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Ecological Indicators 7 (2007) 852–863

ECOLOGICAL INDICATORS

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Molecular characterization of microbial communities in treatment microcosm wetlands as influenced by macrophytes and phosphorus loading

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Received 16 August 2006; received in revised form 13 October 2006; accepted 13 October 2006

Abstract

There has been little understanding of soil microbial community patterns in phosphorus (P) processing in treatment wetlands. The description of soil microbial communities has been implicit, often treated as a 'black box'. Microbes play an important role in P removal by direct P uptake and storage, mineralization of organic P, and direct and indirect control of the redox state of metals important to P removal. We conducted a microcosm wetland study focusing on phosphorus processing in treatment wetlands. The study collected basic data on microbial community structure, attempting to examine the influence of macrophytes and phosphorus loading on microbial communities that is critical in the treatment efficiency of constructed wetlands. Specifically, we fingerprinted soils from microcosms under different conditions of macrophyte and phosphorus loading using length heterogeneity PCR (LH-PCR). We also examined microbial diversity of some of those soils by cloning and sequencing community libraries. Principal coordinate analysis (PCO) and analysis of similarity (ANOSIM) of the fingerprints suggested that high-phosphorus loading has a recognizable impact (global $R = 0.6$, $p = 2.9\%$) in altering overall soil microbial community structure, seemingly decreasing the microbial diversity (i.e., Shannon–Weiner's diversity index, H'). The clone sequence data showed that different bacterial groups were selected for different phosphorus treatments. No significant effects of the presence of macrophytes on soil microbial communities were detected (global $R = -0.15$; $p = 80\%$), likely due to low growth of plants and sampling anomalies. Further study is underway to investigate possible linkages between the soil microbial community patterns and major operational components, including vegetation and phosphorus loading in treatment wetlands, which will help us eventually develop microbial community metrics and/or indicators that wetland engineers and managers can utilize as one tool in managing different operational conditions to achieve optimum phosphorus removal in treatment wetlands.
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Keywords: Microbial community structure; Microbial diversity; Treatment wetland; LH-PCR; Phosphorus

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doi:10.1016/j.ecolind.2006.10.004

1. Introduction

Managing a healthy plant community is crucial in designing, constructing and operating treatment wetlands (Kadlec and Knight, 1996). It has been known in general that plants enhance treatment performance of wetlands through alteration of wetland hydrology and physicochemical features (Kadlec and Knight, 1996; Cronk and Fennessy, 2001). The presence and/or growth of macrophytes influences nutrient dynamics in wetlands through the ability of macrophytes to deliver organic materials and oxidants belowground, which produces dynamic soil redox biogeochemistry (aerobic–anaerobic) inducing chemical gradients for nutrient transformations by microflora (Brix, 1997; Tanner, 2001; Cronk and Fennessy, 2001; Aldridge and Ganf, 2003; Stottmeister et al., 2003). The nutrient removal function of wetlands is also closely related to accumulation and degradation of organic matter, and therefore largely mediated by microbes localized in the surface layers of soils (Kang et al., 1998; Silvan et al., 2003). A few studies recently found higher functional microbial diversity or the development of a new microbial community in rhizosphere soils compared to non-rhizosphere (bulk) soils (Kim et al., 1999; Tam et al., 2001; Vacca et al., 2005), indicating the close connection between plants and soil microbial communities.

Several biogeochemical parameters and associated processes in phosphorus (P) treatment are affected by phosphorus loading, thus controlling the P removal efficiency of treatment wetlands (Kadlec and Knight, 1996; Reddy and D'Angelo, 1997; Nairn and Mitsch, 2000; Ahn et al., 2001). In particular, increased P loading to wetland systems results in a chemical gradient with P non-limiting conditions near the source, and P limiting conditions further from the source (from inflow to outflow in wetlands). Between these two zones, there exists a gradient in quantity and quality of organic matter, nutrient accumulation, and microbial communities, resulting in a diversity of microbial consortia and associated processes (Reddy et al., 1999). However, no studies have investigated the direct effects of phosphorus loading on soil microbial community patterns in treatment wetlands.

There has been little understanding of soil microbial community patterns and their roles in P processing. Microbes play an important role in P removal by direct P uptake and storage, mineralization of organic P, and direct and indirect control of the redox state of metals important to P removal. Nonetheless, the description of soil microbial communities has been implicit in P transformations in wetlands. Therefore, understanding the dynamics of soil microbial communities in relation to the key operational variables of treatment wetlands would be critical in managing the ability of wetlands to retain phosphorus.

LH-PCR fingerprinting is a method used to characterize microbial communities by amplifying the variable regions of SSU rRNA genes from each species and separating the natural variation in the amplicon length on a denaturing polyacrylamide gel. A mix of bacteria can be segregated based on the actual length of these selected amplicons. 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). 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 DGGE and T-RFLP (Dunbar et al., 2001; Mills et al., 2003; Ritchie et al., 2000). LH-PCR is often regarded as a better technique than DGGE because it can identify species and the "abundance" of the species whereas it is difficult to quantify species using DGGE. The LH-PCR fingerprinting is inexpensive and fast with the ability to screen several hundred soil samples a day. Thus, it is routinely used as a survey tool to monitor the dynamics of natural soil microbial communities or to quickly identify samples of interest. There may be more than one species/genus within the same amplicon size in community profiles and thus the peaks in the LH-PCR fingerprints may actually be monitoring the dynamics of more than one genus/species. However, the purpose of any profiling method is to produce an overall pattern of the community, not to identify absolutely each individual species or genus in that community (Mills et al., 2003). It should be noted that microbial functionality cannot be determined directly from LH-PCR fingerprints, but can be inferred from the phylogeny. If needed, cloning and sequencing functional genes can be utilized to

determine functionality such as biogeochemical and phosphorus processing.

The ultimate goal of our study is to develop soil microbial community indicators that sensitively relate to ecological functions of wetlands. LH-PCR molecular fingerprinting technique and cloning have been applied to ecological studies only quite recently, especially in wetlands. Thus, the validation of the use of this relatively new approach to study soil microbial community patterns that have been previously treated as a black box in phosphorus retention function of wetlands deserve to be recognized. The objective of our study presented here was to characterize soil microbial communities and examine the responses of their patterns as influenced by macrophytes and phosphorus loading in treatment wetlands using LH-PCR and cloning. The study was designed as a preliminary test before we launch a large-scale, field mesocosm study over a few growing seasons. We conducted a greenhouse experiment using experimental wetland microcosms.

2. Materials and methods

2.1. Experimental design and treatment

To study the effects of the presence of macrophytes and phosphorus loading in soil microbial community patterns a set of 12 wetland microcosms (~5 gallon plastic buckets, Fig. 1) were positioned in the greenhouse of George Mason University. First, a hole was made on the side of each bucket approximately one inch from the bottom with a clear, plastic tube inserted into it. The tube was approximately 10 in. long and used for draining the microcosm. Inside the bucket, the tube reached to the center of the bucket. The plastic tube end inside each bucket was cut on a diagonal and covered with a 1 in. × 1 in. piece of mesh. Then, all 12 microcosms received 5 cm of non-calcareous river pea gravel (completely covering the drain). Each bucket was lined with a 3 ft × 3 ft piece of black mesh and then about 20 cm of topsoil (~commercially available topsoil) was placed in each wetland microcosm. Two rhizomes of *Schoenoplectus tabernaemontani* (C.C. Gmel) (formerly *Scirpus validus* Vahl.) were planted in half of the microcosms with the other half unplanted. This macrophyte was chosen since it is a typical plant used in constructed wetlands (Kadlec and Knight, 1996). Plants were established throughout the acclimation period. All microcosms were routinely watered with deionized water in the greenhouse maintaining the water level of 7–8 cm. The experiment was launched in Spring 2005 by adding one more treatment, P-spiking, to the half of those microcosms. Low-P microcosms were fed continuously with deionized water and high-P microcosms were fed with deionized water mixed with P_2O_5 (superphosphate). The experimental design of the study then consisted of four different treatment schemes: high-P with plant, high-P with no plant, low-P with plant, and low-P with no plant. Water level was checked daily during the experiment to ensure a similar hydrology to all microcosms with no

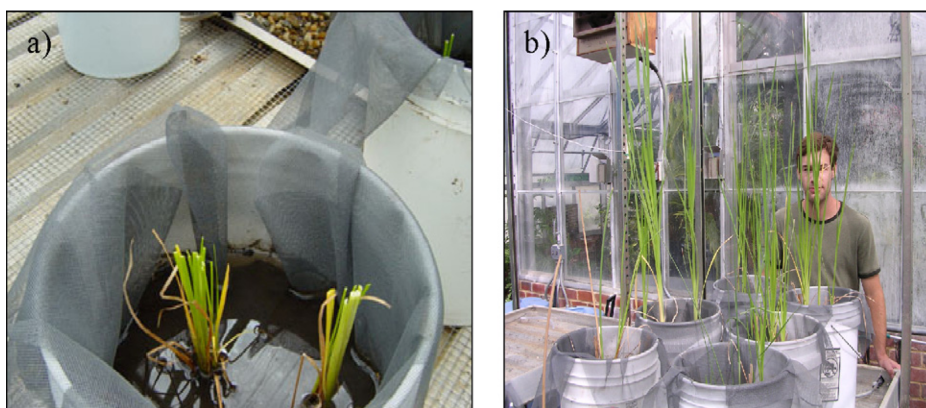

Fig. 1. Microcosm wetlands used for the study in the greenhouse, showing (a) when they were planted, and (b) at the beginning of the experiment.

differences among the treatments. Small amount of distilled water was added to each microcosm to compensate the loss of water by evapotranspiration. The target concentration for high-P inflow was 3–5 mg P L^{-1}, which is a soluble reactive phosphorus concentration of treated wastewater commonly found in constructed wetlands (Ahn and Mitsch, 2002). The experiment lasted from late March through late April 2005.

2.2. Water sampling and analysis

Water quality measurements for the surface water of each microcosm were taken weekly, during the 3-week period of the experiment to assure that two different phosphorus conditions were maintained. A YSI Multiparameter Water Quality Data Transmitter was used on site to measure the pH, temperature, conductivity, redox potential (ORP), and dissolved oxygen (DO). Weekly calibration of the YSI was carried out during the experiments. Surface water and leachate were collected during and at the end of the experiment, respectively. The surface samples were collected from overlying water in the microcosms, and the leachate samples were collected from the outflow tube from each microcosm, which had been folded in half and clamped during the experiment. The water samples were kept at 4 °C for the analysis of phosphorus and later colorimetrically analyzed using a Technicon Autoanalyzer II for soluble reactive phosphorus (SRP) (APHA, 1992). Five prepared standards, a check standard and distilled water blank were run each time that an analysis was conducted. Standards were always within 10% of the prescribed values.

2.3. Soil sampling

Morris and Bowden (1986) reported the top 2 cm of the sediment layer as biogeochemically active layer, especially important for phosphorus. Furthermore, their results suggested that organic matter decomposes quickly within the top 5 cm of sediment compared with deeper sediment in wetlands. At the end of the experiment, three soil samples were taken within the top 5 cm of the microcosms for molecular microbial analysis. A small sub-set of those soil samples was analyzed for 0.5 M $NaHCO_3$-extractable (labile) P by the method of Hedley et al. (1982). The soil samples were taken using a 5 cm^3 syringe with the bottom cut off and the plunger removed. The syringe barrel was forced into the wet soil of the microcosm and the plunger was inserted to facilitate removal of the wet slurry. Rhizosphere soil samples were taken on or immediately next to the plant roots. The samples were stored at −20 °C until usage for DNA extraction and LH-PCR fingerprinting.

2.4. DNA extraction, LH-PCR and cloning

Whole community DNA was extracted using the Bio101 FastDNA SPIN Kit for soil (Q·BIOgene, Inc., Carlsbad, CA). Purified DNA was amplified by PCR using universal bacterial primers as previously described (Mills et al., 2003). Analysis of the community fingerprints was done using the GenoSpectrum software package V2.08 (SpectruMedix LLC.), which converted fluorescent data into electropherograms. Further analysis was performed using custom PERL scripts and visualized by graphs created in Microsoft Excel. Following analysis of community structure, LH-PCR products from the high- and low-phosphorus control groups with no plants were cloned using the TOPO-TA Cloning kit (Invitrogen, Carlsbad, CA). About 144 transformed colonies and a few untransformed control colonies were picked and amplified with M13 forward and reverse primers using standard PCR conditions. The PCR products were then purified using AMPure (Agencourt) magnetic beads according to manufacturer's protocol. A standard sequencing reaction was done using the Big Dye Terminator kit (PE Applied Biosystems, Foster City, CA). The sequencing reactions were run on the SCE 9610 capillary. The sequence data was analyzed using BaseSpectrum program V2.01 (SpectruMedix LLC.) and transferred to Sequencer V4.1 (GenCodes Corp.) for manual base calling when necessary. Then, each clone sequence was used in BLAST searches against GenBank and RDP (NCBI, Rockville, MD). The results of BLAST search were parsed with a PERL script for automatically identifying the clone sequences.

2.5. Data analysis

We used PRIMER-E (Plymouth Routines in Multivariate Ecological Research, v. 5; Clarke and

Gorley, 2001) for principle coordinate analysis (PCO) and analysis of similarity (ANOSIM) to test the effects of plants and phosphorus loading on soil microbial community structure. For diversity, three parameters were used to compare LH-PCR fingerprint patterns. These are richness (S) which is equal to the number of peaks in a samples, the Shannon–Weiner's diversity index (H') which is equal to $\sum(P_i(\ln P_i))$ where P_i is the peak area; evenness (E) which is equal to $H'/\ln S$. For each soil sample under different treatment scheme, we quantitatively assessed these three indices of diversity and compared them using the sign test (Clarke and Warwick, 2001).

3. Results and discussion

3.1. Water and phosphorus chemistry

Table 1 shows physicochemistry and SRP concentrations of surface water in the microcosms were maintained consistently during the experiment except for the difference in phosphorus loading. There was about five times higher SRP concentration (2.4 mg P L^{-1}) under high-P treatment than under low-P treatment (0.5 mg P L^{-1}) (Table 1). Other physicochemical variables were maintained during the experiment regardless of treatment scheme. The leachate and soil labile-P data collected at the end of the experiment showed significantly higher concentrations from high-P microcosms compared to the low-P microcosms (Table 1).

Based on the surface SRP concentrations, we observed up to a 79% decrease of phosphates in high-P microcosms on average over the 1-month experimental period, while there was either no decrease or actually a slight increase of phosphate (~release of P) in low-P microcosms. High-P loading may have facilitated microbial scavenging of phosphates in surface water that would otherwise have been released back to the water due to the anaerobic condition of wetlands. There were no significant differences observed in water chemistry by the presence or absence of plants (Table 1).

3.2. Microbial community fingerprinting

In analyzing the initial community fingerprinting of the microcosms, we used the GenoSpectrum software package V2.08, which converted fluorescent data into electropherograms. The peaks of the electropherograms represent different microbes of various base pair (bp) sizes (Fig. 2). Normalized peak areas were calculated by dividing an individual peak area by the total peak area of the profile. The electropherograms were consistent for each sample under phosphorus treatment across replicate PCRs. The microbial communities profiles were almost identical in soils between planted and unplanted microcosms, but there were a few consistent

Table 1
Physicochemistry and soluble reactive phosphorus (SRP) concentration (mean ± S.E.) of surface water measured in microcosm wetlands weekly during the 3-week period of the experiment under four different treatments

	High-P with plants	High-P w/o plants	Low-P with plants	Low-P w/o plants
During the experiment				
Temperature (°C)	27.9 ± 4.0	28.1 ± 3.7	26.8 ± 3.8	27.6 ± 3.6
DO (mg L^{-1})	9.0 ± 0.24	9.2 ± 0.16	8.12 ± 0.35	9.52 ± 0.23
Conductivity (μS cm^{-1})	690 ± 80	550 ± 80	710 ± 11	670 ± 80
pH	8.3 ± 0.2	8.5 ± 0.2	8.1 ± 0.2	8.7 ± 0.2
Redox potential (mV)	179 ± 3	174 ± 4	180 ± 1	172 ± 3
SRP–surface water (mg L^{-1})	2.47 ± 0.6	2.29 ± 0.59	0.61 ± 0.04	0.40 ± 0.04
At the end of experiment				
SRP-leachate (mg L^{-1})	0.89 ± 0.3	0.93 ± 0.3	0.58 ± 0.07	0.39 ± 0.00
Labile-P in soil (μg P g^{-1})	1003 ± 35	980 ± 20	410 ± 35	338 ± 50
Phosphorus removal (%)	79	77	−14	0.3

Leachate SRP and sediment labile phosphorus were measured from leachate and sediment samples, respectively, taken from microcosms for molecular microbial community analysis at the end of the experiment. Phosphorus removal (%) was calculated based on the differences of SRP concentration of surface water between the beginning and the end of experiment.

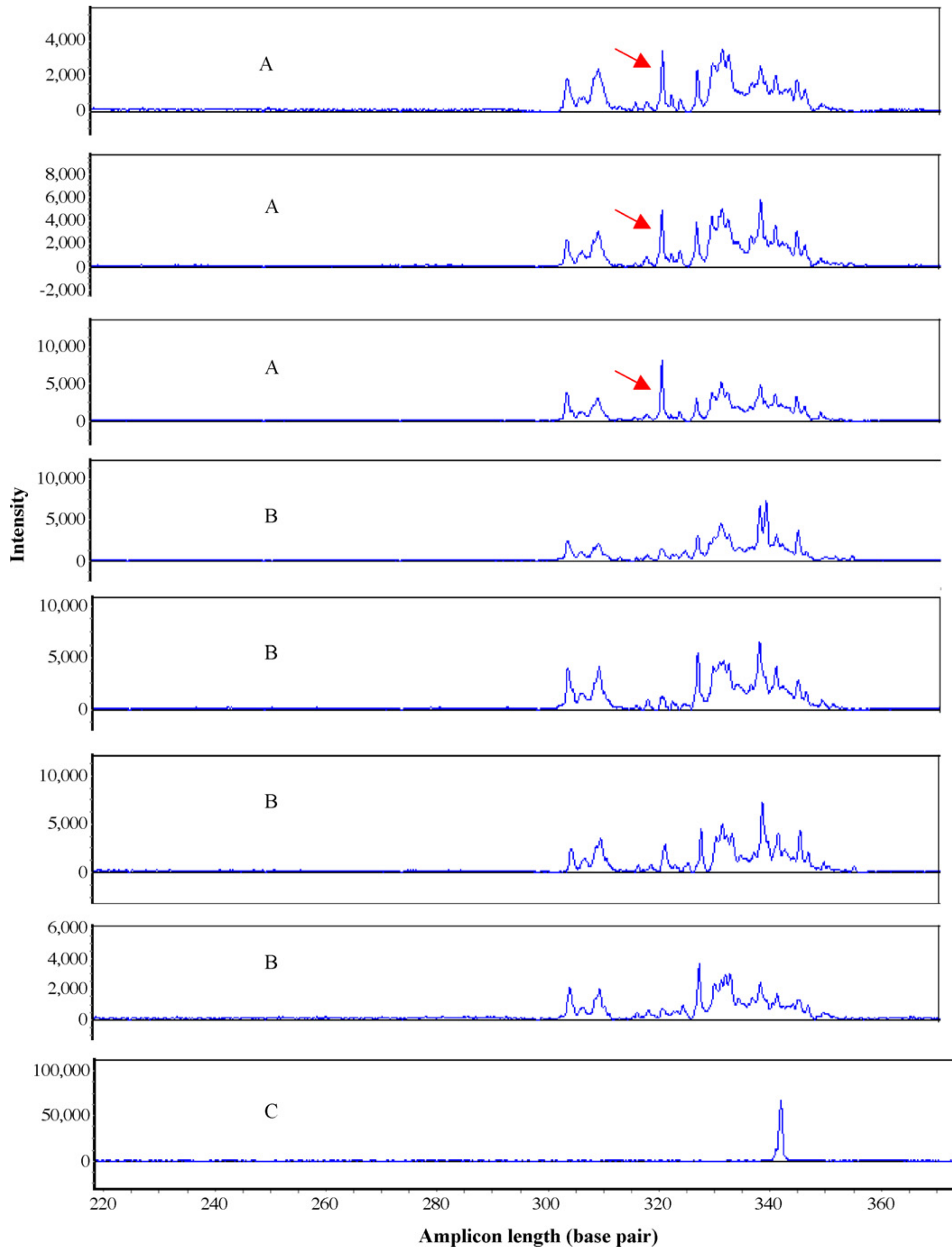

Fig. 2. Electropherograms depicting microbial communities at various amplicon sizes. Panels labeled A represent profiles of high-P microcosms. Panels labeled B correspond to soils from low-P microcosms. Panel C depict the control PCR, which contained genes from only *Escherichia coli*. Arrows point to the peak 323 bp that is introduced with the high loading of phosphorus.

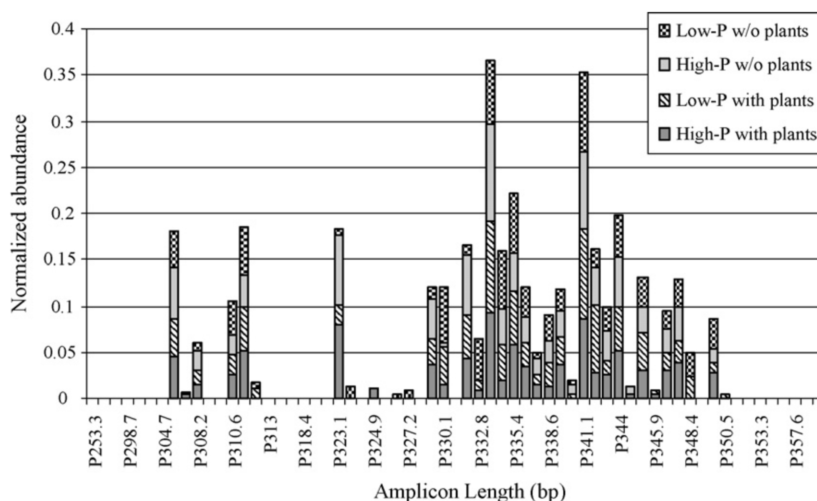

Fig. 3. Microbial community profiles under different plant and phosphorus treatments in microcosm wetlands.

differences in microcosms with the different phosphorus loading, suggesting that high-P treatment did have an effect on microbial community structure. The most marked difference between these community profiles was the addition of a new peak at the 323 bp amplicon size (Fig. 2), suggesting that high-phosphorus loading had facilitated the growth of one or more genus/species within the soil microbial community. To quantitatively compare the relative abundance of each peak in these raw profiles, we averaged the normalized data from triplicate LH-PCRs of the samples representing each treatment and plotted the data as a histogram (Fig. 3). The histogram compares the profiles of the soils from high- and low-P microcosms. Although there were no dramatic differences in abundance in corresponding peaks the ‘‘nascent’’ peak depicted previously by the electropherograms at about 323 bp (Fig. 2) was again shown to be predominantly present only in the high-P treatment (Fig. 3) whereas some peaks were lost due to high-P condition as appeared at about 348.4 bp (Fig. 3).

We ran PCO and ANOSIM on the data from each treatment to see if there was any statistical difference due to phosphorus loading. PCO reduces the data into a series of linear axes that explain the maximum amount of variance in the data (Clarke and Warwick, 2001). The relative position of each sample along the principal component axes is used to describe the degree of community-level similarity between the samples. The three-dimensional PCO representation clearly depicted clusters formed by the high- and low-P groups with no plants (Fig. 4). There was a recognizable difference in soil microbial community patterns between high-P and low-P treatments (global $R = 0.6$, $p = 2.9\%$). However, the macrophyte effects on soil microbial community patterns were not statistically significant (global $R = -0.15$; $p = 80\%$). The presence of plants rather broadened the community compositions as indicated by the broader scattering of these data (Fig. 4) with no statistical difference seen with respect to unplanted microcosms.

The broader community profiles found in the presence of plants may actually be attributed to two possible confounding issues. First, there was a lack of plant growth as the rhizomes were planted at the end of growing season (September) after several months of acclimation under greenhouse condition. The other reason may be the coarse sampling as we did not do any fractionation of the material but simply took a subsurface aliquot of soil near or on the roots. Macrophytes will introduce a network of roots and root hairs into the subsurface soil that will promote the formation of an aerobic boundary around the root system. Although we were careful in our sampling trying to take the rhizosphere soils near or on the root hair, we most likely incorporated both bulk and rhizosphere soils. Truly rhizosphere soil samples affected by the oxygenated layer of plant roots are within only a few millimeters of the roots (Bezbaruah

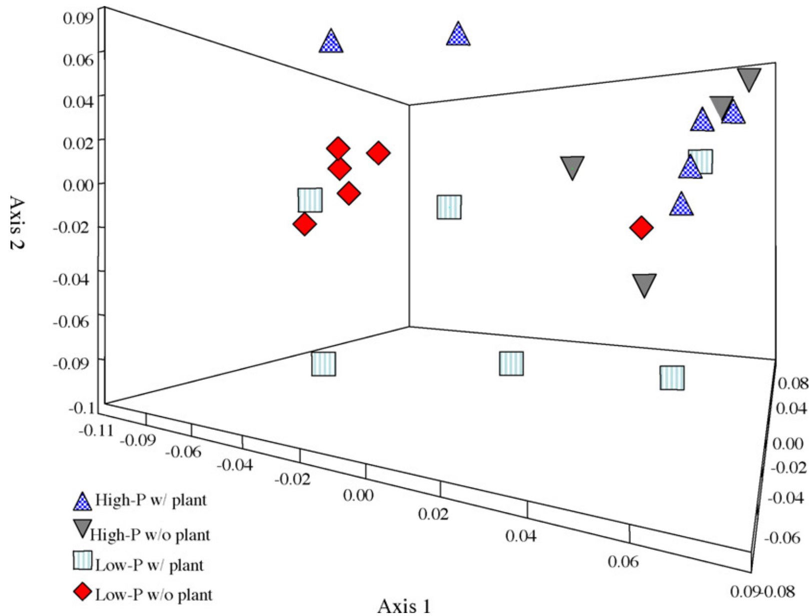

Fig. 4. Principle coordinate analysis (PCO) of the sediment microbial community profiles from four different treatments. This graph clearly depicts clusters formed by the high-P and low-P microcosms, both with no plants. With plants added both high- and low-phosphorus treatment do not form any clusters.

and Zhang, 2004). Vacca et al. (2005) found the effect of plants and filter materials on bacteria removal in pilot-scale constructed wetlands by use of a molecular technique. Some bands that were not present in the bulk soil appeared in the fingerprints of rhizosphere soils in the study, indicating the development of specific communities stimulated or influenced by the macrophyte in the constructed wetlands. Thus, we conclude that our crude sampling by use of a syringe may have precluded the examination of the full effects of plants (and their oxidized rhizosphere) on soil microbial community patterns in this set of experiments. We will have to address this issue in the further studies by conducting a more meticulous sampling of rhizosphere soil relative to bulk soil. We may be able to optimize the sampling regime by subfractioning the samples into root hair mass and particulate soils attached to the root hair mass.

3.3. Diversity of the microbial communities (Shannon–Weiner index, H')

LH-PCR fingerprinting revealed a total of 25–34 bacterial groups based on amplicon enumerations (i.e., length in bp), in the experimental treatment with phosphorus loading and presence of macrophyte (Table 2). The number of microbial species (i.e., species richness) detected was higher in the microcosms with low-P loading and those with plants compared to the ones with high-P loading or no plants (Table 2). The evenness was slightly higher in microcosms fed by high-P loading compared to those fed by low-P loading. No significant differences in Shannon–Wiener's diversity index (H') were found

Table 2
Three diversity parameters used to compare LH-PCR fingerprint patterns of wetland microcosm soil samples from different treatments

Treatment	Richness (S)	Evenness (E)	Diversity (H') ± S.E.
High-P with plants	28	0.88	2.94 ± 0.11
High-P w/o plants	25	0.90	2.89 ± 0.11
Low-P with plants	38	0.86	3.12 ± 0.11
Low-P w/o plants	34	0.87	3.07 ± 0.12

Richness (S) equal to the number of LH-PCR peaks in a sample, Shannon–Weaver diversity index (H') equal to $\sum(P_i(\ln P_i))$ where P_i is the peak area (i.e., a relative abundance of amplicon size), and evenness (E) equal to $H'/\ln S$.

among the treatments although low-P fed microcosms consistently showed relatively high diversity (Table 2), thus suggesting high-P loading may have played as a stress, decreasing the microbial diversity in the microcosms.

3.4. Preliminary cloning

Results from the sequencing of the cloned PCR products were analyzed by a custom PERL script that takes the clone sequence data, trims off any vector sequence, and then performs a MEGABLAST search against the RDP database (Ribosomal Database Project Version 8.1). RDP assignments are based on SSU rRNA homology or phylotype (Cole et al., 2005). This database delineates a hierarchical classification scheme where each component of the number designates a specific level of the hierarchical nomenclature. The classification scheme starts at the domain level and proceeds down to the genus and species names. The MEGABLAST hit table is parsed, the database entry with the highest bit score is selected as the closest relative of the environmental clone, and the RDP number for this closest relative is then assigned to the clone. We then tabulated the identified bacterial groups at the fourth level of the RDP

Table 3
Clone data obtained from the sediments in high-P (C2 and C3) and low-P microcosms (C4 and C5), both with no plants, and tabulated at the fourth level of the RDP hierarchy (Ribosomal Database Project Version 8.1)

RDP level 4 classification	Low-P		High-P		Difference
	Clones	Percent	Clones	Percent	
ACIDOVORAX_GROUP	2	4.4	1	2.5	0.6
BB.BREVIS_GROUP	2	4.4	1	2.5	0.6
DESULFUROMONAS_GROUP	2	4.4	1	2.5	0.6
XANTHOMONAS_GROUP	2	4.4	1	2.5	0.6
ARTHROBACTER_SUBDIVISION	2	4.4	2	5.0	1.1
SPHINGOMONAS_GROUP	2	4.4	2	5.0	1.1
LEGIONELLA_GROUP	1	2.2	1	2.5	1.1
MYXOBACTERIA	1	2.2	1	2.5	1.1
RHIZOBIUM-AGROBACTERIUM_GROUP	5	11.1	7	17.5	1.6
HELICOBACTER_AND_RELATIVES	2	4.4	3	7.5	1.7
BURKHOLDERIA_GROUP	1	2.2	2	5.0	2.3
MC.JANNASCHII_GROUP	1	2.2	2	5.0	2.3
STREPTOMYCES_SUBDIVISION	1	2.2	3	7.5	3.4
ACIDIMICROBIUM_GROUP	4	8.9	0	0.0	Loss
METHYLOMONAS_GROUP	3	6.7	0	0.0	Loss
MYCOBACTERIUM_SUBDIVISION	1	2.2	0	0.0	Loss
PROPIONIBACTERIUM_GROUP	2	4.4	0	0.0	Loss
RHODOPILA_GROUP	1	2.2	0	0.0	Loss
SACCHAROPOLYSPORA_GROUP	1	2.2	0	0.0	Loss
TAR.MARIANENSIS_GROUP	3	6.7	0	0.0	Loss
THIOBACILLUS_GROUP	2	4.4	0	0.0	Loss
ACHOLEPLASMA-ANAEROPLASMA_GROUP	0	0.0	1	2.5	Gain
AZOSPIRILLUM_GROUP	0	0.0	1	2.5	Gain
B.MEGATERIUM_GROUP	0	0.0	1	2.5	Gain
DBB.PROPIONICUS_ASSEMBLAGE	0	0.0	1	2.5	Gain
ENVIRONMENTAL_CLONE_T78_GROUP	0	0.0	2	5.0	Gain
FLX.SANCTI_SUBGROUP	0	0.0	1	2.5	Gain
OCEANOSPIRILLUM_GROUP	0	0.0	1	2.5	Gain
PAE.POLYMYXA_GROUP	0	0.0	1	2.5	Gain
RAL.EUTROPHA_GROUP	0	0.0	1	2.5	Gain
Unassigned	4	8.9	3	7.5	
Total clones	45	100	40	100	

Difference column shows either ‘loss’ or ‘gain’ of several microbial groups over phosphorus treatment.

hierarchical classification scheme (Cole et al., 2005) (Table 3). Overall, low-phosphorus condition displayed a higher number of clones (45) compared to high-phosphorus condition (40). Some groups of bacteria were either lost or gained upon increased P loading, others did not change. There were complete loss in bacteria groups such as Acidimicrobium, Methylomonas, Propionibacterium, Rhodopila, Saccharopolyspora, Tar. Marianensis, and Thiobacillus groups as well as in the Mycobacterium subdivision while several new groups appeared including the Environmental_Clone_T78 over the change in phosphorus treatment. However, it should be noted that this is just a preliminary analysis where we could analyze a few dozen clones only from each sample, thus we were sampling the most abundant members of the community. In this case, the differences of the low abundance taxa may not be significant. Further study should be conducted to complete the analysis of the libraries which will allow us to identify the taxa in the samples down to genus-species level [RDP-level 6].

3.5. Implications and further study

The results from our preliminary microcosm study showed a successful application of LH-PCR technique in studying wetland soil microbial communities and also demonstrated a significant difference in soil microbial community patterns over the phosphorus treatment. This can be the first step toward explicitly investigating soil microbial community patterns that can be used as indicators for phosphorus removal function of treatment wetlands (Fig. 5).

Further study on soil microbial community should be conducted that would involve realistic manipulations of hydrology, vegetation and phosphorus loading that are directly related to phosphorus treatment efficiency of wetlands (Fig. 5). For example, we will need to study the linkage between soil microbial community and vegetation, which may lead to a development of soil microbial community indicator that can be used to manage vegetation in treatment wetlands for sustainable phosphorus treatment.

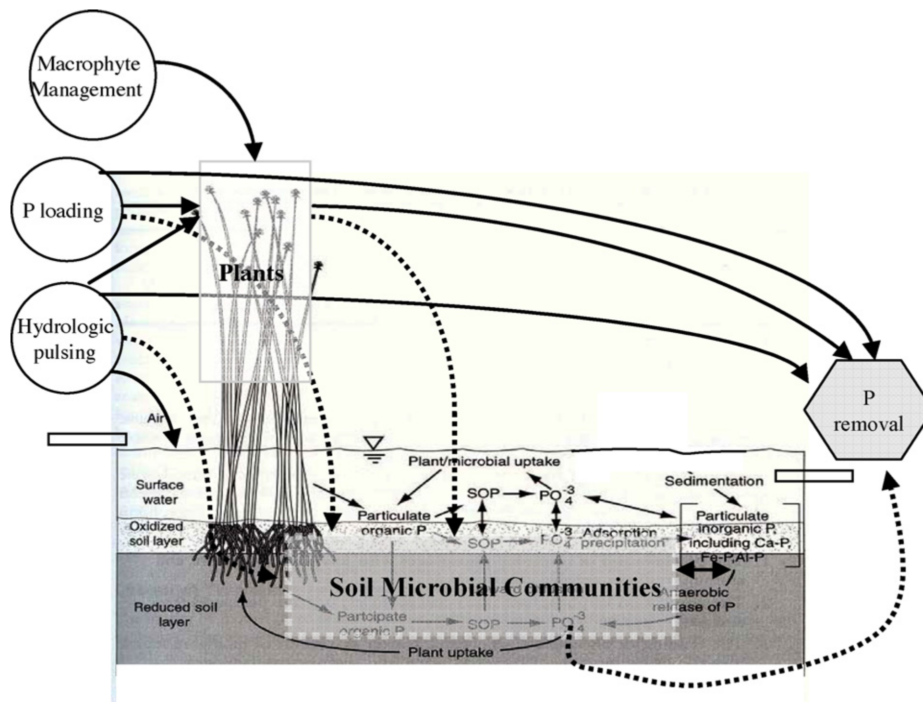

Fig. 5. Phosphorus dynamics in treatment wetlands. Further study should be conducted to more explicitly investigate the implicit linkages (indicated by the dotted lines) between soil microbial communities, and varying conditions of hydrology, phosphorus loading and vegetation that are linked (indicated by solid lines) to phosphorus removal (greatly modified from Mitsch and Gosselink, 2000).

Vegetation management can be critical for achieving and sustaining optimal treatment function, especially due to a variety of problems that may arise as a result of the presence of dense, mature stands of emergent macrophytes in treatment wetlands. Numerous studies have generally correlated high plant productivity with improved phosphorus removal in wetlands (Reddy and DeBusk, 1985; Brix, 1997; Verhoeven and Meuleman, 1999), but most of these studies were conducted when the plants were new and growing, absorbing abundant amounts of nutrients and water as the plants transpired and produced large amounts of biomass (Thullen et al., 2005). Plant growth and biomass production can be amplified especially in treatment wetlands with ample supply of nutrients. In addition, treatment wetlands are often built with one productive macrophyte species such as the one species used in our microcosm study (i.e., *Scirpus* spp.). Sometimes, treatment wetlands with initially multi-species planting become monotypic over time due to their eutrophic environment, which often induce an invasive species (i.e., *Typha* spp.). It is important to know if the role of plants in treatment wetlands can shift as vegetation biomass increases by driving carbon cycling through their senescence while processes driven by active plant growth and production are reduced. No attempts have been made to establish the linkages between the dynamics of soil microbial community patterns and those of vegetation in treatment wetlands, which can provide useful information on identifying the balance between adequate biomass and excessive accumulation of plant detritus for the purpose of vegetation management in treatment wetlands. The information on the dynamics of soil microbial community patterns (e.g., a drastic shift in soil microbial community profiles) along a gradient of plant growth and biomass production can be used as a warning signal or an indicator to determine when to harvest and/or to actively manage vegetation to sustain treatment efficiency.

We are currently focusing on investigating the responses of soil microbial communities in our further study to the manipulation of operational components of treatment wetlands through more realistic field-based wetland mesocosms. We expect the further study will help us understand the extent of microbial community patterns and their functional role in relation to phosphorus removal of treatment wetlands. Moreover, the microbial community data collected over varying conditions of operational components of treatment wetlands will help identify the microbial communities that are sensitive and can serve as an indicator for managing different operational conditions to achieve optimum phosphorus removal in treatment wetlands. Wetland engineers and managers may utilize the microbial community indicator as one tool in managing different operational conditions in treatment wetlands since it takes very small amount of soil samples to examine microbial communities by molecular techniques and that is quite non-destructive, relative to bulk soil and plant biomass sampling that are often adopted to diagnose the system dynamics (Kadlec and Knight, 1996).

Identifying and characterizing microbial community signatures in wetland soil can be potentially applied to wetland delineation and the examination of early development of vegetation and hydrologic conditions in created wetlands, since the signature may be closely related to flooding regimes of the soils and vegetation development of the sites.

4. Conclusions

The study investigated the effects of two variables, vegetation and phosphorus loading, critical in the function of treatment wetlands on the soil microbial community patterns. The LH-PCR fingerprint revealed a recognizable difference made by high-phosphorus loading in soil microbial community profiles relative to low-phosphorus loading in treatment microcosm wetlands, showing great potential for the use of soil microbial communities as an indicator to monitor phosphorus dynamics in treatment process of constructed wetlands. The study was preliminary and it would need a further study in which we will correlate the microbial community patterns with varying conditions of hydrology, vegetation and phosphorus loading that are critical in phosphorus treatment. The outcome will help us develop microbial community measures that can be utilized to manage those operational components of treatment wetlands for sustainable phosphorus removal. Moreover, the soil microbial indicators being developed can be utilized to monitor the dynamics of ecosystem development in newly constructed wetlands.

Acknowledgements

The authors thank Helen Ye and Shera Bender for their help on sample processing for LH-PCR analysis and for chemical analysis of water samples, respectively. Thanks also to G.B. Noe for proof reading the earlier manuscript.

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