IMPACT OF THE PLANT RHIZOSPHERE AND AUGMENTATION ON REMEDIATION OF POLYCHLORINATED BIPHENYL CONTAMINATED SOIL

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Abstract—This study investigated the interactive effects of bioaugmentation, biostimulation, and the rhizosphere during remediation of Aroclor 1242-contaminated soil. Treatments were repeatedly augmented with polychlorinated biphenyl (PCB)-degrading bacteria, inducers (carvone and salicylic acid), surfactant (sorbitan trioleate), minimal salts medium in a 20-cm high soil column, or a combination of these elements. Soils containing a single *Brassica nigra* plant achieved 61% PCB removal in the 0 to 2 and 2 to 6 cm depths after 9 weeks of bioaugmentation, whereas only 43 and 14% PCB removal, respectively, was achieved in unplanted controls. Gas diffusion coefficients of 13.0 and $5.0 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$ were calculated from a methane diffusion assay for planted and unplanted soils respectively, indicating the positive effect of plant roots on gas diffusion into the soil. A second, modified column study removed 87, 73, 63, and 45% of PCB after 12 weeks in the 0 to 5, 5 to 11, 11 to 26, and 26 to 35 cm depths, respectively, in planted-bioaugmented soils, whereas 65, 54, 53, and 47% of PCB was removed from the planted-minimal salts treatment, respectively. Shifts in the soil microbial community structure were demonstrated by denaturing gradient gel electrophoresis of bacterial 16S ribosomal DNA. Results support that *Brassica nigra* directly contributed to accelerated PCB removal by increased oxygen diffusion, amendment infiltration, and microbial enrichment.

Keywords—Phytoremediation Bioremediation Polychlorinated biphenyl Gas diffusion Denaturing gradient gel electrophoresis

INTRODUCTION

Of the xenobiotics found world-wide, polychlorinated biphenyls (PCB) are considered amongst the most recalcitrant. Despite nearly 30 years of research on enhancing in situ PCB biodegradation [1,2], the most common method for remediating PCB-contaminated soil still involves excavation, followed by landfilling or incineration [3,4]. In light of the dearth of available remediation options for PCB-contaminated soils, it is surprising that only a few studies have investigated the potential for plants to assist in its removal [5–8].

Research on bioaugmentation and biostimulation of PCB-contaminated soils has recently focused on the important issue of increasing PCB bioavailability through the addition of surfactants and biosurfactants [9–13]. Based on the work of Lajoie et al. [13] and Gilbert and Crowley [14], Singer et al. [15] developed a bioaugmentation approach utilizing a surfactant, sorbitan trioleate, as a semi-selective growth substrate for two PCB-degrading microorganisms, *Arthrobacter* sp. strain B1B and *Ralstonia eutropha* H850. When cultured in the presence of the two compounds that induce cometabolism of PCBs, carvone and salicylic acid, respectively, nearly twice as many PCBs were removed as compared to identically treated non-surfactant amended soils [14,15]. When the same bioaugmentation regimen was applied using earthworms to promote soil mixing, aeration, and enhanced microbial activity, upwards of 65% PCB removal was achieved in soil to a depth of 6 cm, such that there was 68% greater PCB removal than in similarly treated unmixed soils without earthworms [16].

The aims of this study were to evaluate the efficacy of black mustard (*Brassica nigra*) to enhance subsurface PCB degra-

dation. *Brassica nigra* was chosen because of its hardiness, fast growth, high biomass, extensive root system, and its proven utility in metal contaminated soils [17]. Amendments of the surfactant sorbitan trioleate and two plant-derived inducers, salicylic acid and carvone, were used in two complementary studies that examined the influence of plants on PCB removal. In study 1, a gas diffusion assay was employed to evaluate the impact of plant roots on gas diffusion into the soil profile—a soil with a greater capacity for oxygen replenishment would foster greater aerobic microbial activity thereby potentially hastening PCB-remediation. In study 2, modifications were made to the column design used in study 1 to allow for investigation into the limiting role of oxygen on PCB degradation; evaluation of the potential for mobilization of PCBs in the leachate; examination of PCB degradation at greater depth; and evaluation of the microbial community at five sampling depths using polymerase chain reaction–denaturing gradient gel electrophoresis (DGGE) of 16S ribosomal DNA.

MATERIALS AND METHODS

Soil and mesocosm design

In study 1, 5 kg of air-dry soil (coarse loamy, mixed, thermic Haplic Durixeralf, pH 7.5, 0.21% carbon, 0.01% nitrogen) was acquired from the agricultural plots at the University of California at Riverside (Riverside, CA, USA). Five g of Aroclor 1242 (AccuStandard, New Haven, CT, USA) was dissolved in 275 ml hexane and slowly mixed into 5 kg of sieved (2 mm) air-dry soil. The soil was mixed repeatedly over a 5-d period to ensure volatilization of hexane and subsequently mixed into an additional 45 kg of pristine, sieved soil in an electric cement mixer to make a final concentration of 100 mg Aroclor 1242 kg⁻¹ soil. After 4 d, 0.600 kg (dry wt) of the contaminated soil was added to each of 24 polyvinyl chloride

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columns. The polyvinyl chloride column dimensions were 30-cm length by 2.54-cm radius, and contained 405 cm³-volume of contaminated soil with a 5-cm air reservoir above and below the contaminated soil. Approximately 5 cm of pea gravel was added to the bottom air reservoir of each tube, which was separated from the contaminated soil by glass wool to provide an equal sized upper and lower air reservoir for the gas diffusion study. After preparing the columns, they were allowed to stand for 90 d in a greenhouse at the University of California (26 ± 10°C). One week before the beginning of the study, each column was brought to field capacity ($\theta = 0.12$) with the addition of 140 ml of half-strength minimal salts media (MSM) [18]. The mass of each soil column was noted to assess the extent of water loss between each soil amendment, and to determine the volume of amendment to be added.

The column dimensions in study 2 were 45 cm length by 2.54 cm radius, with 709 cm³ of contaminated soil, overlying a pristine soil layer that was separated from the contaminated zone by a layer of glass wool. The pristine soil layer was intended to capture any leachate and minimize gas diffusion through the base of the column, thereby more closely mimicking natural conditions. Each column was filled with approximately 1.50 kg (dry wt) of soil originating from the same lot used in study 1 after aging for two and one-half years. The soil was aged in closed stainless-steel canisters in a greenhouse at the University of California, Riverside (temperature range 16–36°C, air-dry). The columns were otherwise prepared identical to those used in study 1.

Treatment regimen

Treatments in study 1 consisted of columns with and without plants, which received one of three different amendments. The first treatment employed bioaugmentation with *Ralstonia eutropha* H850 (NRRLB-15940) and *Rhodococcus* sp. ACS. Due to the particular catabolic limitations and congener specificity of PCB-degrading bacteria [19,20], and the wide-range of congeners seen in Aroclor PCB mixtures, it has been suggested a coinoculum approach would achieve more comprehensive PCB degradation [15,21]. The gram-negative strain *R. eutropha* H850 and the gram-positive strain *Rhodococcus* sp. ACS have been previously shown to readily degrade PCBs, in particular, after induction by the plant-derived inducing compounds salicylic acid and carvone [15,22]. The strains also have been shown to grow readily on the surfactant, sorbitan trioleate, which improves PCB bioavailability and biotransformation in soils [15,16]. The bacterial cultures were maintained and prepared as previously described [16]. The *Rhodococcus* sp. ACS culture contained 100 mg/L carvone, and 1,000 mg/L sorbitan trioleate dissolved in MSM. The *R. eutropha* H850 culture contained 500 mg/L salicylic acid and 1,000 mg/L sorbitan trioleate dissolved in MSM. An additional 50 µl of sorbitan trioleate was added to each 100-ml culture prior to application, replacing the depleted surfactant, thereby ensuring sufficient surfactant for desorbing PCBs. After the surfactant was added, the flasks were shaken for 15 min to allow the sorbitan trioleate to dissolve into the medium. Each PCB degrader was added once a week, alternating the isolates at each of the two applications.

The second of the three treatments was a control for the bioaugmentation treatment, in which soils received 100 mg/L carvone, 1,000 mg/L sorbitan trioleate, and 500 mg/L salicylic acid dissolved in MSM. The third treatment investigated the role of essential inorganic nutrients in stimulating PCB bio-

degradation. All amendments were made twice weekly for 9 weeks.

Four replicate columns were prepared for each treatment, totaling 24 in all (i.e., 12 planted and 12 unplanted). In addition to the bacteria and surfactant, which were added to the columns twice weekly, water was applied as needed to maintain the soil moisture at an optimal level for plant growth. The columns were maintained in a greenhouse at the University of California Riverside where the average temperature was 26 ± 10°C. Planted soils contained a single two-week-old seedling of *Brassica nigra* 3 d prior to the first amendment.

Study 2 contained the same treatment regimen as study 1, plus an additional treatment containing MSM without nitrogen (MSM-N). Since the soil was very low in nitrogen (0.01%), a MSM minus nitrogen treatment was added to the study to allow further insight into the potential role of nitrogen-containing plant exudates on microbial activity, diversity and PCB degradation. Four replicate columns were prepared for each treatment, totaling 16 in all. Due to the increased volume of soil, the amendment volumes for study 2 were doubled to 20-ml volumes. The amendments were added twice weekly, and repeated for 12 weeks. With the focus of study 2 being the impact of different treatment regimes on the microbial community structure, the study was carried out in a growth chamber (20°C, 70% relative humidity, and a light intensity of 500 µE m⁻² s⁻¹ with a 16:8-h day:night cycle), thereby minimizing variations in ambient conditions and plant growth.

Analytical procedure

In study 1, soil sampling was conducted 3 d after the eighteenth amendment (9 weeks plus 3 d) by removing the entire soil profile from the polyvinyl chloride pipes and dividing them into three depths, 0 to 2 cm, 2 to 6 cm, and 6 to 20 cm. A 22-g soil sample was randomly removed from each of the thoroughly mixed depths, placed in a 40-ml glass vial with a polytetrafluoroethylene-lined cap, and stored at -20°C until analysis. Soil samples were extracted and analyzed for PCBs by gas chromatograph-flame ionization detector as previously described [16]. Percent recoveries were generated by comparing the solvent-extractable fraction from the treatment to the solvent-extractable fraction from the original untreated Aroclor 1242-contaminated soil. The soil moisture content at the time of sampling was 4% at all depths in planted soils, while unplanted soils were between 10 and 12% water. Soil extractions were normalized to the soil moisture content to account for these discrepancies.

Two replicate columns within each treatment were sampled at 2 depths, 2 to 6 cm and 6 to 20 cm, for water content and carbon/nitrogen analysis [23]. Forty-mg samples were prepared for total carbon and nitrogen analyses as per manufacturer instructions (Carlo Erba Nitrogen Analyzer Model 1500-R/AS 200, Carlo Erba Instruments, Milan, Italy) after being ground into a powder using a mortar grinder (Ratsch Type RM-0, Krefeld, Germany). Plant tissues were carefully removed from the soil profile, divided into the root and shoot tissue, gently washed in deionized water to remove adsorbed soil and stored at -20°C until extraction. The plant tissue was freeze dried and ground into a fine powder in a Wiley mill at which time they were weighed into 2 g portions, and extracted in triplicate 40-ml vials with 10 ml hexane. The solvent extract was removed and passed through a Florisil clean-up cartridge (6 ml LC-Florisil; Supelco, Bellefonte, PA, U.S.A.), that had been preconditioned with hexane. The extract was followed

with 3×1 ml aliquots of hexane. The hexane fraction was analyzed by gas chromatograph-flame ionization detector as described above after concentration [16].

Gas diffusion assay

Representative soil columns from the planted and unplanted treatments in study 1 were subjected to a methane diffusion assay that enabled quantification of the effect of plants on gas diffusion into the soil columns. It was postulated that an increase in gas diffusion would enable greater aerobic biotransformation of PCB. The columns were capped tightly with a rubber pipe cap and fitted with two Teflon® septa per cap. A 2.5 cm³ pulse of methane was injected into the bottom of the sealed soil column through the septum, at which time 2.5 cm³ was removed to relieve any pressure buildup. The top and bottom air reservoirs in the soil column were repeatedly sampled with a gas-tight syringe for a 340-min time period and immediately manually injected onto the gas chromatograph-flame ionization detector (isocratic, 60°C). A gas diffusion coefficient was calculated based on the best fit of a gas diffusion transport model, as previously described [16]. Data from the unplanted column was fitted to the model by assuming zero degradation of methane in the soil during transport. However, the data from the planted column indicated methane depletion over time, necessitating a first-order degradation coefficient to be simultaneously fitted to the data.

Microbial community analysis

The biphenyl-utilizing microbial populations were enumerated from a 1-g soil sample originating from each of the soil depths within each soil column in study 1 [16]; biphenyl is the unchlorinated congener of PCBs. Although biphenyl utilization is not necessarily an indication of a bacterial species' ability to degrade PCBs, all PCB degraders appear to be able to metabolize biphenyl.

To provide an indication of the influence of the different amendments on the indigenous soil microbial population in study 2, the bacterial community 16S ribosomal DNA was extracted and analyzed by DGGE. Soil samples (1g) were processed using a FastPrep bead beater (Bio 101, Vista, CA, USA) for each of the four sample depths (0–5, 5–11, 11–26, 26–35 cm). The total soil DNA was isolated with the Fast DNA kit for soil as described by the manufacturer (Bio 101). The eluted DNA products were stored at –20°C prior to analysis. DNA from each soil sample was subjected to polymerase chain reaction and DGGE as previously described [24].

The DGGE gels were analyzed using Scion Image analysis software (Scion, Frederick, MD, USA). The resulting line profiles for each lane were then analyzed using Peakfit (SPSS Science, Chicago, IL, USA) to determine the band intensities and their positions in the lane relative to the front (Rf). The DNA bands obtained on different gels were normalized using marker DNA run on each gel. After Peakfit analysis, numerical data was exported to an Excel spreadsheet (Microsoft, Redmond, WA, USA) where a summary table (Rf vs intensity) was generated for statistical analyses using Minitab (Minitab, State College, PA, USA). The peak data were subjected to cluster analysis to determine the relatedness of each treatment's microbial community, and to determine the percent similarities in microbial communities between different treatments at each depth (0–5, 5–11, 11–26, 26–35 cm). The cluster diagram presented in this manuscript was generated using the mean values for DNA profiles from four replicate soil columns per

Table 1. Percent polychlorinated biphenyl (PCB) recovered from soil originating from study 1^a

Treatments	Depth (cm)			Total ^b
	0–2	3–6	6–20	
Planted				
Bioaugmented	39 ACa	39 Da	65 Ab	57
Biostimulated	63 Ba	59 CBa	65 Aa	64
Minimal salts	39 Ca	57 CDEa	60 Aa	57
Control				
Bioaugmented	57 ABCa	86 Ab	70 Aab	72
Biostimulated	49 ABCa	84 ABb	79 Aab	77
Minimal salts	48 ABCa	65 BCab	67 Ab	65

^a Soil PCB removal at the same depth are significantly different ($p > 0.05$) if marked with different uppercase letters (two-factor analysis of variance with repeated measure using Student-Newman-Keuls). The PCB removal values within the same treatment are significantly different ($p < 0.05$) if denoted with different lowercase letters (two factor analysis of variance using Student-Newman-Keuls).

^b The total percent PCB removal from treatments was weighted to account for differences in the volume of soil at each depth, as discussed in the text.

treatment to compare the different community profiles at the four soil depths.

RESULTS AND DISCUSSION

Study 1

Planted treatments resulted in removal of 35 to 61% of the PCBs to a depth of 20 cm, as compared to 14 to 52% removal in unplanted soils, irrespective of the treatment and depth (Table 1). No significant differences were detected between planted biostimulated and soils treated only with MSM (39–65% PCB recovery), and their corresponding unplanted treatments (48–84% PCB recovery) at any respective depth. Polychlorinated biphenyl recoveries in the upper 6 cm of the planted bioaugmented soil columns (39% PCB recovery) were significantly lower than PCB recovery in the lower 6 to 20 cm depth (65% PCB recovered). Of particular note is the 2 to 6 cm depth in bioaugmented soils, in which significantly less PCB is recovered in the presence of the plant (61% removal) than in the unplanted treatment (14% removal). This result suggests that the plant rhizosphere contributed to PCB removal through enhanced inoculum penetration into the soil profile, likely by means of preferential flow channels generated from roots. Polychlorinated biphenyl removal observed in this study compares quite favorably with similarly treated PCB-contaminated soil in Singer et al. [16], in which earthworms were evaluated for their ability to stimulate PCB degradation. In this instance, the authors demonstrated 66%, 65% and 50% removal of PCB at depths of 0 to 2, 2 to 6, and 6 to 20 cm, respectively. Total PCB degradation per soil column was calculated by summing the products of PCB degraded from each of the depths by the percent that that depth contributes to the soil in the column. More specifically, in Study 1 the 0 to 2 cm depth contributes 10% to the total volume of soil in the column, while the 2 to 6 cm depth is twice as long, thus representing 20% of the soil in the column, and the 6 to 20 cm depth contributes 70% of the soil in each column. Planted columns removed more PCBs per soil column (36–43% removal) than unplanted treatments (23–35% removal, Table 1).

A previous report demonstrated removal of 77% of Aroclor 1248 in a pot experiment containing eight *Medicago sativa*

L. (alfalfa) using a low organic matter soil, whereas unplanted controls removed only 18% [7]. The same authors conducted a PCB transformation study with two other plants, tall fescue (*Festuca arundinacea* Schreb.) grass and *Sericea lespedeza* (*Lespedeza cuneata* [Dum.-Cours.]) legume species in Aroclor 1248 contaminated soil. In the low organic matter soil, 71% of PCBs were removed in the *Sericea*-planted soil, 66% in the tall-fescue-planted treatment, and 18% in the unplanted control soil [25]. An additional study demonstrated approximately 45% PCB removal of industrially-contaminated soil when planted with *Medicago sativa* L. (alfalfa). The addition of a PCB-degrading rhizobia, *Sinorhizobium meliloti*, to planted treatments was not shown to improve PCB removal in that study [6]. There was no report of PCB degradation on a depth basis in any of these studies. Due to heterogeneous PCB removal (i.e., greater at the surface where volatilization is higher and lower at depth where oxygen is more limiting), dissimilar pot designs, and different methods of assessing PCB removal, comparisons between previous studies and this study can not be accurately made.

Approximately 6.8 g of soil was exposed to the atmosphere at the soil surface (to a depth of 0.5 cm) representing approximately 24% of the 0 to 2 cm depth, and only 1% of the total soil in the treatment. Therefore, volatilization of PCBs is not likely to affect the interpretation of results with respect to the soil column as a whole, however, it certainly played a role in the fate of PCBs in the uppermost soil layer. A measure of the volatilized PCBs may be found in the PCB content of above-ground plant material [26]. Plant leaf material averaged $2.4 \pm 1.5 \mu\text{g PCB g}^{-1}$ plant matter (dry wt). The plant stems contained $2.3 \pm 3.0 \mu\text{g PCB g}^{-1}$ plant matter (dry wt), whereas the plant roots yielded the highest values of $12.8 \pm 2.8 \mu\text{g PCB g}^{-1}$ plant matter (dry wt). Although recovery of PCBs in the above-ground plant matter is only an indication of the amount of PCBs volatilizing from the soil, the measured values would predict low PCB loss due to volatilization. The upper 0 to 2 cm and lower 6 to 20 cm depths in unplanted soils (57% and 70% PCB recovered, respectively) demonstrated significantly more PCB removal than the 2 to 6 cm depth (86% PCB recovery). This curiously high PCB removal at the lowest depth (6–20 cm) is likely an artifact of the column design. The lower air reservoir containing the pebble layer was likely responsible for enhancing oxygen diffusion and possibly PCB volatilization. Therefore, PCB removal in the 6 to 20 cm depth is probably misleadingly higher than would have likely occurred under more natural conditions. However, this occurrence provides evidence for the importance of oxygen in the bioremediation process. An equally important consideration is the possibility of PCB removal due to polymerization to plant-organic matter [27]. Although the soil contained very low organic matter at the outset, analysis of the carbon and nitrogen content of the soils at the termination of the study showed significant deposition of organic matter into the soil. A debate regarding the toxicological significance of the polymerized PCBs might conclude such PCBs as having been removed. An average of 6.0 ± 2.0 times more carbon was recovered from the 2 to 6 cm depth in planted soils ($0.55 \pm 0.07 \text{ mg C g}^{-1}$ soil) than unplanted soils ($0.03 \pm 0.03 \text{ mg C g}^{-1}$ soil). Soils with plants averaged 2.0 ± 0.6 times more carbon ($0.07 \pm 0.04 \text{ mg C g}^{-1}$ soil) in the 6 to 20 cm depth than soils without plants ($0.04 \pm 0.03 \text{ mg C g}^{-1}$ soil). The soil nitrogen content ranged between the limits of detection 0.0001 and 0.271 mg-

Fig. 1. Gas diffusion in the presence and absence of plants. The ratio of the measured methane concentration ($C(t)$) in the inlet (upper series) and outlet (lower series) to the initial methane measurement (C_0) is plotted against time (min). The measured (circles) methane concentrations in the planted column were best fit to the model yielding the predicted (line) and a diffusion coefficient (D) of $0.8 \text{ cm}^2 \text{ min}^{-1}$; unplanted controls yielded $D = 0.03 \text{ cm}^2 \text{ min}^{-1}$.

N g^{-1} soil in the planted soils, whereas unplanted soils ranged between the detection limit and $0.120 \text{ mg-N g}^{-1}$ soil.

The average of three replicate plate counts of colony forming units (CFU) on biphenyl in planted columns ranged from a high of $2.58 \times 10^9 \text{ CFU g}^{-1}$ at the 0–2 cm depth, in bioaugmented soils, to a low of $5.6 \times 10^7 \text{ CFU g}^{-1}$ at the 6–20 cm depth, in MSM-treated soils. The nonplanted columns followed a similar trend, having the highest biphenyl utilizer density in bioaugmented treatments at the 0 to 2 cm depth ($1.22 \times 10^9 \text{ CFU g}^{-1}$) and a low in the MSM treatment at the 6 to 20 cm depth ($2.73 \times 10^7 \text{ CFU g}^{-1}$).

Gas diffusion into the soil column was modeled using a nonconservative methane tracer. The model enabled determination of the diffusion coefficient of planted and unplanted soils. After injection of methane into the lower air reservoir, the methane concentrations in both air reservoirs were repeatedly monitored. Breakthrough of the methane tracer through the 20-cm column was detected after only 2 min in planted soils (filled circles, Fig. 1), while 340 min was required before breakthrough in soils without plants. Using a gas diffusion model, which generated the best fit curve to the data [16], the diffusion coefficients (D) were calculated to be $0.8 \text{ cm}^2 \text{ min}^{-1}$ ($1.3 \times 10^{-6} \text{ m}^2 \text{ s}^{-1}$) and $0.03 \text{ cm}^2 \text{ min}^{-1}$ ($5.0 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$) for planted and unplanted soils, respectively. The diffusion coefficient from planted soils compares favorably to the values obtained in Singer et al. [16], $D = 0.27 \text{ cm}^2 \text{ min}^{-1}$ ($4.5 \times 10^{-7} \text{ m}^2 \text{ s}^{-1}$) which used identical columns containing earthworms instead of plants. Clearly, the presence of plants can greatly influence gas diffusion into a soil profile, upwards of 300% greater than that previously observed for earthworm bioturbation. The increase in the diffusion coefficient favors a significantly higher rate of soil atmospheric oxygen replacement, and in turn can support a more active aerobic microbial population. Best fit of the diffusion data to the model for the planted treatment necessitated a methane consumption constant (upper series line, Fig. 1), whereas data from the unplanted treatment did not. A methane consumption constant (μ) of 0.008 min^{-1} suggested planted soils contained a micro-

Table 2. Percent polychlorinated biphenyl (PCB) recovered from soil originating from study 2^a

Treatments	Depth (cm)					Total ^b
	0–5	5–11	11–26	26–35	Leachate	
Bioaugmented	13 Aa	27 Aab	37 Ab	54 Aab	10 A	36
Biostimulated	34 Aa	42 ABab	49 Aab	53 Ab	1 A	47
Minimal salts	35 Ba	45 Bb	47 Ab	53 Ab	1 A	47
Minimal salts minus N	35 Ba	50 Bb	49 Ab	49 Ab	2 A	47

^a Soil PCB removal values at the same depth are significantly different ($p > 0.05$) if marked with different uppercase letters (two-factor analysis of variance with repeated measures using Student-Newman-Keuls). The PCB removal values within the same treatment are significantly different ($p < 0.05$) if denoted with different lowercase letters (two-factor analysis of variance using Student-Newman-Keuls).

^b The total PCB removal from treatments was weighted to account for differences in the volume of soil at each depth, as discussed in the text.

bial community readily capable of transforming methane, unlike unplanted soils. Increased methane consumption was also previously shown in earthworm-treated soils, suggesting either a shift in the population toward methanotrophs, or possibly an increase in ammonia oxidizing bacteria, which have been linked to methane degradation [16,28]. The methane consumption rate constant in earthworm-treated soils was twice that of planted soils ($\mu = 0.015$). This difference may be attributed to the differing sources of nitrogen being deposited in the soil, thereby enriching for dissimilar microbial communities. Unlike earthworm-treated soils, in which urea is the dominant nitrogen-containing excrement [29], planted soils deposit a range of nitrogen-containing compounds, largely complex in nature, originating from dead plant matter and secondary metabolites [30].

Study 2

All treatments in study 2 contained plants and received one of the following amendments: Bioaugmented, biostimulated, minimal salts medium, and minimal salts medium minus nitrogen. Soil samples from each of the four depths (0–5, 5–11, 11–26, and 26–35 cm) were evaluated for the shifts in the bacterial communities that occurred as a result of the different amendments. The bioaugmented treatment was the most successful treatment in stimulating PCB removal. Polychlorinated biphenyl recoveries in the 0 to 5, 5 to 11, 11 to 26, and 26 to 35 cm depths were 13, 27, 37, and 54%, respectively (Table 2). Total PCB removal per soil column was greatest in the bioaugmented treatment (64% removal) as compared to the three other treatments (53% for each). Although results of study 1 showed that the plant rhizosphere was considerably more effective in remediating PCB than unplanted controls, results from study 2 suggested bioaugmentation with highly competent PCB-degrading microorganisms can facilitate a greater level of remediation than the rhizosphere alone. In the treatment where $\text{NH}_4(\text{NO}_3)_2$ was absent from the medium applied to the soil (MSM-N), no significant differences in PCB removal were observed at any of the 4 depths, which is interesting to note in light of the fact that the biphenyl utilizers from the MSM-N treatment ($8.43\text{--}17.3 \times 10^7 \text{ CFU}^{-1}$) were consistently lower than in all of the other treatments. Biphenyl utilizers in biostimulated soils ranged from 14.5 to $60.3 \times 10^7 \text{ CFU g}^{-1}$, whereas the MSM-treated soil contained between 16.4 and $52.7 \times 10^7 \text{ CFU g}^{-1}$. The higher biphenyl utilizer colony counts in bioaugmented soils ($83.0\text{--}533 \times 10^7 \text{ CFU}^{-1}$) as compared to all other treatments soils suggests there is only a weak positive correlation between the isolation of biphenyl

utilizers and PCB degradation. Moreover, there appears to be a strong positive correlation between the input of carbon and nitrogen (i.e., the organic amendments) and culturable biphenyl utilizers.

The leachate from the bioaugmented column averaged $10 \mu\text{g PCB g}^{-1}$ soil as compared to the other three treatments, which averaged between 1 to $2 \mu\text{g PCB g}^{-1}$ soil ($p > 0.05$). Thus, it can be concluded that much of the observed PCB loss can not be attributed to loss in the leachate. Given that the biostimulated treatment contained identical quantities of surfactant as the bioaugmented treatment, one may conclude that sorbitan trioleate alone, as used in this study, did not increase the risk of PCB leaching. However, since greater PCB-containing leachate was found in the bioaugmented treatment, either metabolic breakdown products of sorbitan trioleate enabled greater PCB mobility or an unknown biosurfactant may be uniquely present in this treatment [12,31].

Evidence already presented suggest that the microbial communities within each treatment were different. Evaluation of the microbial communities present on the DGGE provides evidence of clear shifts in the soil microbial population between each of the treatments. The shift in the bacterial community between treatments is consistently greater than the shift in the bacterial community within a soil column between depths (Fig. 2). The 11 to 26 and 26 to 35 cm depths are consistently more similar to each other within a treatment, most likely because these two depths were the least effected by the amendments, and were more greatly influenced by the plant roots. The 0 to 5 cm depth was consistently most dissimilar from the rest of the soil depths within a soil column, most likely because it had the greatest exposure to the amendments and oxygen, and was the least influenced by the plant roots. It is important to note that in study 2, the pristine soil barrier beneath the contaminated soil profile minimized gas diffusion from below, more realistically simulating subsurface conditions. The MSM and MSM-N treatments showed the highest percent similarity in microbial populations between each of the depths within a column. This may be due to the fact that the MSM or MSM-N treatments did not contain any inoculum or carbon sources that could partition preferentially in the upper soil profile, generating fewer differences by depth. Soil that received the bioaugmentation and biostimulation treatments, on the other hand, had measurable changes by depth, likely due to preferential sorption, filtering, and degradation of the amendments in the upper few centimeters. Cluster analysis of the microbial communities within each treatment clearly demonstrated the presence of two distinct groups of bacterial communities, the

Fig. 2. Cluster analysis of 16S ribosomal DNA similarities for bacterial communities in soil treated using bioaugmentation (BA), biostimulation (BS), minimal salts medium (MSM), and minimal salts medium minus nitrogen (MSM-N). Bacterial communities were analyzed at four depths 0 to 5, 5 to 11, 11 to 26, and 26 to 36 cm.

bioaugmentation and biostimulation treatments; and the MSM and MSM-N treatments (Fig. 2).

This study demonstrates, using multiple lines of evidence, the effect of plants on PCB remediation of contaminated soils. Studies 1 and 2 provided clear evidence in support of the hypothesis that the rhizosphere is capable of supporting a functionally unique community of microorganisms (e.g., differential methane degradation), that are capable of enhanced PCB removal as compared to unplanted controls. The benefits provided by plants originate from their ability to support higher microbial activity through increased oxygen diffusion and release of exudates into the soil. Results from bioaugmented treatments suggested that plant roots allow increased inoculum penetration, enabling greater subsurface PCB removal. Study 2 further demonstrated that despite clear differences in the indigenous bacterial communities, similar PCB removal efficiencies between treatments were observed. This inconsistency may be explained by the presence of a wide range of PCB-degrading microorganisms that can be found in the rhizosphere of *Brassica nigra*. In addition to the observed benefit of the rhizosphere effect, bioaugmentation with highly competent PCB-degrading bacteria also enhanced PCB removal to a similar or greater extent than that obtained with plants alone. On the other hand, further research should be conducted to explore whether certain plant species may be more effective than *Brassica nigra* for enhancing the removal of PCBs by improved gas diffusion, biostimulation of PCB degraders, and increased bioavailability of the contaminant.

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