



Soil bacterial community structure and physicochemical properties in mitigation wetlands created in the Piedmont region of Virginia (USA)

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ABSTRACT

Wetland creation is a common practice for compensatory mitigation in the United States. Vegetation attributes have been used as a quick measure of mitigation success in most post-creation monitoring, while little attention has been paid to soils that provide the substrate for flora and fauna to establish and develop. Created wetland soils are often found not indicative of 'hydric soil' with a lack of development of physicochemical properties (i.e., bulk density, moisture content, and carbon and nitrogen contents) comparable to those in natural wetlands. Moreover, soil bacterial communities are rarely examined though they are integrally involved in biogeochemical functions that are critical for ecosystem development in created wetlands. We analyzed soil physicochemistry and profiled soil bacterial community structure using amplicon length heterogeneity polymerase chain reaction (LH-PCR) of 16S ribosomal DNA in three relatively young wetlands (<10 years old) created in the Piedmont region of Virginia. We examined the data by site and by specific conditions of each site (i.e., induced microtopography and hydrologic regime). Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) showed clear clustering and significant differences both in soil physicochemistry (Global $R=0.70$, $p=0.001$) and in soil bacterial community profiles (Global $R=0.77$, $p=0.001$) between sites. Soil physicochemistry (Global $R=1$, $p=0.005$) and bacterial community structure (Global $R=0.79$, $p=0.005$) of soils significantly differed by hydrologic regime within a wetland, but not by microtopography treatment. A significant association was found between physicochemistry and bacterial community structure in wetland soils, revealing a close link between two attributes ($\rho=0.39$, $p=0.002$). C/N (carbon to nitrogen) ratio was the best predictor of soil bacterial community patterns ($\rho=0.56$, $p=0.001$). The diversity of soil bacterial community (Shannon's H') differed between sites with a slightly higher diversity observed in a relatively older created wetland, and seemed also fairly determined by hydrologic regime of a site, with a relatively dry site being more diverse.

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1. Introduction

Created wetlands often show little evidence of ecosystem development comparable to that of their natural counterparts, and many wetlands created to mitigate wetland losses fail to meet basic success criteria within the time frame legally mandated for monitoring (National Research Council, 2001; Spieles, 2005). In most cases of wetland mitigation, some measure of vegetation is a performance standard, and in many cases, vegetation is the only performance standard in the post-creation monitoring as a quick surrogate for the biogeochemical condition of the wetland and as a measure of success (Spieles, 2005). However, structural attributes of vegetation may be a poor measure of wetland functions that should be in development (Breaux and Serefidin, 1999).

Soil properties are often the least studied indicator of wetland quality (Shaffer and Ernst, 1999; Stolt et al., 2000). Soil physicochemical properties are often found not indicative of 'hydric soils' in created wetlands. Moreover, failures of created/restored wetlands are often attributable to a lack of soil development (Bishel-Machung et al., 1996; Stolt et al., 2000; Cole et al., 2001; Campbell et al., 2002). Soil microbial communities are integrally involved in biogeochemical cycles and their activities are crucial to the functions of wetland systems because they play key roles in energy flows and nutrient transformation (Batzer and Sharitz, 2007), but are rarely examined in assessing ecosystem development in created wetlands. Ecological engineers often assume that microbes are passively reacting to abiotic and biotic stimuli rather than controlling soil processes explicitly, and have typically treated them as a "black box" (Nichols, 1983; Busnardo et al., 1992; Jordan et al., 2003). Yet these subsurface domains are now known to contain higher species diversity than the aboveground world (Beare et al., 1995; Pace, 1997; Kennedy, 1999; Emmerling et al., 2002). Moreover, there is increasing evi-

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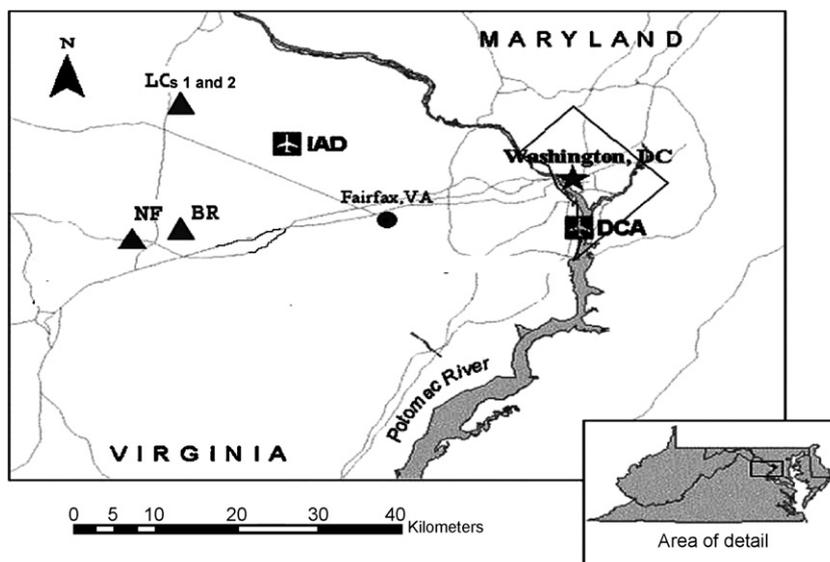


Fig. 1. Locations of four created mitigation wetland sites for the study (LC1, LC2, BR, and NF) in the Piedmont region of Virginia.

dence that the patterns of microbial communities are related to soil processes (Bossio and Scow, 1995; Fierer et al., 2003; Zak et al., 2003; Merkley et al., 2004; D'Angelo et al., 2005; Edwards et al., 2006; Gutknecht et al., 2006; Hunter et al., 2006; Mentzer et al., 2006; Zul et al., 2007; Ahn et al., in press).

The trend in microbial ecology has recently been to rely more on culture-independent methods, such as phospholipid fatty acid analysis (PLFA) and molecular methods, which are more inclusive of slow growing and highly specialized microorganisms that are not competitive in culture media. Length heterogeneity polymerase chain reaction (LH-PCR) fingerprinting is a method used to characterize microbial communities by amplifying the variable regions of small subunit (SSU) rRNA genes from each species and separating the natural variation in the amplicon length on a denaturing polyacrylamide gel (Ritchie et al., 2000). The peak area in the profile is proportional to the abundance of that amplicon in the community and it has been successfully used to estimate the diversity present in bacterioplankton (Suzuki et al., 1998) and in soil bacteria (Ahn et al., 2007). Studies have also evaluated the robustness of this method and found it to be highly reproducible yielding good representation of communities that have been assessed by alternative methods such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) (Dunbar et al., 2001; Mills et al., 2003; Ritchie et al., 2000). For characterizing bacterial communities, DNA is first extracted from soil samples, and the 16S rRNA gene operon is amplified using universal bacterial primers. The heterogeneity in length of the amplicon is taken as the representation of a distinct operational taxonomic unit (OTU) (Ritchie et al., 2000).

The objective of the study was to assess soil physicochemical properties and bacterial community structure in mitigation wetlands created in the Piedmont region of Virginia. No previous studies have assessed soil bacterial communities in these created wetlands. The LH-PCR fingerprinting was applied as a first-step screening of the bacterial community patterns of soils. We hypothesized that soil bacterial community patterns and diversity would differ by different hydrologic conditions (within a site of the same age) and carbon contents of the sites (among the sites of varying ages). We also investigated if there was any association or specific relationship between soil bacteria community patterns and soil physicochemical properties measured.

2. Methods

2.1. Site description

Field research was carried out in summer 2007 at three created wetlands in northern Virginia, USA (annual precipitation 99.75 cm, mean annual temperature min. 2.0°C/max. 24°C). Created wetlands were Loudoun County (LC) (39°02.05'N, 77°36.5'W), Bull Run (BR) (38°51.3'N, 77°36.05'W), and North Fork (NF) (38°49.4'N, 77°40.2'W) mitigation banks, all built by Wetland Studies and Solutions, Inc. (WSSI) in 1999, in 2002, and in 2006, respectively. There were specific conditions to note for LC. The LC wetland consists of two contiguous sites (i.e., LC1 and LC2) separated by a berm with a drainage trench, and LC1 approximately 0.4 m higher in position than LC2. This design primarily causes LC1 to drain quickly leaving it inundated only for very short periods during and after precipitation, while LC2 remains under standing water (i.e. <12 cm) for several months. With the difference in hydrologic regime we decided to treat LCs 1 and 2 as separate sites, resulting in four study sites in total. All sites are located in the Piedmont physiogeographic province of northern Virginia, just about 40 km southwest of Washington, DC (Fig. 1). The Piedmont is generally characterized by rolling terrain underlain by igneous and metamorphic rock (Hook et al., 1994). LC is a 32-acre wetland complex, located within the floodplains of Big Branch Creek (to the east), a tributary of Goose Creek in Loudoun County. BR is a 50-acre wetland complex in Prince William County. NF is a 125-acre created wetland complex constructed on former cattle pastureland in Prince William County. Soils are generally silt loams and silty clay loams over Newark Supergroup basalt. The wetland design for the sites includes 0.2 m topsoil atop 0.3 m or greater thick low permeability subsoil layer resulting in perched water table that fluctuates with precipitation with no or negligible groundwater exchange. The sites were hydro-seeded with commercially available wetland plant seed mixes appropriate for the region and the intended hydrology (e.g., wetland meadow as opposed to obligate wetland). Although the created wetlands are intended to mitigate the loss of palustrine forested wetlands, all planted trees are currently small saplings, so these wetlands can best be characterized as palustrine scrub/shrub wetland.

In addition, study plots in LCs 1 and 2 were separated into two groups (disked: Y vs. non-disked: N) due to the specific disk-

ing treatment applied during the construction as part of another research project that investigates the effects of disking on early vegetation development in created wetlands. Microtopographic variation induced by disking has been correlated with increased water retention, species richness, and diversity in these wetlands (Moser et al., 2007). BR and NF were all disked during their construction the same way as in the disked plots of LC sites.

2.2. Soil sampling

For the study, we randomly established 23 plots (10 m × 10 m each) throughout the wetlands (i.e., 6 plots in LC1, 4 plots in LC2, 7 plots in BR, and 6 plots in NF). Soil samples were collected at the end of the growing season, September–October 2007. A soil probe/auger was used to collect the top 10 cm of soil, excluding surface litter. The plots were divided into quadrants and a 1 m² grid was randomly tossed to select the sampling points within each quadrant. Triplicate soil cores were taken per quadrant, combined in a polyethylene bag and transported to the lab on ice in a cooler. In the laboratory, samples were manually homogenized for each quadrant and visible roots and rocks were removed prior to further processing. From these homogenized samples, approximately 2 g were taken and placed in sterile 2 mL culture tubes for LH-PCR analysis. The tubes were kept at –20 °C for 48 h, and then transferred to a –80 °C freezer until usage for DNA extraction and LH-PCR fingerprinting. All other samples were processed immediately for physicochemical analysis.

2.3. Soil physicochemical analysis

Bulk density and percent soil moisture were measured following Blake and Hartge (1986). Sub-samples were oven-dried at 105 °C for 48 h and used to determine moisture content for each sample. Dried sub-samples were passed through a 2 mm sieve to remove small rocks and large organic debris, and ground with a mortar and pestle before analysis for total carbon (%C) and total nitrogen (%N) (percent dry weight) using a Perkin-Elmer 2400 Series II CHNS/O Analyzer (Perkin-Elmer Corporation, Norwalk, CT, USA).

2.4. Soil bacterial community fingerprinting

Whole community DNA was extracted using the Bio101 FastDNA[®] SPIN Kit for soil (Q-MP Biomedicals, Inc., Carlsbad, CA) and subsequently stored at –20 °C. The extracted DNA was diluted (1:10) and 2 µL were used as template for LH-PCR in duplicate reactions. The purified DNA was amplified by PCR using universal bacterial primers (Mills et al., 2006): fluorescently labeled forward primer 27F (5'-[6FAM]-AGAGTTTGATCCTGGCTCAG-3') and unlabeled reverse primer 355R (5'-GCTGCCTCCGCTAGGAGT-3'). The reactions were performed using 20 µL mixtures containing a final concentration of: 1× PCR-GOLD buffer, 25 mM MgCl₂, deoxynucleoside triphosphates at a concentration of 200 µM each, primers at the concentration of 0.5 µM and 0.5 U (5 units/µL) of AmpliTaq[™]-GOLD DNA polymerase (Applied Biosystems, Foster City, California). GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) was programmed for initial denaturation and polymerase activation at 95 °C for 11 min. It was followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 48 °C for 30 s, and extension at 72 °C for 2 min (+ 5 s/cycle), with a final extension step at 72 °C for 30 min to ensure the complete extension of all the fragments. PCR reactions for each sample were visualized on a 1% agarose gel with ethidium bromide. They were stored at 4 °C until used in fingerprinting. A 1:10 dilution of the PCR products was made and mixed with 1/20 ratio of internal size standard (ILS 600, Promega) and HiDi Formamide (Applied Biosystems, Foster City, CA) and ran on an SCE 9610 capillary (Spectrummedix LLC, State College, PA) for fingerprinting. Analysis of the community

fingerprints was done using the GenoSpectrum (Spectrummedix LLC, State College, PA) software package (version 2.08), which converted fluorescent data into electropherograms. Reproducibility was tested by comparing duplicate PCRs for each sample. Further analysis was performed using custom PERL scripts that normalized peak areas by dividing an individual peak area for each amplicon by the total peak area of the profile in order to quantitatively compare the relative abundance of each peak.

2.5. Data analysis

All data were pooled together and averaged by site for soil physicochemistry as we found no significant difference between disked and non-disked plots in both LCs 1 and 2. The site averages were then compared by using a modification of Bonferroni 95% confidence intervals for all pair-wise combinations by running Welch's *t*-tests (to reduce impact of unequal variance), using the Minitab (ver. 15) statistical software.

The LH-PCR profiles of soil physicochemistry and soil bacterial community were analyzed by non-metric multidimensional scaling (MDS) conducted on dissimilarity matrices created from both Euclidian and Bray-Curtis similarity analysis, respectively, to compare sample assemblages both between sites and between different conditions/treatments (e.g., disking for microtopography and hydrologic regime). We also used a multivariate analysis of similarities (ANOSIM) (Clarke and Warwick, 2001) to compare assemblages of both physicochemical and bacterial community data by sites. ANOSIM is a nonparametric permutation procedure that tests whether differences in dissimilarity *between* groups exceed differences *within* groups (Clarke and Warwick, 2001). The ANOSIM *R* statistics (i.e., Global *R*) fall between –1 and 1 with *R*=0 indicating completely random grouping while *R*=1 indicates that all replicates of a sampling site or a treatment are more similar to each other than to any replicates of another site or treatment. Significant Global *R* values indicate the *R* value is significantly different from zero, suggesting the compared sites or treatments are significantly similar or dissimilar from one another. Both MDS and ANOSIM were performed using PRIMER software (Clarke and Gorley, 2001). In order to test the linkage between physicochemical characteristics and bacterial community fingerprints of soils we used RELATE and BEST (i.e., BIO-ENV) procedures in PRIMER, equivalent to conducting Mantel test (Clarke and Warwick, 2001). BIO-ENV calculates the Spearman rank correlations (ρ) between two similarity matrices (i.e., those of soil bacterial community and soil physicochemistry). The significance of this correlation is then tested by a randomization/permutation test to create a Global *R* value similar to that of ANOSIM.

Three diversity indices were also calculated to compare LH-PCR fingerprint patterns of soils: (1) Richness (*S*) which is equal to the number of peaks (~amplicons) in a samples; (2) the Shannon–Weiner Diversity Index (*H'*) which is equal to $\sum(p_i \ln p_i)$ where p_i is the peak area (i.e., a relative abundance of amplicon size) in the *i*th species, and (3) Evenness (*E*), which is equal to $H'/\ln S$. Averages of the three indices were compared by sites using one-way ANOVA.

3. Results and discussion

3.1. Soil physicochemical properties

The carbon content (%) of soils in four wetland sites ranged between 1.1 and 2.2% (Table 1). The nitrogen content (%) followed the same pattern as in the carbon content. There seemed a slight increase in the contents of soil carbon and nitrogen over the age of the wetland with NF (8-year old) being significantly higher in its

Table 1

Soil physicochemical properties measured for three created wetlands (mean \pm SE). Pair-wise comparisons between sites were performed using Welch's *t*-statistic and overall *p*-values calculated based on Bonferroni scheme. Means in each column followed by the same letter are not significantly different across the sites at the $p < 0.05$ level.

Site	%C	%N	C/N	%Moisture	Bulk density (g/cm ³)
LC1	2.05 \pm 0.10c	0.20 \pm 0.01c	10.3 \pm 0.12a	8.7 \pm 0.87a	0.92 \pm 0.05a,b
LC2	1.14 \pm 0.09a	0.06 \pm 0.01a	29.8 \pm 3.62c	25.8 \pm 1.01c	1.21 \pm 0.05c
BR	1.43 \pm 0.08a	0.14 \pm 0.01b	10.4 \pm 0.36a,b	14.5 \pm 1.62b	0.76 \pm 0.11a
NF	1.70 \pm 0.05b	0.16 \pm 0.00b	11.1 \pm 0.21a	17.3 \pm 1.24b	1.16 \pm 0.14b,c
	<i>p</i> = 0.006	<i>p</i> = 0.003	<i>p</i> = 0.054	<i>p</i> = 0.003	<i>p</i> = 0.018

carbon content than in both BR (5-year old) and LC2 (1-year old). However, the carbon content in LC1 (1-year old) was highest among the four sites sampled, which was unusual as the youngest site since it usually takes a long time for a created wetland to accumulate carbon to a substantial level (Giese et al., 1999; Anderson et al., 2005; Bruland and Richardson, 2006; Fajardo, 2006). Right before our soil sampling in the designated study plots there was an operation of organic amendment in LC1 by the banker (i.e. WSSI) that was beyond our control. Although they claimed that they applied the organic material right outside our plots staked out, some of the application may have affected our study plots in LC1, increasing the carbon content of the soils sampled and analyzed from those plots. Carbon-to nitrogen (C/N) ratio lie between 10:1 and 30:1, which is fairly common in forested wetland soils although total nitrogen levels were slightly lower compared to other natural forest and/or forested wetlands (Selmants et al., 2005; Stoeckel and Miller-Goodman, 2001). The ratio was significantly higher in LC2 than in the rest of the sites due to the significantly lower nitrogen content of that site. Percent soil moisture was drastically different between LC1 (8.7%) and LC2 (25.8%), reflecting the difference in hydroperiod and hydrologic regime of the two sites. Soil moisture content (%) was comparable between BR and NF with no difference, but being higher than in LC1 and lower than in LC2 (Table 1). Mean bulk densities of the four sites ranged between 0.9 and 1.2 on average (Table 1), typical of mineral soils in young created wetlands (Mitsch and Gosselink, 2000). The soil bulk densities were relatively higher in LC2 and NF than in LC1 and BR (Table 1).

MDS and ANOSIM were performed on the soil physicochemical data to examine if the soil physicochemical patterns differed by sites. ANOSIM results showed significant differences in soil physicochemistry between sites (Global $R=0.70$, $p=0.001$) with all the pair-wise comparisons being significantly different. The MDS plot of soil physicochemistry (Fig. 2a) shows closer clustering within each site than between the sites, clearly separating the sites based on their soil physicochemical characteristics. The stress levels of MDS ($=0.07 < 0.2$) indicates that the ordination provides an acceptable representation of the overall structure of the physicochemical data set (Clarke and Warwick, 2001). LC, the newly created wetland, formed a cluster that was fairly distant from BR and NF. Especially, the data of LC2 that was significantly wetter than the other sites clustered around the bottom right corner in the MDS plot, far from those in LC1 that was dry (Fig. 2a), indicating the effect of different hydrologic regime on soil physicochemical properties developed in a newly created wetland. We ran additional ANOSIM on the data with site and microtopography as factors to test if any differences either by microtopography (i.e., Y vs. N) or by hydrologic regime (i.e., LC1 vs. LC2). There was no significant difference in soil physicochemistry between the plots mechanically disked and the plots non-disked across the sites (i.e., Y vs. N) (Global $R=-0.2$, $p=0.8$), but there was significant difference between LC1 and LC2 by hydrologic regime (Global $R=1$, $p=0.005$) (Fig. 2a). Moser et al. (2007) documented the rationale of inducing microtopography and its significant contribution on vegetation development and its inevitable relations to some of the soil nutrients, but we did not find any differ-

ence in the early development of soil physicochemical properties by induced microtopography in this study.

3.2. Soil bacterial community structure

MDS and ANOSIM were performed on the soil bacterial community data to examine if the soil bacterial community patterns differed by sites. ANOSIM results showed significant differences in soil bacterial communities between the sites (Global $R=0.77$, $p < 0.001$). The MDS plot of soil bacterial communities displayed clear clustering (Fig. 2b) between the sites with the stress levels of MDS ($=0.15 < 0.2$) indicating that the ordination provides an acceptable representation of the overall structure of the soil bacterial community data set (Clarke and Warwick, 2001). Although the data in each site clustered more closely than between the sites the distance between LC2 and the other created wetland sites was striking (Fig. 2b). This indicates hydrologic regime may have great effects on the structure of soil bacterial community. Additional ANOSIM conducted with site and microtopography as factors, revealed that

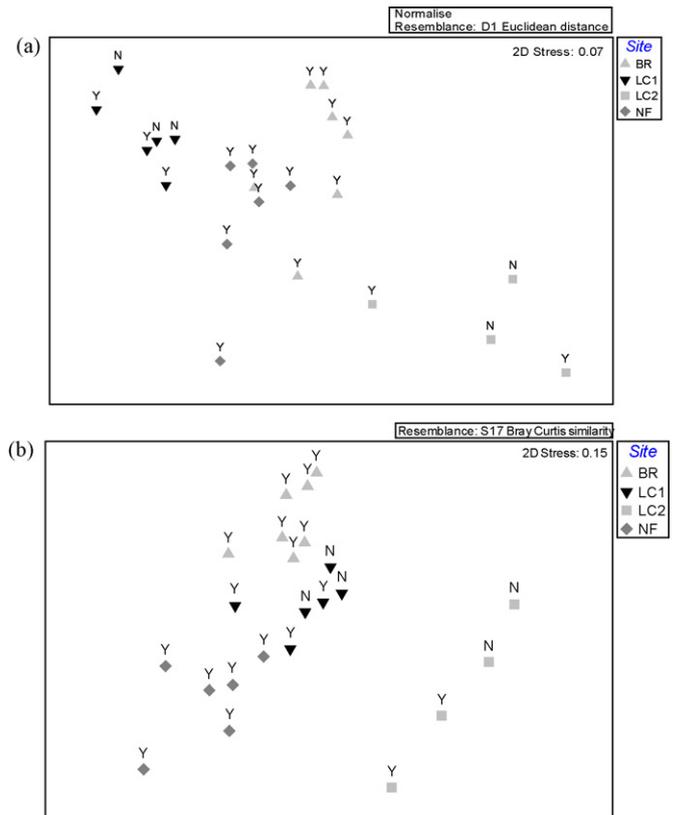


Fig. 2. Non-metric, multidimensional scaling (MDS) plots of: (a) soil physicochemistry; and (b) assemblages of LH-PCR profiles of soil bacterial communities, both in four created mitigation wetland sites. Letters refer to whether site was mechanically disked (i.e., Y-disked; N-not disked).

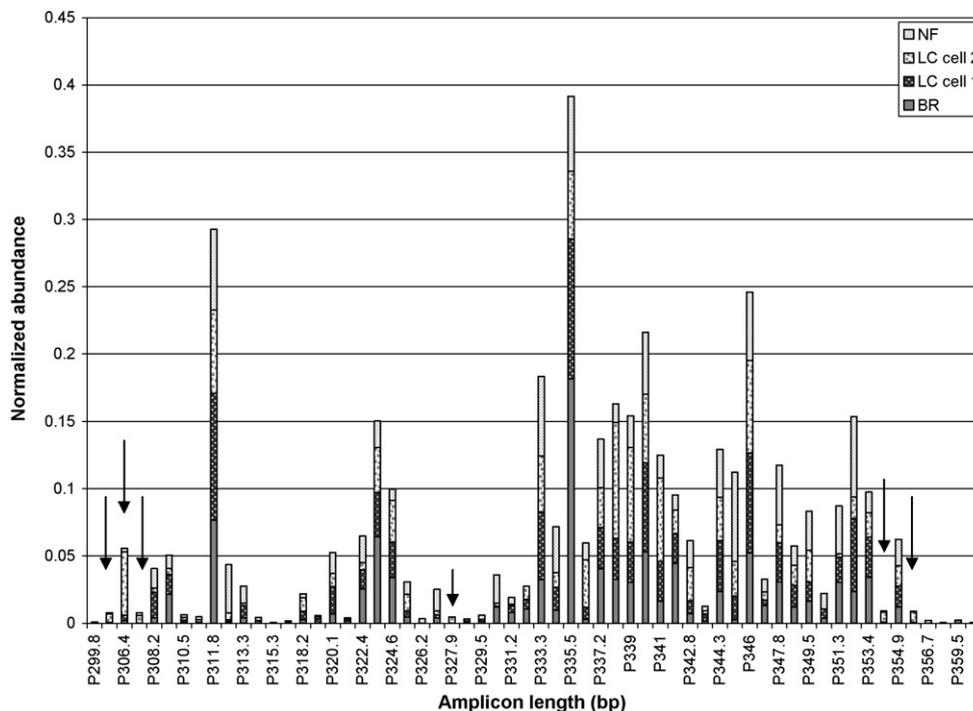


Fig. 3. Normalized abundances of LH-PCR amplicons of soil bacterial communities in four created wetland sites, including LC1 (i.e., dry cell) and LC2 (i.e., wet cell). Arrows point to the amplicons unique to LC2 (i.e., wet cell).

microtopography treatment (i.e., Y vs. N) did not induce any significant difference in soil bacterial community (Global $R=0.06$, $p=0.36$) whereas hydrologic regime (i.e., LC1 vs. LC2) within a wetland did (Global $R=0.79$, $p=0.005$), confirming the visually evident separation of LC2 from LC1 in the MDS plot (Fig. 2b).

The normalized abundances of LH-PCR amplicons of soil bacterial communities were plotted in a histogram to visually identify the differences between sites (Fig. 3). Fig. 3 clearly shows certain OTUs that were specific to a certain site and/or condition; 305.7, 306.4, 306.9, 327.9, 354.3, and 356 were predominantly present in LC2, the wetter site (Fig. 3). The results indicate that hydrology and/or hydrologic regime may have played an important role in determining the bacterial community structure in the site.

3.3. Association between physicochemical properties and bacterial community profiles in wetland soils

We investigated if there was any significant association between soil physicochemical properties that are often measured to examine the condition and/or progress of created wetlands, and soil bacterial community patterns. The idea behind this was to test the utility of simple LH-PCR profiles of soil bacterial community as an indicator in monitoring the development of soils and vegetation in created wetlands (Ahn et al., 2007). A significant correlation was found between soil physicochemical properties and soil bacterial community structure ($\rho=0.36$, $p=0.004$), showing a clear association between the two. This confirms the visual resemblance of the two MDS plots (Fig. 2a and b). All the possible combinations of five soil physicochemical variables (%C, %N, C/N, %moisture, and bulk density) were significantly correlated with the patterns of soil bacterial community across the sites (BEST result: ρ ranged from 0.38 through 0.56, $p=0.001$ in all combinations). C/N ratio as a single variable was the best descriptor of overall soil bacterial community patterns observed throughout the sites ($\rho=0.56$, $p=0.001$). The C/N ratio was also found highly, negatively correlated with % soil moisture across the sites ($r=-0.76$, $p<0.05$), indicating the

role of hydrologic regime in structuring soil bacterial community. Although % soil moisture was presented and used as a quantitative value, it is more of a qualitative indicator of hydroperiod and/or hydrologic regime. It may vary seasonally or with a rainfall event, but all the sites under our study were close to each other (i.e., within 15–20 km), relatively under the same climate condition. Soil moisture content is inevitably linked to water retention and has been found significantly correlated with soil carbon contents of these sites (unpublished manuscript). It can work as a useful indicator for hydrologic regime of created palustrine scrub/shrub sites such as ours, which rarely have standing water during the year.

3.4. Diversity of the bacterial communities (Shannon–Weiner index, H')

Species richness, Shannon–Weiner's diversity index (H'), and evenness were calculated based on the observed OTUs in the fingerprints of wetland soils. No significant difference was found in the community diversity measures by microtopography treatment, so the measures were pooled together by each site. One-way ANOVA revealed significant differences in H' and evenness between the sites (Table 2). Shannon's diversity and evenness of soil bacterial community in NF were found to be significantly higher than those in the other two younger sites (i.e., LC2 and BR). However, the diversity measures in NF were more comparable with those in LC1 (Table 2). There were also significant differences between LC1 and LC2 among the diversity measures calculated on the communities. The dry site (LC1) had consistently higher diversity and evenness than the wet site (LC2), suggesting that hydrologic regime may play a bigger role in determining the structure of soil bacterial community than the age of a site.

We may need to be cautious in interpreting the differences of diversity measures between the sites. It seems that these indices of community diversity based on OTUs are relatively not as sensitive given the fact that ANOSIM showed a distinctive difference in bacterial community patterns by site and by hydrologic regime within

Table 2

Richness, diversity (Shannon–Weiner H') and evenness of soil bacterial community fingerprints of created wetlands (mean \pm SE). One-way ANOVA results are reported in the bottom row. Means in each column followed by the same letter are not significantly different across the sites at the $p < 0.05$ level.

Site	Richness (S)	Diversity (H')	Evenness (E)
LC1	20 \pm 0.6	2.81 \pm 0.04b	0.95 \pm 0.004b
LC2	18 \pm 0.7	2.63 \pm 0.04a	0.92 \pm 0.003a
BR	19 \pm 0.7	2.68 \pm 0.04a	0.92 \pm 0.007a
NF	22 \pm 1.5	2.93 \pm 0.08b	0.95 \pm 0.006b
	$F = 3.95, p = 0.24$	$F = 5.70, p = 0.006$	$F = 5.04, p = 0.01$

Richness (S) = number of peaks at each sample; diversity (H') was measured using the Shannon–Weiner's diversity index = $-\sum p_i(\ln p_i)$, where p_i is equal to the relative peak area; evenness (E) = $H'/(\ln S)$.

a site. Moreover, there may be more than one species/genus within the same amplicon size in the bacterial community profiles and thus the peaks in the LH-PCR fingerprints may actually be monitoring the dynamics of more than one genus/species. Mills et al. (2006) points out that microbial ecologists are often faced with the dilemma of deciding if these traditional indices (i.e., S , H' , and E), as used in this study, are appropriate measures for microbial community profiles since they are designed for discrete macro-community analyses. The traditional ecological indices such as H' that are based on the clear definition and ecological description of an individual species as an entity are often difficult to define in microbiology (Mills et al., 2006).

4. Conclusions

We conducted LH-PCR as a quick examination of bacterial community of soils in four created mitigation wetland sites in the Piedmont region of Virginia along with their physicochemical measurements. Both physicochemical properties and bacterial community structure of soils significantly differed between the study sites. The difference between two newly created sites that were drastically different in their hydrologic regime was striking. Moreover, there was a clear association between physicochemistry and bacterial community patterns of soils, suggesting soil bacterial community dynamics may serve as an indicator for physicochemical changes in soils, which has a practical implication for post-construction monitoring of ecosystem development in created wetlands. Further studies are needed to identify specific bacterial groups observed along with specific site conditions (e.g., unique peaks observed in LC2). Cloning and sequencing of such specific groups are necessary to fully characterize the community down to genus/species level. With the identification of species and functional genes we should be able to not only better explain differences observed in the community profiles, but study their roles in the ecosystem development of created/restored wetlands.

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