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ARTICLE

The Influence of Vegetation on Microbial Enzyme Activity and Bacterial Community Structure in Freshwater Constructed Wetland Sediments

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Abstract Microorganisms play important roles in wetland ecosystems, but little is known about the influence of wetland plants on microbial community structure and activity. A greenhouse experiment was conducted to study the short-term influence of wetland vegetation on the sediment microbial community. Mesocosms were either planted with *Juncus effusus*, *Carex lurida*, or *Dichanthelium acuminatum var. acuminatum* or remained unvegetated. After eight weeks, sediment samples were taken and assayed for the activity of five microbial extracellular enzymes associated with carbon, nitrogen, and phosphorus cycling. β-1,4-glucosidase, phosphatase, and N-acetylglucosaminidase exhibited similar activity for all vegetation treatments, while the activity of the phenolic-degrading enzymes phenol oxidase and peroxidase was higher in sediments with no vegetation. Denaturing gradient gel electrophoresis and sequencing of partial 16S rRNA genes indicate differences in the sediment bacterial community associated with each plant regime. Acidobacteria, Firmicutes and Proteobacteria were the dominant phyla, although unvegetated sediments contained proportionally fewer Firmicutes and Alphaproteobacteria. This study provides insights into the structure of wetland bacterial communities and suggests that vegetation can influence both bacterial community structure and specific enzyme activity in wetland sediments. Moreover, these influences can occur over a relatively short time and could occur within just a few months of vegetation changes.

Keywords Bacterial community structure · Microbial enzymes · DGGE · Wetland sediments

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Introduction

Wetlands are productive ecosystems that act as ecotones between terrestrial and aquatic systems (Holland et al. 1990; Mitsch and Gosselink 2000). Emergent plants are essential components of wetland ecosystems and are responsible for maintaining many of the ecological functions of these systems (Bouchard et al. 2007; Hollis et al. 1988). The concept of constructed wetlands is engineered based on the ability of a wetland to retain pollutants (Vymazal 2007). Constructed wetlands remove nutrients through a variety of physical, chemical, and biological processes (Vymazal 2007). Wetland plants remove pollutants via direct assimilation into their tissue and also by providing surfaces for the growth and development of microbial populations that can transform pollutants (Brix 1993). The efficiency of a wetland, natural or constructed, is driven by the combined activity between microbes and filter material; complemented by wetland plants (Faulwetter et al. 2009; Truu et al. 2009; Zedler and Kercher 2005). Soil microorganisms are important degraders of contaminants and hence play a significant role in determining the nutrient removal efficiency of constructed wetlands (Stottmeister et al. 2003).

The cycling and processing of nutrients and organic matter by microorganisms depends on the production of extracellular enzymes (Burns 1982). For example, cellulose and chitin are degraded by different suites of hydrolytic enzymes, while lignin and polyphenolic compounds are degraded by the oxidative enzymes (Burns 1982). Hence,

many of the key ecosystem functions of constructed wetlands likely depend on the aggregate activity of a variety of microbial enzymes produced by various sediment microbial populations (Nannipieri et al. 2003). However, only a limited number of studies have focused on the microbial enzyme activities in constructed wetlands (Tao et al. 2007; Truu et al. 2005). Factors such as pH, sediment organic matter and moisture content, nutrient concentrations, and the overall hydrology of the system have been shown to affect microbial enzyme activities in wetlands (Akiyama et al. 2010; Shackle et al. 2000). Plants also modify the sediment microclimate by controlling substrate concentrations, excreting exogenous enzymes, oxygen and exudates into the sediment; all of which influences the microbial enzyme activity (Caravaca et al. 2005; Singh and Kumar 2008). Plants are a major source of organic carbon, a universal electron donor for which heterotrophic microbes compete (Neubauer et al. 2005). Therefore, changes in the presence or type of vegetation could also lead to changes in soil environmental factors (like sediment oxygen levels, redox potential, nutrient availability, and organic matter content) that could also affect the sediment microbial community (Faulwetter et al. 2009; Sutton-Grier and Megonigal 2011).

It is well established that the selection of plant species is critical for increasing the treatment efficiency of a constructed wetland but it is still poorly understood how the manipulation of plants could be important for the establishment of desirable functional microbial groups in a constructed wetland (Faulwetter et al. 2009). Therefore, the efficiency of constructed wetlands to function as sinks for pollutants depends on the ability of plant species to influence the sediment microbial community and microbial enzyme activities. Thus, the knowledge of the functional microbial communities associated with plants would benefit in improving the management strategies for these systems. In this study, we examine the plant differential (presence or change in species) effects of three wetland plants- *Juncus effusus*, *Carex lurida* and *Dichanthelium acuminatum var. acuminatum* on sediment microbial community and microbial enzyme activity in constructed wetlands. These species are native macrophytes found in constructed wetlands (Brisson and Chazarenc 2009; Zazo et al. 2008; White et al. 2012; USDA and NRCS 2007) but little is known about their influence on the sediment microbial enzyme activity and bacterial community structure in these systems.

Materials and Methods

Experimental Set-up and Sampling

A greenhouse-based mesocosm experiment was conducted to test our hypothesis that the presence as well as the type of plant species affects the sediment microbial enzyme activity and bacterial community structure in constructed wetlands. A mesocosm facility was used in order to control factors beyond vegetation. Mesocosm studies are relevant to microbial ecology as the assemblages of bacteria found in these systems are often similar to those in the field (Eller et al. 2005; Ranjard et al. 2006). Mesocosms were established in June 2007 by filling 16 plastic barrels (approximately 0.30 m diameter and 0.5 m tall, total volume of 105 l) with sediment (mixture of sand and clay) taken from unvegetated constructed wetlands at the University of Mississippi Field Station (UMFS), Abbeville, MS, USA. The constructed wetlands at University of Mississippi Field Station (UMFS) are artificial ponds with clay lining, fed by groundwater and mainly used for research purposes. Groundwater at UMFS is routinely tested and has below detectable levels of nitrate, ammonia, and phosphorus. The constructed wetland from which sediment was used for this study has not been previously used for experiments and was inundated by ground water. Sediment was mixed thoroughly before filling the mesocosms. Mesocosms were filled with UMFS groundwater to 10 cm depth above the sediment surface and left to acclimatize for 2 weeks in the UMFS greenhouse facility. The water depth in each mesocosm was restored to 10 cm once per week to account for loss through evapotranspiration so that throughout the study, water levels fluctuated between approximately 8 and 10 cm. The 10 cm line was marked in the barrels and the water levels were measured before replenishing it to the 10 cm line (once every week) and the lowest level was at 8 cm. Mesocosms were maintained under the lighting regime of 14 h of day light and 10 h of darkness.

Juncus effusus (Family-Juncaceae), *Carex lurida* (Family-Cyperaceae) and *Dichanthelium acuminatum var. acuminatum* (Family-Poaceae) were chosen for the study as they are all perennial plants commonly found in wetlands of the South-Central US (USDA and NRCS 2007). Individual plants were collected from constructed wetlands at the UMFS and sediment removed from roots by spray washing with groundwater before plants were transplanted into the mesocosms. Mesocosms were either planted (monoculture) with 33 individual plants of each plant species (either *Juncus effusus* or *Carex lurida* or *Dichanthelium acuminatum var. acuminatum*) or were left unvegetated (four replicates for each plant species and unvegetated mesocosms). The plant height of all the 33 plants in each mesocosm was measured using a measuring tape and an average was taken for each species, and mesocosms were left undisturbed to allow plants to become established. After 8 weeks (August 2007), plant height was measured again, and the growth rate calculated as the increase in height from initial planting in relation to initial height and expressed as growth percentage.

After 8 weeks, pH of the water in each mesocosm was measured using a portable pH meter (Accumet AR 25 dual-

channel pH meter) and three sediment samples were taken from each mesocosm (48 samples total). Sediment samples were taken from the top 10 cm of sediment using a soil core sampler (1.9 cm diameter). For each sample, roots were removed by hand picking, and the sample was then mixed and divided into four subsamples. The first subsample (5 g) was taken for immediate determination of microbial enzyme activity. A second subsample (0.3 g) was frozen (−20 °C) for subsequent molecular analysis of bacterial community structure. The third subsample (approximately 10 g) was weighed, dried (65 °C, 48 h), and ashed (500 °C, 2 h) to determine sediment moisture content and organic matter content (as ash free dry mass). A final subsample (1 g) was analyzed on a Dionex ion chromatograph for total Kjeldahl nitrogen (TKN) and total phosphorus (TP) concentrations using the ammonia persulfate digestion procedure (APHA 1998; Murphy and Riley 1962).

Assays of Microbial Enzyme Activity

Each sample was analyzed for the activity of five extracellular enzymes: β-1,4-glucosidase (EC 3.2.1.21), phosphatase (EC 3.1.3.2), N-acetylglucosaminidase (NAGase; EC 3.2.1.52), phenol oxidase (EC 1.10.3.2) and peroxidase (EC 1.11.1.7). These enzymes are involved in the decomposition of cellulose (β-1,4-glucosidase), the mineralization of phosphorus (phosphatase), the decomposition of chitin (NAGase), and the degradation of phenolic compounds (phenol oxidase and peroxidase). A known mass of sediment material (approximately 5 g) was homogenized in pH5.0 50 mM acetate buffer (Jackson et al. 2006) to yield 10 mL slurries. For each enzyme assay, four 150 μl replicates of each of the sample slurries were incubated with 150 μl of the appropriate artificial substrate solution for 2–4 h. Substrates for β-1,4-glucosidase, phosphatase, and NAGase were linked to *p*-nitrophenol and activity for these enzymes was determined from absorbance at 410 nm in the presence of 0.067 M NaOH, as described previously (Jackson and Vallaire 2007, 2009). L-3,4-dihydroxyphenylalanine (L-DOPA) was the substrate used to assay both phenol oxidase and peroxidase activity, with peroxidase assays also receiving hydrogen peroxide to 0.015 %. Activity for these assays was determined from absorbance at 460 nm (Jackson and Vallaire 2007). Substrate controls (in duplicate) consisted of incubations of the substrate for each enzyme sample without the addition of sediment slurry, while duplicate controls for each sample consisted of 150 μl of the sediment slurry incubated without any substrate. Final enzyme activities were expressed as nmol substrate consumed $h^{-1} g^{-1}$ dry weight of sediment.

Molecular Analyses

DNA was extracted from 0.3 g of each sediment sample using a Power Soil DNA kit (Mo Bio, Carlsbad, CA, USA). DNA from the three replicate samples taken from each mesocosm was pooled prior to amplification to reduce random bias (Wagner et al. 1994). Bases 1070–1392 (*Escherichia coli* numbering) of the 16S ribosomal rRNA (16S rRNA) gene were amplified under conditions described previously (Ferris et al. 1996; Jackson et al. 2001) and analyzed using denaturant gradient gel electrophoresis (DGGE). Denaturant concentrations were 40–70 % urea–formamide in 8 % acrylamide, and electrophoresis conditions were 88 V for 18 h at 60 °C. Approximately 700 ng of amplified product from each sample was loaded onto DGGE gels. Following electrophoresis, gels were stained with SYBR Green 1 and images captured using a Kodak Gel Logic 200 running Molecular Imaging Software 4.0 (Eastman Kodak, Rochester, NY, USA). Banding patterns were detected digitally using Kodak Molecular Imaging Software version 4.0.5 (Eastman Kodak Company, Rochester, NY) and converted to binary data form (the absence or presence of specific bands) for statistical analysis.

Bac 8f and Univ 1492r primers were used to amplify a larger portion of the 16S rRNA gene for sequencing analysis, following reaction procedures described previously (Jackson et al. 2001). DGGE results showed that bacterial communities in replicate mesocosms with the same vegetation treatment were more similar to each other than to those under a different treatment. Therefore, PCR products obtained from replicate mesocosms with the same vegetation treatment were pooled to produce an overall sample for each treatment type. These mixed PCR products were cloned into artificial plasmid vectors (TA TOPO Cloning, Invitrogen, Carlsbad, CA, USA) and a clone library was generated for each vegetation treatment (*J.effusus*, *C. lurida*, *D. acuminatum*, unvegetated). For each clone library, the first 500–600 bp in the inserts from 48 randomly sampled clones were sequenced. Sequences were aligned and classified using Greengenes (DeSantis et al. 2006). Alignments were checked manually, and aligned sequences were incorporated into an existing 16S rRNA gene phylogenetic tree of >8,600 16S rRNA gene sequences (Hugenholtz 2002) using the 'quick add by parsimony' function in ARB software (Ludwig et al. 2004). For tree visualization, 16S rRNA sequences in the existing tree that were irrelevant for this study were subsequently removed while maintaining accurate tree topology.

Statistical Analyses

Differences in plant growth rate, abiotic factors (pH, TKN, TP, organic matter, sediment moisture), and sediment enzyme activity were tested using analysis of variance (ANOVA) with Tukey's HSD test as a follow-up procedure to test for specific differences between vegetation treatments. Community profiles obtained as binary data from DGGE were used to generate a similarity matrix (Bray-Curtis distance measure)

comparing different mesocosms. Similarity scores were used to visualize groupings of mesocosms by both cluster analysis (nearest neighbor group linkage method) and non-metric multidimensional scaling (NMDS) (Ramette 2007). All multivariate analyses were performed using Primer 6 software (PRIMER–E, Ivybridge, PL, UK). Species richness was estimated from the sequences detected in each clone library as the non-parametric diversity index S_{Chao1} (Chao 1987) and was calculated using a web based application http://www.aslo.org/lomethods/free/2004/0114a.html (Kemp and Aller 2004). Multivariate correlation analysis was carried out to determine relationships among different abiotic parameters, enzyme activities and bacterial communities using JMP (SAS, version 10).

Nucleotide Sequence Accession Numbers

The partial 16S rRNA gene sequences obtained in this study have been deposited in the GenBank database under accession numbers HM535008-535190.

Results

Mesocosm Characteristics

After 8 weeks (August 2007), there was a clear increase in plant height indicating that the plants were generally well established in the mesocosms. Mean (± standard error, average of the 4 replicate mesocosms) increase in height was greatest for *J. effusus* (52 %±7 %) followed by *C. lurida* (25 %±4 %) and *D. acuminatum* (10 %±1 %). Growth of *J. effusus* and *C. lurida* was significantly greater ($p<0.01$) than that of *D. acuminatum*. The pH of the water in unvegetated mesocosms (mean 6.5±SE 0.28) was significantly higher ($p<0.05$) than in mesocosms vegetated with *J. effusus* (5.1±0.02) or *C. lurida* (5.0±0.02), but similar to that in mesocosms vegetated with *D. acuminatum* (6.0±0.03). Sediment moisture content did not significantly differ between treatments ($p>0.05$, Fig. 1a), nor did the organic matter content of sediments ($p>0.05$, Fig. 1b), which was generally low and accounted for less than 2.5 % of the total sediment dry weight. Total Kjeldahl nitrogen (TKN) was significantly lower in mesocosms vegetated with *J. effusus* or *C. lurida* than those vegetated with *D. acuminatum* or left unvegetated ($p<0.01$, Fig. 1c). Total phosphorus concentration varied from 0.002 to 0.003 $mgPL^{-1}$ and did not differ significantly between the vegetation treatments ($p>0.05$, Fig. 1d).

Microbial Extracellular Activity

Activities of the hydrolytic enzymes β-glucosidase, phosphatase and NAGase did not significantly differ between treatments ($P>0.05$, Fig. 2). Oxidative enzyme activity showed more defined patterns with vegetation type; phenol oxidase activity was higher ($P<0.05$) in sediments of unvegetated mesocosms and those planted with *D. acuminatum* than those containing *C. lurida or J. effusus* (Fig. 2d). Peroxidase activity showed a similar pattern, with the lowest activity being observed in sediments collected from mesocosms planted with *J. effusus* and *C. lurida*. However, activity of this enzyme was also lower in mesocosms containing *D. acuminatum* than in unvegetated mesocosms, which had significantly higher peroxidase activity ($P<0.05$) than those planted with any type of vegetation (Fig. 2e).

Molecular Analysis of Bacterial Community Structure

Across all samples, a total of 79 distinct bands were observed in DGGE profiles of amplified bacterial 16S rRNA gene fragments. DGGE profiles for sediment samples collected from individual mesocosms showed a mean richness of 25 (SE±2) bands for *J. effusus*, 24 (SE±2) bands for *C. lurida*, 20 (SE±0.5) for *D. acuminatum*, and 24 (SE±1) for the unvegetated treatment (Fig. 3). When binary DGGE profiles (presence-absence of specific bands) were used to examine similarity in sediment bacterial community structure between vegetation types, samples taken from different mesocosms with the same vegetation treatment tended to group together, whether analyzed by hierarchical cluster analysis or NMDS (Fig. 4). This was especially pronounced for mesocosms vegetated with *C. lurida* and *D. acuminatum*, and less so for those planted with *J. effusus* or those left unvegetated. However, even the sediment bacterial communities in unvegetated mesocosms could be separated from those with vegetation when visualized through NMDS (Fig. 4b).

The frequency of different 16S rRNA gene sequences in clone libraries was used to derive estimates of species richness as S_{Chao1}. Following this index, the most diverse (number of species) bacterial communities appeared to be in sediments planted with *C. lurida* ($S_{Chao1}=163$). Richness estimates for communities in the other mesocosms were more similar to each other, with the highest score for unvegetated sediments ($S_{Chao1}=104$) followed by those vegetated with either *D. acuminatum* or *J. effusus* ($S_{Chao1}=87$ and 86, respectively). Sequences in the 16S rRNA gene clone libraries generated from each mesocosm type were predominantly affiliated with the Acidobacteria, Proteobacteria and Firmicutes (Table 1). Within the Proteobacteria, representatives of the Alphaproteobacteria, Betaproteobacteria and Deltaproteobacteria were found in all of the sediment samples, while the Gammaproteobacteria were not detected in the *D. acuminatum* sample (and were the least prevalent Proteobacteria in the other samples). The most dominant

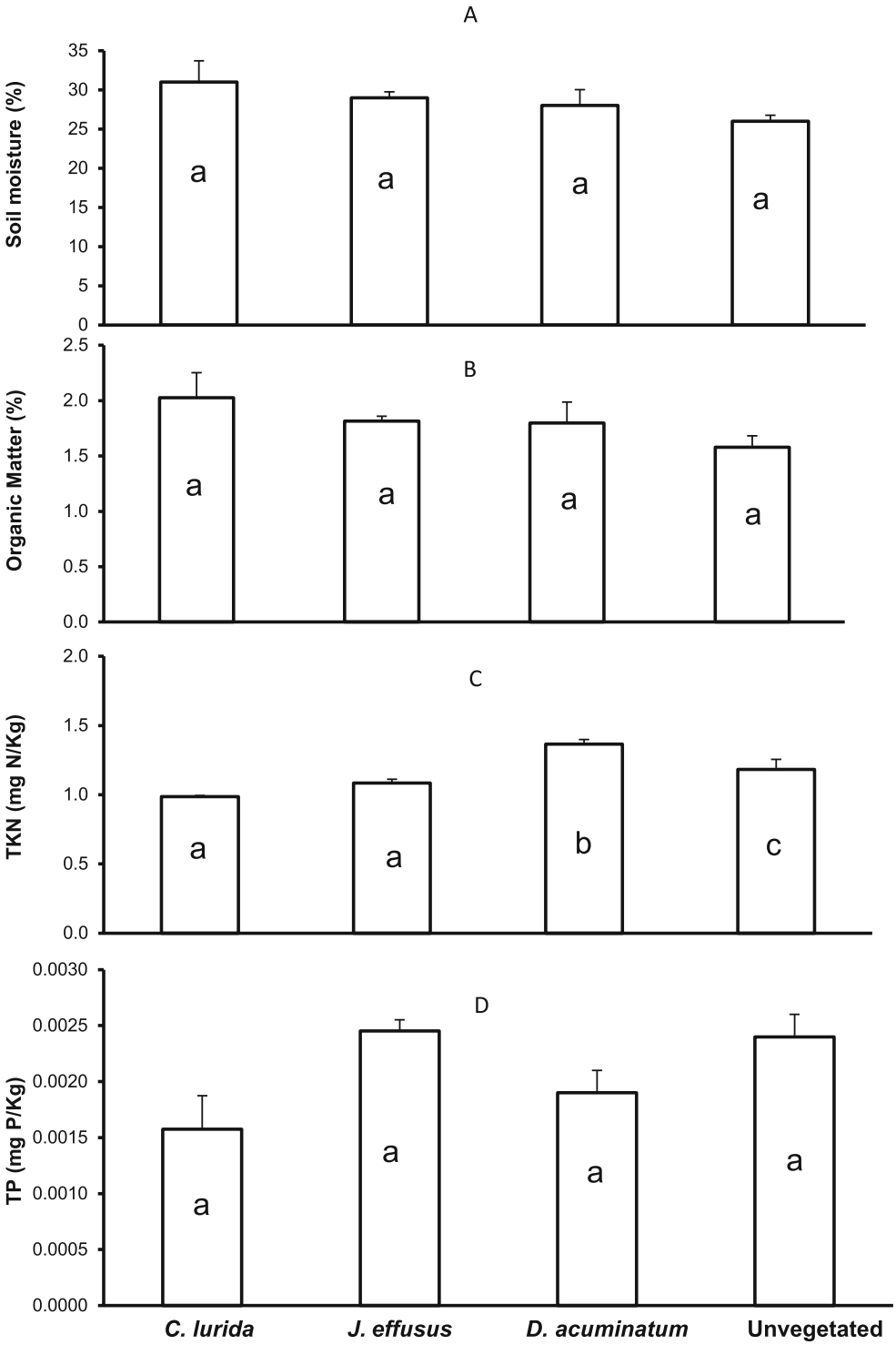

Fig. 1 Sediment moisture (**a**), organic matter content (**b**), total Kjeldahl nitrogen (**c**) and total phosphorus (**d**) for freshwater wetland mesocosms planted with *Carex lurida*, *Juncus effusus*, *Dichanthelium acuminatum var. acuminatum,* or left unvegetated. Sediment moisture is shown as the percentage of total sediment weight; organic matter content is as ash free dry mass as a percentage of sediment dry weight. Values are the mean with standard error (n=12). *Letters* indicate treatments showing statistically significant (P<0.05) differences

families among the Proteobacteria were the *Bradyrhizobiales* (Alphaproteobacteria) and *Geobacter* (Deltaproteobacteria). These groups were more dominant in the vegetated treatments (particularly *D. acuminatum* and *C. lurida,* respectively) compared to unvegetated mesocosms. Sequences affiliated with the Firmicutes and the Alphaproteobacteria were less prevalent in clone libraries generated from unvegetated sediment; each lineage accounting for just 4 % of the clones sequenced from the unvegetated sample compared to 11–19 % (Firmicutes) and 11–15 % (Alphaproteobacteria) for the different vegetated treatments. Other lineages that accounted for substantial proportions of most of the clone libraries included the Chloroflexi, Planctomycetes and Verrucomicrobia (Table 1).

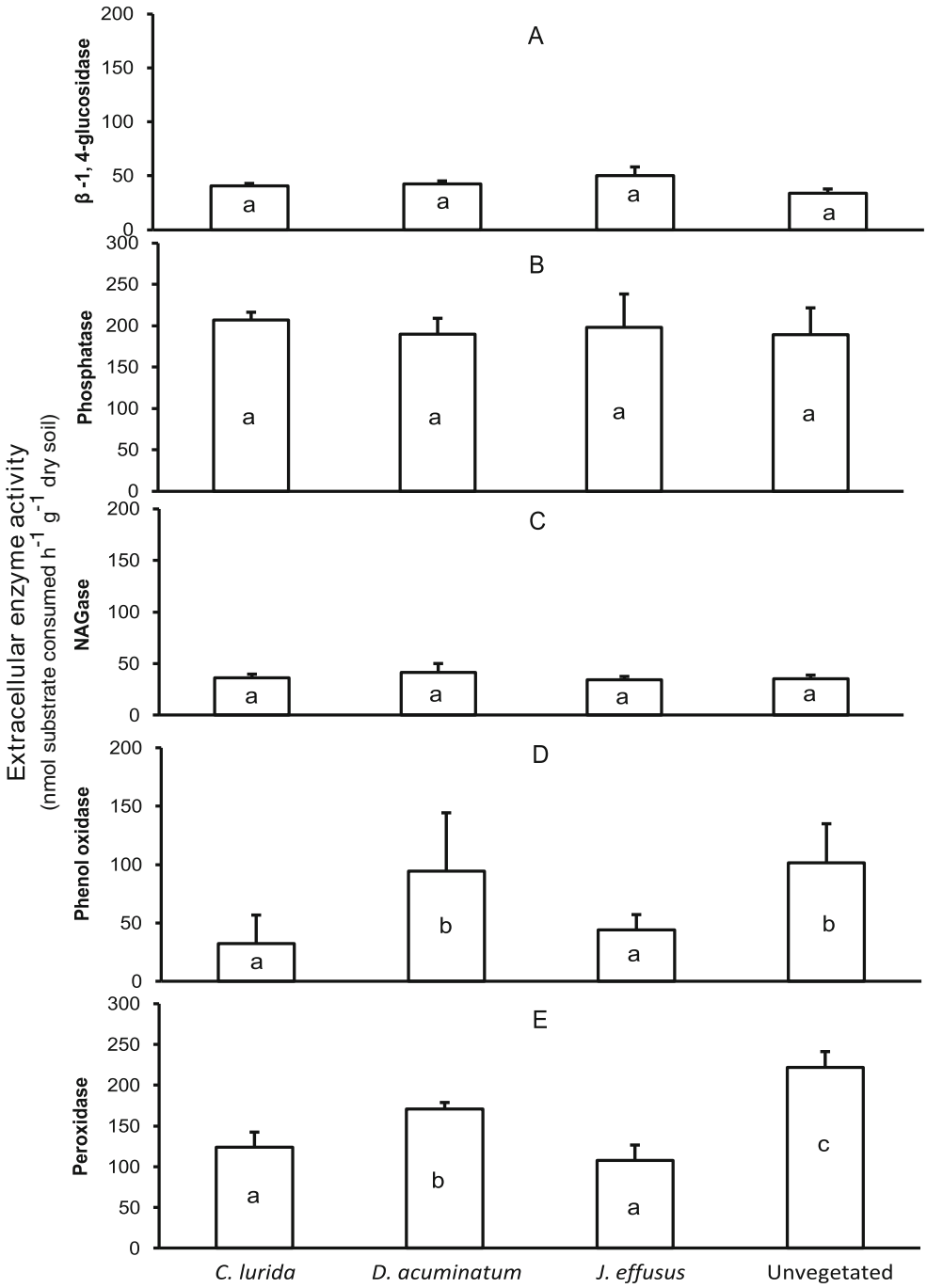

Fig. 2 Activities of β-1,4-glucosidase (**a**), phosphatase (**b**), N-acetylglucosaminidase (**c**), phenol oxidase (**d**), and peroxidase (**e**) in sediments taken from freshwater wetland mesocosms planted with *Carex lurida*, *Juncus effusus*, or *Dichanthelium acuminatum var. acuminatum*, or left unvegetated. Enzyme activities are in nmol substrate consumed $h^{-1} g^{-1}$ dry soil. Values are means (with standard error) of four mesocosms per vegetation type with four replicate sediment samples assayed for enzyme activity for each mesocosm. *Letters* indicate treatments showing statistically significant ($P<0.05$) differences in enzyme activity

Even though the libraries from all mesocosm types generally contained more 16S rRNA gene sequences affiliated with the Proteobacteria, Firmicutes, and Acidobacteria than other bacterial taxa, the proportion of sequences affiliated with each of these lineages varied by treatment. For example, while phylotypes affiliated with the Proteobacteria and the Firmicutes were the most common sequences obtained from the clone library generated from *C. lurida* sediment (accounting for 39 % and 19 % of sequenced clones, respectively), the clone libraries generated from sediment that was vegetated with *J. effusus* or *D. acuminatum*, or left unvegetated, were dominated by phylotypes belonging to the Acidobacteria. This lineage accounted for 32–43 % of the clones sequenced from all three of those treatments, but only 17 % of the clones sequenced from the *C. lurida* samples. Because the Acidobacteria were the most dominant lineage in the majority of samples, we examined this phylum at a more detailed phylogenetic level. Most of the Acidobacteria sequences were affiliated with Acidobacteria subdivisions 1, 3, 4, 6, and 8, and were generally most closely related to uncultured bacteria

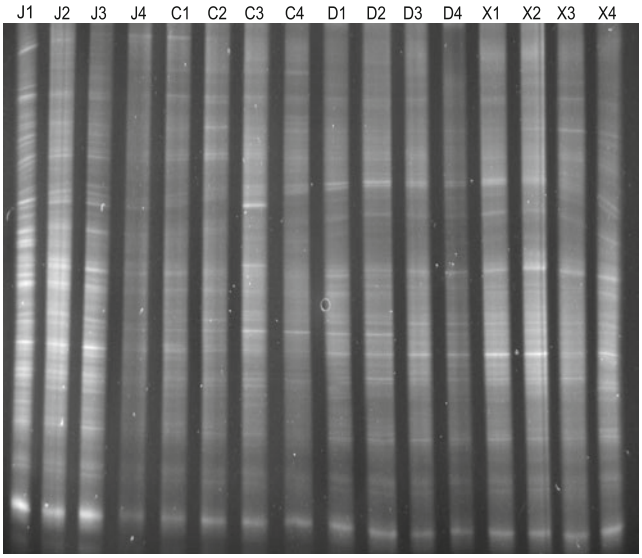

Fig. 3 DGGE of 16S rRNA gene fragments amplified from bacterial communities in sediments taken from freshwater wetland mesocosms vegetated with either *Juncus effusus* (J), *Carex lurida* (C), *Dichanthelium acuminatum var. acuminatum* (D), or left unvegetated (X). *Numbers (1–4)* indicate replicate mesocosms of each treatment and each sample represents a pool of three replicate sediment samples taken from each mesocosm

detected through 16S rRNA gene sequencing of diverse environments ranging from metal-contaminated sediments to forest soils (Fig. 5).

Multiple correlations comparing abiotic and biotic variables indicated several interesting patterns. The pH was negatively correlated to the activity of the three hydrolytic enzymes ($R>-0.77$, $p<0.05$), soil moisture ($R=-0.90$, $p<0.05$), organic matter ($R=-0.83$, $p<0.05$), and the amount of plant growth ($R=-0.96$, $p<0.05$). Among the hydrolytic enzymes, phosphatase activity was positively correlated with both β-glucosidase ($R=0.80$, $p<0.05$) and NAGase activity ($R=0.80$, $p<0.05$) and negatively correlated with phenol oxidase activity ($R=-0.97$, $p<0.05$). Relating enzyme activities to community composition, negative correlations were found between the proportion of Actinobacteria sequences in clone libraries and the activities of β-glucosidase ($R=-0.94$), phosphatase ($R=-0.90$, $p<0.05$), and NAGase ($R=-0.94$, $p<0.05$). The activity of these three hydrolytic enzymes was positively correlated with the proportion of Firmicutes ($R>0.85$, $p<0.05$ for all), and the proportion of this lineage was also negatively correlated with activity of the oxidative enzymes, phenol oxidase ($R=-0.90$, $p<0.05$) and peroxidase ($R=-0.91$, $p<0.05$). The proportion of Acidobacteria sequences in clone libraries was positively correlated to phenol oxidase activity ($R=0.82$, $p<0.05$) and negatively correlated to phosphatase activity ($R=-0.82$, $p<0.05$).

Discussion

Constructed wetlands are critical for pollution control and for improving water quality (Cooper and Moore 2003; Vymazal 2007). Nutrient removal and carbon processing likely depend on the activity of microbial enzymes in wetland sediments and this activity is potentially affected by biological factors, soil variables, as well as prevailing climatic conditions (Duarte et al. 2009; Shackle et al. 2000). By using a mesocosm approach, we were able to control soil and climatic factors, so that we could specifically examine the influence of three plant species, *Juncus effusus*, *Carex lurida* and *Dichanthelium acuminatum var. acuminatum*, on both the sediment microbial enzyme activities and bacterial community structure. We allowed the vegetation in mesocosms to grow for just 8 weeks, but differences in both oxidative enzyme activity and sediment community structure developed over this time.

The major impact of plant species on microbial enzyme activity was seen for phenol oxidase and peroxidase, as certain types of vegetation (in this case *C. lurida* or *J. effusus*) decreased the activity of these enzymes compared to unvegetated soil. Both phenol oxidase and peroxidase are involved in the mineralization of lignin and phenolic materials. Factors known to influence phenol oxidase and peroxidase activities include the concentration of soluble phenolic compounds, the lignin content of plant litter, soil pH, and nitrogen availability (Sinsabaugh 2010). Phenol oxidase activity is also positively related to the availability of oxygen and it's activity is enhanced by availability of molecular oxygen to act as an electron acceptor (Sinsabaugh 2010). It has been shown that the oxygen release rate by plants is strongly correlated to above ground biomass (Wießner et al. 2002). Therefore, plant growth may result in greater sorption of phenolics because wetland plants release oxygen from their root systems (Cronk and Fennessy 2001; Inderjit 1997) and thereby limit the phenolics availability in the sediment (Freeman et al. 2004). *D. acuminatum* exhibited the least growth amongst the three plants used for the study, increasing by just 10 % over the experimental period, and did not show the lower phenol oxidase activity seen for the other vegetation types (although they did show slightly lower peroxidase activity than unvegetated sediments). This supports the suggestion that differences in plant growth might indeed account for patterns in oxidative enzyme activity. Therefore, the observed reductions in oxidative enzyme activity in vegetated mesocosms may actually reflect lower substrate availability due to presence of vegetation. A previous study also suggested that plants have a negative correlation with the phenol oxidase activity in constructed wetlands (Zhang et al. 2010). However, we

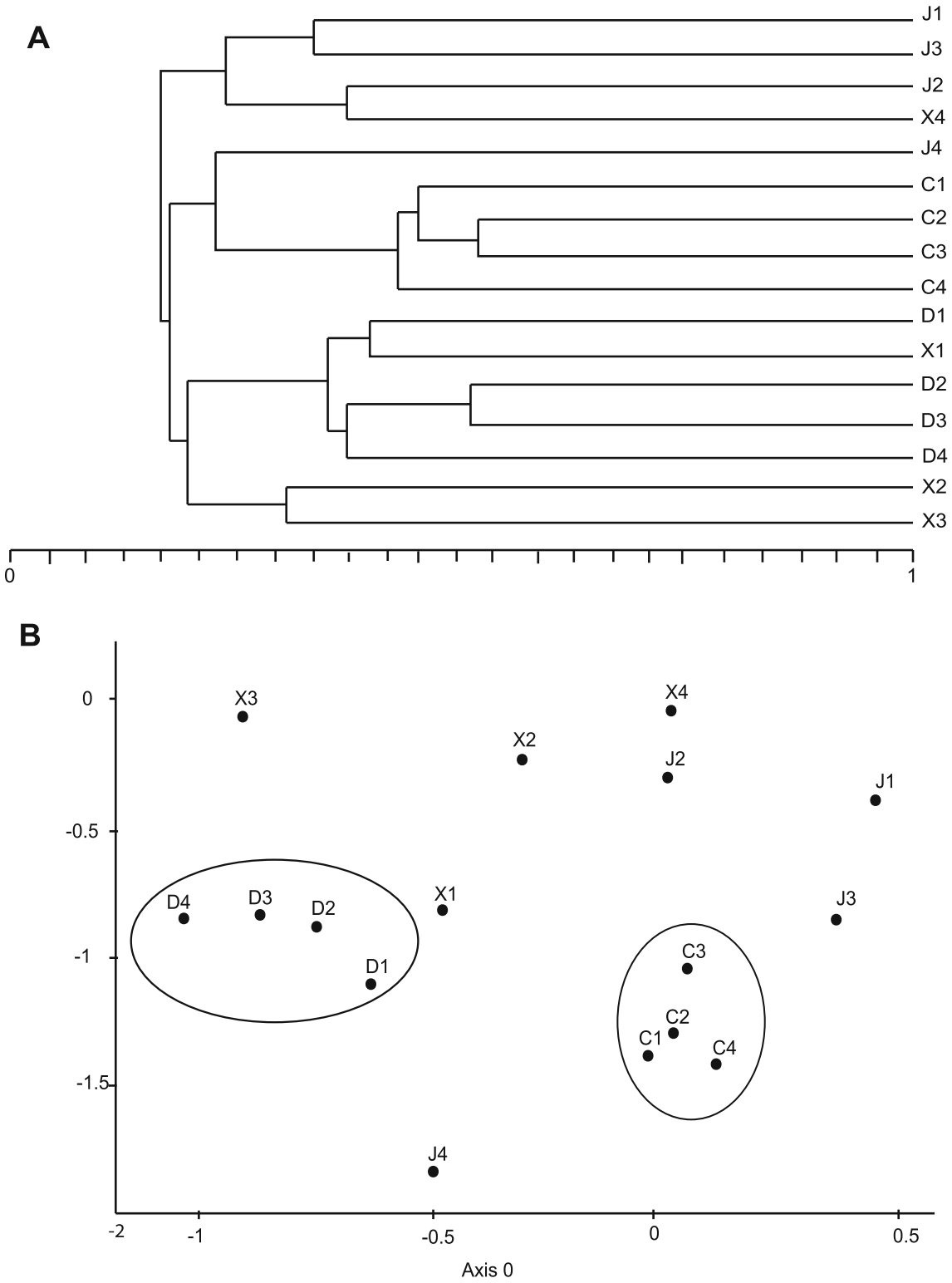

Fig. 4 Similarity of bacterial communities in freshwater wetland sediments vegetated with either *Juncus effusus (J), Carex lurida (C)*, *Dichanthelium acuminatum var. acuminatum (D)*, or left unvegetated (X) as derived from DGGE profiles of 16S rRNA gene fragments. *Numbers (1–4)* indicate replicate mesocosms planted with each vegetation type. Similarity is shown as a hierarchical dendrogram obtained from cluster analysis of DGGE profiles (**a**), and as a two dimensional plot of multidimensional scaling (NMDS; stress = 0.3) of DGGE profiles (**b**). *Points* that are closer in the NMDS plot indicate samples with more similar bacterial community structure

Table 1 Percentage of 16S rRNA gene sequences affiliated with different phylogenetic groups of Bacteria in clone libraries generated from DNA recovered from wetland sediment in mesocosms vegetated with either *J. effusus*, *C. lurida*, *D. acuminatum*, or left unvegetated

Phylogenetic group	*C. lurida*	*J. effusus*	*D. acuminatum*	Unvegetated
Alphaproteobacteria	15	11	13	4
Betaproteobacteria	9	5	4	8
Deltaproteobacteria	9	3	9	10
Gammaproteobacteria	6	3	0	4
Acidobacteria	17	32	43	38
Actinobacteria	0	0	2	6
Bacteroidetes	4	0	2	6
Chloroflexi	2	8	4	2
Cyanobacteria	0	5	0	2
Firmicutes	19	16	11	4
Gemmimonas	2	0	0	0
Planctomyces	2	5	9	4
Uncultured	6	5	2	4
Verrucomicrobia	9	8	0	6

did not quantify the amount of phenolics or oxygen concentration in the soils and hence it is difficult to make definitive conclusions about the mechanism of reduced phenol oxidase activity in vegetated soils.

Oxidative enzyme activities were also positively correlated with greater prevalence of *Actinobacteria* sequences in clone libraries, and negatively correlated with phosphatase activity, sediment moisture and organic matter content in this study. *Actinobacteria* are known to produce peroxidase enzymes but little is known about the ecological roles of the group found in our study, the Acidimicrobidae. These results indicate that it is important to consider the presence/type of vegetation together with the abiotic factors and potentially the presence of specific bacterial groups like the *Actinobacteria* to increase the degradation of phenolic compounds in constructed freshwater wetlands. Further research is needed to study the relationship between vegetation and phenol oxidase activity since it might be a crucial factor in determining the efficiency of constructed wetlands treating phenolic compounds.

Despite the differences in the oxidative enzyme activities, there were no differences between treatments in hydrolytic enzyme activities. The activities of β-glucosidase, phosphatase, or NAGase enzymes are influenced by the supply of carbon and nutrients to the soil via plant litter production and root turnover (Alvarez and Guerrero 2000; Jackson and Vallaire 2009; Shackle et al. 2000). In our study, we found that β-glucosidase activity was positively correlated to sediment organic matter and sediment moisture content. Plants can influence the soil enzyme activity by excreting exogenous enzymes, and can affect species composition and diversity of microbes by releasing exudates and oxygen into the rhizosphere that indirectly affect enzyme activity (Kong et al. 2009). They can also reactivate free enzymes which may be inactivated and preserved by tannins and other chemicals in bulk anaerobic soil, by oxygenating the anaerobic substrate with their expanding root system (Neori et al. 2000). Also, enzyme activity in soil may increase either when suitable microbial substrates are at a premium or when the growth of the microbial population as a whole is stimulated (Shackle et al. 2000). Our results showed that the activities of the hydrolytic enzymes β-glucosidase, phosphatase and NAGase did not differ significantly due to the presence of plants or between plant species. This might be because of the similar concentrations of nutrients and organic matter between all of the mesocosms, overall low sediment organic matter, or the short-term nature of the study. Furthermore, plant-microbial interactions change with time, mostly depending on the plant species establishment period and other parameters like organic matter accumulation and plant litter characteristics (Chazarenc and Merlin 2005). Hence, we hypothesize that as the constructed wetland system matures, these factors will be more distinct and there will be more profound changes in the hydrolytic enzyme activities with the presence/change in plant species. Future long term studies are needed to monitor the changes in the hydrolytic enzyme activities in the presence of plants in these systems.

While there was no difference in activity of the hydrolytic enzymes, the activity was correlated to an increased proportion of sequences related to both *Alphaproteobacteria* and *Firmicute*s within the clone libraries. *Bradyrhizobiales*, a family of Alphaproteobacteria found in our study, are known to be associated with nitrogen fixation in agricultural systems and have also been found in peat soils (Dedysh 2011; Sawada et al. 2003). This is interesting since the modulation of these microbial populations might enhance the activity of the hydrolytic enzymes and in turn improve the treatment efficiency of constructed wetlands. Indeed, further studies are needed to correlate the function and

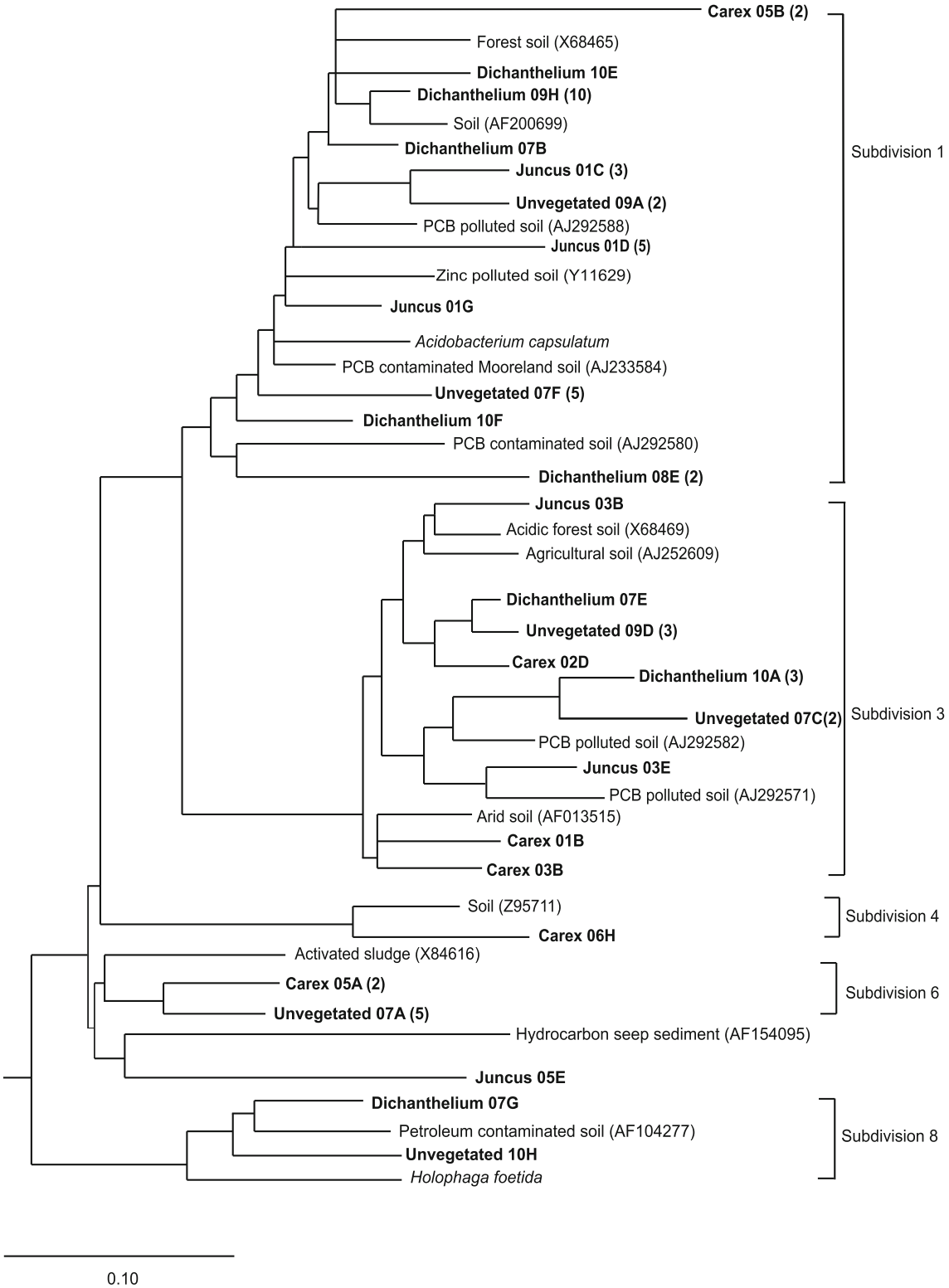

Fig. 5 Phylogenetic tree showing 16S rRNA gene sequences affiliated with the Acidobacteria that were detected in clone libraries generated from sediments in freshwater mesocosms vegetated with either *Carex lurida*, *Juncus effusus*, *Dichanthelium acuminatum var. acuminatum*, or left unvegetated. *Sequences in bold* were found in this study and are designated by the vegetation treatment that they were associated with and a number relating to the specific clone within a library. *Numbers in parentheses* indicate multiple clones in a library were detected with that sequence. Related sequences are shown for reference and are represented by GenBank accession numbers if uncultured. Major subgroups of Acidobacteria are shown and the scale bar indicates 0.10 changes per base pair

structure of this bacterial group and their changes over time in response to vegetation and nutrients in constructed wetlands.

In this study, the bacterial community structure was examined through two 16S rRNA gene approaches: DGGE and cloning/sequencing. For each sediment type, bacterial diversity derived from DGGE gels suggest lower richness values than the richness estimate, S_{Chao1}, that was derived from the frequency of phylotypes in clone libraries. This is consistent with the concept that DGGE gives only a crude assessment of community structure and often underestimates diversity for complex microbial communities (Nakatsu 2007). DGGE does, however, provide a useful means of comparing the overall structure of different communities, and NMDS and hierarchical cluster analysis of DGGE profiles showed that mesocosms with the same vegetation type tended to contain similar bacterial communities. This suggests that both the presence and type of vegetation influences the structure of the sediment bacterial community in constructed wetlands, and supports previous studies where plants have been suggested as a major factor in determining the bacterial community structure (Calheiros et al. 2009; Faulwetter et al. 2012; Truu et al. 2009).

Previous studies have reported Acidobacteria and Proteobacteria to be the dominant lineages of bacteria present in freshwater natural and constructed wetlands (Hartman et al. 2008; Jackson and Vallaire 2009; Peralta et al. 2013; Wang et al. 2012) and sequences affiliated with these groups, along with the Firmicutes, were the most common sequences detected in this study. Other major bacterial lineages detected in the clone libraries (e.g. the Verrucomicrobia, Planctomycetes, and Chloroflexi) have typically been found in surveys of other wetlands (Ibekwe et al. 2003; Jackson and Vallaire 2009), and likely form a consistent but non-dominant part of the core bacterial community in many aquatic sediments. Proteobacteria was the most dominant group associated with *C. lurida*. *Nitrospina gracilis* (a nitrifying species within the Deltaproteobacteria) was also found associated with *C. lurida* which suggests that *C. lurida* might be a potential candidate for treatment wetlands involved in nitrogen mitigation. Acidobacteria was the most dominant group in sediments planted with *J. effusus*, *D. acuminatum* and in unvegetated sediment. Firmicutes were predominately associated with *C. lurida* and *J. effusus*. Absence of vegetation has been shown to reduce the abundance of Alphaproteobacteria in terrestrial systems (Thomson et al. 2010) and our results show a similar pattern, as the representation of Alphaproteobacteria in the clone library generated from unvegetated sediment was lower compared to the other clone libraries. Overall, these data indicate that presence and type of plant species have an effect on the dominant phyla and in turn on the microbial community structure.

Acidobacteria was another dominant group found in our study and they represent one of the most abundant groups bacteria found in all soil and sediment ecosystems (Arroyo et al. 2013; Iasur-Kruh et al. 2010; Jones et al. 2009; Peralta et al. 2013). Higher concentrations of organic carbon, pH and restoration are other factors known to influence the relative abundance of Acidobacteria in wetland soils (Hartman et al. 2008). Within Acidobacteria, only subdivision 1 is relatively well represented by cultured and characterized strains, many of which were isolated from *Sphagnum*-dominated wetlands (Dedysh 2011). In our study, phylotypes affiliated with Acidobacteria subdivision 1 were particularly prevalent in the clone libraries generated from sediment taken from *D. acuminatum* and *J. effusus* mesocosms, but were not detected in the *C. lurida* sample, whereas phylotypes affiliated with Acidobacteria subdivision 3 were found in the clone libraries generated from all treatments. Fewer phylotypes were detected that were affiliated with Acidobacteria subdivisions 4, 6, and 8. The overall representation of Acidobacteria sequences in clone libraries was positively correlated to the nitrogen content of sediments, suggesting that nitrogen availability may influence the distribution of this phylum. Future studies are needed to determine the factors that influence specific microbial lineages in wetlands, as well as to determine what functional roles these specific groups are playing.

The influence of plant species on soil microbial community structure may be linked to the process of rhizodeposition (Marschner et al. 2001; Stottmeister et al. 2003; Vacca et al. 2005). Certain plants exude organic acids which can lead to the selection of microorganisms that are tolerant to acidic conditions (Ohwaki and Hirata 1992). *Carex* species are commonly characterized by short lateral roots (dauciform cluster roots) that can release organic acids and impact soil microbial populations (Davies et al. 1973). Similarly, *J. effusus* is known to release chemicals such as indole acetic acid through its roots (Halda-Alija 2003). While these differences would be more pronounced in the rhizosphere (Marschner et al. 2001), differentiation between the rhizosphere and bulk sediment was difficult in this study as the roots of all of the plants used are fibrous and diffuse. Densities of the root mats were high in all mesocosms so that rhizodeposition effects would likely have extended into most of the sediment. In this study, the pH of all of the vegetated mesocosms was slightly lower than unvegetated sediments, implying that the differences in bacterial community composition in the vegetated sediments (particularly *Carex* and *Juncus*) may be associated with differences in chemicals deposited by rhizodeposition. However, while we measured the pH of the surface water, we did not measure sediment pH and further studies may be required to determine the effect of root exudates from these plants on the sediment and surface water pH and bacterial community composition.

The change in the plant functional groups present is known to impact the plant- and microbial-mediated functions in freshwater wetlands (Bouchard et al. 2007). Wetland plants have the ability to determine the outcome of microbial competition for resources by influencing both the availability of electron donors and electron acceptors (Megonigal et al. 2003; Sutton-Grier and Megonigal 2011). Therefore, the efficiency of a constructed wetland to function as sinks for pollutants may be related to the ability of plant species to influence soil microbial enzyme activities and bacterial community structure. Regardless of the mechanism of effect, results from our study indicate that the presence and type of the plant species influences the sediment microbial community and oxidative enzyme activity in constructed wetlands. It was beyond the scope of this study to determine the source of the bacterial communities that developed in each mesocosm, whether they came from the plants themselves or whether the plants increased the growth of certain microbial populations already present in the sediment. Accounting for the differences in the representation of the bacterial groups in the clone libraries obtained from the different vegetation treatments is difficult, but any factors that resulted in changes in microbial enzyme activity or overall community structure could also have led to changes in specific bacterial lineages. Future studies are required to identify the influence of these plant species on specific phenotypic and metabolic functions of the associated microbial populations. As well as vegetation type, factors like water quality, filter material, nutrient concentrations, and pollutant concentration may also influence microbial enzyme activity and community structure in constructed wetlands (Peralta et al. 2013). Therefore, we suggest that future studies examine the influence of environmental factors, together with plant species composition, on the structure and function of the sediment microbial community. These studies would be beneficial in order to develop and optimize effective management strategies in constructed wetlands.

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