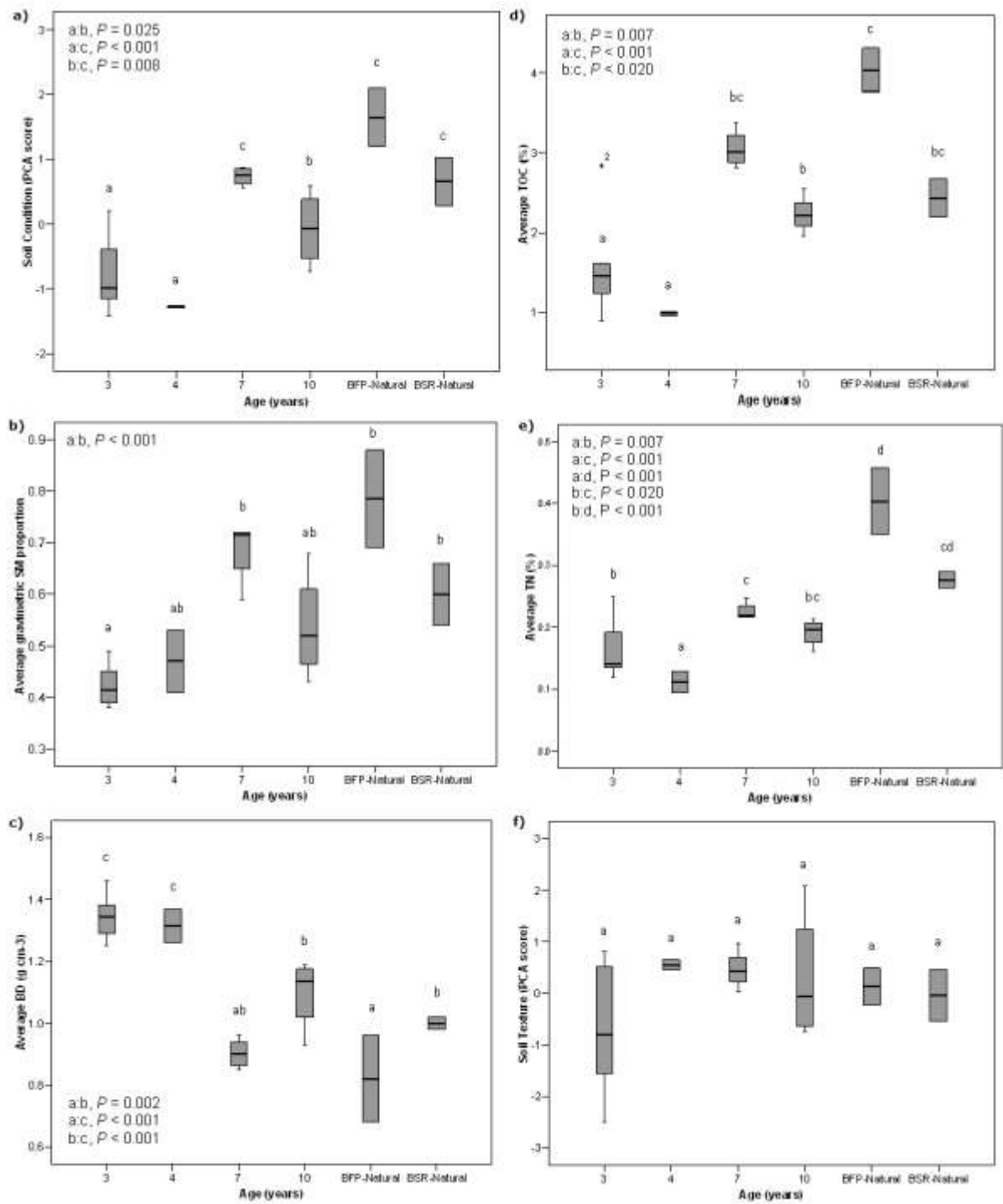


Figure 2

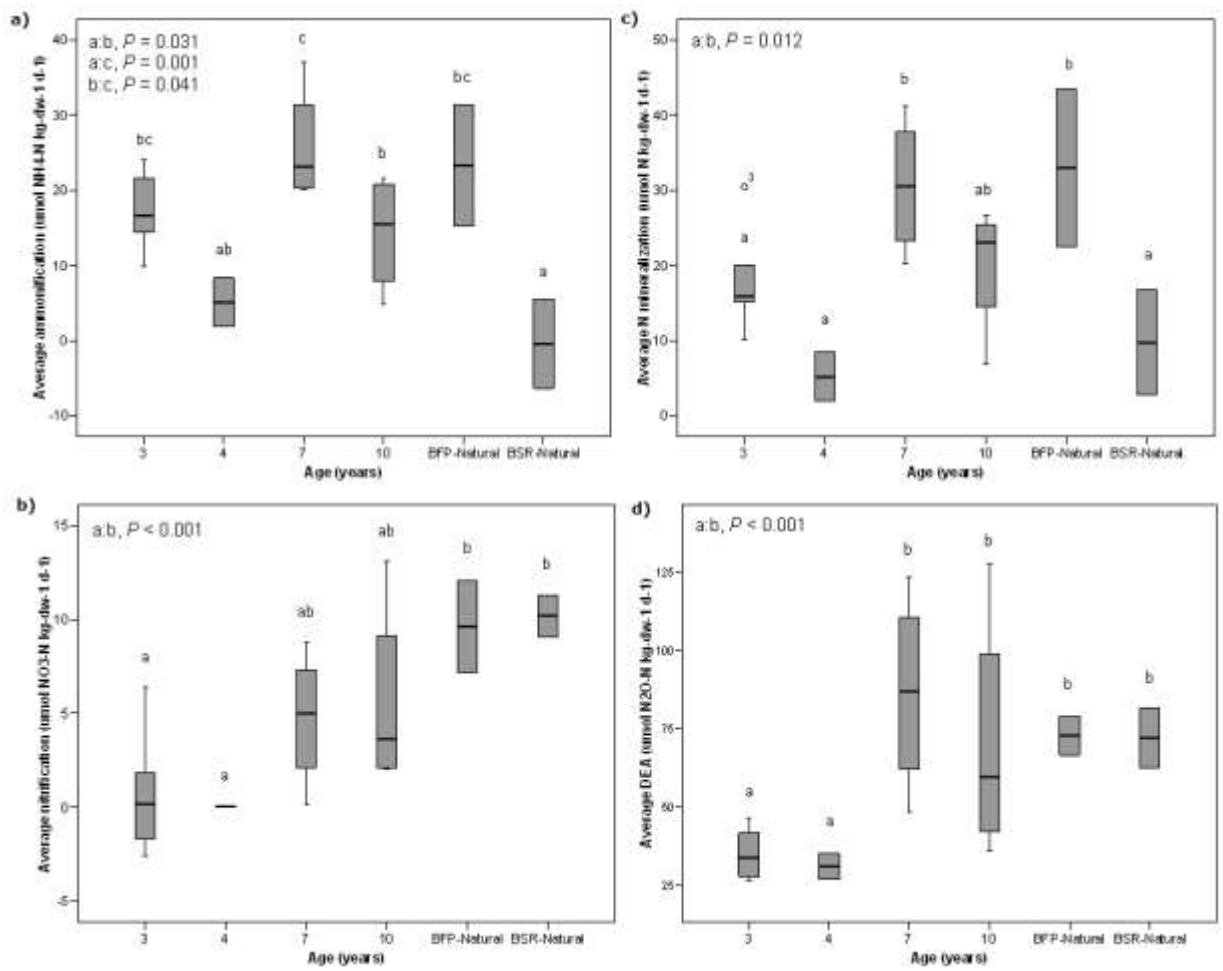


Figure 3



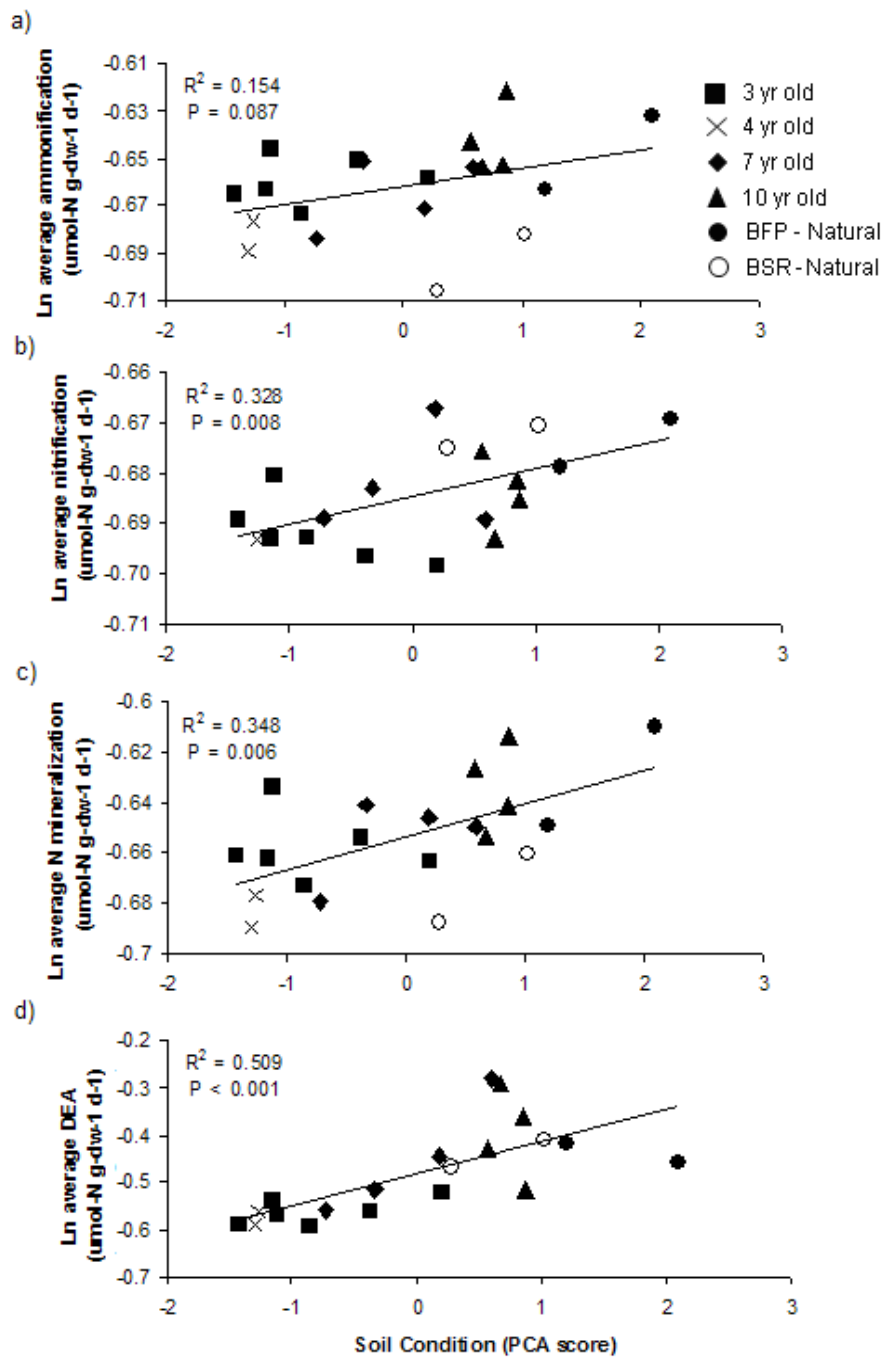


Figure 4

### **Chapter 3: Microtopography enhances nitrogen processing and removal in created wetlands**

#### **Abstract**

Natural wetlands often have a heterogeneous soil surface topography, or microtopography (MT), that creates microsites of variable hydrology, vegetation, and soil biogeochemistry. Created mitigation wetlands are designed to mimic natural wetlands in structure and function, and recent mitigation projects have incorporated MT as one way to attain this goal. Microtopography may influence nitrogen (N) cycling in wetlands by providing adjacent areas of aerobic and anaerobic conditions and by increasing carbon storage, which facilitates N cycling and removal. This study investigated three created wetlands in the Virginia Piedmont that incorporated disking-induced MT during construction, including one that had paired disked and undisked plots, in order to understand how this design feature influences N flux rates. Microtopography was measured using conventional survey equipment along a 1-m circular transect and was described using two indices: tortuosity (T), describing soil surface roughness and relief, and limiting elevation difference (LD), describing soil surface relief. Ammonification, nitrification, and net N mineralization were determined with *in situ* incubation of modified ion-exchange resin cores and denitrification potential was determined using denitrification enzyme assay (DEA). Results demonstrated that disked plots had

significantly greater T than undisked plots one year after construction. Autogenic sources of MT (e.g. tussock-forming vegetation) in concert with variable hydrology and sedimentation maintained and in some cases enhanced MT in study wetlands. Tortuosity and LD values remained the same in one wetland when compared over a two-year period, suggesting a dynamic equilibrium of MT-forming and -eroding processes at play. Microtopography values also increased when comparing the original induced MT of a one-year old wetland with MT of older created wetlands (five and eight years old) with disking-induced MT, indicating that MT can increase by natural processes over time. When examined along a hydrologic gradient, LD increased with proximity to an overflow point as a result of differential sediment deposition and erosion during flood events. Nitrification increased with T and denitrification potential increased with LD, indicating that microtopographic heterogeneity enhances coupled N fluxes. Findings of this study support the incorporation of MT into the design and regulatory evaluation of created wetlands in order to enhance N cycling and removal.

## **1. Introduction**

Wetland mitigation banks have become the preferred method of compensatory mitigation (National Research Council 2001) and as such have become the standard practice for mitigating the loss of natural wetlands. The practice of wetland mitigation banking attempts to create and restore wetlands to parallel the structure and function of natural wetlands (U.S. Army Corps of Engineers 2010), although their ability to do so has been questioned (Erwin 1991, Kentula 1992, Zedler and Calloway 1999, Hoeljte and

Cole 2007). In an attempt to rectify the structural and functional disparities between created and restored wetlands and the natural wetlands they were meant to replace, it has been recommended that natural reference wetlands be used as a template for mitigation wetlands (National Research Council 2001). Considering this approach, certain features of natural wetlands could be incorporated into mitigation wetlands in an attempt to mimic naturally-occurring structure that, in turn, supports beneficial natural wetland functions.

One feature of natural wetlands that has recently been incorporated into created and restored wetlands is microtopography (Price et al. 1998, Whittecar and Daniels 1999, Norfolk District Army Corps of Engineers and Virginia Department of Environmental Quality 2004, Bruland and Richardson 2005). Microtopography (MT) is the variation of soil surface roughness and relief at the approximate scale of 1 cm to 1 m (Moser et al. 2007) and has been shown to influence wetland hydrology (Kamphorst 2000, Tweedy et al. 2001, Day et al. 2007), soil physicochemistry (Stoeckel and Miller-Goodman 2001, Bruland and Richardson 2005, Ahn et al. 2009, Stribling et al. 2006, Moser et al. 2009), and vegetation (Vivian-Smith 1997, Bedford et al. 1999, Spieles 2005, Moser et al. 2007, Alsfeld et al. 2008, Rossel et al. 2009). Natural wetlands have been shown to have greater MT than created wetlands with and without induced MT (Moser et al. 2007 and Stolt et al. 2000, respectively) as a result of animal burrowing, plant rooting and litter fall, preferential hydrologic flowpaths, heterogeneous sedimentation, and other natural processes. Failures of mitigation wetlands have been partly attributed to the flat homogenous soil surfaces (Kunz et al. 1988) associated with soil cutting, scraping, and compaction by the heavy machinery used during construction (Stolt et al. 2000, Bruland

and Richardson 2005), and mitigation projects often proceed without proper consideration of microtopography (Bledsoe and Shear 2000). These construction practices, paired with the siting of created wetlands on former agricultural and pasture land that is characteristically homogeneous (Robertson et al. 1993), often result in a soil matrix with low vertical stratigraphy, muted horizontal variability (Bruland and Richardson 2005), and high bulk density (Bishel-Machung et al. 1996, Campbell et al. 2002, Hossler and Bouchard 2010).

Considering the impact of MT on wetland soil properties, it follows that biogeochemical processes within created and restored wetland soils should also be affected by the roughness and relief of the soil surface. Incorporating MT into created wetlands creates adjacent areas of aerobic hummocks and anaerobic hollows (Bruland et al. 2006) that may be optimal for supporting coupled nitrification and denitrification, respectively (Groffman and Tiedje 1988, Neill 1995). Hummocks that have greater oxygen concentrations facilitate the oxidation of ammonium ( $\text{NH}_4^+$ ) to nitrate ( $\text{NO}_3^-$ ), the high mobility of  $\text{NO}_3^-$  the soil causes it to leach downwards in the soil into oxygen poor hollows, where it can be removed as dinitrogen gas ( $\text{N}_2$ ) via denitrification. The proximal gradient of redox conditions that a heterogeneous soil surface topography creates should facilitate nitrogen (N) processing and removal. In addition to fluctuating redox conditions, the N cycle also requires both a labile carbon energy source and an organic and inorganic N substrate source available within the soil matrix to function in its entirety (Reddy and Patrick 1984). Microtopography regulates the distribution of organic matter along the soil surface (Waddington and Roulet 2000, Stoeckel and Miller-Goodman

2001) and determines the flowpath of water containing both organic and dissolved inorganic materials; consequently, it may also regulate the contact and time that N processing microbes have with energy and N substrate sources.

Understanding the relationship between induced MT and N cycling is important because incorporating MT by disking or mounding is a relatively simple and inexpensive way to increase soil surface heterogeneity which may, in turn, affect the retention, transformation, and removal of excess N from the wetland. On this premise, created and restored wetlands could not only mirror the soil surface structure of natural wetlands, but also attain their N cycling functional capacity, and by doing so, improve the water quality of the surrounding landscape. This study investigated the effects of disking-induced MT on N dynamics in three non-tidal freshwater created wetlands in the northern Virginia Piedmont physiographic province. Microtopographic indices representing soil surface roughness and relief were analyzed for their relationship to soil ammonification, nitrification, N mineralization (ammonification + nitrification), and denitrification potential. Soil physicochemical properties and hydrologic variables were analyzed to determine their role in the potential effects of MT on the coupled N cycle constituents.

The study focused on the following research questions:

- (1) How does MT differ between disked and undisked plots, vegetation types, and along hydrologic gradients in created wetlands?
- (2) How does MT affect N cycling in created wetlands and what soil and hydrologic properties influence their relationship?

## **2. Site Descriptions**

### *2.1 General setting*

All study sites were non-tidal freshwater wetlands located in the northern Virginia Piedmont physiographic province (mean annual precipitation 40 cm, mean temperature min 6°C / max 19°C; Figure 1). The wetland mitigation banks were created to mitigate for various local construction projects that impacted a mixture of bottomland forested floodplain, shrub/scrub, and emergent wetlands and open water ponds. All of the created wetlands were under 11 years old and had predominantly herbaceous cover, with some open water aquatic areas, shrub-scrub areas, and/or young stands of trees.

### *2.2 Created wetlands*

Loudoun County Mitigation Bank (LC) is a 32-acre wetland and upland buffer complex, constructed by Wetland Studies and Solutions, Inc. (WSSI) in the summer of 2006 in Loudoun County, Virginia (39°1'58.98" N, 77°36'26.10" W). LC contains disked areas that were tilled with a disk roller during construction and undisked areas that were designed as adjacent paired plots for comparison (Ahn and Dee, in review). The site is enclosed within a berm on the floodplain of Big Branch Creek and Goose Creek. The wetland contains two contiguous areas (cell 1 and cell 2) that are separated by a central

berm. Cell 2 receives flow from an unnamed tributary of Goose Creek through a head race attached to a cross vane structure with flow impacts regulated by an Agridrain structure and gate valve. LC also receives surface water runoff from an upland housing development and forested buffer, as well as minor groundwater inputs from toe-slope intercept seepage. Vegetation is currently dominated by herbaceous plants with small, container-grown, planted woody vegetation interspersed throughout.

Bull Run Mitigation Bank (BR) is a 50-acre wetland and upland buffer complex, constructed by WSSI in 2002 in Prince William County, Virginia (38°51'12.74" N, 77°32'58.52" W). The site receives water from Bull Run from an AgriDrain structure that routes water via a central ditch through the wetland, as well as overbank flow from Bull Run (average of one overflow event per year; WSSI 2007), which sharply bends around the corner of the site. The wetland receives limited surface water runoff from uplands and negligible groundwater. Vegetation is predominantly herbaceous, with small, planted woody vegetation throughout and the entire wetland was disked during construction.

North Fork Wetlands Bank (NF) is a 125-acre wetland and upland buffer complex, constructed by WSSI in 1999 in Prince William County, Virginia (38°49'31.53" N, 77°40'9.17" W). With the exception of minor contributions from toe-slope intercept seepage, the site is disconnected from the groundwater by an underlying clay liner and is hydrologically divided into four areas: main pod area – fed by upland surface water runoff and a tributary of the North Fork of Broad Run that is controlled by an artificial dam; overbank flow area – fed by overbank flow from the tributary; vernal pool area – located in the southwest quadrant of the wetland and fed solely by precipitation; and



depressional wetland area – fed by surface runoff from the adjacent Boy Scout campgrounds. Vegetation is mostly herbaceous, interspersed with young tree saplings and shrubs in projected forested areas, patches of shrub/scrub and emergent vegetation, and aquatic plants in the open water area. The entire wetland was disked during construction.

The study wetlands contain at least a 0.3 m low permeability subsoil layer covered with 0.2 m of commercially available topsoil. This design creates a perched, surface-driven water table close to the soil surface and limits groundwater exchange in the wetlands (Ahn and Peralta 2009). The three wetlands were disked using the same method during construction.

### **3. Methods**

#### *3.1 Sampling design*

Study plots (10 x 10 m) at the three created wetlands were selected so that typical hydrology, vegetation, and any experimental manipulation (disking) of the wetland site was represented. At LC three disked plots (LC BB from cell 1 and LC DD and LC EE from cell 2), paired with three undisked plots (LC B from cell 1 and LC D and LC E from cell 2) were selected for a site total of six plots. At NF two plots in the main pod area (NF 40 and NF 41) and two plots in the vernal pool area (NF 14 and NF 15) were selected. At BR four plots arranged perpendicularly to the overbank flowpath were selected (BR 3,

BR 4, BR 5, and BR 6; plot numbers increasing with distance from the corner of the site that receives overbank flow), with the central ditch separating BR 5 and BR 6.

Microtopography surveys were conducted at the 14 wetland plots in the summer of 2007. Soil sampling for physicochemical properties and N flux rates occurred over a two-day period the second week of every month from July 2008 to July 2009.

### *3.2 Microtopography*

Microtopography was measured using conventional surveying equipment (Sokkia SET4110 total station, Olathe, Kansas, USA) at each wetland plot in the Summer of 2007. Survey conditions were generally dry so as to avoid any alterations in existing MT. Elevation coordinates were taken every 10 cm along a 1-m diameter circular transect that was randomly placed in the 10 x 10 m plot. Microtopography was quantified using tortuosity (T) and limiting elevation difference (LD) indices. Tortuosity was calculated as the ratio of over-surface distance to straight-line distance of the measurement interval and was used as a unitless metric of soil surface roughness and relief (Kamphorst et al. 2000, Moser et al. 2007). Limiting elevation distance was defined by the limit of elevation change over the measurement interval and was used as an elevation metric (cm) of soil surface relief (Linden and Van Doren 1986).

### *3.3 N mineralization*

Soil net N mineralization was measured *in situ* using a modification of the DiStefano and Gholz (1986) resin core technique for use in wetlands that was developed by Noe (in press). The method incubates in place a soil core with six ion-exchange resin bead bags, three placed above and three below the soil core, to quantify inorganic N loading to the soil core (two outer resin bags) and production from inside the intact soil core (two inner resin bags + soil core) in hydrologically dynamic wetlands soils. The two middle bags were used as a quality control check to ensure that inner and outer bags were not saturated with ions and incapable of trapping nutrients. A preliminary study has shown that the outer and inner bags are sufficient for removing all dissolved inorganic N from water entering and leaving the modified resin core in wetland soils (Noe, in press).

Study plots were sampled by randomly placing a 1-m<sup>2</sup> quadrat at the beginning of the study that divided the sampling area into 100 10-cm<sup>2</sup> cells. The surficial soil (0-5 cm) of two adjacent 10 cm<sup>2</sup> cells were randomly sampled each month each with beveled, thin-walled, PVC core tubes, 7.8 cm in diameter and 11 cm in length, that were driven into the ground until the soil surface was 3 cm below the top of the tube. Cores were then removed, and the excess 3 cm of soil on the bottom of the core was scraped out, resulting in a soil core depth of 5 cm and a soil volume of 251.3 cm<sup>3</sup> in the middle of the core tube. The first core was processed as an initial core and placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within 48 hours to yield initial 2M KCl extractable soil NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> concentrations (Keeney and Nelson 1982). The initial core was also used for soil characterization and denitrification flux measurements (see below). The second core was processed as a resin core by placing 3 resin bags each

on the top and bottom of the extracted soil core. Each resin bag was constructed using an undyed nylon stocking (Kayser-Roth, Inc., Greensboro, North Carolina, USA) stretched over a 7.6 outer diameter, nitrile O-ring (Macro Rubber & Plastics Products, Inc., North Andover, Massachusetts, USA), filled with mixed-bed ion-exchange resin beads (Rexyn I-300, Fisher Scientific, Pittsburgh, Pennsylvania, USA), and closed with two stainless steel staples. The two outer and middle bags each contained 20 g of resin and the two inner bags contained 30 g of resin (necessitated by the higher rate of capture by inner bags in a pilot study). Each bag was sealed into the core along its outer edge with a silicone caulk free of anti-microbial agents (Silicone I rubber sealant, General Electric Co., Fairfield, Connecticut, USA) to prevent leakage and flow bypass around the resin. Wire supports were added over the outer bags on both the top and bottom of the core tube to keep the bags in place, and the resin core was inserted back into the core hole and made flush with the surrounding soil surface. Resin cores were then incubated *in situ* for approximately one month (range of 26 to 34 days) and then harvested, placed in a polyethylene bag, stored on ice during transport, then stored at 4°C, and analyzed within one week. Monthly estimates of net ammonification and nitrification were calculated as the sum of extractable  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , respectively, in the soil core and two inner bags in the resin core minus the initial core. Net N mineralization was calculated as the sum of ammonification and nitrification relative to the dry mass of soil in the resin core. We present cumulative annual flux calculated as the sum of monthly incubations: (resin core soil  $\mu\text{mol}$  + 2 inner resin bags  $\mu\text{mol}$  – initial core soil  $\mu\text{mol}$ ) / ((total incubation days x

(plot mean bulk density ( $\text{kg-dw cm}^{-3}$ ) x volume of soil core ( $251.3 \text{ cm}^3$ )) for  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and  $\text{NO}_3^- + \text{NH}_4^+$  (N mineralization) expressed as  $\mu\text{mol-N kg-dw}^{-1} \text{ d}^{-1}$ .

### *3.4 Denitrification enzyme assay (DEA)*

Monthly denitrification potential was determined for each initial core using the denitrification enzyme assay (DEA) procedure (Smith and Tiedje 1979; Tiedje et al 1989; Groffman 1999). Assays were run in duplicate and consisted of a slurry of 25 g of homogenized field moist soil and a 25 mL solution of 1 mM glucose, 1 mM  $\text{KNO}_3$ , and 1  $\text{g L}^{-1}$  chloramphenicol mixed in a 125 ml Erlenmeyer flask. Flasks were sealed with rubber septa, made anaerobic by bubbling the slurry for 10 min followed by a 1 min headspace flush with  $\text{N}_2$  gas, and evacuated with a vacuum pump. Assays were brought to atmospheric pressure with  $\text{N}_2$  gas and injected with  $\text{H}_2\text{SO}_4$  scrubbed acetylene (modified from Hyman and Arp 1979) to 10% of the volume of the flask headspace. Flasks were then incubated on a rotary shaker table with gas samples taken at 30 and 90 min. Gas samples were stored in freshly evacuated 2 mL glass vacutainer vials (Tyco Healthcare Group LP, Mansfield, MA, USA) until they could be analyzed for  $\text{N}_2\text{O}$  on a Shimadzu 8A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with electron capture detection, generally within three days of sampling. DEA was calculated as:  $M = C_g * (V_g + V_l \times \beta)$  where, M = total amount of  $\text{N}_2\text{O}$  in water plus gas phase ( $\mu\text{g N}_2\text{O-N}$ ),  $C_g$  = concentration of  $\text{N}_2\text{O}$  in the gas phase ( $\mu\text{g N}_2\text{O-N/L}$ ),  $V_g$  =

volume of the gas phase (L),  $V_1$  = volume of liquid phase (L), and  $\beta$  = Bunsen coefficient (0.544 @ 25°C) and expressed as  $\mu\text{mol N}_2\text{O-N kg-dw}^{-1} \text{d}^{-1}$ .

### *3.5 Soil physicochemical properties*

#### *3.5.1 Laboratory analysis*

Gravimetric soil moisture (GSM) was determined for each initial core and resin core by removing a ~40 g dry-weight equivalent (dw-eq) subsample of homogenized soil, recording initial field-moist weight, and drying at 60°C until a constant weight was achieved. Bulk density (BD) was determined for each core by first weighing the entire field-moist core, converting to dry weight based on GSM percentage, and dividing by the total volume of the soil in the core (251.3 cm<sup>3</sup>). Volumetric (VSM) was calculated as BD x (GSM / density of water, assuming 1.0 g-H<sub>2</sub>O mL<sup>-1</sup>). Water-filled pore space (WFPS) was calculated as  $\text{VSM} / [(1 - (\text{BD} / \text{quartz parent material density, assuming } 2.65 \text{ g cm}^{-3}))]$ . Total soil carbon and N was determined by dry combustion of oven-dried, ground subsamples from each core on a 2400 Series II CHN/O elemental analyzer (Perkin-Elmer, Waltham, Massachusetts). Extractable NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> was determined for each initial and resin soil core and resin bag, within 48 hours of collection, by adding 40 ml of 2 M KCl to a 4 g dw-eq subsample of soil or 2 g ww subsample of resin beads, agitating on a shaker table for 1 h, and centrifuging (soil samples only) at 2500 rpm for 5 minutes. The supernatant was then filtered through a syringe with a 0.2  $\mu\text{m}$  polyethersulfone filter

tip (Pall Corporation, Port Washington, New York, USA) and analyzed on an Astoria 3020 series segmented flow autoanalyzer (Astoria-Pacific International, Clackamas, Oregon, USA). External reference standards and sample blanks (empty extraction vessel for soil blanks or extraction vessel with new resin for resin bead blanks) were included in each monthly run to validate results and to correct incubated resin and soil extractable concentrations, respectively.

### *3.5.2 Field analysis*

Redox potential was measured each month by inserting a RE 300 ExStik<sup>®</sup> ORP meter (Extech Instruments Corporation, Waltham, Massachusetts, USA) to a depth of approximately 3 cm into the soil between the initial core and resin core sampling locations. Redox potential was recorded after drift was sufficiently stabilized (approximately 1 min). Ceramic sedimentation tiles (20 x 20 cm) were installed monthly at an adjacent location that was representative of each plot, yet avoided any microtopographic or vegetative irregularities. Deposited sediment (excluding coarse woody debris and litter free of mineral sediment) was harvested from the tiles during each collection period for subsequent elemental analysis.

### *3.6 Statistical analysis*

All data were reviewed for normality and those variables failing tests for normality were transformed, usually by natural log transformation. One-way analysis of variance (ANOVA) was used to test for significant mean differences in T and LD in

disked and undisked plots in LC and between main pod and vernal pool plots of NF. Pearson Product-moment correlation matrices were used to investigate T and LD with distance from the overflow point (measured in the field using a meter tape) along a hydrologic gradient in BR, as well as the relationship between MT indices and N fluxes and MT indices and soil and hydrologic variables and their temporal coefficients of variation (CV). Linear regression analysis was performed on MT indices and N fluxes to determine whether T or LD significantly predicted N fluxes. All statistical tests were performed using SPSS version 15 (SPSS 2006), and tests were considered significant at  $\alpha = 0.05$ , unless otherwise noted.

## 4. Results

### *4.1 How does MT differ between disked and undisked plots, vegetation types, and along hydrologic gradients in created wetlands?*

Plot T and LD show variability between sites and hydrologic designations within sites (Figure 2). In LC, disked plots demonstrate significantly higher average T than undisked plots at  $\alpha = 0.10$  (one-way ANOVA,  $df = 5$ ,  $P = 0.051$ , Figure 2), but did not differ in LD ( $P = 0.858$ ). In NF, plots in the main pod area have significantly higher average LD than vernal pool plots ( $df = 3$ ,  $P = 0.002$ , Figure 2), but did not differ in T ( $P = 0.254$ ). Tortuosity and LD values for NF main pod were the essentially the same in 2007 as they were in 2005 and average MT indices for BR (5 years old in 2007) and NF main pod (8 years old in 2007) are greater than those of LC (1 year old; Table 1). In BR,



LD had a strong negative correlation (with low statistical power) with distance from the overflow point (Pearson Product-Moment Correlation,  $n = 4$ ,  $r = -0.938$ ,  $P = 0.062$ ), where BR 3 was the closest to and BR 6 was the farthest from the overflow point. Tortuosity, however, showed no correlation with distance from the overflow point ( $r = 0.000$ ,  $P = 1.0$ ).

#### *4.2 How does MT affect N cycling in created wetlands and what soil and hydrologic properties influence their relationship?*

Ammonification was not significantly predicted by T (Linear regression,  $n = 14$ ,  $R^2 = 0.054$ ,  $P = 0.423$ ) or LD ( $R^2 = 0.073$ ,  $P = 0.349$ ). Nitrification significantly increased with T ( $R^2 = 0.439$ ,  $P = 0.010$ , Figure 3a), but was not significantly associated with LD ( $r = 0.043$ ,  $P = 0.884$ ). Nitrogen mineralization was not significantly predicted by T ( $R^2 = 0.223$ ,  $P = 0.088$ ) or LD ( $R^2 = 0.124$ ,  $P = 0.216$ ). Denitrification potential significantly increased with LD ( $R^2 = 0.503$ ,  $P = 0.004$ , Figure 3b), but was not associated with T ( $r = 0.362$ ,  $P = 0.201$ ). Tortuosity was positively correlated with gravimetric soil moisture variability (Pearson Product-Moment Correlation,  $n = 14$ ,  $r = 0.532$ ,  $P = 0.050$ ). Limiting elevation distance was positively correlated with gravimetric soil moisture ( $r = 0.638$ ,  $P = 0.014$ ), volumetric soil moisture ( $r = 0.630$ ,  $P = 0.016$ ), total carbon ( $r = 0.632$ ,  $P = 0.015$ ), total N at  $\alpha = 0.10$  ( $r = 0.527$ ,  $P = 0.053$ ), and carbon to nitrogen ratio (C:N) ( $r = 0.692$ ,  $P = 0.006$ ) and negatively correlated with bulk density ( $r = -0.554$ ,  $P = 0.040$ ). Tortuosity and LD were positively correlated ( $r = 0.536$ ,  $P = 0.048$ ).

## 5. Discussion

### *5.1 How does MT differ between disked and undisked plots, vegetation types, and along hydrologic gradients in created wetlands?*

Tortuosity describes soil surface roughness and relief (Figure 4) and was significantly higher in disked plots than undisked plots in the Loudoun County site one year after the wetland was created (Figure 2). While this is a relatively short time frame by which to judge MT establishment and persistence, it is a critical period during which the wetland may be particularly vulnerable to loss of soil surface topography. During its first year this wetland had very little established vegetation to hold surficial soil in place, making the wetland susceptible to degradation of its topographic heterogeneity by erosion from precipitation, overland surface flow, and flooding (Braskerud 2001) from the nearby tributary of Goose Creek. Microtopography could also have been susceptible to smothering effects by sedimentation (Huenneke and Sharitz 1986, Werner and Zedler 2002). The fact that MT was maintained during this period of vulnerability further supports its incorporation into newly created wetlands.

Limiting elevation difference describes soil surface relief (Figure 4) and was significantly higher in the main pod plots than the vernal pool plots in the North Fork site (Figure 2). However, both the main pod and vernal pool plots were disked when the wetland was created in 1999. Consequently, the differences in MT present eight years after construction must be due to natural processes that decrease or increase MT over time. Main pod plots are fed by a permanent creek tributary that has a dispersive flow

(creating small channels that contribute to microrelief) throughout the main pod as it shallows before flowing into an open ponded area of the wetland. This low velocity, sheet-like flow creates a variable hydroperiod with low sedimentation that supports a diversity of vegetation, including tussock-forming vegetation like Woolgrass (*Scirpus cyperinus* L.), Common Rush (*Juncus effusus* L.), and sedges (*Carex* sp. L.) that create and maintain soil surface relief. *Carex* communities in wetlands have been found to increase soil surface area in Midwestern meadows (Peach and Zedler 2006) and form rough topography that reduces water velocity and provides short-term water storage (Cole 2002). The vernal pool plots, in contrast, receive only precipitation and overland surface flow and are hydrologically less dynamic than main pod plots. Vernal pool plots support non-tussock forming plants, predominantly Beggarticks (*Bidens* L.), that do not substantially contribute to soil surface relief. While all of NF was hydroseeded after construction, variable hydrology within the wetland has caused preferential establishment of certain plant communities that persist due in part to the autogenic maintenance of tussock-forming vegetation. These vegetation-propagated hummocks create drier microhabitats where plants that are less tolerant of flooding can germinate and grow (Schlesinger 1978, Titus 1990, Chimner and Hart 1996, Roy et al. 1999, Duberstein and Connor 2009), as well as heterogeneous MT and hydroperiod within the wetland that supports species richness and diversity and may require less hydrology management (Vivian-Smith 1997). Autogenic MT has been reported in European and North American bogs (Foster et al. 1988), in models of boreal peatlands (Nungesser 2003), and as a result of mesofaunal activity in Asian wetlands (Haitao et al. 2010). Vegetation-propagated MT

has also been found in interior tidal marshes of the Chesapeake Bay (Stribling et al. 2007) and may be an important element of self-design (Mitsch and Wilson 1996) for created and restored wetlands.

Another important trend noted for the NF study site is the persistence of MT over time within the main pod area. Tortuosity and LD values remained essentially the same over this two-year period (Table 1). The soil surface topography may have reached a state of dynamic equilibrium in which the possible MT-diminishing effects of sediment imported by the Broad Run tributary are counter-balanced by the autogenic enhancement of MT through tussock-forming vegetation. When comparing MT across wetland ages, average T and LD are greater in the older created wetlands (5 year old BR and 8 year old NF main pod) than the younger wetland (1 year old LC; Table 1). Microtopography was measured in LC when the wetland was sparsely vegetated by *Juncus effuses* tussocks, non-tussock-forming herbaceous plants, and annual rye grass (*Lolium L.*) seeded for erosion control. There were also patches of bare soil where vegetation had not yet colonized. Consequently, the low MT indices for LC, although still significantly different between disked and undisked plots, were likely the result of its early developmental stage. These results provide further evidence that MT can persist during vegetation establishment and increase with age in created wetlands.

While no significant correlation existed between T or LD and distance from the overflow point in BR, a trend of decreasing LD is apparent as plots get farther from the overflow point (Figure 2). The lack of a significant correlation between LD and distance from overflow point is likely due to low statistical power, as the high *r*-value indicates a

strong relationship. Sedimentation rate, while not directly correlated with T or LD, also decreased with distance from the overflow point, and the most proximal plot (BR 3) was noted in a related study for its exceptionally high sedimentation rate (Wolf et al., in review). The dynamic hydrology that BR 3 experiences may enhance LD through differential sediment deposition and scouring during high velocity overflow events. While greater MT at this plot may be partially attributable to larger textured sediment deposits, BR 3 is richly vegetated with exceptional N cycling capacity (Wolf et al., in review). As the distance from the overflow point increases, flooding velocity decreases and floodwaters disperse across a larger area, depositing sediment more uniformly across the more distal BR plots. Considering that BR 4, BR 5, and BR 6 have similar vegetation (predominantly *Juncus effuses* tussocks) the continued, albeit variable, trend of decreased LD with distance from the overflow point may indicate that the greater sedimentation rate of BR 4 and BR 5 when compared to BR 6 is at least partially responsible for their enhanced soil surface relief. This trend indicates that sedimentation has not smothered soil surface topography, as it has in other studies (Huenneke and Sharitz 1986, Werner and Zedler 2002), but rather has potentially enhanced MT in study plots.

*5.2 How does MT affect N cycling in created wetlands and what soil and hydrologic properties influence their relationship?*

Net ammonification rates were not significantly predicted by MT indices, probably due to the dynamic relationship between ammonification and the soil moisture variables influenced by MT. Limiting elevation difference and T demonstrate a clear

positive relationship with soil moisture and soil moisture temporal variability, respectively; the relationship of these variables with ammonification, however, may not be apparent due to the competing effects of ammonification and nitrification. Drier aerobic conditions increase organic matter breakdown and  $\text{NH}_4^+$  production, but also increase  $\text{NH}_4^+$  loss by nitrification and typically lead to net  $\text{NH}_4^+$  loss. In contrast, wetter anaerobic conditions inhibit organic matter breakdown and  $\text{NH}_4^+$  production, but decrease  $\text{NH}_4^+$  loss by nitrification and lead to net gains in  $\text{NH}_4^+$  (Brinson et al. 1981, Reddy and Patrick 1984, Bridgham et al. 1998). If  $\text{NH}_4^+$  production and loss are occurring at a similar rate (as a result of fluctuating redox conditions), the effects of greater MT on net ammonification would not be detectable by our measurement of net (as opposed to gross) ammonification using *in situ* incubation of modified resin cores. In a study of similar wetlands, Moser et al. (2007) found that  $\text{NH}_4^+$  concentrations were higher in plots with greater MT (disked) than those with lower MT (undisked). Similarly, Calderon and Jackson (2002) found that tilling increased soil  $\text{NH}_4^+$  concentrations. However, these findings provide a snapshot of  $\text{NH}_4^+$  concentration at the time of sampling and, while informative, they do not readily compare with ammonification flux rates.

Nitrogen mineralization was not significantly predicted by T or LD. In the study wetlands ammonification comprised a much larger percentage of total N mineralization (~87%) than nitrification (~13%). As a consequence, N mineralization follows an expectedly similar pattern as ammonification and its lack of relationship to MT indices is similarly explained.

Nitrification significantly increased with greater T (Figure 3a). As the soil surface roughness and relief increase, the presence of hummocks may facilitate the conversion of  $\text{NH}_4^+$  to  $\text{NO}_3^-$  due to higher soil redox potential (Ehrenfeld 1995). Tortuosity was positively correlated with soil moisture temporal variability, and while soil moisture variability was not directly related to nitrification, fluctuating hydrology may play an indirect role as increasing moisture content during wet periods retains  $\text{NH}_4^+$  substrate for nitrification and decreasing moisture content during dry periods facilitates  $\text{NO}_3^-$  production. While this study quantified MT on a continuous scale, results are similar to Bruland and Richardson (2005) who found higher concentrations of soil  $\text{NO}_3^-$  in hummocks than in flats or hollows.

Denitrification potential significantly increased with greater LD (Figure 3b). As LD increases, the enhanced relief of the soil surface enables greater water retention capacity and soil moisture content, as demonstrated in this study and others (Moser et al. 2007). Limiting elevation difference was also positively correlated with total carbon and N. This relationship may also be explained by the greater soil moisture and an anaerobic soil environment, as the rate of organic matter decomposition decreases under these conditions, and more carbon and N are retained. Additionally, greater soil surface relief collects and retains organic matter and carbon in topographic depressions as demonstrated by Waddington and Roulet (2000) and Rossel et al. (2009) who found differential carbon storage based on soil surface topography. Greater soil moisture, carbon, and N content, in turn, facilitate denitrification by providing the anaerobic conditions, energy, and N substrate that denitrifying microbes require to reduce  $\text{NO}_3^-$  to

N<sub>2</sub> (Reddy and Patrick 1984). The positive correlation of LD and soil C:N ratio is likely due to both the increase in carbon and the removal of NO<sub>3</sub><sup>-</sup> by denitrification with enhanced MT. These results differ from Bruland and Richardson (2005) that found that DEA was not significantly influenced by MT when comparing hummocks, flats, and hollows. Defining MT indices on a continuous rather than categorical scale may have allowed the relationship between MT and DEA to become apparent.

One unexpected result of this study is that T and LD did not have the same effect on nitrification and denitrification. Previous studies of these wetlands have shown a close coupling between nitrification, denitrification, and the soil and hydrologic variables that influence these processes (Wolf et al., in review). Consequently, one would expect that an increase in T and/or LD would increase both N processes, rather than greater T increasing nitrification and greater LD increasing denitrification. These results may be explained by differences between the MT indices and the soil variables with which they are correlated. The greater elevation range, described by LD as the maximum elevation change along the transect, may indicate the presence of more established or permanent hummocks. The tops of hummocks are more consistently above the water table and have higher oxygen concentrations that support nitrification (Figure 4). Likewise, plots that exhibit greater LD may also have permanent hollows, which are consistently saturated and maintain an anaerobic condition that supports a robust denitrifying community. Furthermore, LD was positively correlated with greater soil moisture and total carbon and N, which indicates that hollow conditions may be ideal to support heterotrophic denitrifying bacteria. Limiting elevation difference was negatively correlated with soil



bulk density, which would facilitate greater oxygen infiltration for nitrification, as well as greater  $\text{NO}_3^-$  mobility. If greater LD does in fact enhance coupled nitrification and denitrification by facilitating these processes simultaneously, any  $\text{NO}_3^-$  that is produced in hummocks and transported vertically into anaerobic hollows would be denitrified and a negligible net nitrification rate would result. Tortuosity, in contrast, encompasses both soil surface roughness and relief, as described by the ratio of overland surface to straight line distance. As T increases, so do soil surface area and oxygen availability in the soil that is elevated above the water table (Figure 4). Plots with greater T, however, may lack the permanent, well-defined hummocks and hollows that characterize those with greater LD. A lack of established hollows would diminish the ability of the soil surface to collect and retain water, carbon, and N and remove  $\text{NO}_3^-$  through denitrification.

## **6. Conclusions**

Disking during construction increased the roughness and relief of the soil surface in created wetlands. Disking-induced MT can be self-maintained in created and restored wetlands during their vulnerable first years of establishment, when a lack of soil stabilizing vegetation can degrade soil topography. Autogenic changes in the original induced MT can be seen in created wetlands with distinct hydrologic regimes, as differences in hydrology and the establishment of tussock-forming vegetation led to enhancement of soil surface topography and heterogeneity eight years after wetland creation. Microtopography persists over time in created wetlands and may eventually reach a state of dynamic equilibrium as microtopographic-building and -eroding forces

proceed simultaneously. Microtopography can also increase with age in created wetlands as vegetative development enhances originally induced MT. Variation in MT can be seen along a hydrologic gradient of overbank flow, where areas of the wetland proximal to overflow can increase soil surface relief as a result of heterogeneous deposition and erosion during high velocity flood events. Finally, the incorporation of MT into created and restored wetlands increases N cycling: soil surface roughness and relief increase nitrification by enhancing soil surface area and aeration, and soil surface relief increases coupled nitrification and denitrification by providing adjacent areas of aerobic and anaerobic conditions and by enhancing soil moisture, carbon, and N storage. This finding links the action of inducing MT through disking with the functional attributes of N cycling and removal potential. These findings support the incorporation of MT into created and restored wetlands as a relatively simple, cost-effective, and low maintenance means of increasing their capacity to process and remove N and provide the important ecosystem service of water quality improvement.

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Table 1. Mean tortuosity (T) and limiting elevation difference (LD) microtopographic indices for disked and undisked plots of Loudoun County mitigation bank (LC), Bull Run mitigation bank (BR), and main pod and vernal pool plots in North Fork mitigation bank measured in 2007. Wetland age is reported at the time microtopography was surveyed in 2007. North Fork main pod microtopography was measured in 2005 by Moser et al. (2007).

	LC		BR	NF		
	1 year old		5 years old	8 years old		
	Disked	Undisked		Vernal Pool	Main Pod 2007	Main Pod 2005
T	1.007	1.003	1.105	1.005	1.024	1.015
LD (cm)	1.651	1.559	2.753	1.086	3.345	3.40

## Figures Legend

Figure 1. Site map of wetland locations in the northern Virginia Piedmont, USA.

Figure 2. Scatter-plot of tortuosity vs. limiting elevation difference for Loudoun County (LC), Bull Run (BR), and North Fork (NF) mitigation bank plots.

Figure 3. Linear regression of (a) tortuosity vs. nitrification and (b) limiting elevation difference vs. denitrification potential (DEA). Symbols represent individual wetland plots and their hydrologic designations within the wetland where applicable. See Figure 2 legend for a definition of the site labels.

Figure 4. Schematic diagram of hypothetical soil surface cross-sections illustrating increasing tortuosity (T) and limiting elevation difference (LD). As soil surface roughness (T) increases, so does soil oxygen and nitrification. As soil surface relief increases (LD), so does coupled aerobic / anaerobic processes and denitrification potential (DEA). Figure modified from Moser et al. (2007).

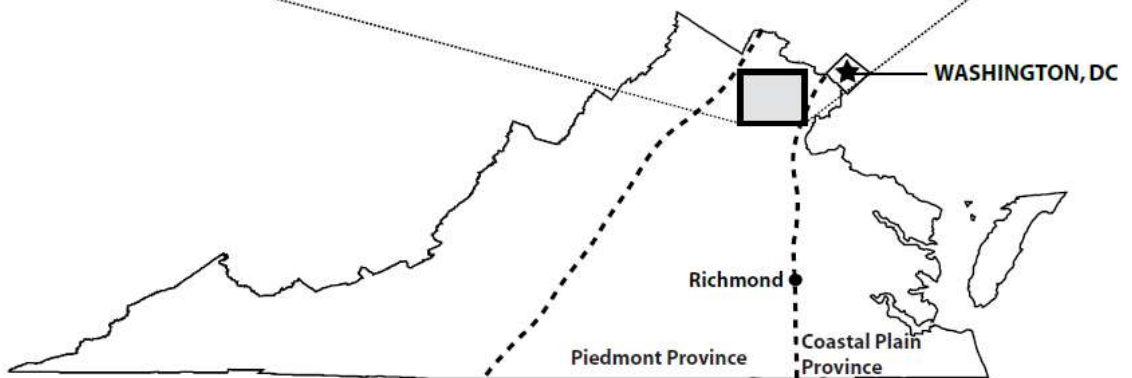
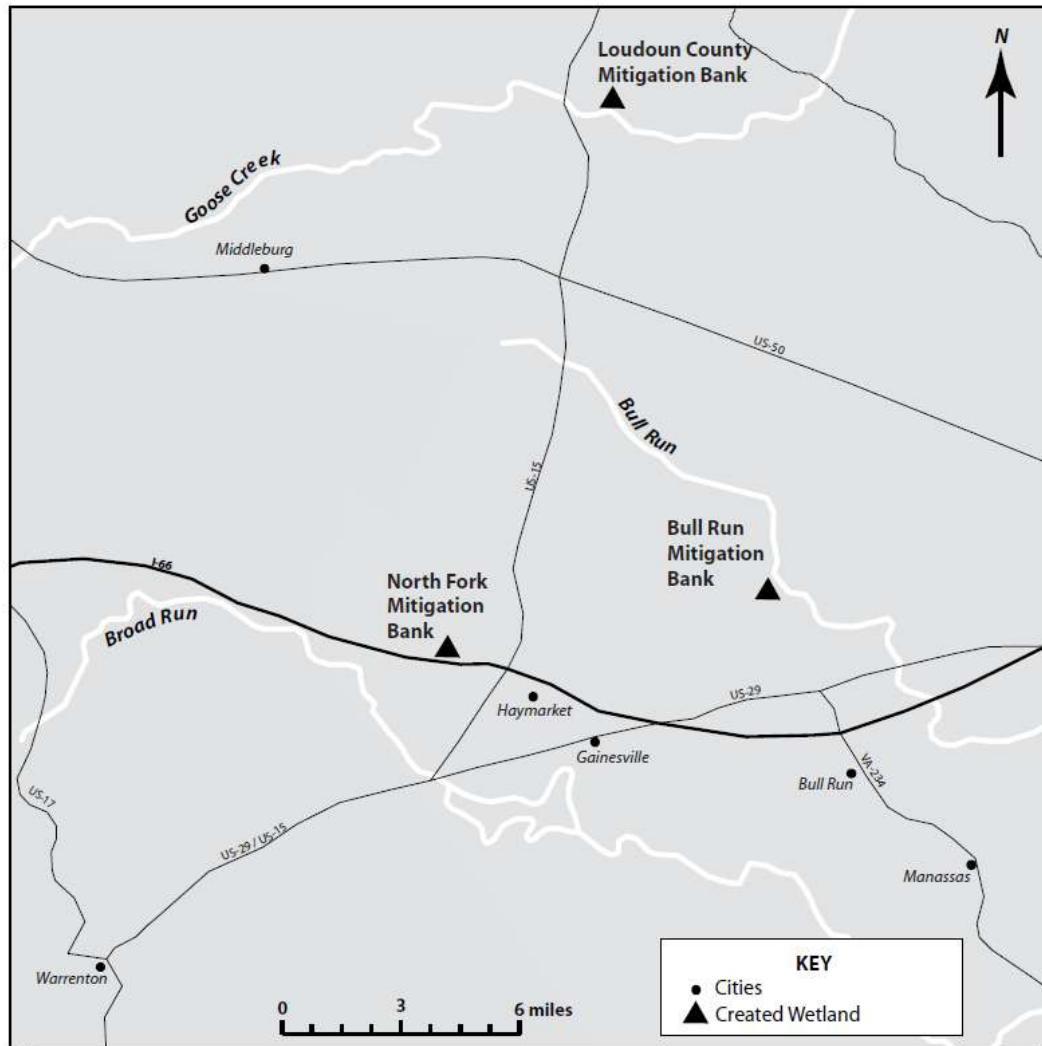


Figure 1

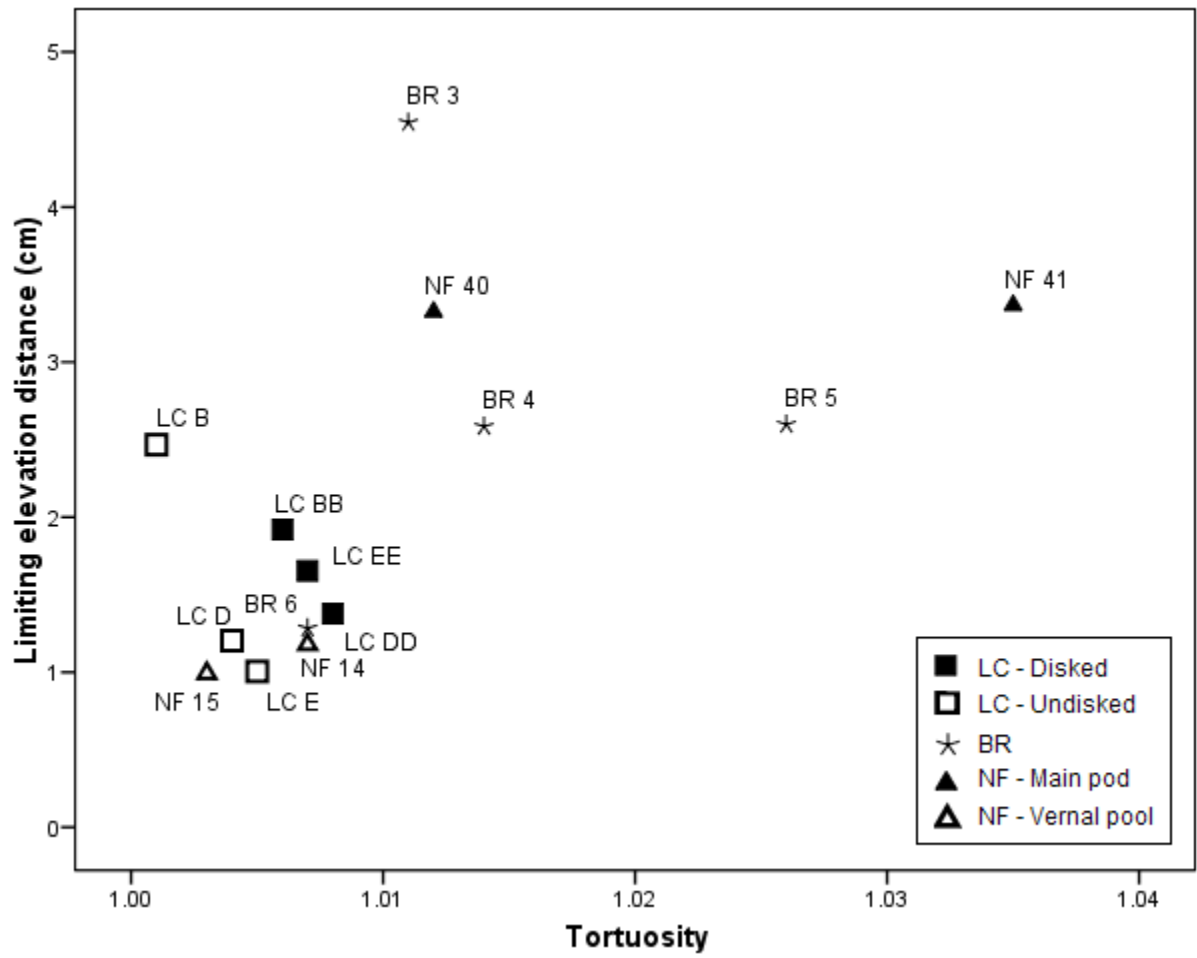


Figure 2

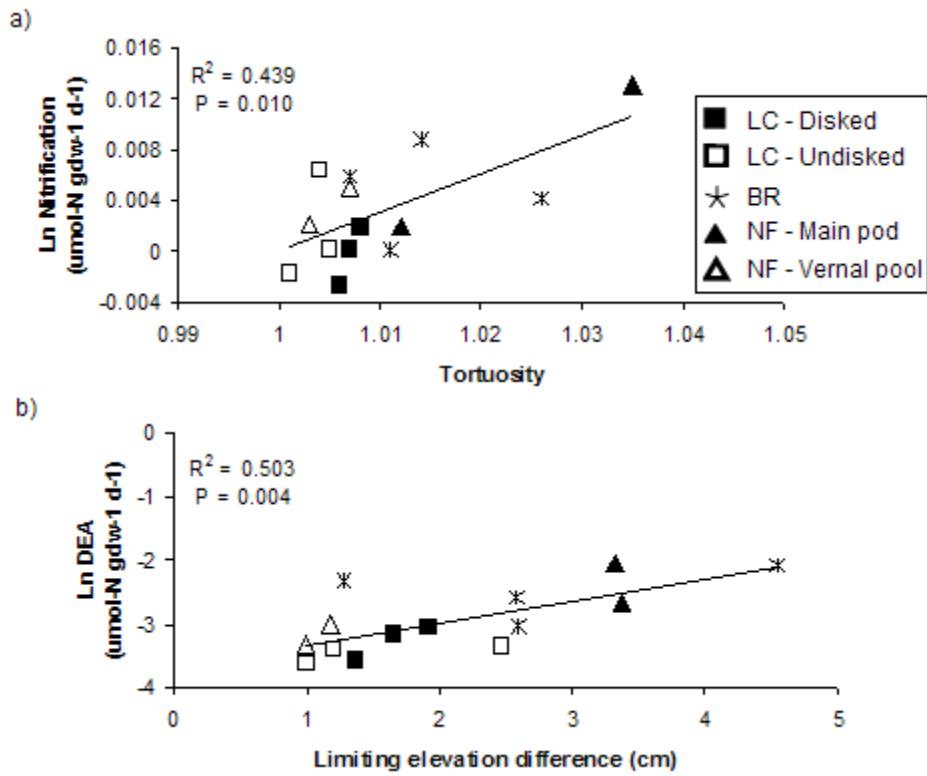


Figure 3

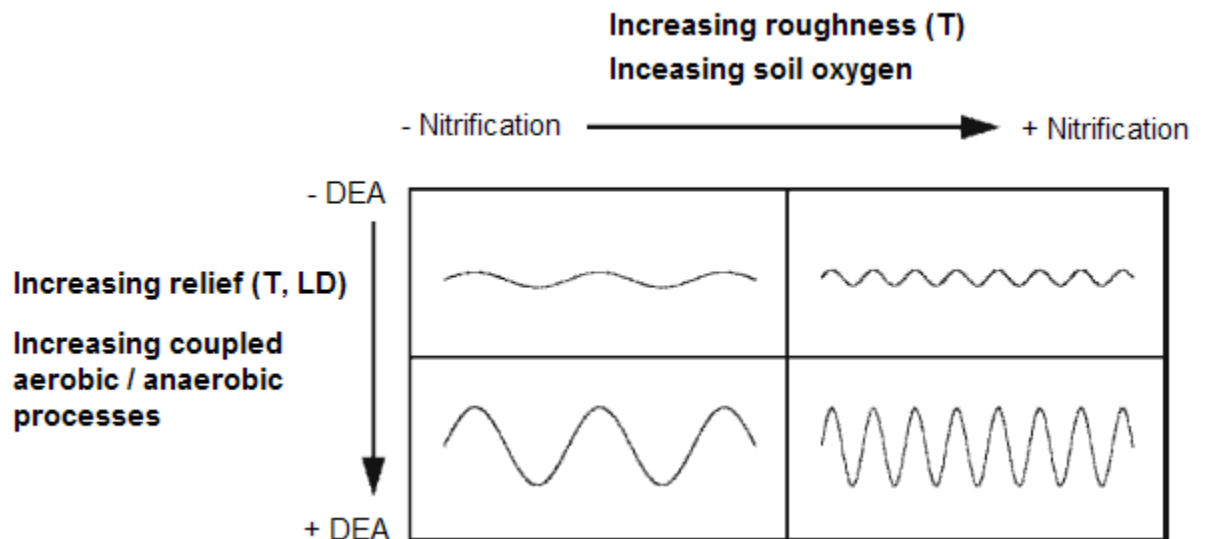


Figure 4

## **Curriculum Vitae**

Kristin Wolf graduated from Mt. Lebanon High School in the great city of Pittsburgh, Pennsylvania. She earned her B.S. in Biology with a concentration in Vertebrate Physiology from Penn State University in 2003. While at Penn State, she also received a minor in International Studies, earned for a semester abroad in Vienna, Austria, where she studied Viennese art, architecture, and history. Kristin earned her M.S. in Environmental Science and Management from Duquesne University, finishing her final semester at the University of Cologne, Germany in 2005. She received grants from The Cosmos Foundation Young Scholars Program, Society of Wetlands Scientists, and the Washington Field Biologist Club for this dissertation project. While at George Mason University, Kristin was a research assistant for the U.S. Geological Survey in Reston, Virginia.