Biodegrader Metabolic Expansion during Polyaromatic Hydrocarbons Rhizoremediation

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Root-microbe interactions are considered to be the primary process of polyaromatic hydrocarbon (PAH) phytoremediation, since bacterial degradation has been shown to be the dominant pathway for environmental PAH dissipation. However, the precise mechanisms driving PAH rhizostimulation symbiosis remain largely unresolved. In this study, we assessed PAH degrading bacterial abundance in contaminated soils planted with 18 different native Michigan plant species. Phenanthrene metabolism assays suggested that each plant species differentially influenced the relative abundance of PAH biodegraders, though they generally were observed to increase heterotrophic and biodegradative cell numbers relative to unplanted soils. Further study of > 1800 phenanthrene degrading isolates indicated that most of the tested plant species stimulated biodegradation of a broader range of PAH compounds relative to the unplanted soil bacterial consortia. These observations suggest that a principal contribution of planted systems for PAH bioremediation may be via expanded metabolic range of the rhizosphere bacterial community.

Key words: Rhizostimulation Symbiosis, Phenanthrene Degraders

Introduction

Polyaromatic hydrocarbons (PAHs) are a class of environmentally persistent pollutants with low aqueous solubility and a strong affinity for soil organic matter. Some PAH compounds are considered high priority pollutants by the US Environmental Protection Agency due to their tendency to bioaccumulate to hazardous concentrations at higher trophic levels (EPA, 2003). Extensive research has been conducted on microbial PAH degradation at the molecular, biochemical, and ecological levels (Cerniglia, 1993; Harayama, 1997; Wilson and Jones, 1993). Natural PAH biodegradation rates are highly dependent on PAH physicochemical properties, geochemical and other site environmental conditions, and microbial community structure (Shuttleworth and Cerniglia, 1995). Nonetheless, microbial biodegradation has been pursued as an attractive alternative to engineering-based approaches for remediation of PAH contaminated sites.

Phytoremediation, the use of plants to remove and/or detoxify environmental pollutants, is an emerging ecologically compatible remediation technology. Plants do not readily uptake hydrophobic pollutants such as PAHs into their root tissues (Briggs *et al.*, 1982), though they may contain or secrete enzymes potentially capable of transforming some aromatic pollutants (Schaffner *et al.*, 2002). Microbial metabolism is considered to be the primary mechanism for environmental PAH degradation (Heitkamp *et al.*, 1988; Reilley *et al.*, 1996). Numerous bacterial strains have been shown to utilize low molecular weight PAHs such as naphthalene, phenanthrene, and anthracene as a sole carbon source (Cerniglia, 1992; Kastner *et al.*, 1994). Environmental PAH biodegradation may be limited by the low abundance of PAH degrading bacteria typically found in contaminated soils (Kastner *et al.*, 1994). Laboratory studies demonstrated that root released nutrients and organic compounds enhance microbial cells numbers and PAH biodegradation (Daane *et al.*, 2001; Reilley *et al.*, 1996). Plants have been shown to accelerate endogenous biodegradation rates for various organic pollutants, including polychlorinated bi-

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phenyls and PAHs (Banks *et al.*, 2000; Donnelly *et al.*, 1994; Muratova *et al.*, 2003; Nichols, 1997). The role of plants for enhanced organic compound biodegradation likely derives from co-metabolic induction of bacterial degradation activity by plant secondary compounds, such as phenolics and terpenoids, found in root exudates (Fletcher and Hegde, 1995; Miya and Firestone, 2001; Singer *et al.*, 2003).

Different plant species produce vastly different amounts of root exudates with highly varied chemical composition, which are further influenced by habitat growing conditions and plant growth stage (Grayston *et al.*, 1998; Smalla *et al.*, 2001). Mulberry root phenolic compounds were shown to stimulate the growth of PCB (polychlorinated biphenyls) degrading bacteria (Hegde and Fletcher, 1996), which further doubled during root mortality at the end of the growing season (Leigh *et al.*, 2002). Conversely, root exudation may negatively impact xenobiotic degradation by preferential enrichment of non-biodegrading bacteria (Susilawati, 2003), production of antimicrobial compounds (Gleba *et al.*, 1999), or increased soil sorption reducing contaminant bioavailability (Jordahl *et al.*, 1997). Despite these well-studied phenomena, there remains limited understanding of many fundamental aspects of plant-microbe interactions during PAH phytoremediation.

The goal of this study was to observe the differential influence of a wide range of plant species on PAH biodegrader abundance and metabolic capability. A phytoremediation field experiment was conducted in a treatment facility constructed at the Allen Park Clay Mine (Allen Park, MI). This project utilized only plant species native to the Michigan-Great Lakes area in order to evaluate the potential for combined site remediation and original habitat restoration at regional brownfield sites. PAH-impacted soils were planted with a variety of individual plant species in mono-specific plots and monitored for PAH reduction under planted versus unplanted conditions (Wan, 2002). After two growing seasons of phytoremediation treatment, rhizosphere soil samples were collected and analyzed for total culturable and PAH biodegrader cell densities as well as metabolic range analysis of biodegrader isolates from various plant species vegetated and non-vegetated treatments.

Materials and Methods

Phytoremediation field site design

The phytoremediation field trial facility was constructed at the Allen Park Clay Mine (Allen Park, Michigan, USA) by URS Corporation (Willow Grove, Pennsylvania, USA) in the summer of 2000. Polyaromatic hydrocarbon (PAH) contaminated soils were obtained from the grounds surrounding the coke oven facility at the Ford Rouge Manufacturing Complex (Dearborn, Michigan, USA). Coke oven soils were amended with composted yard litter (10% v/v; Ypsilanti County, Michigan, USA) and composted poultry manure (5% v/v; Herbruck's Poultry Farm, Saranac, Michigan, USA) to produce a final volume of approx. 200 cubic yards of Rouge soil mix. The amended Rouge soil mix was distributed into an excavated pit (6.1 m × 30.5 m × ~ 0.6 m depth) lined with a 40 mil high-density polyethylene (HDPE) barrier sheet. The soil bed was overlaid by a grid of walk boards to divide the surface into ~ 1.5 m × 1.5 m plots, or "cells", to form the phytoremediation treatment study site. The phytoremediation treatment site contains 60 plots planted in September 2000 as follows: 54 plots were planted with 18 selected, native Michigan species; 3 plots left unplanted as treatment controls; and 3 plots were not used due to the presence of utility fixtures (Wan, 2002). Each vegetated cell was planted with 12 plugs of a single species with 3 replicate cells for each of the 18 different plant species (Table I). Selected plants were obtained from Wildtype Nursery (Mason, Michigan, USA) and included grasses, herbaceous dicots, and woody shrubs, all perennials with the exception of the biennial pasture thistle (*Cirsium discolor*). The treatment plot was fertilized with a 475 ppm N-P-K solution (Scott's, Marysville, Ohio, USA) bi-weekly for the first year (2001) and on two occasions during each of the 2002 growing season. Soil PAH levels in the treated soils were observed to be reduced ~ 25–40% across the various treatments one year after planting (Wan, 2002).

Soil sample collection for microbial analysis

Soil samples were collected from the phytoremediation field site in July 2002 or about midsummer of the second growing season. A 5.1 cm diameter soil probe was used to obtain 2 cores from 5–25 cm depth of each treatment plot. This depth was observed to be densely rooted for all

planted treatments at the time of sampling. Soil samples were transferred to 125cc amber jars and kept in coolers on ice in the field until storage in a 4 °C walk-in cold room. Soil samples were prepared for analysis by sieving with a stainless steel 20.3 cm diameter 2.36 mm sieve (Gilson Co., Worthington, Ohio, USA) to remove root and stone debris and combined to homogenize the core samples for a specific plot. Sieved samples were stored dark in 125cc amber jars with Teflon-lined caps at 4 °C. PAH contaminated soil was collected from the Rouge coke oven area and included in this study as an unamended, untreated control, except for sieving and storage at 4 °C.

Total soil bacterial and PAH biodegrader quantification

We utilized a spray plate assay technique for direct observation of PAH degrading bacterial colonies (Ahn *et al.*, 1999; Kiyohara *et al.*, 1982) in a separate composite soil for each of 20 different soil treatments, including 18 plant species, 1 unplanted, and untreated Rouge coke oven area soils. Soil bacteria were extracted by mixing 1.0 g of soil with modified SPP buffer: 0.1% tetrasodium pyrophosphate (w/v; Sigma, St. Louis, Missouri, USA), 0.1 mM EGTA (Sigma), 0.4 mM Tween20 (Bio-Rad Laboratories, Hercules, California, USA), 0.01% Bacto Peptone (w/v; Becton Dickinson, Sparks, Maryland, USA), and 0.007% yeast extract (w/v; Becton Dickinson). The soil suspension was extracted in conical centrifuge tubes containing 10 ml SPP using a vortex mixer set at maximum speed for 30 s, paused for 30 s, and re-vortexed for 30 s. Serial dilutions were prepared in SPP and plated at dilution levels to achieve ~ 150–200 colony forming units (CFU) per plate on YEPG agar plates adjusted to pH 8.0 to match the Rouge soil mix pH value. For each planted and unplanted treatment, twenty individual YEPG plates were inoculated with a 0.1 ml soil extract aliquot, incubated for 5 d at 25 °C, and visible colonies counted to calculate total CFU. For each soil treatment, 15 pre-grown plates (~ 120–200 colonies each) were sprayed in a fume hood cabinet with phenanthrene (Sigma) in acetone solution (1% w/v) using a thin layer chromatography (TLC) sprayer connected to the exhaust port of a vacuum pump (~ 20 psi). Phenanthrene sprayed plates were incubated for 5 d at 28 °C and then scored for phenanthrene degradation. Sprayed colonies producing clear rings in the cloudy phenanthrene residue, or "zone forming units" (ZFUs), were scored as PAH degrading bacteria (Bogardt and Hemmingsen, 1992; Kiyohara *et al.*, 1982). Soil extract CFU and ZFU values were obtained by multiplying plated colony counts by the extract dilution factor and soil moisture correction factor to obtain cell counts per g soil dry weight (*e.g.* CFU/g soil DW). Phenanthrene was used as the PAH substrate in this experiment for the following reasons: a) it generates an easily observable cloudy residue on the plates during spray application making cleared zones easy to observe; b) it has a relatively low vapor pressure (0.018 Pa at 25 °C), so does not evaporate too quickly, in contrast to naphthalene (Marlowe *et al.*, 2002); c) the phenanthrene chemical structure contains both bay- and K-regions and is subsequently used as a model compound for carcinogenic PAHs such as benzo[*a*]pyrene, benzo[*a*]-anthracene, and chrysene (Samantha *et al.*, 1999).

Biodegrader confirmation and broad-spectrum PAH screening

Approx. 2100 putative phenanthrene-degrading isolates from the soil bacteria quantification experiment were transferred to YEPG plates at ~ 25 isolates/plate, except for fast growing colony types (~ 5 isolates/plate). At least 100 bacterial isolates from each planted or unplanted treatment were replica patch-plated using sterile toothpicks on fresh YEPG plates. After 5 d incubation at 25 °C to allow colony development, replica patch-plates were tested using the Spray-Test for 6 different PAHs: phenanthrene (1% w/v), anthracene (1% w/v; Sigma), fluoranthene (1% w/v; Aldrich Chemical Co., Milwaukee, Wisconsin, USA), pyrene (0.5% w/v; Aldrich Chemical Co.), chrysene (0.5% w/v; Aldrich Chemical Co.; Milwaukee, Wisconsin USA), and benzo[*a*]pyrene (0.5% w/v; Fluka Chemical, Steinheim, Switzerland). Patch-plated cultures were sprayed with a single acetone-solubilized PAH compound and incubated at 28 °C. Phenanthrene-sprayed plates were scored for clear zone formation after 5 d incubation, while other PAH-sprayed plates were observed at 1, 2, or 4 weeks until clear zone development. CFU and ZFU calculations were conducted as previously described.

Statistical analysis of the data

For statistical analysis of broad-spectrum PAH metabolism between the different planted or unplanted treatments, we conducted comparisons between the species and the compounds for the total degrader percentages from the whole data set. In this analysis, the experimental design was considered to be a two-way factorial with the plant species and the compounds representing the two factors. Since a single observation for number of degraders was available at each species-compound combination, we assessed the significance of the main factor effects by using the interaction between the factors as an error term, hence assuming the interaction to be negligible. Problems with non-normality and unequal variances in the data were addressed by using the generalized linear model approach for count data with Poisson distribution and log link. The analysis was conducted using GLIMMIX macro of SAS (Littell *et al.*, 1996).

Results and Conclusion

Soil microbial analysis

A survey of soil microbes from the 18 different plant species treatments in the phytoremediation field study displayed varied numbers for both total heterotrophic bacteria (CFU) and phenanthrene-degrading ($Phen^D$) bacteria (ZFU) (Table I). In general, most planted treatments possessed higher CFU counts relative to the unplanted treatment. CFUs in planted treatments ranged from 89.7×10^6 to 163.4×10^6 with CFUs for the unplanted treatment 97.7×10^6 and untreated Rouge soil 57.0×10^6. The numbers of $Phen^D$ ZFUs in planted treatments ranged from 1.7×10^6 to 13.7×10^6, the ZFU value for the unplanted treatment was 4.9×10^6 and untreated Rouge soil was 3.9×10^6. The relative abundance of $Phen^D$ bacterial cells (% ZFU/CFU) was quite variable among the treatments (1.8 to 9.3%), with the unplanted soil treatment (5.0%) near the mean ratio value and the

Table I. Soil treatments and preliminary bacterial density quantification in the phytoremediation field study plot. Shown are the species and common names for each treatment. Trmt ID is the abbreviated planted or unplanted treatment identification used in figure labeling and some text references. Heterotrophic (CFU) and phenanthrene degrader (ZFU) bacterial cell densities are shown for soil samples from phytoremediation study plots or from coke oven soil (UC). CFU, colony forming units ($\times 10^6$) per g soil dry weight; ZFU, zone forming units ($\times 10^6$) per g soil dry weight; % ZFU, ZFU/CFU $\times$ 100. High and low values for each bacterial density category are shown in italic type in respective columns.

Planted treatments	Common name	Trmt ID	CFU (10^6)	ZFU (10^6)	% ZFU
Amorpha canescens	leadplant	AC	125.0	5.5	4.4
Andropogon gerardii	big bluestem	AG	89.7	2.4	2.7
Andropogon scoparius	little bluestem	AS	96.5	*1.7*	*1.8*
Aster novae-angliae	New England aster	AN	106.8	5.1	4.8
Carex sprengelii	sprengel sedge	CS	117.8	2.6	2.2
Ceanothus americanus	New Jersey tea	CA	109.2	7.9	7.3
Cirsium discolor	pasture thistle	CD	89.9	3.5	3.9
Eupatorium perfoliatum	boneset	EE	118.5	5.9	5.0
Eupatorium purpureum	joe-pye weed	EU	106.3	9.4	8.9
Geum triflorum	prairie smoke	GT	147.9	*11.9*	8.0
Hystrix patula	bottlebrush grass	HP	116.0	10.8	*9.3*
Lobelia cardinalis	cardinal flower	LC	93.8	2.2	2.4
Physocarpus opulifolius	ninebark	PO	115.8	7.8	6.7
Scirpus atrovirens	green bulrush	SA	93.0	3.6	3.8
Silphium teribinthinaceum	prairie-dock	ST	96.3	7.4	7.7
Spartina pectinata	prairie cordgrass	SP	109.7	5.9	5.4
Spirae alba	meadowsweet	SL	109.3	6.9	6.3
Viburnum dentatum	arrowwood viburnum	VD	*163.4*	13.7	8.4
Control treatments					
Unplanted control	unplanted	UT	97.7	4.9	5.0
Untreated control	untreated coke oven	UC	*57.0*	3.9	6.9

percentage of degraders in the Rouge soil relatively high (6.9%).

Positive identification and isolation of phenanthrene degrading bacteria required a secondary, confirming spray test, which eliminated approx. 10% false positives of the putative degrader isolates from the primary analysis. This result was expected given the densely plated primary spray test plates making precise ZFU isolation difficult. These over-crowded plates were from less diluted extracts, which were not used in the primary CFU and ZFU calculations (shown in Table I).

Broad-spectrum PAH biodegrader analysis

Concurrent with 2° $Phen^D$ confirmation testing, replica patch plates of the 1° $Phen^D$ isolates were also tested via spray-test analysis for other PAH compounds, including anthracene, fluoranthene, pyrene, chrysene, and benzo[*a*]pyrene. All 1° $Phen^D$ isolates found to be negative in the 2° $Phen^D$ screen were also observed to be negative in spray tests for the other tested PAH compounds. The sets of $Phen^D$ isolates from each of the specific planted and unplanted treatments possessed widely different biodegrader numbers for the other PAH compounds (Fig. 1). Only 13.1% of the $Phen^D$ isolates were observed to degrade the other tested 3-ringed PAH compound, anthracene. All but one set of planted treatment $Phen^D$ isolates (ranging from 4.9 to 29.7%) were observed to have higher percentages of anthracene degrader ($Anth^D$) colonies than for the unplanted treatment (5.8% $Anth^D$) and all phyto study treatments had higher $Anth^D$ values than the untreated soil extract isolates (2.2% $Anth^D$). In spray tests with fluoranthene, a heterocyclic, 4-ringed compound containing a bay-region structural motif, 22.1% of the $Phen^D$ isolates were observed to also be capable of fluoranthene degradation ($Flra^D$). $Flra^D$ numbers ranged from 5.9 to 46.2% of tested $Phen^D$ colonies among the different plant species, compared to 18.6% $Flra^D$ for the unplanted and 2.2% $Flra^D$ of untreated soil colonies. Approx. 20% of the $Phen^D$ isolates were observed to degrade pyrene, a 4-ringed, clustered PAH containing K-region structural motifs. Pyrene degrader ($Pyre^D$) values ranged from 4.5 to 42.9% for planted treatment $Phen^D$ isolate sets, versus 17.4% $Pyre^D$ and 3.3% $Pyre^D$ of the unplanted and untreated soil isolates, respectively. In spray assays with chrysene or benzo[*a*]pyrene very few spray positive colonies (~ 0.1% each) were observed among the $Phen^D$ isolates (data not shown). For the three PAH compounds displaying substantial numbers of spray assay positives from the $Phen^D$ bacteria (*i.e.* $Anth^D$, $Flra^D$, and $Pyre^D$), the most frequent broad-spectrum PAH degrader isolates were always from the cardinal flower (*L. cardinalis*) treated soils (LC) and the most limited-spectrum ($Phen^D$-only) isolates were from the untreated Rouge soil (UC) (Table II).

Plant species observed to enrich for broad-spectrum PAH biodegraders among the $Phen^D$ isolates relative to unplanted soils were *A. gerardii* (big bluestem), *A. novae-angliae* (New England aster), *C. sprengelii* (sprengel sedge), *C. americanus* (New Jersey tea), and *L. cardinalis* (cardinal flower) (Table II). Compared to an overall

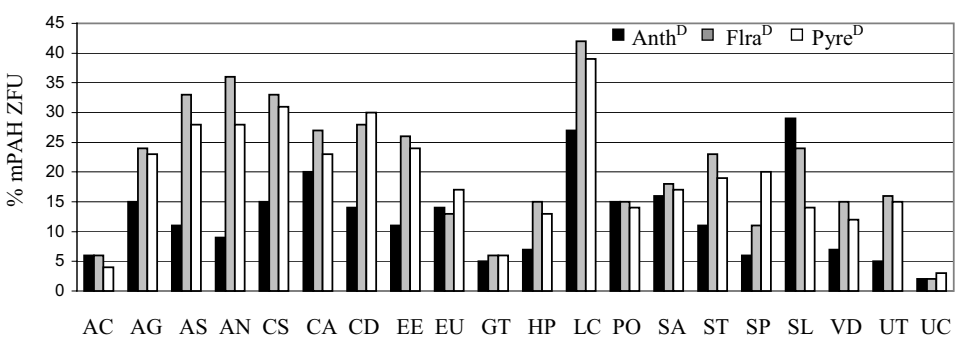

Fig. 1. Percentage of $Phen^D$ bacterial isolates from each of the planted and unplanted soil treatments capable of metabolizing multiple PAH compounds (% mPAH ZFU). The specific treatments represented by the 2-letter IDs along the *x*-axis are listed in Table I. Data bars represent the percentages of specific treatment pools of $Phen^D$ isolates capable of degrading anthracene ($Anth^D$, black bar), fluoranthene ($Flra^D$, gray bar), or pyrene ($Pyre^D$, white bar) in spray test assays.

Treatments	ZFU cell density		Statistical tests		
Planted	Phen^D ZFUs (Total 1869)	% mPAH^D	A	B	C
A. canescens	88	9.1	abc		
A. gerardii	77	44.2	ef	*	* *
A. scoparius	85	31.8	cdef	*	
A. novae-angliae	87	43.7	ef	*	* *
C. sprengelii	96	35.4	def	*	*
C. americanus	91	31.9	cdef	*	
C. discolor	94	34.0	def	*	*
E. perfoliatum	94	29.8	bcdef	*	
E. purpureum	101	20.8	bcdef	*	
G. triflorum	102	7.8	ab		
H. patula	103	15.5	abcd		
L. cardinalis	91	49.5	f	*	* *
P. opulifolius	100	18.0	abcde	*	
S. atrovirens	91	26.4	bcdef	*	
S. teribinthinaceum	98	28.6	bcdef	*	
S. pectinata	94	22.3	bcdef	*	
S. alba	107	30.8	cdef	*	
V. dentatum	86	19.1	abcde	*	
Controls					
Unplanted soil	90	19.8	abcde	*	
Untreated Rouge	94	3.3	a		
	Mean	25.8			

Table II. Multiple PAH degrader isolates. Shown are the percentages of phenanthrene degrader isolates (Phen^D ZFUs) observed to be capable of metabolizing at least one or more additional PAH compounds (% mPAH^D), *i.e.* Anth and/or Phen and/or Flra. Statistical analyses were performed as described in Materials and Methods. Column A shows different letters (a–f) for treatment total mPAH^D values shown to be different from other treatments ($p = 0.05$), *i.e.* same letters indicate statistically similar values. Column B shows treatments with total mPAH^D values greater than untreated Rouge soil (*$p = 0.05$). Column C shows treatments with total mPAH^D values greater than unplanted soil (**$p = 0.05$; *$p = 0.15$).

average of 25.8% multiple PAH^D colonies for all bacterial Phen^D isolates, the broad-spectrum PAH metabolic capabilities of the isolates from the unplanted soils were relatively limited (19.8%) and extremely restricted for untreated Rouge soil isolates (3.3%). A vast majority (15 of 18) planted treatments as well as the unplanted, composted control soil treatment enhanced the PAH biodegradative range relative to the bacterial consortia from the unamended, unplanted Rouge soil material (Table II, Column B). The non-vegetated soil treatment displayed superior microbial enrichment compared to some of the planted treatments in addition to the untreated Rouge soil. The increased cell counts and metabolic range of the unplanted soil consortia was likely due to the addition of 15% (v/v) organic compost to the phytoremediation test soils, including the unplanted cells. Compost amendments have been shown to effectively enhance *in situ* PAH bioremediation treatments (Haderlein *et al.*, 2001). However, composting alone does not provide the ancillary benefits of hydraulic control, erosion mitigation, or soil aeration gained from vegetative cover. In addition, some composts and biosolids may possess other contaminants, including pesticides (Lee *et al.*, 2003), polyaromatics and biphenyls (Beck *et al.*, 1996), which should be determined prior to use in remediation applications.

Possible mechanisms of rhizosphere-enhance bioremediation

Notable among the planted treatments was the very high metabolic versatility demonstrated by the bacterial consortia from the *L. cardinalis* planted soils, despite this species' low phenanthrene degrader cell numbers relative to the other plant species in the initial survey. This observation indicates that phenanthrene is likely not the "preferred" metabolic substrate for analysis of all planted rhizosphere communities. It may be that *L. cardinalis* root systems, for example, secrete secondary compounds that stimulate bacterial cometabolism of a different PAH compound(s), which would only be evident if that compound rather than phenanthrene was utilized in the primary spray test. Since the bacterial isolate pools in this experiment were initially selected for phenanthrene metabolism, it would be informative to conduct primary screens of the soil bacterial extracts with a diverse array of different PAH compounds to gain a more comprehensive understanding of biodegrader diversity. Use of a single, "model" compound, such as phenanthrene, limits

characterization of the soil PAH biodegrader community to a subset of the full PAH metabolic capability. To diagnose the full degradative potential of the culturable soil bacterial consortia, an extensive matrix of a number of polycyclic aromatic hydrocarbon compounds should be tested.

It is not clear how plants differentially enhance the metabolic range of the soil bacterial consortia. Plants have been previously demonstrated to stimulate bacterial cell numbers and biodegradative capabilities against target PAH compounds and mixtures (Corgie *et al.*, 2003; Fiorenza *et al.*, 2000; Liste and Alexander, 2000; Muratova *et al.*, 2003). PAH rhizosphere-assisted degradation has been typically characterized at the bacterial community level, with limited analysis of the metabolic capabilities of representative pools of isolates from planted soils. Wetland plant species were shown in one study to increase the diversity and range of a very limited number of PAH degradative microbes from vegetated sediments (Daane *et al.*, 2001). Rhizostimulation may enhance microbial activation of broad-range PAH dioxygenase enzymes or alternatively for multiple genes encoding narrow-range dioxygenases (Hamann *et al.*, 1999). In addition, bacterial PAH dioxygenase genes are found on plasmids or chromosomes (Herrick *et al.*, 1997). Specific rhizosphere environments may promote horizontal transfer of PAH degrader plasmid elements, proliferation of degrader-competent cells, or a combination of these two means of enhancing biodegrader abundance.

Plants may influence the prospective bacterial community in a variety of ways during phytoremediation of persistent organic contaminants. We analyzed a suite of native Michigan plant species in an experimental phytoremediation field trial as part of a strategy for concurrent native habitat restoration and soil phytoremediation at a PAH impacted brownfield facility. We examined nearly 2000 individual bacterial isolates from 20 different soil treatments to elucidate the vegetative influence on the biodegradative capability of the rhizosphere bacterial community across a range of PAH compounds. In this study, we observed that plants generally enhanced both PAH biodegrader cell numbers and metabolic range in contaminated soils relative to unplanted or untreated soils. Of particular interest were plant species that failed to increase total heterotrophic or phenanthrene biodegrader cell counts, though they did enhance broad-spectrum PAH metabolism among the selected soil bacterial consortia. The exceptional metabolic versatility observed for the *L. cardinalis*-treated soil isolates in spite of their low abundance in the initial phenanthrene assay suggests that the standard spray test screening method may not fully characterize the range of rhizosphere response of the PAH degrading bacterial consortia. Additional biochemical and physiological analyses are warranted to better understand and subsequently exploit beneficial plant-microbe interactions for effective *in situ* PAH phytoremediation applications.

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