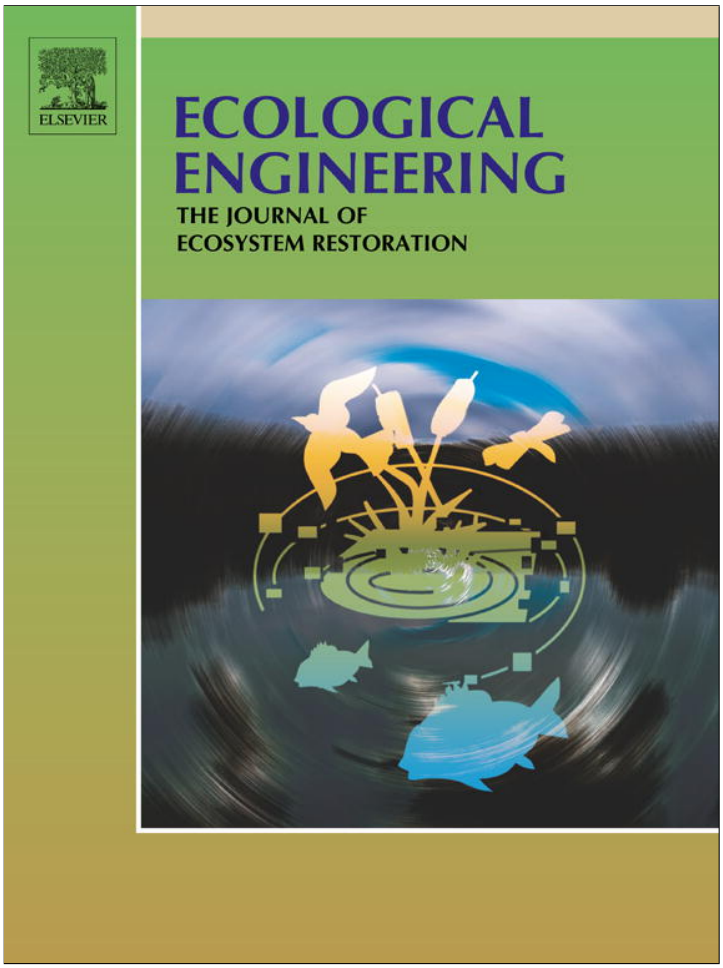

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Soil properties are useful to examine denitrification function development in created mitigation wetlands

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ABSTRACT

We investigated structural soil attributes, the development of denitrification potential (DP), and their relations in created and natural non-tidal freshwater wetlands in Virginia. Soil attributes included soil organic matter (SOM), total organic carbon (TOC), total nitrogen (TN), pH, gravimetric soil moisture (GSM), and bulk density (Db). A subset of soil attributes were analyzed across the sites, using Euclidean cluster analysis, resulting in three soil condition (SC) groups of increasing wetland soil development (i.e., SC1 < SC2 < SC3 less to more developed) as measured by accumulation of TOC and TN, the increase of GSM, and the decrease of Db. Denitrification enzyme activity (DEA) was measured for DP. DEA rates were somewhat different by wetland site, but with no age-based trajectory. No significant difference was seen in DEA rates by sampling period ($p = 0.06$). However, DEA rates were clearly differentiated by SC groups, with the highest rates in SC3 followed by SC1 and lowest in SC2. The lowest DEA rates in SC2 seemed associated with a higher soil pH (~6.6) in the group than that (~5.3) of the other SC groups, but it needs a further investigation. The principal component analysis (PCA) of soil physicochemical properties and average DP showed the association between the development of denitrification function and the maturation of soil conditions in wetlands. The outcome of the study suggests that the use of a suite of simple soil properties may be useful to examine the development of denitrification function in created mitigation wetlands. The inclusion of soil properties in post-construction monitoring should be required to enhance our understanding and prediction of the functional development of created mitigation wetlands.

1. Introduction

Denitrification is one of the key ecological functions of natural wetlands extensively studied (Groffman, 1994; Hunter and Faulkner, 2001; Hill and Cardaci, 2004). Denitrification requires anoxic conditions and organic matter that are often associated with hydric soils characteristic of most natural wetlands (Mitsch and Gosselink, 2000). Numerous studies have investigated the factors controlling denitrification in an attempt to better understand the process, mostly focusing on the roles of NO_3^- availability, O_2, and pH (Firestone et al., 1979; Weier et al., 1993; Thomas et al., 1994).

Denitrification is difficult to quantify due to its high spatial and temporal variability and because the N_2 produced by denitrification is difficult to measure in the presence of ambient concentrations of atmospheric N_2 (Tiedje et al., 1989). Denitrification can be assessed with surrogate measurements such as denitrification potential (DP). Denitrification enzyme activity (DEA) has been used as an index of DP in numerous studies (Groffman, 1994; Jordan et al., 2007; Hopfensperger et al., 2009). The DEA represents the activity of denitrifying enzymes *in situ* (i.e., enzymes active at the time of sampling), and therefore is indicative of the historic site conditions (Tiedje et al., 1989). The rate represents not only enzyme activity, but also the environmental factors that control enzyme expression (O_2 content, C availability, and NO_3^- concentration). In anaerobic environments without carbon limitations, the amount of enzyme produced is proportional to the concentration of nitrate available, and the rate of N_2O production is proportional to the enzyme content (Tiedje et al., 1989). The DEA is useful for site comparisons since it offers a method by which the DP can be compared across different soil types and conditions (Groffman, 1994; Jordan et al., 2007). In addition, studies have also showed that DEA may be useful in estimating field denitrification rates (Groffman and Tiedje, 1989; Hopfensperger et al., 2009).

With increasing age and additional plant growing seasons, the soil properties of a created wetland should mature and develop, which is critical in both structural and functional ecosystem development of mitigation wetlands created and/or restored. Previously, many mitigation projects have been generally unsuccessful in meeting the performance criteria that have been legally mandated (e.g., vegetation community development) (National Research

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Council, 2001). Moreover, even successful cases of mitigation wetlands often fail or turn out to be slow in developing soil properties that are critical for the development of more complex functional attributes of wetlands (Wolf et al., 2011). An excellent indicator of soil development and quality is soil organic matter (SOM) (Howard and Howard, 1993; Schaffer and Ernst, 1999; Bruland and Richardson, 2005), as it is a major source of nutrients (especially N) (Sollins et al., 1999), except in peat-accumulating soils. SOM provides both organic N, the substrate of mineralization, and organic carbon (OC), which is a required energy source of both mineralizing and heterotrophic denitrifying microbes (Beauchamp et al., 1989; Groffman, 1994; Hill and Cardaci, 2004). The greater accumulation of OC may increase denitrification rates.

Dee and Ahn (2012) found that soil condition (SC) attributes such as SOM, bulk density (Db), and gravimetric soil moisture (GSM), all related to the maturation of a created wetland, were significantly associated with the development of structural and functional vegetation attributes in created mitigation wetlands. The study also reported that soil properties varied greatly within sites than between sites in a spatially heterogeneous manner, suggesting that site age does not necessarily equate with the overall maturity of mitigation wetland. Soil properties are interdependent with higher SOM or soil organic carbon (SOC) displacing compacted soil and reducing Db, in addition to providing an absorptive substrate for water retention, thus increasing soil moisture (Ballantine and Schneider, 2009; Bruland and Richardson, 2005; Ehrenfeld et al., 2005; Reddy and DeLaune, 2008). Little, however, is known whether and how SCs are associated with the development of a biogeochemical function (i.e., denitrification) critical to water quality ecosystem services provided by wetlands.

We studied soil properties and DEA rates in both created mitigation and natural wetlands, all located in the Virginia piedmont. The objective of this study was to investigate if soil properties (i.e., SC groups) were associated with a functional property (i.e., DEA rates). The inclusion and use of soil properties in post-construction monitoring and management may be useful to track the functional maturity of created mitigation wetlands.

2. Methods

2.1. Site descriptions

Five non-tidal freshwater wetlands located in the Piedmont physiographic region of northern Virginia were chosen for this study (mean annual precipitation 109 cm, mean temperature min 7 °C/max 18 °C) (see Wolf et al., 2011). Three of the wetlands were mitigation wetlands created by Wetland Studies and Solutions Inc. (WSSI) on old farmland with a predominantly herbaceous cover with little difference (Dee and Ahn, 2012). The other two were natural wetlands and include bottomland riparian forested wetlands and open herbaceous wetlands.

All created wetlands contain at least a 0.3 m low permeability subsoil layer covered with the original topsoil from the site that was supplemented with commercially available topsoil to a depth of 0.2 m. This design creates a perched, precipitation-driven water table close to the soil surface and limits groundwater exchange in the wetland. Loudoun County Mitigation Bank (LC) is a 12.9 ha wetland and upland buffer complex, constructed in the summer of 2006 in Loudoun 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. LC consists of two wetland basins (LCs 1 and 2). LCs 1 and 2 are two contiguous sites separated by a berm and connected by a drainage channel with LC1 approximately 0.4 m higher in elevation than LC2. This design causes LC1 to drain more quickly leaving it inundated for shorter periods after precipitation than LC2, while LC2 can remain under standing water (e.g., <∼12 cm) for longer periods. Bull Run Mitigation Bank (BR) is a 20.2 ha wetland and upland buffer complex, constructed in 2002 in Prince William County, Virginia (38°51′N, 77°32′W). The site may receive 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 by WSSI 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.

Manassas National Battlefield Park (BFP), established in 1940, is a 2000 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 occasional overbank flooding from Goose Creek. Vegetation is mature bottomland forest with little understory.

2.2. Soil sampling

Soil samples for this study were collected in October and December 2010 and April and June 2011, totaling four times. A total of 16 study plots in the created wetlands (e.g., LC1, LC2, BR and NF) and 4 plots in the natural wetlands (e.g., BN and BP) were selected. Each plot was 100 m^2 (10 m × 10 m) and was divided into four 5 m × 5 m quadrants. Within each quadrant, three soil samples were taken at random at the depth of 5–10 (targeting at 7.5 cm) cm from the top using an auger (1 1/4″ diameter) and combined in a polyethylene bag. Soils were mostly saturated with little standing water (<2 cm). All samples were kept in a cooler with ice packs to slow bacterial activity until further processing in the laboratory. At the laboratory, each bag was homogenized manually to mix all three samples for each quadrant. Any visible root or plant material was removed prior to homogenization.

2.3. Soil physicochemical analyses

To determine SOM, total organic carbon (TOC), total nitrogen (TN) and pH, soils were air dried. Once air dried, soils were macerated using a mortar and pestle and large constituents (e.g., rocks and large organic debris) were removed. A Perkin-Elmer 2400 Series II CHNS/O Analyzer (Perkin-Elmer Corporation, Norwalk, CT, USA) was used to analyze TOC (∼TC) and percent TN. Sub-samples (2–3 g of air dried soil) were separated for SOM, loss on ignition (LOI) method, and oven dried at 105 °C for 24 h, weighed and placed at 405 °C for 16 h. SOM (%) was measured using weight loss on ignition method (Wilson and Sander, 1996). For gravimetric soil

moisture (GSM), field-wet mass was measured and samples dried at 105 °C for 48 h. GSM was calculated by the difference between field moist mass and oven dried mass [(wet mass − dry mass)/(dry mass) × 100] (Gardner, 1986). For pH determination, 10 g air dried soil samples were combined with 10 mL of deionized water, swirled and left to stabilize for 10 min prior to measurement (Thomas, 1986). Bulk density (Db) was determined for each core, 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 (200.2 cm^3). Soil temperatures were measured using ibuttons (Embedded Data Systems Inc.) for each sampling periods, including a week before and after the period. The ibuttons are computer chips that contain temperature sensors and are encased in portable button sized capsules. All ibuttons were buried at each plot at a soil depth of 5–10 cm.

2.4. Denitrification potential (DP)

The potential rate of the initial denitrification phase was quantified using the denitrification enzyme activity (DEA) assay with the acetylene block technique modified from Smith and Tiedje (1979) and Groffman et al. (1999). Field moist soil samples for each quadrant were homogenized after collection and kept refrigerated until DEA assay. Replicate assays were performed per quadrant in 125 mL Erlenmeyer flasks with airtight stoppers (Thermo Fisher Scientific) containing 25 mL of DEA media (1 mM glucose, 1 mM KNO_3 and 1 g per L chloramphenicol) and 25 g of soil. The resultant soil slurries were bubbled with N_2 gas for 11 min (including 1 min headspace flush), sealed with airtight septa centered stoppers, purged with N_2 for 1 min, vacuumed for another 1 min, pressurized with N_2 and vented prior to addition of 10 mL of scrubbed acetylene (Hyman and Arp, 1987). To ensure equal distribution of acetylene and N_2O, flasks were placed on a rotary shaker table at 125 rpm and samples were taken at 30 and 90 min. Gas samples were stored in 2 mL airtight Monojet vials and kept in a cool dry place until analyzed using a Shimadzu GC-8a ^{63}Ni electron capture detector gas chromatograph (Shimadzu Corporation, Columbia, MD, USA) equipped with a Hayesep Q, 80/100-mesh column (320C injector/detector temperature, 80 °C oven temperature and 300 kPa carrier gas flow). Standards ranging from 0.229 to 4293.75 μg N_2O-N/L were generated from 1% N_2O in N_2 balance (Air Liquide, Houston, TX, USA). Denitrification rates were calculated using the difference between N_2O concentrations at the two sampling times based on calibration curves.

2.5. Statistical analyses

SC groups were determined by cluster analysis at 70% similarity of soil physicochemical parameters that included pH, GSM, Db, TOC and TN of all four sampling periods. Statistical significance of the SC groups was verified by applying a similarity profile test (SIMPROF) which performs permutation tests at each node of the cluster analysis dendrogram. SIMPROF thus determines whether each cluster set has significant evidence of a multivariate pattern different from the rest (Clarke and Gorley, 2006). DEA rates were below our detection limit for the month of October 2010 at all sampling plots. Further discussion of DEA rates and relationship to soil properties was thus limited to the samplings conducted in the other three sampling periods. PCA was used to visualize 'best fit' of plots along soil physicochemical properties, and DEA rates. All test described thus far were performed using PRIMER 6, version 6.1.5 (Primer-E Ltd., Plymouth, UK). Analysis of variance (ANOVA) was used to compare soil physicochemical variables and DP between soil condition groups. *Bonferroni* pairwise *t*-tests for uneven variances were carried out for each ANOVA to determine between-group differences. Spearman rank correlations were performed between soil physicochemical attributes and DP to determine significant relationships between factors. ANOVAs and correlations were all conducted using SYSTAT 12 (Cranes Software International Ltd.).

3. Results

3.1. Soil properties and DEA rates by wetland site

We did not observe an age-related maturation of soils in the study sites. Soils from the forested natural wetland and one of the youngest created wetlands (e.g., BN and LC1) contained the highest TOC (1.9–2.1%) and TN (0.18–0.20%). NF, the 11-year-old created wetland, contained SOM comparable to LC1 and BN, which ranged between 4.4 and 5.3% (Table 1). Two of the younger created wetlands (e.g., LC2, BR) and the natural wetland (e.g., BP) had the lowest SOM (2.8–3.2%), TOC (0.9–1.2%) and TN (0.08–0.11%) (Table 1). Soil pH was significantly higher in NF (6.5) and lower (4.3) in BP (Table 1). Soil pH in the rest of the wetlands (e.g., LC1, LC2, BR and BN) ranged from 5.1 to 5.6, with no significant differences between these sites (Table 1). GSM was expectedly higher in the three wetlands with the highest SOM content. BN had the highest mean GSM (43%) followed by NF (39%) and LC1 (34%). There were, however, no significant differences between BN and NF or NF and LC1 (Table 1). DEA rates were highly variable within each site as observed by the high standard error rates, but less so between sites (Table 1). Significantly higher DEA rates were observed in LCs 1 and 2 (228 and 143 μg N-N_2O/kg soil/h on average, respectively) than the rest of the sites with the lowest rate observed in the soils of NF (41 μg N-N_2O/kg soil/h on average; Table 1).

3.2. Soil properties and DEA rates by sampling periods

Differences in SOM, TOC and TN were also found by sampling period (Table 2). SOM, TOC and TN were highest in December, averaging 4.5%, 1.6% and 0.14%, respectively (Table 2). The mean soil pH for April and June ranged from 5.7 to 5.8, which was higher than that in December soils (i.e., 5.3 on average) (Table 2). GSM levels were significantly higher in December (40%), followed sequentially by those in April (35%) and in June (30%) (Table 2). Temperature means for the sampling periods in December, April and June were 1.3, 12.8 and 20.5 °C, respectively, being significantly different, which reflected a natural seasonal variation of soil temperature. However, DEA rates were a little bit higher in summer months (i.e., April and June) than in the winter (December), but not significantly different ($p = 0.06$) (Table 3).

3.3. Soil properties and DEA rates by SC groups across the sites

We derived three SC groups from a cluster analysis of five soil physicochemical attributes; TOC, TN, Db, GSM and pH. SC groups showed progressive soil development/maturation (e.g., SC1 < SC2 < SC3 less to more developed), irrespective of site (Table 3). SC1 soils were characterized by the lower SOM (3.1%), TOC (1.0%), TN (0.1%), GSM (32%) and higher Db (1.4 g cm^{-3}). The more developed (or matured) soils of SC3 had higher SOM (4.9%), TOC (1.9%), TN (0.2%), GSM (38%) and lower Db (1.2 g cm^{-3}). SC2 consisted entirely of plots at NF, the oldest created mitigation wetland in the study. SC2 had intermediate soil characteristics between SC1 and SC3, but was characterized distinctively by higher soil pH (6.6 on average) than those in the other SC groups (Table 3). Overall, soil attributes indicative of wetland soil maturity (e.g., higher SOM and GSM, and lower Db) in SC2 were more similar to SC3 (Table 3).

Table 1
Site level soil physicochemical attributes and denitrification rate (mean ± standard error).

	Created wetlands				Natural wetlands			
Site:	LC1	LC2	BR	NF	BP	BN	*F*	*p*
Age (years):	4	4	8	11				
SOM (%)	4.9 ± 0.29^{a}	2.8 ± 0.16^{b}	3.2 ± 0.28^{b}	4.4 ± 0.16^{a}	3.0 ± 0.19^{b}	5.3 ± 0.70^{a}	14.8	*
pH	5.3 ± 0.09^{b}	5.1 ± 0.09^{b}	5.3 ± 0.11^{b}	6.5 ± 0.07^{a}	4.3 ± 0.09^{c}	5.6 ± 0.06^{b}	91.5	*
TOC (%)	1.9 ± 0.11^{a}	0.9 ± 0.10^{b}	1.1 ± 0.14^{b}	1.2 ± 0.06^{b}	1.1 ± 0.07^{b}	2.1 ± 0.38^{a}	10.6	*
TN (%)	0.18 ± 0.009^{a}	0.09 ± 0.009^{b}	0.11 ± 0.013^{b}	0.11 ± 0.004^{b}	0.08 ± 0.007^{b}	0.20 ± 0.03^{a}	15.2	*
GSM (%)	34 ± 2.9^{bc}	33 ± 1.4^{c}	31 ± 2.1^{c}	39 ± 1.5^{ab}	31 ± 1.5^{c}	43 ± 3.4^{a}	5.95	*
DEA rate (μg N-N_2O/kg soil/h)	228 ± 39^{a}	143 ± 42^{ab}	123 ± 34^{b}	41 ± 11^{c}	124 ± 51^{b}	115 ± 48^{bc}	3.22	*

Different letters between SC groups indicated significance at $\alpha < 0.05$ after Bonferroni pairwise *t*-tests.
* $p < 0.05$.

Table 2
Soil physicochemical attributes and denitrification rate (mean ± standard error) differences by sampling periods.

	December	April	June	*F*	*p*
SOM (%)	4.5 ± 0.22^{a}	3.6 ± 0.26^{b}	3.4 ± 0.13^{b}	*7.17*	*
pH	5.3 ± 0.10^{b}	5.8 ± 0.12^{a}	5.7 ± 0.13^{ab}	*4.12*	*
TOC (%)	1.6 ± 0.11^{a}	1.2 ± 0.11^{b}	1.1 ± 0.07^{b}	*5.84*	*
TN (%)	0.14 ± 0.01^{a}	0.1 ± 0.01^{b}	0.1 ± 0.01^{b}	*6.42*	*
GSM (%)	40 ± 1.6^{a}	35 ± 1.3^{b}	30 ± 1.0^{c}	*12.8*	*
Temperature (°C)	1.3 ± 0.2^{c}	12.8 ± 0.4^{b}	20.5 ± 0.3^{a}	*631*	*
DEA rate (μg N-N_2O/kg soil/h)	75 ± 18^{a}	139 ± 16^{a}	119 ± 26^{a}	2.87	*0.06*

Different letters between SC groups indicated significance at $\alpha < 0.05$ after Bonferroni pairwise *t*-tests.
* $p < 0.05$.

Table 3
Summary of the soil physicochemical attributes and denitrification rate (mean ± SE) by wetland soil condition (SC).

	SC1 ($n = 10$)	SC2 ($n = 5$)	SC3 ($n = 5$)	*F*	*p*
LC1			2		
LC2	3				
BR	4		1		
NF	1	5			
BP	2				
BN			2		
SOM (%)	3.1 ± 0.2^{b}	4.3 ± 0.2^{a}	4.9 ± 0.4^{a}	12.2	*
pH	5.2 ± 0.2^{b}	6.6 ± 0.1^{a}	5.4 ± 0.1^{b}	14.6	*
TOC (%)	1.0 ± 0.1^{b}	1.2 ± 0.0^{b}	1.9 ± 0.2^{a}	18.2	*
TN (%)	0.1 ± 0.01^{b}	0.1 ± 0.00^{b}	0.2 ± 0.02^{a}	24.9	*
GSM (%)	32 ± 1.0^{b}	39 ± 2.4^{a}	38 ± 2.1^{a}	6.82	*
Db (g/cm^3)	1.4 ± 0.03^{a}	1.2 ± 0.02^{b}	1.2 ± 0.06^{b}	9.6	*
DEA rate (μg N-N_2O/kg soil/h)	119 ± 16^{b}	32 ± 6^{c}	177 ± 25^{a}	17.4	*

Different letters between SC groups indicated significance at $\alpha < 0.05$ after Bonferroni pairwise *t*-tests.
* $p < 0.05$.

DEA rates were clearly discernible when analyzed by SC group. DEA rates were significantly higher (177 μg N-N_2O/kg soil/h) in SC3 with highest TOC, TN and lowest Db (Table 3). The DEA rates showed the lowest (32 μg N-N_2O/kg soil/h) in SC2 which differed significantly for its soil pH than the rest of SC groups. A PCA of all soil physicochemical attributes and DEA rates was conducted to further tease out the association between DEA rates and soil condition variables. Since TOC, TN and SOM were found to be strong covariates (Table 4), we omitted TN and SOM from the PCA. PCA of the data identified two components with eigenvalues greater than 1 (i.e., 2.49 and 1.58), which accounted for over 81% of the data variability. Explaining 50% of the variability, PCA component 1 had highest factor loadings for pH (0.503), GSM (0.542), and Db (−0.54). Component 2 accounted for 32% of the data variability, with highest component loadings for TOC (−0.616) and DEA rate (−0.673) (Fig. 1). The both axes clearly separated SC3, the most mature soil condition, from SC1, the least mature one. It also seems that soil pH separates SC2 from SCs 1 and 3 (Fig. 1).

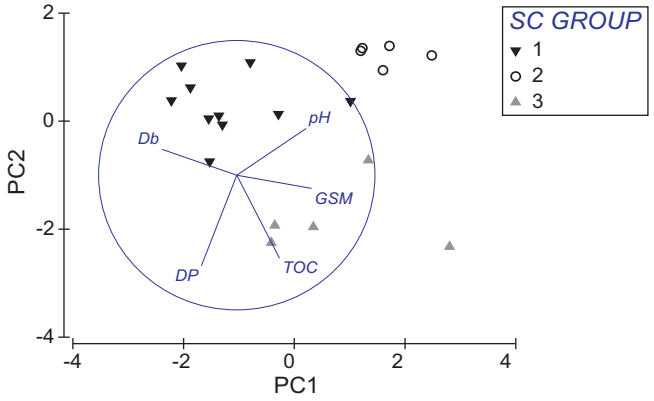

Fig. 1. Principal component analysis of soil properties (Db, pH, GSM, and TOC) and average denitrification potential (DP) of soils collected in the study during all four sampling periods. Symbols represent the plots at the indicated wetland soil condition (SC). SC1 < SC2 < SC3 less to more developed.

Table 4
Spearman rank correlation among measured soil attributes and denitrification potential rates (i.e., DEA rates).

	SOM	pH	TOC	TN	GSM
SOM (%)	–				
pH	0.352	–			
TOC (%)	**0.767**	0.022	–		
TN (%)	**0.728**	−0.006	**0.902**	–	
GSM (%)	**0.574**	0.268	0.415	0.446	–
DEA rate (μg N-N_2O/kg soil/h)	−0.053	−0.258	0.147	0.156	0.179

Values in boldface indicate that correlation is significant at $\alpha < 0.01$ level and values underlined indicate that the correlation is significant at $\alpha < 0.05$.

3.4. Correlations between soil properties and DEA rates

Spearman rank correlations were performed to relate soil properties and DEA rates irrespective of the SC, site or sampling period (Table 4). TOC and TN were found to be covariates ($\rho = 0.90$) and together positively correlated to SOM contents ($\rho > 0.7$). GSM was positively related to SOM ($\rho = 0.57$), TOC ($\rho = 0.42$) and TN ($\rho = 0.45$). Soil pH demonstrated a positive relationship with SOM ($\rho = 0.35$) and GSM ($\rho = 0.27$). DEA rates were not found to be significantly correlated with any of the structural soil properties, but displayed a negative correlation with soil pH ($\rho = -0.26$; Table 4).

4. Discussion

4.1. Use of SC groups in examining the progress of soil development in created wetlands

Although the mitigation of wetland losses by creating wetlands has become a common practice, there continues to be uncertainty in regards to the degree of functional ecosystem development in created wetlands (Bishel-Machung et al., 1996; Stolt et al., 2000; Campbell et al., 2002). In this study we focused on a suite of SC attributes as a potential indicator for the development of a biogeochemical function (i.e., denitrification) in created mitigation wetlands.

Soil provides the structural matrix in which many biogeochemical processes occur. Spatial variability of soil attributes in both created and restored wetlands were found and discussed in previous studies (Bruland and Richardson, 2005, 2006) where they found relatively lower spatial variability of those in created wetlands compared to natural wetlands. The outcome of the study showed no relationship between site age and soil development, as shown in similarly higher SOM contents of the natural wetland (e.g., BN), the oldest created wetland (e.g., NF) and one of the youngest wetlands (e.g., LC1) (Table 1). SOM is naturally accumulated through autochthonous (e.g., seasonal plant senescence) and allochthonous or allogenic (e.g., sediment brought by flooding or runoff) inputs of organic matter over time, being a good indicator for soil maturation (Wolf et al., 2011). In addition, wetland soils are generally characterized by their high water holding capacity which is largely due to high SOM content (Reddy and DeLaune, 2008).

SC grouping managed to sort out the study plots that were physicochemically similar across wetland sites, with directionality from less to more developed (i.e., SC1 < SC2 < SC3). This study revealed that a functional attribute (i.e., DEA rate) was highest in SC3 (Table 3) characterized by greater TOC and TN, lower Db, and higher GSM, all indicative of maturity in wetland ecosystem development (Table 4). SC groups can be viewed as a measure of soil developmental variation within a created wetland, with some groups lagging others and thus maturing at different rates. The finding that SC can be used to examine the development of biogeochemical functions in created wetlands has potential application for future monitoring, created wetland design, and post-creation refinement.

4.2. Soil properties and DEA

Denitrification is a biochemical processes accomplished by the metabolic activity of soil microorganisms under anaerobic conditions. DEA rates represent potential enzyme activity, where O_2 is limited while C and NO_3^- concentrations are non-limiting. Therefore, the rate of N_2O production is proportional to the enzyme content extant within each soil (Tiedje et al., 1989). In our study, DEA rates were negatively associated with soil pH and positively with TOC regardless of the wetland site (Fig. 1). Among the study plots with similar soil pH, DEA rates were higher in soils with higher SOM, TC, TN, GSM and lower Db, indicating a functional development is clearly associated with the development of soil properties (Table 1 and Fig. 1).

The pH values of the soils collected from the wetlands ranged between 4.3 and 6.5 (Table 1), showing a typical, acidic characteristic of the soils of Virginia Piedmont (Farrel and Ware, 1991). The Piedmont soils can also be described as clay rich, highly weathered, low base saturation soils (Farrel and Ware, 1991). This indicates a preponderance of acidic soils in this physiographic region that has developed over a long period of time through regional land use changes (Sherwood et al., 2010). DEA rates were found to have a negative association with soil pH (Table 4) in this study, but it is not clear how slightly higher soil pH in SC2 led to a significantly lower DEA rates at the group since we did not study the mechanism(s) by which soil pH within the observed range influences or alters DEA. The relationship between pH and denitrification has been extensively researched, with most studies finding higher denitrification rates at circumneutral pH (Wijler and Delwiche, 1954; Van Cleemput et al., 1975; Thomsen et al., 1994). The DEA rates might have been influenced by some other factor or a set of factors that was not measured in this study. SC2 was composed with four study plots at NF, the oldest created wetland, that were fed by a large open water area. The design of NF and its resulting propensity to flooding might have induced more flooded soil conditions as reflected in higher GSM, leading to a reduction in N mineralization, as observed for this site by Wolf et al. (2011), and limiting the availability of NO_3 substrate known to constrain denitrification in freshwater wetlands (Groffman and Tiedje, 1989; Martin and Reddy, 1997). Simek and Cooper (2002), in a thorough review of the last half century of the relationship between soil pH and denitrification, concluded that soil pH can influence different parts of the denitrification process in different ways. They mentioned that DEA rates can be influenced by soil pH and are less in acidic soils than neutral or slightly alkaline soils, which does not support our results. It would not be proper to generally interpret our DEA rates and their association with soil pH based on Simek and Cooper (2002) since our DEA rates came from soils of which pH were all under 7, being acidic. They cautioned that due to the gap in specific knowledge it is misguided to assume that there is an optimum pH for denitrification. Since the relation between soil pH and potential denitrification as determined by a variety of incubation methods remains unclear, with results being affected both by original conditions in soil samples and by unknown changes

during incubation (Simek and Cooper, 2002) a further study for the relationship between soil pH and DEA rates is necessary.

4.3. Tracking functional development in post-construction of mitigation wetlands

Time is reasonably considered the master variable in soil development (e.g., SOM or SOC accumulation) in created wetlands. However, SC groups highlighted that soil development can also be affected by other variables leading to heterogeneity within a wetland (Table 3). BR consisted of five plots, four of which were classified in SC1 and one in SC3, showing variability in SC at the same wetland created at the same time. A trajectory of soil development was apparent with the higher SC group (i.e., more mature soil properties), showing higher TOC and DEA rates that are indicative of mature structural and functional development of created wetlands. Thus measuring soil physicochemical variables and determining SC differences can better identify ways to improve site-level functional development examination. The association between SC and functional development (e.g., denitrification) was indirectly explored by Sutton-Grier et al. (2011), where the focus was on vegetation influence on DEA rates. In that study, variations in soil resources were found to be important in determining the level at which the plant community contributed to functional development (Sutton-Grier et al., 2011). The denitrification function of wetlands is an essential ecosystem service that natural wetlands provide, and as such, should be required to be attained within the monitoring period of mitigation wetlands. Since the outcome of the study showed a clear linkage between DP development and soil maturation it is recommended that soil properties be included in post-construction monitoring of created mitigation wetlands.

5. Conclusion

The study demonstrates that denitrification function that is important for the ecosystem service of water quality improvement by wetlands is associated with the conditions and/or developmental stages of wetland soils. Site age does not necessarily equate with soil maturation with functional developmental rates varying both within and among sites. SC groups turned out to be useful in examining the functional development of created mitigation wetlands. Maturity in SC with accumulation of TOC and TN, the increase of GSM, and the decrease of Db seemed positively associated with DP development. The ranges of soil pH found in this study were fairly limited within the characteristic ranges of acidic soils in the Virginia Piedmont, but a further study is necessary to investigate the impacts of soil pH in acidic wetland soils on DEA rates and the relations among the SC attributes explored in this study. Nonetheless, the inclusion of soil properties in post-construction monitoring of created mitigation wetlands seems necessary to enhance our understanding and prediction of the development trajectory of an important biogeochemical function (i.e., denitrification).

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