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ARTICLE

Development of Soil Properties and Nitrogen Cycling in Created Wetlands

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Abstract Mitigation wetlands are expected to compensate for the loss of structure and function of natural wetlands within 5–10 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 attain the water quality ecosystem service of nitrogen (N) cycling over time. Soil condition component and its constituents, gravimetric soil moisture, total organic carbon, and total N, generally increased and bulk density decreased with age of the created wetland. 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 and natural reference wetlands. Results show a clear age-related trajectory in coupled soil condition and N cycle development, which 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.

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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. The practice of compensatory mitigation assumes that created and restored wetlands will replace losses in wetland structure and function within the 5–10 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 comparable 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 et al. 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 over consecutive growing seasons, as vegetative seasonal dieback and litter fall contribute autogenic materials to the soil (Reddy and D'Angelo 1997). Bishel-Machung et al. (1996) and Shaffer and Ernst (1999), however, found no relationship between organic matter and time 3–5 or 6 years after creation, respectively, and Ballentine and Schneider (2009) found no significant increases in soil organic matter until 35 years after creation. The lack of organic carbon (C) accumulation, in particular, has been found to be characteristic in many created wetlands under 10 years old (Whittecar and Daniels 1999; Stolt et al. 2000; Hossler and Bouchard 2010), which has implications for vegetation development (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: ammonification, nitrification, and denitrification, require an energy source in the form of labile C and their respective N substrates (Reddy and Patrick 1984), both of which must be available in the soil. N processing also requires fluctuating redox conditions, specifically, aerobic conditions for expedited ammonification and nitrification, followed by anaerobic conditions for NO_3^- removal by denitrification (Ponnamperuma 1972; Reddy and Patrick 1984).

Considering the obvious link between soil properties and coupled N processes, the design, construction, and maturation of mitigation wetlands may have a cumulative impact on these elements. 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 influencing 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 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 and natural non-tidal freshwater wetlands in the Virginia Piedmont physiographic province (Hunt 1967) (Washington D.C. area mean annual precipitation 109 cm, mean temperature min 7°C / max 18°C) (Weatherbase 2011). Soil properties and N fluxes were compared among two natural reference wetlands and four created wetlands, aged 3, 4, 7, and 10 years, that 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 (Fig. 1). The influence of soil properties on N fluxes was investigated. 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?

Site Descriptions

Created Wetlands

Loudoun County Mitigation Bank (LC) is a 12.9 ha wetland and upland buffer complex, constructed in the summer of 2006 (3 years old during study year) in Loudoun

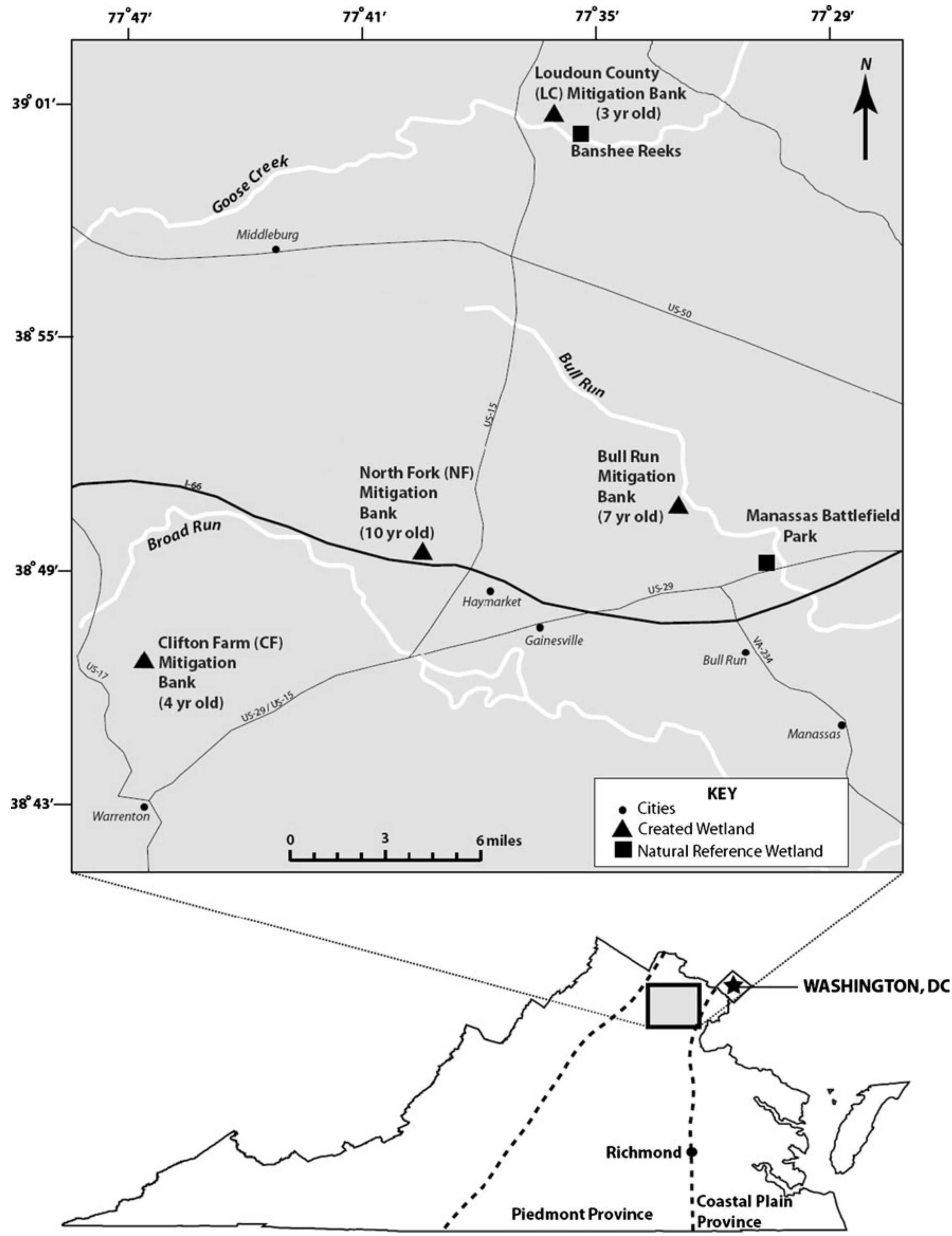

Fig. 1 Site map of wetland locations in the Virginia Piedmont, USA

County, Virginia (39°1′ N, 77°36′ 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.

Clifton Farm (CF) is a 0.9 ha mitigation wetland, constructed in 2005 (4 years old during study year) in Fauquier County, Virginia (38°46′ N, 77°47′ W). The site receives groundwater from a small upland reservoir and surface water runoff, but has no stream connection.

Bull Run Mitigation Bank (BR) is a 20.2 ha wetland and upland buffer complex, constructed in 2002 (7 years old during study year) in Prince William County, Virginia

(38°51′ N, 77°32′ W). The site receives water from Bull Run from a culvert structure 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 wetlands and negligible groundwater.

North Fork Wetlands Bank (NF) is a 50.6 ha wetland, constructed in 1999 (10 years old during study year) in Prince William County, Virginia (38°49′ N, 77°40′ W). With the exception of minor contributions from toe-slope intercept seepage, the site is disconnected from the groundwater by an underlying clay liner. Study plots are located in two created hydrologic regimes: 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.

The LC, BR, and NF wetlands contain at least a 0.3 m low permeability subsoil layer covered with the stockpiled original topsoil from the site that was supplemented with commercially available topsoil to a depth of 0.2 m. This design creates a perched, precipitation-driven water table close to the soil surface and limits groundwater exchange in the wetland (Ahn and Peralta 2009). Vegetation in the created wetlands is mostly herbaceous, interspersed with young tree saplings and shrubs in projected forested areas.

Natural Wetlands

Manassas National Battlefield Park (BFP), established in 1940, is a 2,000 ha site with areas of natural wetland coverage located in Prince William County, Virginia (38°49′ N, 77°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 290 ha site with areas of seep and riparian wetlands located in Loudoun County, Virginia (39°1′ N, 77°35′ W). These floodplain riparian wetlands receive water from groundwater springs, surface water runoff, and overbank flooding from Goose Creek. Vegetation is mature bottomland forest with little understory.

Methods

Sampling Design

A total of 20 study plots across 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. Soil sampling in the wetlands occurred over a two-day period the second week of every month from July 2008 to July 2009.

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 modified design includes three mixed-bed ion-exchange resin bags located above and below soil incubating inside a core tube. The two inner resin bags adjacent to the soil capture NH_4^+ and NO_3^- transported out of the soil during incubation, the two outer resin bags remove inorganic nutrients transported into the modified resin core, and the two middle bags serve as quality-control checks on the function of the inner and outer resin bags. The sampling, extraction, and analysis of soil cores and resin beads followed the same methodology as Noe (2011), but increased the amount of resin in the two inner bags by 10 g as necessitated by the higher rate of capture by inner bags in a pilot study. Study plots were sampled by randomly placing a 1 m^2 quadrat at the beginning of the study that divided the sampling area into 100 10 cm^2 cells. The surficial soil (0–5 cm) of two adjacent 10 cm^2 cells were randomly sampled each month. The first cell sampled was processed as an initial core and was immediately analyzed for NH_4^+ and NO_3^- concentrations, and the second cell sampled was processed as a resin core (described above) incubating it in situ for approximately 1 month (range 26–35 days) before nutrient analysis. Monthly estimates of net ammonification and nitrification were calculated as the sum of extractable NH_4^+ and 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 (1 sample per plot per month for 1 year): (resin core soil μmol+2 inner resin bags μmol - initial core soil μmol) / ((total incubation days×(plot mean bulk density (kg-dw cm^{-3})×volume of soil core (251.3 cm^3)) for NH_4^+, NO_3^-, and $NO_3^-+NH_4^+$ (N mineralization) expressed as μmol-N kg-dw^{-1} d^{-1}.

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) with a 1 mM glucose, 1 mM KNO_3, and 1 g L^{-1} chloramphenicol amendment, as detailed in Wolf et al. (in press). 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_2O on a Shimadzu 8A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) with electron capture detection, generally within 3 days of sampling. DEA was calculated as: $M = C_g^*(V_g + V_l \times \ )$ where, M = total amount of N_2O in water plus gas phase (μg N_2O-N), C_g = concentration of N_2O in the gas phase (μg N_2O-N/L), V_g = volume of the gas phase (L), V_l = volume of liquid phase (L), and ß = Bunsen coefficient (0.544 @ 25°C) and expressed as μmol-N kg-dw^{-1} d^{-1}.

Soil Physicochemical Properties

Field Analysis

Redox potential was measured each month by inserting a RE 300 ExStik® 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 stabilized (approximately 1 min).

Lab 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^3). Volumetric soil moisture (VSM) was calculated as BD×(GSM / density of water, assuming 1.0 g-H_2O mL^{-1}). Water-filled pore space (WFPS) was calculated as VSM / [(1-(BD/quartz parent material density, assuming 2.65 gcm^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, MA). 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). Soil texture analysis was conducted using a LISST-100X laser particle size analyzer (Sequoia Scientific, Inc., Bellevue, WA, USA) on a mixture of 0.02 g of well-mixed soil (combusted at 550°C for 4 h to remove organics and then sieved to ≤250 μm), 10 mL sodium hexametaphosphate solution (50 g NaHMP/L), and 90 mL deionized water for a final soil concentration of 200 mg/L. This mixture was placed in an ultrasonic bath for 5 min and then agitated on a shaker table at 100 rpm for 16 h in order to breakup soil aggregates before being analyzed on the LISST fitted with a stirring mixing chamber (Gee and Bauder 1986). The LISST reported particle volume concentrations for 32 log-spaced size classes ranging in size from 1.2 to 250 μm. Median particle size (D50) was interpolated from the cumulative size distribution of LISST output. The percent clay (<2 μm), silt (2–50 μm), and fine sands (50–250 μm) were determined from their appropriate LISST 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-μm sieve.

Statistical Analysis

All data were reviewed for normality and those variables failing tests for normality were transformed 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. Identification of the PCA components enabled the investigation of the combined effect of numerous soil variables on each N flux rate and characterized the relationship that individual variables had with each other within their component. 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 SC and TXT components between wetlands. The PCA 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 α=0.05, unless otherwise noted.

Results

Identification of Soil Condition and Texture Components

The PCA resulted in a two component model that explained a cumulative 83% of total variance in soil structural variables (Table 1). The SC component explained 45.5%

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

	Soil Condition component (SC)	Soil Texture component (TXT)
	Avg total organic carbon (.935)	Avg particle size diameter (.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%

of total variance and was characterized by higher TOC, TN, and gravimetric soil moisture and lower bulk density. The TXT component explained 37.5% of total variance and was characterized by higher sand percentage and higher redox potential, larger median particle diameter, and lower clay content. Soil texture classification was determined as silt loam for all wetland plots, with the exception of one NF plot and one LC plot, which were classified as sandy loam and silty clay loam, respectively.

How Do Soil Properties in Created Wetlands Develop Over Time?

Natural wetlands and the 7 year old wetland had the highest SC scores (one-way ANOVA, df=5,14, Fig. 2a), followed by the 10 year old wetland, and then the 3 and 4 year old wetlands, which had significantly lower SC scores than the other wetlands. A 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 year old and natural wetlands, followed by the 10 year old wetland, and then the 3 and 4 year old wetlands, which had the lowest values (Fig. 2b, c, and d, respectively). Bulk density followed the opposite pattern in that 3 and 4 year old wetlands had the highest bulk density, followed by the 10 year old and BSR natural wetlands, and finally the 7 year old and BFP natural wetlands, which had the lowest bulk density (Fig. 2e). The TXT component scores and variables that comprised the TXT component (P>0.05, data not shown) showed no significant differences across wetland ages (Table 2, Fig. 2f).

How Do N Flux Rates Differ between Created Wetlands of Different Ages and between Created and Natural Wetlands?

Soil net ammonification rates were highly variable along the age trajectory to natural wetlands. Seven year old and BFP natural wetlands had the highest ammonification rates, followed by 10, 3, and 4 year old wetlands, and BSR natural wetland, which had the lowest ammonification rate (one-way ANOVA, df=5,14, Fig. 3a). Net N mineralization, the sum of net ammonification and net nitrification, showed the same type of pattern as ammonification among the wetland sites (Fig. 3b). The similarities and differences in pattern between ammonification and net N mineralization were 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 (Fig. 3c). Natural wetlands had the highest nitrification rates, followed by 7 and 10 year old wetlands, and 3 and 4 year old wetlands, which had the lowest nitrification rates (Fig. 3b). Potential denitrification rates had a step pattern with age with 3 and 4 year old wetlands demonstrating significantly lower DEA rates than 7 and 10 year old and natural wetlands (Fig. 3d).

How Do These Age-Related Soil Properties Influence N Flux Rates in Created and Natural Reference Wetlands?

Ammonification was marginally influenced by SC (Fig. 4a) and was positively correlated with TOC (Table 3). Ammonification was also positively correlated with TC sedimentation at α=0.10 (Table 3). The SC component was a significant positive predictor of nitrification (Fig. 4b). Nitrification was positively correlated with gravimetric soil moisture, TOC, and TN negatively correlated with bulk density (Table 3). Nitrification was also positively correlated with redox potential. Soil condition was a significant positive predictor of N mineralization (Fig. 4c). Net N mineralization was significantly positively correlated with TOC and TN (Table 3). Soil condition was a significant positive predictor of DEA (Fig. 4d). Denitrification potential was positively correlated with gravimetric soil moisture, TOC, and TN and negatively correlated with bulk density (Table 3). The TXT component was not predictive of any of the N cycling rates (P>0.05, data not shown).

Discussion

Soil Condition and Texture Components

The separation of soil variables into the PCA components SC and TXT indicates that soil quality (TOC and TN),

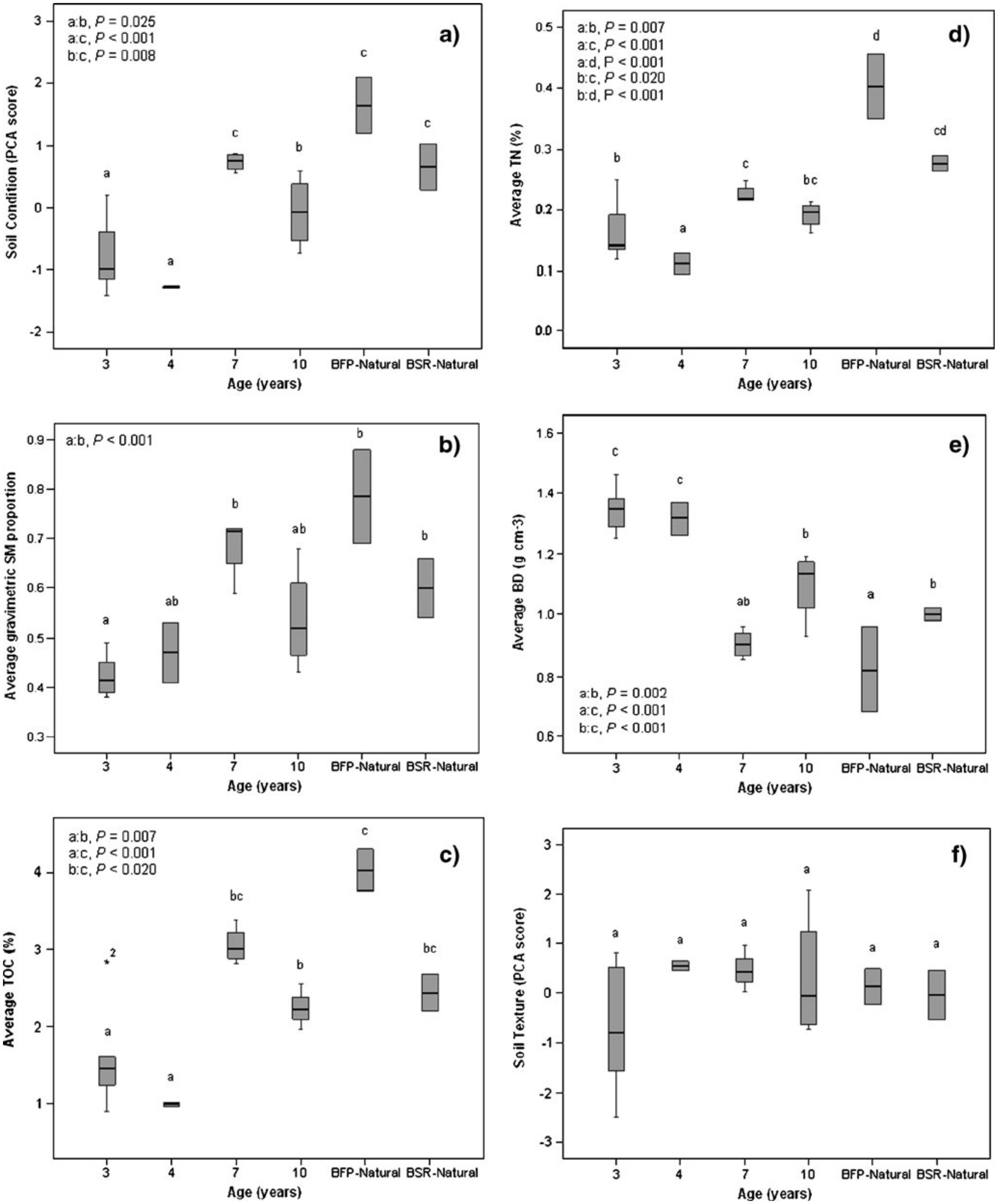

Fig. 2 Boxplots showing average **(a)** soil condition (SC) component score, **(b)** gravimetric soil moisture, **(c)** total organic carbon (TOC), **(d)** total nitrogen (TN), **(e)** bulk density (BD), and **(f)** soil texture (TXT) component score for 3, 4, 7, and 10 year old created wetlands and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

Table 2 Soil texture, redox potential, and carbon and nitrogen sedimentation by wetland age (mean ± one standard error)

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

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

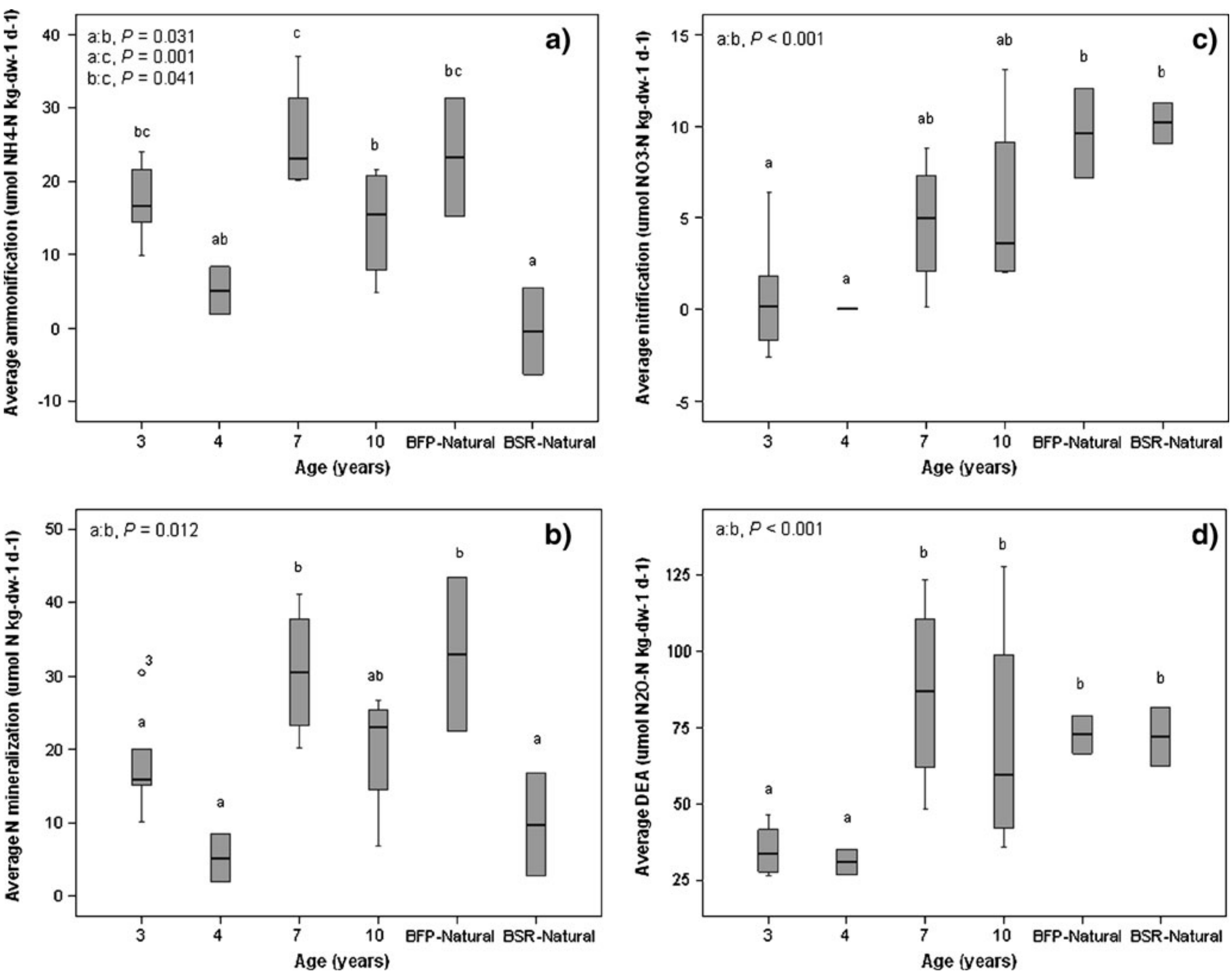

Fig. 3 Average **(a)** ammonification, **(b)** nitrogen mineralization, **(c)** nitrification, and **(d)** denitrification potential (DEA) rate for 3, 4, 7, and 10 year old created wetlands and BFP and BSR natural wetlands. Letters **(a–c)** represent significant differences between wetland plots for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

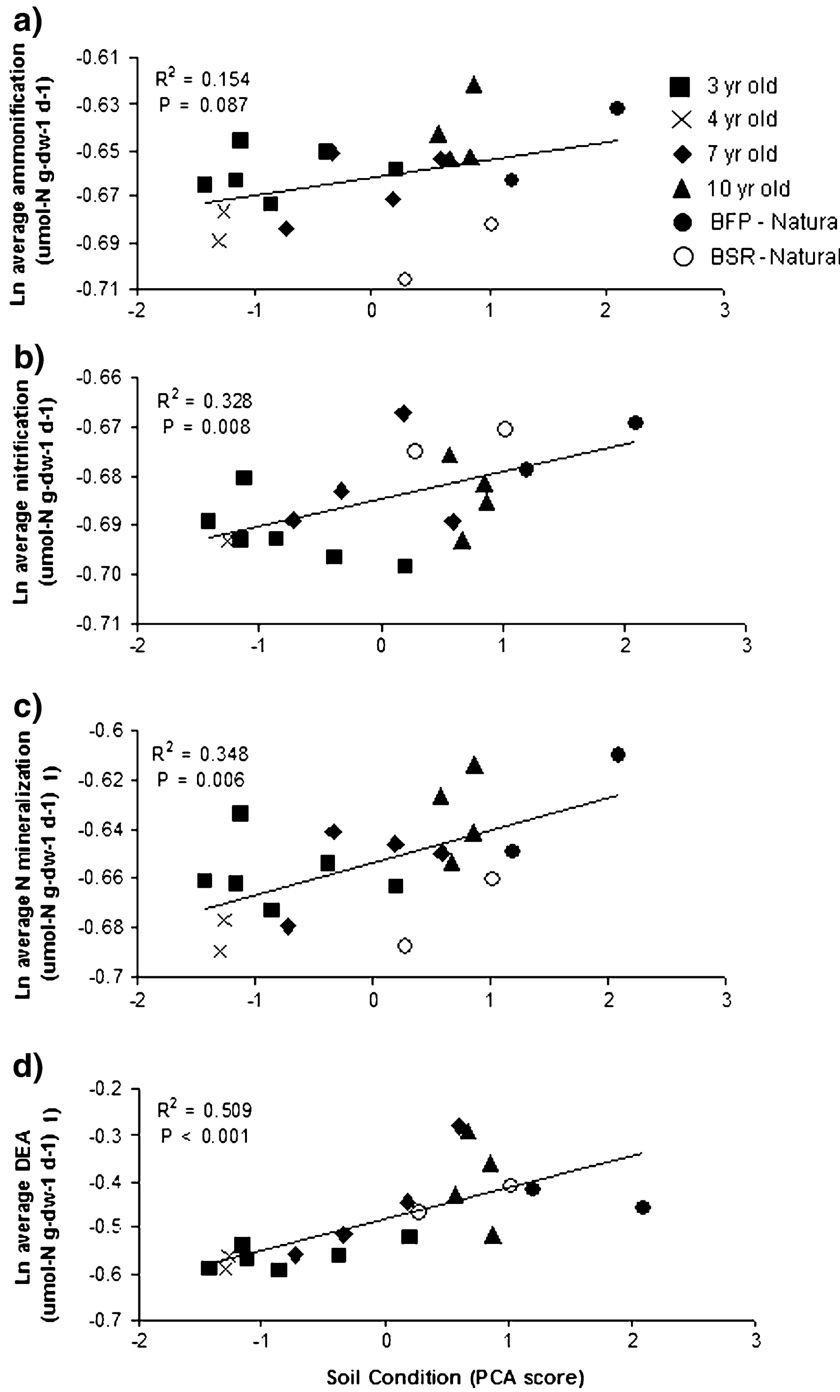

Fig. 4 Linear regression of soil condition component vs. **(a)** ammonification, **(b)** nitrification, **(c)** nitrogen mineralization, and **(d)** denitrification potential (DEA)

2008). Variables within the TXT gradient are similarly interconnected, as higher percentages of sand indicate larger average particle diameter, lower percentages of clay, and higher redox potential. While variables within the TXT component should influence and be influenced by SC variables (i.e. higher clay content increases bulk density, higher bulk density decreases redox, etc.), TXT variables did not vary substantially between wetlands as SC variables did (Fig. 2f, 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. The age of the wetland had a negligible effect on the texture of study 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).

Table 3 Pearson product-moment correlations (n=20) for nitrogen flux rates and soil properties, including gravimetric soil moisture (GSM), soil total organic carbon (TOC), soil total nitrogen (TN), bulk density (BD), redox potential (redox), and total carbon sedimentation (TC sed).

	GSM	Soil TOC	Soil TN	BD	Redox	TC sed
Ammonification	r=0.201	r=0.449	r=0.168	r=−0.223	r=−0.280	r=0.435
	P=0.395	P=0.047	P=0.479	P=0.344	P=0.232	P=0.056
Nitrification	r=0.453	r=0.513	r=0.547	r=−0.571	r=0.509	r=−0.009
	P=0.045	P=0.021	P=0.013	P=0.009	P=0.022	P=0.971
N Mineralization	r=0.453	r=0.549	r=0.481	r=0.418	r=−0.139	r=0.259
	P=0.045	P=0.012	P=0.032	P=0.066	P=0.558	P=0.271
DEA	r=0.776	r=0.771	r=0.647	r=−0.774	r=0.322	r=−0.200
	P<0.001	P<0.001	P=0.002	P<0.001	P=0.166	P=0.397

Created Wetland Soil Properties Compared by Age and Compared to Natural Reference Wetlands

A consistent pattern of SC and variables therein was apparent across wetland ages (Fig. 2). Created wetlands that were 3 and 4 years old showed markedly less soil development, specifically less gravimetric soil moisture, TOC, and TN 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 a soil that is low 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 et al. 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 et al. 1996; Campbell et al. 2002; Hunter et al. 2008; Hossler and Bouchard 2010) than natural reference wetlands.

Seven and 10 year old created wetlands generally had values for SC and its constituent variables that were intermediate between the younger (3 and 4 year old) created wetlands and natural wetlands (Fig. 2). The 10 year old wetland had significantly lower bulk density and higher TOC than 3 and 4 year old wetlands, but lower TOC, TN, and overall SC score than natural wetlands. The 7 year 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, TOC, bulk density, and overall SC, and differed only by having lower soil TN. The fact that the 7 year old wetland soils were more similar to those of natural wetlands than the 10 year old wetland soils may be the result of the higher TC and TN sedimentation rate in the 7 year 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 year old wetland soil to better resemble the soil properties of the natural wetlands. The 7 year 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. unpublished data).

The natural wetlands, while demonstrating higher SC than the 10 year old and similar SC to the 7 year old wetland, have different individual soil properties when compared to each other. The BSR plots are located on a forested floodplain along a highly incised portion of Goose Creek that received little flooding and sedimentation during the study year (Table 2). The BFP plots, in contrast, are located in an herbaceous, culvert-fed floodplain of Bull Run that is well-connected to the stream and thus had a higher occurrence of flooding and sedimentation (Table 2). This may explain the lower gravimetric soil moisture, TOC, and TN, as there was less opportunity for allochthonous nutrient import at BSR, as well as less standing vegetation to be incorporated into the soil as organic matter.

A trajectory of soil development was apparent with the older created wetlands having similar soil condition as the natural wetlands (Fig. 2a). 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, mixed wetland types, 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 et al. 1996; Shaffer and Ernst 1999; Campbell et al. 2002). It should be noted that 3 of the 4 created study wetlands received commercial topsoil during construction, in contrast to created wetlands of cited comparison studies which did not, suggesting that the addition of topsoil may be necessary for proper soil development. Study results also indicated that created wetlands may (7 year old wetland) or may not (10 year old wetland) accumulate natural levels of organic C within 10 years in order to 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).

The Effects of Age-Related Soil Properties on N Flux Rates

Ammonification was positively correlated with both soil TOC and TC sedimentation (Table 3). The conversion of organic N to NH4+ 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. Soil TOC 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 TOC and more than 9 times the amount of TC sedimentation as BSR natural wetland, owing to the greater hydrologic connectivity of the BFP site floodplain. 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 year old created wetlands had lower ammonification rates than 7 and 10 year old wetlands, which were similar to BFP natural wetland, but higher than rates for BSR natural wetlands (Fig. 3). This relationship can be explained by TC sedimentation (and TN sedimentation although its relationship to ammonification was not significant), as well as the age-related effects of TOC incorporation into the soil over time. These results support the findings of Pinay et al. (1995) that ammonification is stimulated by allochthonous import of organic material in sediments.

Nitrification increased with greater SC (Fig. 4b) and was positively correlated with soil TOC and TN (Table 3). This relationship is to be expected as microbial nitrification requires inorganic N substrate in the form of NH4+ (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 negatively correlated with bulk density and positively correlated with gravimetric soil moisture and redox potential (Table 3). 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. The influence of oxygen availability on nitrification has also been found by Strauss et al. (2004) who found that oxygen penetration into the sediments of the Upper Mississippi regulated nitrification, as well as Neill (1995) and Zak and Grigal (1991) who found higher nitrification rates in more oxygen-rich environments, including 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 year old wetlands having the lowest rates, 7 and 10 year old wetlands having intermediate rates, and natural wetlands having the highest rates (Fig. 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 increases with age, indicating that this trend is not limited to wetlands ecosystems and may be linked to successional processes.

Nitrogen mineralization increased with SC (Fig. 4c) and was positively correlated with TOC and TN (Table 3). These patterns are 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 the created and the BFP natural wetland is similar to patterns found in constructed salt marsh soils with consistently low redox potentials (Langis et al. 1991). Nitrogen mineralization in the BSR natural wetland, in contrast, was dominated solely by nitification contributions (200%), which were offset by the net negative ammonification rate at the site (−100% of total N mineralization) and were more similar to patterns found in riparian wetland soils in semi-arid Northwest Colorado (Adair et al. 2004). Like ammonification patterns, 3 and 4 year old created wetlands had lower N mineralization rates than 7 and 10 year old wetlands, which were similar to BFP natural wetland, but higher than rates for BSR natural wetlands (Fig. 3). Results are similar to Verhoeven et al. (1990) and Fickbohm and Zhu (2006) who 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.

Much like the patterns for nitrification, DEA increased with SC (Fig. 4d) and was positively correlated with soil gravimetric soil moisture, TOC, and TN and negatively correlated with bulk density (Table 3). 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 NO_3^-. While denitrification requires anaerobic conditions, it is usually limited by the availability of NO_3^- substrate (Martin and Reddy 1997), 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., unpublished data), 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 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 (Tiedje et al. 1989; Pinay et al. 2000; Hefting et al. 2004).

Nitrogen flux rate results demonstrate that N cycling increases with greater SC. These results highlight the relationship between the structural maturation of soils, such as increased TOC and TN and decreased bulk density, and the functional development of N cycling within the created wetland. The increased availability of N substrate and C energy sources, along with the moisture retention capability and fluctuating redox conditions enabled by a more porous, organic matter-rich soil support the various processes of the N cycle from its breakdown of organic N to its removal of N2 through denitrification.

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 year old) showed significantly less soil development than older created wetlands (7 and 10 year old), while older created wetlands showed less (10 year old) or similar (7 year old) soil development to that of natural wetlands. Local site variability in created wetlands, notably hydrologic connectivity and its resultant nutrient subsidies, likely explain the greater soil development of the 7 year old wetland as compared to the 10 year old. 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 10 years of creation, it is not guaranteed, indicating that environmental factors independent of time 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 how well created wetlands function in comparison to the natural 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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