ENVIRONMENTAL BIOTECHNOLOGY

Microbial community analysis of switchgrass planted and unplanted soil microcosms displaying PCB dechlorination

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Abstract Polychlorinated biphenyls (PCBs) pose potential risks to human and environmental health because they are carcinogenic, persistent, and bioaccumulative. In this study, we investigated bacterial communities in soil microcosms spiked with PCB 52, 77, and 153. Switchgrass (*Panicum virgatum*) was employed to improve overall PCB removal, and redox cycling (i.e., sequential periods of flooding followed by periods of no flooding) was performed in an effort to promote PCB dechlorination. Lesser chlorinated PCB transformation products were detected in all microcosms, indicating the occurrence of PCB dechlorination. Terminal restriction fragment length polymorphism (T-RFLP) and clone library analysis showed that PCB spiking, switchgrass planting, and redox cycling affected the microbial community structure. Putative organohalide-respiring *Chloroflexi* populations, which were not found in unflooded microcosms, were enriched after 2 weeks of flooding in the redox-cycled microcosms. Sequences classified as *Geobacter* sp. were detected in all microcosms and were most abundant in the switchgrass-planted microcosm spiked with PCB congeners. The presence of possible organohalide-respiring bacteria in these soil microcosms suggests that they play a role in PCB dechlorination therein.

Keywords *Chloroflexi* · *Geobacter* · *Clostridia* · qPCR · T-RFLP · Redox cycling · Phytoremediation · Switchgrass

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Introduction

Polychlorinated biphenyls (PCBs), widely used in industry in the last century, encompasses a set of 209 congeners. Although their production has been banned since the 1970s, they remain persistent in the environment and pose potential risk to public health because they are toxic and carcinogenic and bioaccumulate in the food web (Ross 2004). The bioremediation of PCB-contaminated soils and sediments is challenging because of the tendency of PCB congeners to be recalcitrant in the environment (Agarwal et al. 2007; Amend and Lederman 1992). Traditional methods such as soil excavation and dredging with subsequent landfill processing or high temperature incineration are energy-consuming and are thus considered not sustainable (Vasilyeva and Strijakova 2007).

Microorganisms can degrade and detoxify PCBs via two major pathways—anaerobic PCB dechlorination and aerobic PCB oxidation (Borja et al. 2005; Gibson and Parales 2000; Pieper and Seeger 2008; Wiegel and Wu 2000). Under anaerobic conditions, certain bacteria use PCB congeners as electron acceptors, which lead to reduction of the PCB molecule and removal of chlorine atoms from the biphenyl ring. Anaerobic reductive dechlorination of PCBs can be growth supporting or co-metabolic (Wiegel and Wu 2000; Wu et al. 1998). All confirmed PCB dechlorinators are members of the phylum *Chloroflexi*, including the *Dehalococcoides* spp. and the o-17/DF-1 group (Adrian et al. 2009; Fagervold et al. 2007; Fennell et al. 2004; Wang et al. 2014; Yan et al. 2006). Reductive dechlorination of PCBs by *Dehalobacter* was confirmed in an enrichment culture but not yet in pure culture (Yoshida et al. 2009). Under aerobic conditions, certain microorganisms oxidize PCBs via the upper biphenyl pathway, generating chlorobenzoates (Gibson and Parales 2000; Macková et al. 2010). Chlorobenzoates can be further metabolized by other microbial community members (Pavlů et al. 1999).

Several studies have demonstrated the effect of plants on enhancing PCB degradation and increasing microbial PCB-degrading populations (Leigh et al. 2006; Macková et al. 2006; Slater et al. 2011). Plants play an important role in promoting oxygen diffusion and regulating soil water in the rhizosphere (Schnoor et al. 1995). Plant-released compounds such as flavonoids and terpenes were found to support PCB-degrading microbial populations and induce PCB degradation (de Cárcer et al. 2007; Donnelly et al. 1994; Gilbert and Crowley 1997; Hernandez et al. 1997; Leigh et al. 2002). Some plant species, such as Austrian pine (*Pinus nigra*) and goat willow (*Salix caprea*), were able to increase the abundance of microbial PCB degraders in their root zones (Leigh et al. 2006).

However, most studies have focused on how plants influence aerobic PCB degradation while anaerobic dechlorination in the root zone is rarely investigated. There is a recent report of PCB dechlorination in the switchgrass rhizosphere (Meggo and Schnoor 2013); however, the microbial community and its potential roles in PCB dechlorination was not investigated. In the present study, we developed an independent set of soil microcosms and aimed to characterize microbial communities in switchgrass planted and unplanted PCB-dechlorinating soil microcosms, identify potential PCB-dechlorinating community members, and investigate the effect of plants and soil zone water flooding on PCB-dechlorinating communities.

From a remediation technologies perspective, plant-promoted PCB degradation could be useful in instances where PCB-contaminated sediments are dredged from freshwater lakes, rivers, estuaries, and coastal marine areas. These sediments are typically transferred to a confined disposal facility (CDF) for long-term storage (Liang et al. 2014a). Planting dredged, PCB-contaminated sediments with hardy plant species such as switchgrass could accelarate remediation and facilitate reclamation of these sediments for beneficial purposes.

Materials and methods

Soil microcosm setup

The soil microcosm setup procedure has been described previously (Liang et al. 2014b; Meggo and Schnoor 2013). Briefly, a mixture of PCB 52, PCB 77, and PCB 153 (99 % pure) (Accustandard Inc., New Haven, CT) was dissolved in hexane and then applied to the PCB-free soil (Nodaway series, silt loam from Middle Amana, Iowa, USA) at a target concentration of 500 ng g^{-1} each (wet weight). The three congeners were selected because they are considered more difficult to degrade in comparison to other congeners (Bedard et al. 1986). PCB 52 and PCB 77 are tetrachlorinated, and

PCB 153 is hexachlorinated and thus relatively more resistant to aerobic oxidation than lesser chlorinated congeners (Bedard et al. 1986). The *ortho* chlorines in PCB 52 and PCB 153 are difficult for enzymes to attack (Dai et al. 2002). PCB 77 has a dioxin-like structure and is one of the most toxic congeners (Van den Berg et al. 2006). Finally, the three congeners are frequently detected in the environment (Lammel and Stemmler 2012; Martinez et al. 2010). The PCB-spiked soil was homogenized and aged for 8 weeks at 25 °C in the sealed tubs to facilitate sequestration of PCB congeners into the soil matrix.

Four soil microcosms were constructed by filling plastic containers (33.8 cm×21.6 cm×211.9 cm) with 2,500 g of PCB-spiked soil each, prepared as described above. Two microcosms remained unplanted (UP), and the remaining two microcosms were planted with switchgrass (*Panicum virgatum*) (SG) seeds (Adams-Briscoe Seed Co., Jackson, GA) (Figure S1). Besides the four soil microcosms, an unplanted "blank" microcosm (BLK), which contained 2,500 g of soil that was not spiked with PCBs, was also prepared. All microcosms were covered with aluminum foil and incubated for 8 weeks in a plant growth chamber at 25 °C and 60 % humidity. Each day consisted of a 16-h light period (200 mmol m^{-2} s^{-1} intensity) and an 8-h dark period. After the initial 8-week incubation, one UP microcosm and one SG microcosm was subjected to alternating cycles of complete soil saturation (i.e., "flooding"—hereafter referred to as UPF and SGF microcosms). Two weeks of flooding with deionized water was followed by 2 weeks where extra water was withdrawn with a sterile syringe, and soil drying was allowed to occur (i.e., non-flooding). Four complete flooding cycles were performed during the experiment. This flooding/non-flooding procedure caused cycling of the redox potential in the soils (Fig. S2) and is thus hereafter referred to as "redox cycling."

Volumetric soil moisture content was monitored in each microcosm with Vernier soil moisture sensors interfaced with the Vernier LabQuest data collection system (Vernier Software & Technology, Beaverton, OR). The redox potential was measured with a portable pH/ORP meter (Hanna Instruments, Woonsocket, RI). A sterile syringe (5 ml) with the narrow tip removed was used to sample vertical soil cores randomly from the microcosms. The core samples were homogenized before PCB congener and microbial community analysis. Soil samples for PCB congener analysis were taken at week 12 and week 24. Soil samples for microbial community analysis were taken every 2 weeks starting at week 8.

PCB congener analysis

The PCB extraction method, described previously (Liang et al. 2014b; Meggo and Schnoor 2013), was performed in triplicate for each soil sample. Briefly, soil (5 g) was mixed

with 1:1 hexane/acetone (3 ml g^{-1}). The surrogate standards including PCB14, deuterated PCB65, and PCB166 (Cambridge Isotope Laboratories, Inc.) were added into the samples (50 ng of each surrogate) to determine the extraction recovery. Surrogate recoveries were 101.8±17.5 % (PCB 14), 100.4± 15.2 % (deuterated PCB 65), and 99.8±18.9 % (PCB 166).

The soil and hexane/acetone mixture was sonicated for 1 h and then subjected to two rounds of centrifugation (1,500×g, 5 min). The combined supernatant was evaporated to dryness by rotary evaporation and dissolved in hexane. Lipids and other polar substances were removed by double extraction with concentrated sulfuric acid and hexane. A nitrogen stream was used to concentrate the hexane extract down to 0.5 ml. The concentrate was eluted with 10 ml hexane through a filter consisting of 0.1 g of silica (70–230 mesh, Fisher Scientific, Inc.), 0.1 g of anhydrous sodium sulfate, and 0.9 g silica gel acidified with sulfuric acid (silica/sulfuric acid 2:1).

The PCB congener analysis procedure was described previously (Hu et al. 2010). PCB 204 (2,2′,3,4,4′,5,6,6′- octachlorobiphenyl; 100 ng) was added to the concentrated extracts as an internal standard. PCB congeners were analyzed and quantified via a modified EPA method 1668 A gas chromatography with mass selective detection (GC-MS/MS) method (US EPA 1999). An Agilent 6,890 N gas chromatograph was used with an Agilent 7,683 series autosampler coupled to a Waters Micromass Quattro micro GC mass spectrometer (Milford, MA, USA). The mass spectrometer was operated under electron impact (EI) positive mode at 70 eV and multiple reaction monitoring (MRM), and the trap current was 200 μA. The retention windows were defined by PCB parent/daughter ion pairs from mono- to deca-homologs which were 188/152, 222/152.10, 255.96/186, 291.92/222, 325.88/255.90, 359.84/289.90, 393.80/323.90, 427.76/ 357.80, 461.72/391.83, 497.68/427.70, respectively. For each congener, the limit of quantification was defined as six times the standard deviation from three samples from the blank microcosm. Congeners with a concentration lower than the limit of quantification (as determined by analysis of the blank) were removed from the PCB profiles in PCB-spiked microcosms. The molar percentage of initial concentration for PCB 52, 77, and 153 after 12 and 24 weeks of incubation in the microcosms is shown in Fig. S3. The unplanted and switchgrass-planted PCB congener data (Fig. S3) was previously presented in Liang et al. (2014b) and is shown here for the purposes of comparison with the other treatments.

Nucleic acid extraction and quantitative PCR (qPCR)

DNA was extracted from 2-g soil (wet weight) immediately after sampling using the DNA Elution Accessory Kit attached with the RNA PowerSoil kit (Mobio, Carlsbad, CA) and stored at −80 °C before further analysis. The abundances of total bacteria, *Geobacteraceae*, and putative dechlorinating

Chloroflexi were estimated with qPCR targeting bacterial 16S ribosomal RNA (rRNA) gene (primer set 16SU f/r) (Nadkarni et al. 2002), *Geobacteraceae* 16S rRNA genes (Wei and Finneran 2011), and putative dechlorinating *Chloroflexi* 16S rRNA genes (primer set dhc793f/946r) (Yoshida et al. 2005), respectively (Table S1). PCR conditions were as follows: 10 min at 95 °C, 40 cycles of 15 s at 95 °C, and 1 min at 60 °C followed by a dissociation step. Each 25 μl qPCR contained 12.5 μl Power SYBR Green PCR Master Mix (Invitrogen, Carlsbad, CA), various amount of primers, and DNA templates (Table S2). Bovine serum albumin (500 ng) was added to reduce possible PCR inhibition (Kreader 1996).

For qPCR targeting total bacterial 16S rRNA genes, the standard DNA template was PCR amplified from *Burkholderia xenovorans* strain LB400 with primer set 8F/ 1492R (Grabowski et al. 2005). For qPCR targeting putative dechlorinating *Chloroflexi* 16S rRNA gene and *Geobacteraceae* 16S rRNA genes, the standard curves were prepared from pCR 2.1-TOPO vectors containing a PCR product amplified from soil DNA with primer set dhc793f/ 946r and Geo494f/825r, respectively. All qPCRs were performed in triplicate or replicate with an ABI 7000 Sequence Detection System (Applied Biosystems, Grand Island, NY), and fluorescence data was analyzed by ABI 7000 System SDS Software (Applied Biosystems, Grand Island, NY) at the Iowa Institute of Human Genetics Genomics Division. With each primer set, the target gene was not detected in no template (DI water) controls (Ct value >35). Additional qPCR information, including qPCR linear range, qPCR efficiency range of the standard curves, and *y*-intercepts are provided in Table S2, in accordance with MIQE guidelines (Bustin et al. 2009).

qPCR quality assurance

Several published qPCR primer sets targeting putative dechlorinating *Chloroflexi* 16S rRNA gene and *Geobacteraceae* were tested. Primer sets chl348f/884r (Fagervold et al. 2005), dhc1f/264r (Grostern and Edwards 2006), dhc793f/946r (Yoshida et al. 2005), dhc1154f/1286r (Krzmarzick et al. 2012), and Geo494f/825r (Wei and Finneran 2011) each yielded a single band of expected size with the soil DNA template. To verify the specificity of these primer sets, clone libraries were constructed from the amplification products of DNA extracted from soil with redox cycling. From the dhc793f/946r PCR product clone library, five unique sequences were obtained from 12 clones and 92 % of these sequences were identified as *Dehalogenimonas* sp. by RDP classifier (Table S3) (Cole et al. 2007). Primer sets chl348f/884r, dhc1f/264r, and dhc1154f/1286r also amplified sequences from the class *Anaerolineae* (phylum *Chloroflexi*) and from the *Actinobacteria*, both of which are not associated with dechlorination (Table S3). From the Geo494f/825r PCR

product clone library, 12 unique sequences were obtained from 14 clones. Seventy nine percent of these sequences were identified as *Geobacteraceae* sp. and the rest as unclassified *Deltaproteobacteria* by RDP classifier (Table S3). Thus, the primer set dhc793f/946r and Geo494f/825r was used to quantify the putative dechlorinating *Chloroflexi* 16S rRNA gene and *Geobacteraceae* 16S rRNA gene in soil samples. Primer set dhc793f/946r is not expected to amplify sequences from the *o*-17/DF-1 group. However, the primer set 14f/dehal1265r, which targets the *o*-17/DF-1 group, did not generate amplicons, suggesting that the *o*-17/DF-1 group was not present in our soil samples (Watts et al. 2005). The specificity of the SYBR green-based qPCR was also validated by dissociation curve analysis, which showed similar melting temperatures for each qPCR experiment with these primers.

Terminal-restriction fragment length polymorphism (T-RFLP) analysis

T-RFLP analysis was performed on the bacterial 16S rRNA gene as described previously (Liang et al. 2014a). Briefly, PCR was performed with fluorescent-labeled primer set 6-FAM 8Fm/533R (Schütte et al. 2008) with 1 ng DNA as template (Table S1). The restriction enzyme *Hae*III (New England BioLabs, Inc., Ipswich, MA) was used to digest PCR products. Digested DNA was precipitated, recovered by centrifugation, resuspended in distilled water, and sent to the Iowa Institute of Human Genetics Genomics Division at the University of Iowa for sizing.

Terminal restriction fragment (TRF) sizes were estimated by Peak Scanner software (Applied Biosystems, Carlsbad, CA). The TRF size matrix containing 84 samples (rows) and 439 unique 16S rRNA gene TRFs (columns) was produced by T-REX software after filtering background peak noises and rounding fragment sizes to the nearest whole number (Culman et al. 2009). TRF profiles were analyzed by non-metric multidimensional scaling (NMDS) in R (da C Jesus et al. 2009). The Bray-Curtis dissimilarity index was calculated with a random starting configuration, and a two-dimensional solution was reached after two runs. The final stress was 12.77. The TRF composition differences among groups were assessed by multiresponse permutation procedure (MRPP), which evaluates grouping with real and randomized data based on Bray-Curtis distances and 5,000 permutations, and permutational multivariate analyses of variance (PERMANOVA) based on Bray-Curtis distances with 5,000 permutations.

PCR amplification, cloning, and sequencing of bacterial 16S rRNA genes

Clone libraries were constructed with bacterial 16S rRNA genes amplified from unplanted soil, switchgrass-planted soil, unplanted and cycled soil, and switchgrass planted and cycled soil at 24 weeks. The cloning procedure was described previously (Liang et al. 2014a). Bacterial 16S rRNA genes were amplified using primer set 8F/1492R (Grabowski et al. 2005) (Table S1). Purified PCR products were cloned into the pCR 2.1-TOPO vector using the TOPO TA cloning Kit (Invitrogen Corp., Carlsbad, CA) and transformed into One Shot TOP10 chemically competent *Escherichia coli* cells (Invitrogen Corp., Carlsbad, CA). Recombinant *E. coli* were plated on Luria Broth agar containing kanamycin (50 mg l^{-1}) and X-gal (0.4 mg plate^{-1}) and incubated overnight at 37 °C to check for successful DNA insertion via blue-white screening. Clones were partially Sanger-sequenced at the Iowa Institute of Human Genetics Genomics Division initially with M13F primers (Table S1). A second round of sequencing was performed with M13R primers (Table S1) if the M13F sequence revealed that the 16S gene was cloned in the opposite orientation. Chimeric sequence identification was performed by DECIPHER (Wright et al. 2012). Sequence identification and classification were performed by RDP classifier (Cole et al. 2007). The sequences were subjected to in silico TRF production using TRiFLE (Junier et al. 2008). Sequences collected in this study were submitted to Genbank (Accession Nos. KJ766128–KJ766299).

Statistical analyses

PCB congener data was analyzed with independent sample *t* test using R. The total bacterial 16S rRNA gene copies, putative dechlorinating *Chloroflexi* 16S rRNA gene copies, and *Geobacteraceae* 16S rRNA gene copies were analyzed with a two-factor analysis of variance after log transformation using R. Pearson's *r* was calculated in R to evaluate the correlation between gene abundances and environmental variables (Rieu and Powers 2009).

Results

Soil PCB congener analysis

Parent PCB congener concentrations decreased in all microcosms by 30–40 % after 24 weeks of incubation with greater overall removal of PCB 77 and PCB 153 than PCB 52 observed (Fig. S3). The occurrence of PCB dechlorination was further verified by detecting the expected transformation products of PCB52, PCB77, and PCB153. These included PCB3, PCB15, PCB 26/29, PCB 35, PCB48, and PCB 118 in all soil microcosms (unplanted (UP), switchgrass planted (SG), and redox-cycled soils (UPF and SGF)) (Figs. 1 and 2). Redox potential measurements (Fig. S2) suggest that soil microcosms that were not subjected to periods of flooding were still possibly experiencing "anoxic" conditions (<350 mV) (Setter and Waters 2003). PCB 101 and PCB 118 are plausible

Fig. 1 Transformation products detected after 12 and 24 weeks of incubation in unplanted microcosm (*UP*), switchgrass-planted microcosm (*SG*), unplanted microcosm with redox cycling (*UPF*), and switchgrass-planted microcosm with redox cycling (*SGF*). *Error bars* indicate the standard deviation of three soil subsamples from the same microcosm
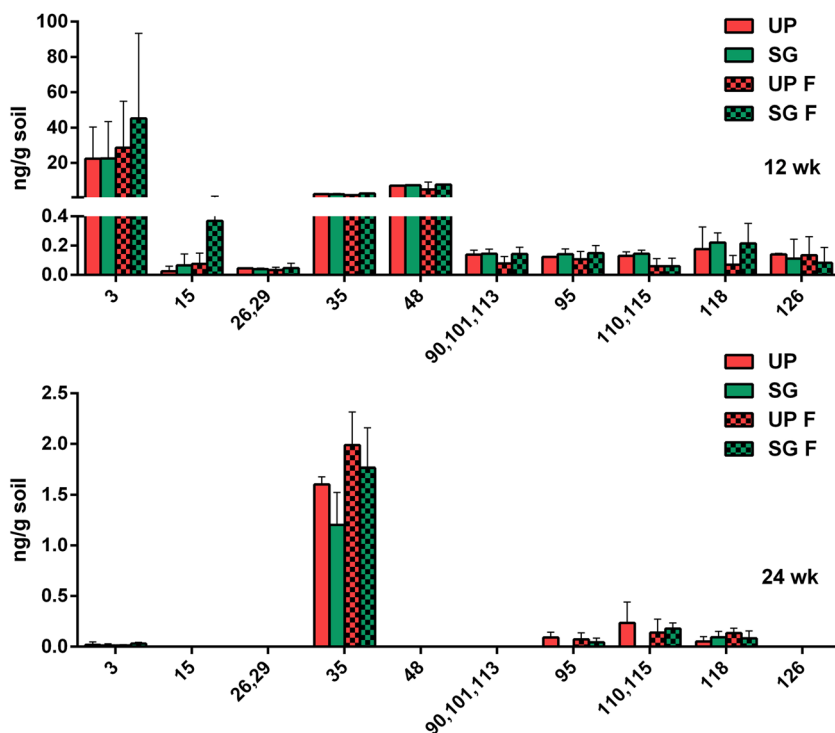
dechlorination products of PCB 153 (Fig. 2). PCB 101 could be further transformed into PCB 52 by *para* chlorine removal, and PCB 118 can be further dechlorinated to PCB 77 by *ortho* chlorine removal (Fig. 2). PCB 95 and 110/115 were also detected at low concentrations in PCB-spiked soils but not in the blank soil (i.e., soil without PCB spiking) (Fig. 1). PCB 35 is a plausible dechlorination product of PCB 77 by *para* chlorine removal, and PCB 26 could be generated from PCB 52 by *ortho* chlorine removal (Fig. 2). PCB dechlorination in a similar system has been previously reported (Meggo and Schnoor

Fig. 2 Schematic of the possible dechlorination pathways for PCB 52, 77, and 153. *Dotted arrows* indicate possible dechlorination pathways where the intermediate was not observed in our experiments
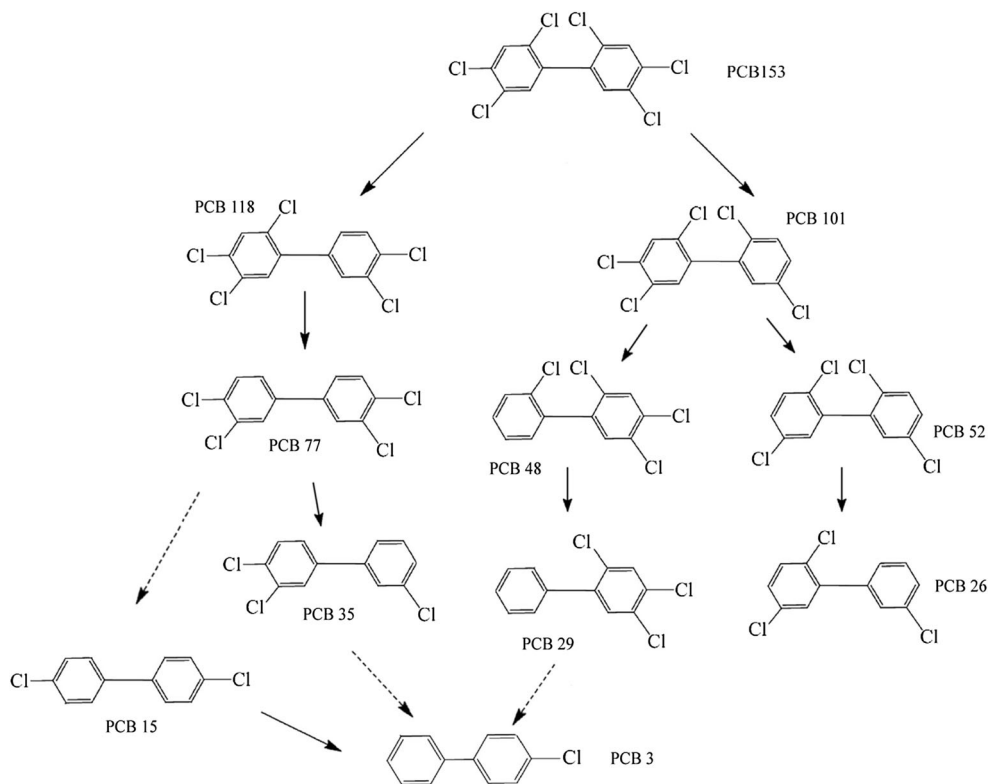
2013), but the mediating microbial community was not investigated. Therefore, in this study, we focused on comparative microbial community analysis and searched for possible PCB dechlorinators in the microcosms in an effort to delineate the role of microorganisms in the observed biotransformations.

Influence of PCB spiking, switchgrass planting, and redox cycling on microbial community structure in microcosms

With evidence of PCB dechlorination, we next sought to better characterize the microbial communities in the microcosms. We hypothesized that a comparative microbial community analysis would allow identification of potentially important community members that might be contributing to PCB dechlorination in the soil microcosms. To achieve this, T-RFLP analysis was performed to delineate the overall microbial community structure of the microcosms and track changes in the communities with time in response to different treatments. NMDS ordination of the T-RFLP profiles revealed that at the beginning of the experiment, the BLK and UP microcosms shared similar bacterial community structures (Fig. 3). Interestingly, the subsequent soil treatments resulted in community structures that diverged significantly from the original community structure. The combined effect of PCB spiking, switchgrass planting, and periodic flooding appeared to have the greatest impact (Fig. 3). The differences between T-RFLP profiles in microcosms with different treatments were confirmed by MRPP and PERMANOVA ($p = 0.001$).

The composition of the bacterial community in soil microcosms with different treatments after 24 weeks of incubation
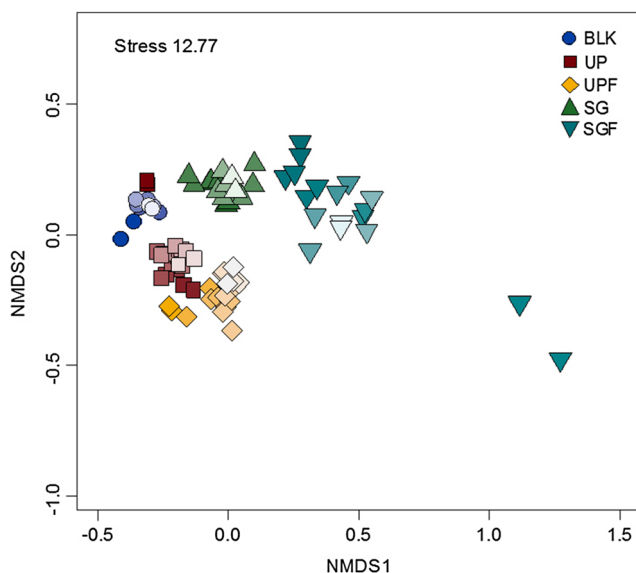
Fig. 3 NMDS ordination of T-RFLP profiles from the blank microcosm (*BLK*), unplanted microcosm (*UP*), switchgrass-planted microcosm (*SG*), unplanted microcosm with redox cycling (*UPF*), and switchgrass-planted microcosm with redox cycling (*SGF*). The *symbols* are shaded with respect to incubation time, with lighter shading indicating longer incubation time at sampling. The final stress was 12.77

was assessed by sequencing a total of 162 clones from four 16S rRNA gene clone libraries (UP, SG, UPF, and SGF). Each of the soil communities were dominated by *Proteobacteria* (47.7 ± 12.9 % of total clones) and *Acidobacteria* (15.4 ± 7.8 % of total clones) (Fig. 4). The remaining phylotypes detected were affiliated with *Bacteroidetes, Actinobacteria, Chloroflexi, Chloroplasts, Firmicutes, Gemmatimonadetes, Nitrospira, OP11, Planctomycetes,* and *Verrucomicrobia*.

Redox cycling had significant effects on the microbial community structure in comparison to the other treatments (Fig. 3). Analysis of clone libraries from UPF and SGF microcosms indicated that *Proteobacteria* increased by 21.2 %, and in particular, *Gammaproteobacteria* increased by 18.1 % in comparison to the UP and SG microcosms. Conversely, *Acidobacteria* abundance decreased in the UPF and SGF microcosms by 11.7 %. The 203 and 215 bp TRFs, which were more abundant in switchgrass-planted soils ($p < 0.05$; Figure S4), corresponded to *Geobacter* sp. as determined by T-RFLP of the clones themselves (Table 1). Of the 42 clones sequenced from the UPF microcosm, two were classified as *Geobacter* sp., a genus containing known sulfate, sulfur, and Fe(III) reducers (Methe et al. 2003) as well as dechlorinators (Sung et al. 2006). Another clone that would produce a 203-bp TRF is from the same order of *Desulfuromonadales* (Table 1). Cloned 16S rRNA gene sequences that generated 288 and 298-bp TRFs were classified as *Clostridium* sp. These TRFs comprised 0.3 and 0.5 % of all TRFs detected in each microcosm (Figure S4).

Sequences classified as *Geobacter* sp. and *Clostridium* sp. were not highly abundant in the four clone libraries (both sequences were only 1.2 % of the total sequences retrieved), although these libraries were not extensively sequenced (162 clones sequenced in total). Members of the *Chloroflexi* detected in 16S rRNA gene clone libraries from the SG, UPF, and SGF microcosms were classified as members of the *Anaerolineae* and *Caldilineae* (Table 1), both of which are not currently associated with reductive dechlorination processes (Gupta et al. 2013).

qPCR analysis of total bacterial 16S rRNA genes, putative dechlorinating *Chloroflexi*, and *Geobacteraceae* 16S rRNA genes in soil microcosms

Because the *Geobacteraceae* have previously been implicated in reductive dechlorination processes (Sung et al. 2006) and certain members of the *Chloroflexi* are the only confirmed anaerobic PCB dechlorinators (Adrian et al. 2009; Fennell et al. 2004; Yan et al. 2006), we quantified putative dechlorinating *Chloroflexi* and *Geobacteraceae* 16S rRNA genes in all microcosms using more specific qPCR primers (Table S1). We also quantified total 16S bacterial rRNA gene abundance over the course of the experiment (Table S1, Fig. S6). We found that switchgrass treatment significantly

Fig. 4 Clone library analysis of bacterial 16S rRNA genes displayed at the **a** phylum level and also **b** proteobacterial subdivision level in unplanted microcosm (*UP*), switchgrass-planted microcosm (*SG*), unplanted microcosm with redox cycling (*UPF*), and switchgrass-planted microcosm with redox cycling (*SGF*)
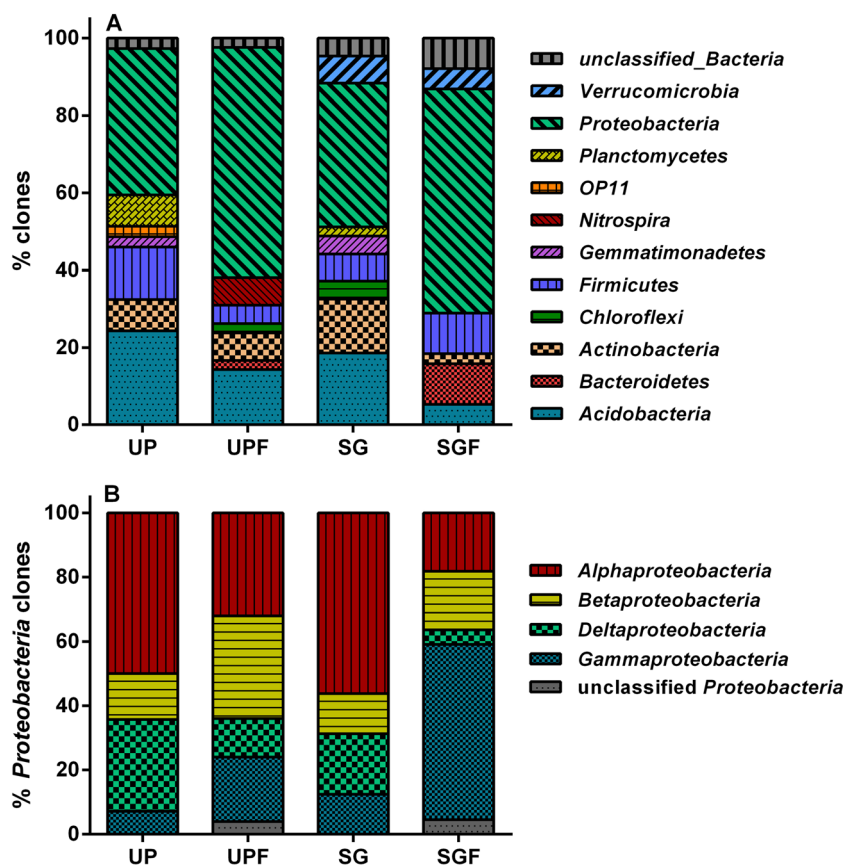
increased the total bacterial 16S rRNA gene abundance; however, this parameter was not affected by redox cycling ($p > 0.05$) (Figure S6).

Initially, putative dechlorinating *Chloroflexi* 16S rRNA gene abundance was below the qPCR quantification limit (<30 genes per reaction) in all microcosms and remained below quantification in the BLK, UP, and SG microcosms for the duration of the experiment. However, after 2 weeks of flooding, putative dechlorinating *Chloroflexi* 16S rRNA gene abundance increased to between 10^5 and 10^6 genes per gram soil in the UPF and SGF microcosms before leveling off at week 12 (Fig. 5). Despite repeated flooding and subsequent draining periods, putative dechlorinating *Chloroflexi* 16S rRNA gene abundance remained stable for the remainder of the experiment and was not correlated to redox potential or moisture content change (Pearson's r, $p > 0.05$). Interestingly, the abundance of putative dechlorinating *Chloroflexi* 16S rRNA genes in the SGF microcosm was significantly higher than in the UPF microcosm ($p < 0.05$).

Clone libraries were constructed with the UPF and SGF qPCR products in an effort to identify the *Chloroflexi* members we detected by qPCR. Each of the 12 sequences obtained from the clone libraries were classified as *Dehalogenimonas* sp. (Table S3). Our sequences share 98–100 % similarity with the *Dehalogenimonas* sequence retrieved from a PCB-dechlorinating enrichment culture CG3,

which can couple its growth with PCB dechlorination (Wang and He 2013b), as visualized by phylogenetic tree analysis (Figure S5). Overall, putative dechlorinating *Chloroflexi* 16S rRNA genes were not abundant with respect to total bacterial 16S rRNA gene abundance (0.0028 ± 0.0008 % in UPF and 0.0029 ± 0.0025 % in SGF).

PCB addition did not significantly affect *Geobacteraceae* abundance ($p > 0.05$, Fig. 6), while the highest *Geobacteraceae* 16S rRNA gene abundances were observed in the SG microcosm (Fig. 6). Redox cycling treatment did not affect *Geobacteraceae* abundance significantly ($p > 0.05$). Overall, the *Geobacteraceae* and putative dechlorinating *Chloroflexi* represented only a small fraction of the total bacteria 16S rRNA genes (1.01 to 1.43 % in the four microcosms, respectively).

Discussion

Detection of PCB congener transformation products verified the occurrence of PCB dechlorination in the soil microcosms. PCB dechlorination is typically observed in more strongly reducing anaerobic environments, as typified by a higher negative redox potential (Bedard et al. 1996; Krumins et al. 2009; Quensen et al. 1990). However, it is plausible that within soil microenvironments, pockets of strongly reducing conditions
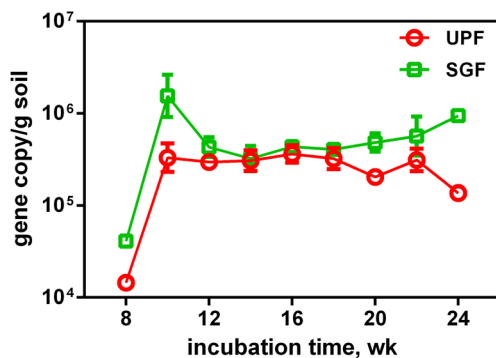
Table 1 Summary of 16S rRNA gene clones recovered from unplanted microcosm (UP), switchgrass-planted microcosm (SG), unplanted microcosm withredox cycling (UPF), switchgrass-planted microcosm with redox cycling (SGF)

Observed TRF (bp)	Predicted TRF (bp)	Closet classified relative (% certainty)	NMDS axis 1	NMDS axis 2
71	75	*Sorangium* (95 %)	−0.010	−0.165
187	194	Unclassified *Betaproteobacteria* (100 %)	−0.156	0.065
195	196	*Luteolibacter* (100 %)	−0.045	−0.331
197	200	*Variovorax* (100 %)	0.267	−0.229
196	200	Unclassified *Betaproteobacteria* (96 %)	−0.004	0.307
203	204	*Geobacter* (100 %)	0.295	−0.429
203	204	Unclassified *Desulfuromonadales* (100 %)	0.295	−0.429
205	206	*Rhodanobacter* (100 %)	−0.198	−0.014
210	214	*Dokdonella* (94 %)	0.567	−0.109
213	214	*Caldilinea* (100 %)	0.561	−0.168
213	216	*Gp3* (100 %)	0.561	−0.168
215	217	*Geobacter* (100 %)	1.389	0.242
216	219	Unclassified *Comamonadaceae* (100 %)	0.154	−0.056
225	227	*Novosphingobium* (95 %)	−0.176	0.230
228	230	*Gracilibacter* (100 %)	−0.109	0.178
229	231	*Bacillus* (100 %)	−0.045	−0.114
232	234	*Gp6* (100 %)	−0.286	0.046
235	237	Unclassified *Deltaproteobacteria* (97 %)	−0.298	−0.129
248	250	Unclassified *Ruminococcaceae* (100 %)	0.307	−0.187
260	262	*Flavisolibacter* (99 %)	0.085	0.047
288	290	*Clostridium XI* (100 %)	0.433	−0.141
298	300	*Clostridium sensustricto* (100 %)	−0.142	−0.028
314	316	Unclassified *Anaerolineaceae* (100 %)	0.247	−0.051
328	331	*Nitrospira* (100 %)	−0.348	−0.298

The percent certainty is reported as determined by RDP classifier

existed that favored the utilization of PCB congeners as alternative electron acceptors by microorganisms.

The detection of PCB 118 was notable, because it requires *ortho* chlorine removal from PCB 153 which is known to be difficult in other systems (Bedard et al. 2006; Quensen et al.

Fig. 5 qPCR analysis of putative dechlorinating *Chloroflexi* 16S rRNA gene in unplanted microcosm with redox cycling (*UPF*) and switchgrass-planted microcosm with redox cycling (*SGF*). *Error bars* indicate the range of two soil subsamples from the same microcosm. Putative dechlorinating *Chloroflexi* 16S rRNA gene abundance in unplanted (*UP*) and switchgrass-planted microcosms (*SG*) were below the quantification limit

1990). In this system, PCB 153 is potentially dechlorinated to PCB 52 and 77, congeners which were also spiked into the soils initially. The reduction of PCB 153 to PCB 52 is more likely, which can be achieved by removing two *para*-chlorines, while PCB 77 production requires *ortho* dechlorination, which is considered more difficult (Tiedje et al. 1993).

PCB 95 and 110/115 are not expected dechlorination products of the three spiked PCB congeners and were possibly produced by chlorine rearrangement, although the mechanism is unclear. Chlorine rearrangement of PCB congeners has been observed previously in poplars, switchgrass, and maize (Liu et al. 2009; Wang et al. 2011).

Overall, the PCB transformation products were less than 5 % on a molar basis with respect to the amount of parent PCB congeners added and did not accumulate significantly, possibly because of further sequential dechlorination and subsequent aerobic oxidation (Master et al. 2001). Evidence that supports the occurrence of aerobic PCB oxidation in these systems (Figure S3) was also presented previously (Liang et al. 2014b). Our results were also somewhat in contrast to a similar study, which indicated greater accumulation of dechlorination products in redox-cycled soils (Meggo and

Fig. 6 qPCR analysis of *Geobacteraceae* 16S rRNA genes in the blank microcosm (*BLK*), unplanted microcosm (*UP*), switchgrass-treated microcosm (*SG*), unplanted microcosm with redox cycling (*UPF*), and switchgrass-treated microcosm with redox cycling (*SGF*). *Error bars* indicate the range of two soil subsamples from the same microcosm
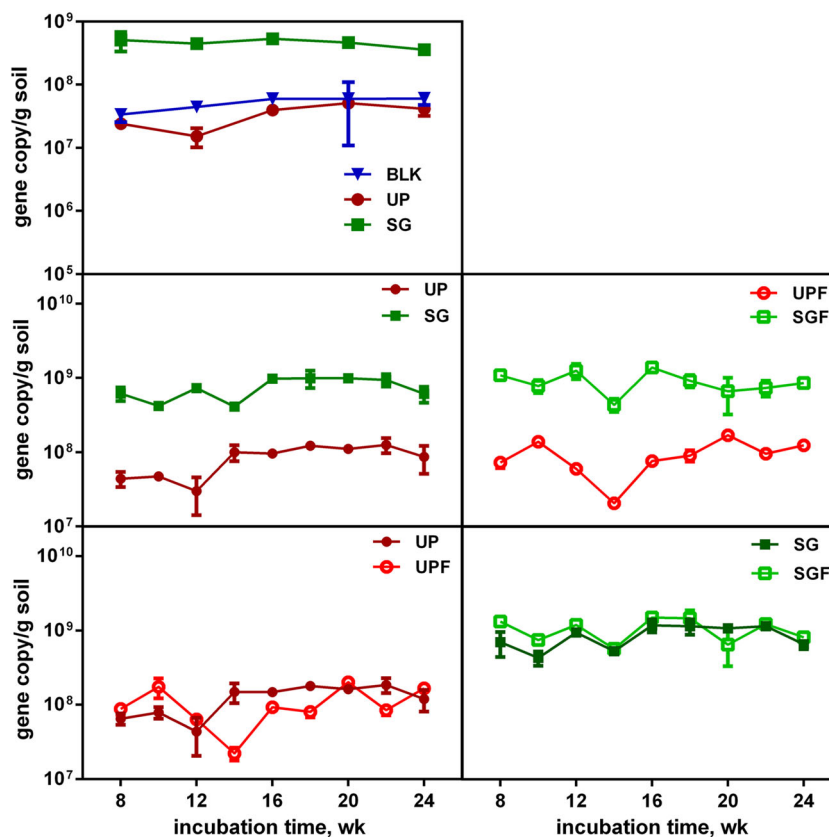
Schnoor 2013). The variability in PCB dechlorination among similarly constructed and treated microcosms is not clear but might have resulted by a differential development of soil microbial communities between the two experiments.

Proteobacteria are commonly the dominant bacteria in soils and sediments (de Cárcer et al. 2007; Petric et al. 2011; Spain et al. 2009). Many known aerobic PCB degraders are *Proteobacteria* (e.g., *Pseudomonas*, *Sphingomonas*, *Acinetobacter*, *Comamonas*, and *Burkholderia* (Nováková et al. 2002)); thus, it is possible that these same groups could participate in aerobic PCB degradation in the soil microcosms. In a previous study, we quantified the biphenyl dioxygenase alpha subunit gene (*bphA*) from the UP and SG microcosms (Liang et al. 2014b). The BphA gene encodes the enzyme catalyzing the first step of the upper biphenyl pathway. Additional potential aerobic PCB degraders other than *Proteobacteria*, such as *Bacillus* and *Rhodococcus* sp., were also identified (Masai et al. 1995; Shimura et al. 1999). A high abundance of *Acidobacteria* was previously noted in other PCB-contaminated soils and sediments (Nogales et al. 1999; Petric et al. 2011); however, there is no evidence linking them to PCB degradation.

It is possible that the bacterial groups represented by the TRFs identified as important in the UP microcosm are stimulated by PCB spiking and play a role in PCB degradation in the soil. The relatively low abundance of these groups with respect to the total abundance of the microbial community, plus the

relative low coverage of the clone libraries (162 clones) are probably what prevented us from identifying these TRFs.

Putative dechlorinating *Chloroflexi* were not detected in our bacterial 16S clone libraries, possibly because of their low relative abundance in the microcosms with respect to total bacterial 16S rRNA gene sequences. However, using a different set of qPCR primers, we were able to verify the presence of putative dechlorinating *Chloroflexi* and identify them as probable *Dehalogenimonas* sp. Strains of *Dehalogenimonas* are reported to reductively dechlorinate polychlorinated aliphatic alkanes (Maness et al. 2012; Yan et al. 2009). A *Dehalogenimonas* strain was recently implicated in growth-coupled PCB dechlorination in the sediment-free culture, CG3 (Wang and He 2013b). Dechlorinating *Chloroflexi*, while found in our microcosms, were more abundant in previously reported PCB-dechlorinating sediment-free cultures. For example, *Dehalococcoides* was 3.74 % of total bacterial community in highly enriched sediment-free JN culture (Bedard et al. 2006). *Dehalogenimonas* sp. represented 2.16 % of a sediment-free culture that dechlorinated Aroclor 1260 (Wang and He 2013b). Despite this, the apparent growth of *Dehalogenimonas* sp. in the UPF and SGF microcosms strongly suggests that they played a role in PCB dechlorination therein.

Although we observed putative dechlorinating *Chloroflexi* in soil microcosms via qPCR, they were only present in the UPF and SGF microcosms at relatively low abundance. This

suggests that additional microbial groups were involved in PCB dechlorination in the soil microcosms (UP and SG especially). Within the *Proteobacteria*, the *delta*- and *gamma*-*proteobacteria* contain some strains known to degrade chlorinated compounds via organohalide respiration or co-metabolic reductive dehalogenation, such as *Desulfuromonas* spp. (Krumholz 1997), *Geobacter lovleyi* (Sung et al. 2006), and *Shewanella putrefaciens* (Picardal et al. 1995). Certain *Geobacter* sp. can dechlorinate trichloroacetic acid and tetrachloroethene (de Wever et al. 2000; Strycharz et al. 2008; Sung et al. 2006) and have been found in sediment-free PCB-dechlorinating enrichment cultures (Bedard et al. 2006; Bedard et al. 2007; Holoman et al. 1998). Notably, a recent study found that *Geobacteraceae* enriched in cultures that completely degraded trichloroethene under iron-reducing conditions, suggesting that *Geobacter* may be able to dechlorinate under less strict anaerobic conditions (Wei and Finneran 2011). Our results suggest that the presence of switchgrass enriched for certain *Geobacter* sp. or related *Desulfuromonadales* (Figure S4), perhaps by releasing root exudates as carbon and energy sources (Bais et al. 2006; Baudoin et al. 2003)

Clostridium is another genus we identified as responding to redox cycling with the potential to participate in PCB dechlorination. For instance, certain *Clostridia* dechlorinate tetrachloroethene (Chang et al. 2000; Okeke et al. 2001). *Clostridium* sp. were detected in a PCB-dechlorinating enrichment culture derived from St. Lawrence River sediment (Oh et al. 2008) and sediment-free PCB-dechlorinating culture AD14 (Wang and He 2013a). In another study, *Clostridium* sp. were abundant in sediments where *para*- and *meta*-PCB dechlorination was occurring (Hou and Dutta 2000). *Clostridium* sp. can also produce hydrogen, an important and common electron donor for reductive dechlorination processes (Bowman et al. 2009; Nandi and Sengupta 1998). The presence of such species in soil microcosms where PCB dechlorination had occurred indicates that their possible role in PCB degradation should be considered in future studies.

In this study, we investigated the microbial communities in soils displaying PCB dechlorination. The occurrence of dechlorination was verified by the detection of transformation products of parent PCB congeners in all soil microcosms, some of which were under anoxic conditions. Putative dechlorinating *Chloroflexi* populations were enriched after the first 2 weeks of flooding of the redox cycling treatment, which sequences were classified as *Dehalogenimonas* sp., a potential PCB-dechlorinating species. *Geobacter* were identified in all soil samples and were more abundant in switchgrass-planted soil with PCB spiking. Overall, the potential organohalide-respiring bacteria were about 1 % of the total bacteria, but their presence in the soils suggests a dechlorination potential of the indigenous microbial community and their role in PCB dechlorination.

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