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## Microtopography enhances nitrogen cycling and removal in created mitigation wetlands

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### 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 together facilitate N cycling and removal. This study investigated three created wetlands in the Virginia Piedmont that incorporated disking-induced MT during construction. One site had paired disked and undisked plots, allowing an evaluation of the effects of this design feature on 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 LD 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. The resulting N flux patterns may be explained by the increase in oxygen availability elicited by greater T (enhancing nitrification) and by the adjacent zones of aerobic and anaerobic conditions elicited by greater LD (enhancing coupled nitrification and denitrification potential). 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.

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### 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 compensates for impacts to the structure and function of natural wetlands by creating and restoring wetlands (U.S. Army Corps of Engineers, 2009), although the efficacy of this practice has been called into question (Erwin, 1991; Kentula et al., 1992; Zedler and Callaway, 1999; Hoeltje 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 wet-

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lands 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 et al., 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). This MT is a result of natural processes such as animal burrowing, plant rooting and litter fall, preferential hydrologic flowpaths, and heterogeneous sedimentation. 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). In many situations these construction practices are paired with the homogenizing legacy effects of past land use (e.g. agricultural and pasture lands) (Robertson et al., 1993), which often results in a soil matrix of 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 ( $\text{NO}_3^-$ ) via nitrification. 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, N mineralization and denitrification also require a labile carbon energy source and an organic and inorganic N substrate source available within the soil matrix (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 might 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 measured and their relationships to soil ammonification, nitrification, N mineralization (ammonification + nitrification), and denitrification potential were quantified. Soil physicochemical properties and hydrologic variables were also 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, between vegetation types, and along hydrologic gradients in created wetlands?
- (2) What is the relationship between MT and N cycling and how do soil and hydrologic properties influence this 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 109 cm, mean temperature min 7°C/max 18°C; Fig. 1). The wetland banks were created to mitigate impacts to a mixture of bottomland forested floodplain, shrub/scrub, and emergent wetlands and open water ponds, during local construction projects. All created wetlands were <11 years old and were 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 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 press). 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 regulated by a culvert structure and gate valve. Cell 1 occasionally receives flow from a central ditch that connects it to cell 2. 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 in 2002 in Prince William County, Virginia (38°51'12.74"N, 77°32'58.52"W). The entire wetland was disked during construction. The site receives water from Bull Run from a culvert that routes water via a central ditch through the wetland, as well as overbank flow from Bull Run, 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.

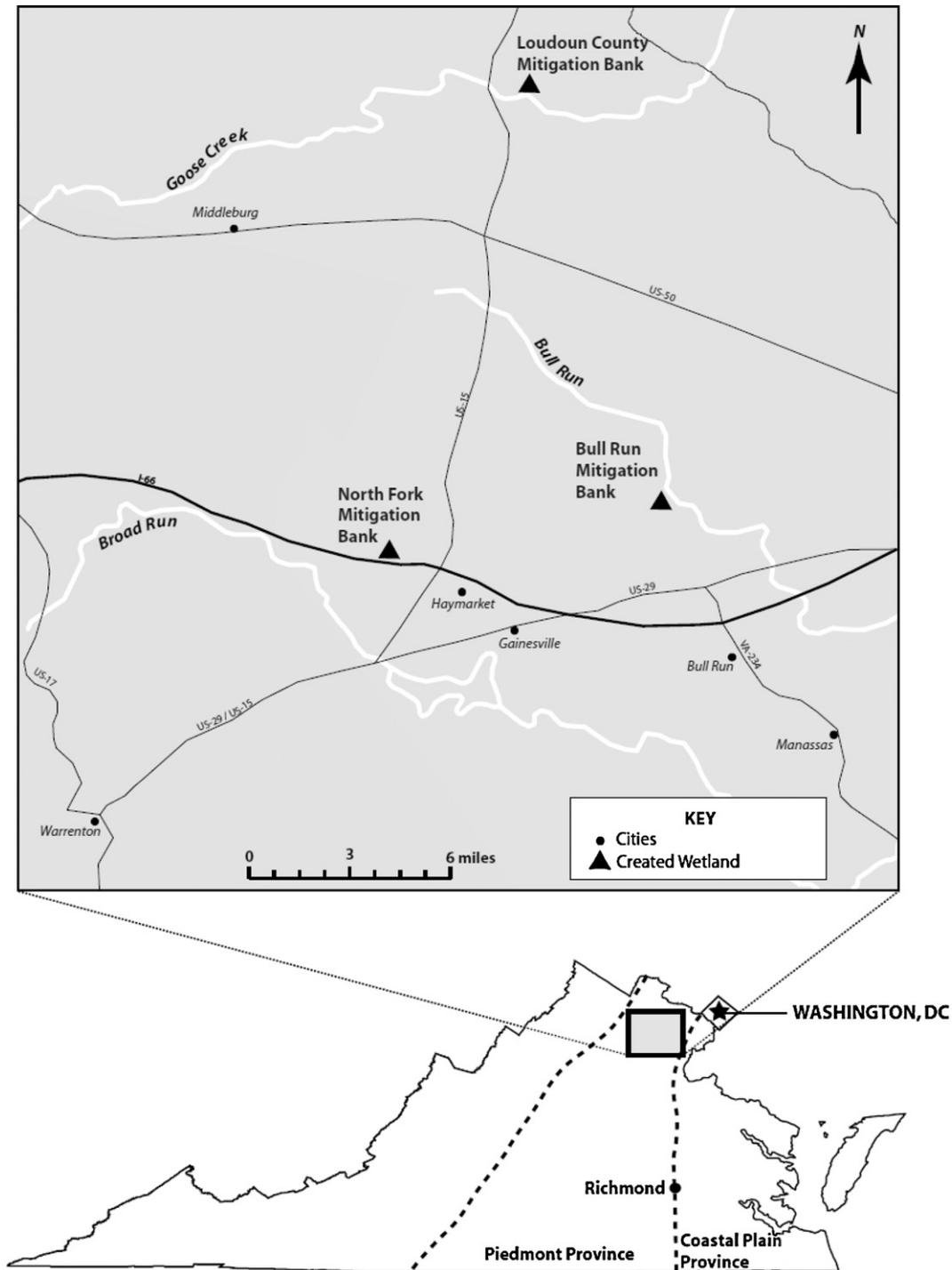


Fig. 1. Site map of wetland locations in the northern Virginia Piedmont, USA.

North Fork Wetlands Bank (NF) is a 125-acre wetland and upland buffer complex, constructed in 1999 in Prince William County, Virginia (38°49'31.53"N, 77°40'9.17"W). The entire wetland was disked during construction. With the exception of minor contributions from toe-slope intercept seepage, the site is disconnected from the groundwater by an underlying clay liner. This site was sampled in two hydrologically distinct 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 and; vernal pool area – located in the southwest quadrant of the wetland and fed solely by precipitation. Vegetation is mostly herbaceous, inter-

persed 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 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 during construction with roller disking equipment that cut and turned the soil surface (to a depth of approximately 40 cm) in order to increase soil surface roughness and relief.

### 3. Methods

#### 3.1. Sampling design

Study plots (10 × 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 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 increase with distance from the corner of the site, which 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, KS, 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 × 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 two-dimensional, unitless metric of soil surface roughness and relief (Kamphorst et al., 2000; Moser et al., 2007). Limiting elevation difference was defined as the maximum elevation change over the measurement interval. The LD index was used as an elevation metric (cm) of soil surface relief (Linden and Van Doren, 1986) and has been used to estimate depressional storage (Bertuzzi et al., 1990; Kamphorst et al., 2000).

#### 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 (2011). 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, 2011).

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 was 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 mid-

dle 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 h to yield initial 2 M 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 (Kaysar-Roth, Inc., Greensboro, NC, USA) stretched over a 7.6 outer diameter, nitrile O-ring (Macro Rubber & Plastics Products, Inc., North Andover, MA, USA), filled with mixed-bed ion-exchange resin beads (Rexyn I-300, Fisher Scientific, Pittsburgh, PA, 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, CT, 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 26–34 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 NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>, 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 μmol + 2 inner resin bags μmol – initial core soil μmol)/(total incubation days × (plot mean bulk density (kg dw cm<sup>-3</sup>) × volume of soil core (251.3 cm<sup>3</sup>))) for NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, and NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup> (N mineralization) expressed as μmol N kg dw<sup>-1</sup> d<sup>-1</sup>.

#### 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 et al., 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, 1987) 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  $M$  is the total amount of N<sub>2</sub>O in water plus gas phase (μg N<sub>2</sub>O-N),  $C_g$  is the concentration of N<sub>2</sub>O in the gas phase (μg N<sub>2</sub>O-N/L),  $V_g$  is the volume of the gas phase (L),  $V_l$  is the volume of liquid phase (L), and  $\beta$  is the Bunsen coefficient (0.544 @ 25 °C) and expressed as μmol N<sub>2</sub>O-N kg-dw<sup>-1</sup> d<sup>-1</sup>.

### 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 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  $VSM / [1 - (BD / \text{quartz parent material density, assuming } 2.65 \text{ g cm}^{-3})]$ . Total soil carbon and N were determined by dry combustion of oven-dried, ground subsamples from each core on a 2400 Series II CHN/O elemental analyzer (PerkinElmer, Waltham, MA). 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 h 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 min. The supernatant was then filtered through a syringe with a 0.2 μm polyethersulfone filter tip (Pall Corporation, Port Washington, NY, USA) and analyzed on an Astoria 3020 series segmented flow autoanalyzer (Astoria-Pacific International, Clackamas, OR, 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, MA, 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 × 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 fall 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; those variables that failed normality tests – gravimetric and volumetric soil moisture, total carbon and nitrogen, carbon to nitrogen ratio, bulk density, mass sedimentation, and all N fluxes – were transformed by natural log transformation. One-way analysis of variance (ANOVA) was used to test for significant mean differences in T and LD between disked and undisked plots at LC and between the main pod and vernal pool plots at 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 at 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.

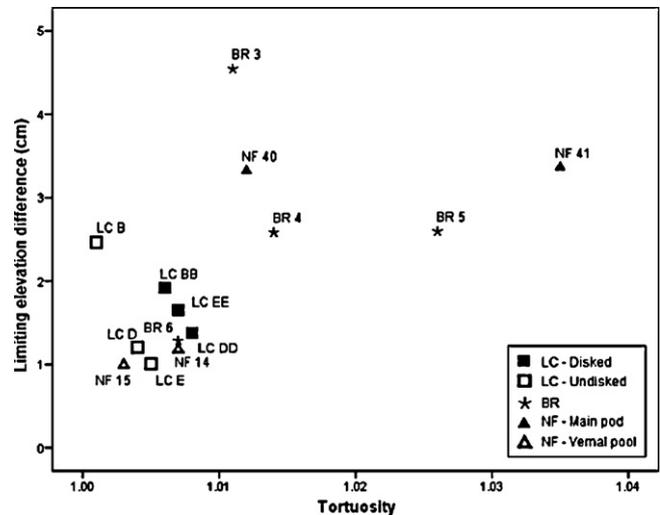


Fig. 2. Scatter-plot of tortuosity vs. limiting elevation difference for Loudoun County (LC), Bull Run (BR), and North Fork (NF) mitigation bank plots. Single letters for LC plots denote undisked plots (B, D, and E) and double letters denote paired disked plots (BB, DD, and EE).

## 4. Results

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

Plot T and LD showed variability between sites and hydrologic designations within sites (Fig. 2). In LC, disked plots demonstrated marginally higher average LD than undisked plots (one-way ANOVA,  $df = 5$ ,  $P = 0.051$ , Fig. 2), but did not differ in T ( $P = 0.858$ ). In NF, plots in the main pod area had significantly higher average LD than vernal pool plots ( $df = 3$ ,  $P = 0.002$ , Fig. 2), but did not differ in T ( $P = 0.254$ ). Tortuosity and LD values for the NF main pod did not differ from measurements made in 2005 by Moser et al. (2007) (Table 1). Average MT indices for BR (5 years old in 2007) and NF main pod (8 years old in 2007) were greater than those for LC (1 year old; Table 1). In BR, LD had a marginally 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. This negative trend, however, was variable as BR 4 and BR 5 demonstrated similar LD values (Fig. 2). Sedimentation also showed a negative correlation with distance from the overflow point ( $r = -0.953$ ,  $P = 0.047$ ), although it showed no correlation with LD ( $r = 0.491$ ,  $P = 0.075$ ). Tortuosity showed no correlation with distance from the overflow point ( $r = 0.000$ ,  $P = 1.0$ ). Tortuosity and LD were positively correlated ( $r = 0.536$ ,  $P = 0.048$ ).

### 4.2. What is the relationship between MT and N cycling and how do soil and hydrologic properties influence this 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 ( $R^2 = 0.439$ ,  $P = 0.010$ , Fig. 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 ( $R^2 = 0.503$ ,  $P = 0.004$ , Fig. 3b), but was not associated with T ( $r = 0.362$ ,  $P = 0.201$ ). Tortuosity was positively correlated with gravimetric soil moisture (Pearson product-moment correlation,  $n = 14$ ,  $r = 0.532$ ,  $P = 0.050$ ).

**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 1 year old  |                  | BR 5 years old | NF 8 years old      |                  |  |
|---------|----------------|------------------|----------------|---------------------|------------------|--|
|         | Disked (n = 3) | Undisked (n = 3) | (n = 4)        | Vernal pool (n = 2) | Main pod (n = 2) | Main pod 2005 (n = 2, Moser et al., 2007)* |
| T       | 1.007 ± 0.001  | 1.003 ± 0.001    | 1.105 ± 0.004  | 1.005 ± 0.002       | 1.024 ± 0.012    | 1.015 ± 0.003                              |
| LD (cm) | 1.65 ± 0.016   | 1.559 ± 0.458    | 2.753 ± 0.672  | 1.086 ± 0.093       | 3.345 ± 0.021    | 3.400 ± 0.469                              |

Limiting elevation difference 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 ( $r=-0.554$ ,  $P=0.040$ ).

## 5. Discussion

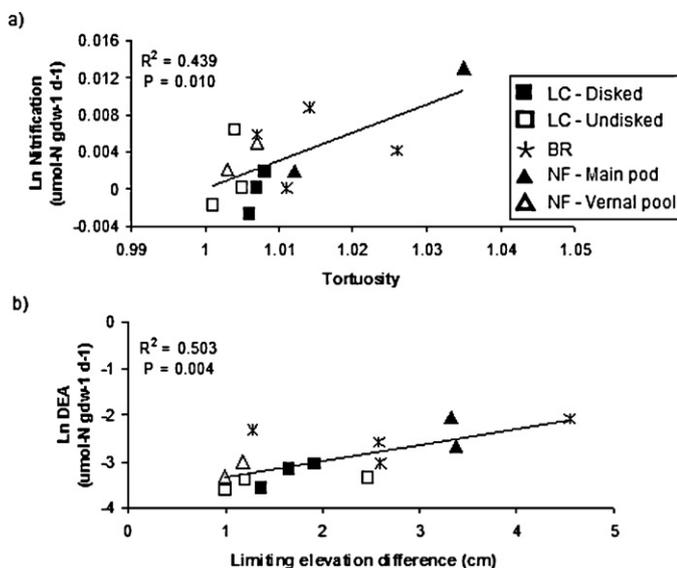
### 5.1. How does MT differ between disked and undisked plots, vegetation types, and along hydrologic gradients in created wetlands?

Limiting elevation difference was significantly higher in disked plots than undisked plots in the LC site one year after the wetland was created (Fig. 2). While one year is a relatively short time frame by which to judge MT establishment and persistence, it is a critical period during which a created wetland may be particularly vulnerable to loss of soil surface topography. During its first year the LC 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 that fills microtopographic depressions (Huenneke and Sharitz, 1986; Werner and Zedler, 2002). The fact that the enhanced MT of the disked plots was maintained during this period of vulnerability, indicates that MT can persist in newly created, sparsely vegetated wetlands.

Limiting elevation difference was significantly higher in the NF main pod plots which received overland surface and stream flow than the vernal pool plots which were fed by precipitation and overland surface flow (Fig. 2). The differences in LD at the NF site present eight years after construction must be due to natural processes that decrease or increase MT over time, because both the main pod and vernal pool plots received uniform topsoil amendments and were disked when the wetland was created in 1999. 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 (Ahn and Dee, in press). *Carex* communities in wetlands were found to increase soil surface area in Midwestern meadows (Peach and Zedler, 2006) and to 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 uniformly 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; Chimmer and Hart, 1996; Roy et al., 1999; Duberstein and Connor, 2009). They also create heterogeneous MT and a 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 was the persistence of MT over time within the main pod area. When compared with Moser et al. (2007) values, T and LD values remained essentially the same from 2005 to 2007 (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. This finding again demonstrates the importance autogenic MT in maintaining soil surface roughness and relief over time.

When comparing MT across wetland ages, average T and LD were greater in the older created wetlands (5 year old BR and 8



**Fig. 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.

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 effusus* tussocks, non-tussock-forming herbaceous plants, and annual rye grass (*Lolium* L.) seeded for erosion control (Ahn and Dee, in press). 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 vegetative establishment and increase with age in created wetlands. It should also be noted that MT indices for created wetlands in this study were within range and in some cases exceeded values measured at the same 1-m scale (T: 1.001–1.012, LD: 0.9–3.2 cm) for a natural wetland in Virginia (Moser et al., 2007).

While no significant correlation existed between T or LD and distance from the overflow point in BR, LD averages generally decreased as plots got farther away from the overflow point (BR 3 being closest to the overflow point and BR 6 being farthest away) (Fig. 2). The lack of a significant correlation between LD and distance from overflow point was likely due to low statistical power. 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., unpublished data). The dynamic hydrology that BR 3 experiences might have enhanced 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, similar to the unvegetated sand mounds described by Owen et al. (1989) in Wisconsin restoration sites, BR 3 is richly vegetated with exceptional N cycling capacity (Wolf et al., in press). 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 effusus* 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 may be at least partially responsible for their enhanced soil surface relief. This trend indicates that sedimentation has not smothered soil surface topography, as it has been shown to do in other studies (Huenneke and Sharitz, 1986; Werner and Zedler, 2002), but rather has potentially enhanced MT in study plots.

### 5.2. What is the relationship between MT and N cycling and how do soil and hydrologic properties influence this 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 slow organic matter breakdown and  $\text{NH}_4^+$  production, but decrease  $\text{NH}_4^+$  loss by nitrification, leading 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 or across a redox gradient within soil particles), the effects of greater MT on net ammonification would

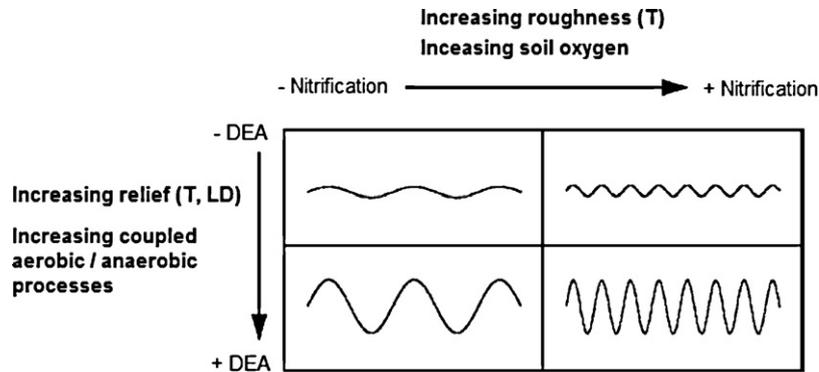
not be detectable by our measurement of net (as opposed to gross) ammonification using *in situ* incubation of modified resin cores. In a study of similar wetlands, Moser et al. (2007) found that  $\text{NH}_4^+$  concentrations were higher in plots with greater MT (disked) than those with lower MT (undisked). 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 presented here.

Nitrification significantly increased with greater T (Fig. 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. In addition, 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.

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.

Denitrification potential significantly increased with greater LD (Fig. 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, with greater soil surface relief, organic matter and carbon are retained 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  $\text{N}_2$  (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  $\text{NO}_3^-$  by denitrification with enhanced MT. These results differ from Bruland and Richardson (2005) who 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 in this study.

One unexpected result of this study was 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., unpublished data). 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 might 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, might indicate the presence of more established or permanent hummocks. The tops of hummocks are more consistently



**Fig. 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).

above the water table and have higher oxygen concentrations that support nitrification (Fig. 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. It should also be noted that the DEA provided optimized denitrification potential rates and likely overestimated the actual denitrification rate. This is in contrast to nitrification rates, which were measured *in situ*, and thus, may influence the correlation between these two processes.

Tortuosity 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 (Fig. 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 MT 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 likely 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 may also increase with age in created wetlands if vegetative development enhances originally induced MT. The effect of sedimentation on MT in created wetlands is variable; 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, thus, sedimentation does not degrade MT in all cases. Finally, the incorporation of MT into created and restored wetlands increases N cycling: soil surface roughness and relief increase nitrification likely 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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