Bioremediation and Biodegradation

Enhanced Phenanthrene Biodegradation in Soil by Slender Oat Root Exudates and Root Debris

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ABSTRACT

To investigate the mechanisms by which slender oat (*Avena barbata* Pott ex Link) enhances phenanthrene biodegradation, we analyzed the impacts of root exudates and root debris on phenanthrene biodegradation and degrader community dynamics. Accelerated phenanthrene biodegradation rates occurred in soils amended with slender oat root exudates as well as combined root debris + root exudate as compared with unamended controls. Root exudates significantly enhanced phenanthrene biodegradation in rhizosphere soils, either by increasing contaminant bioavailability and/or increasing microbial population size and activity. A modified most probable number (MPN) method was used to determine quantitative shifts in heterotrophic and phenanthrene degrader communities. During the first 4 to 6 d of treatment, heterotrophic populations increased in all amended soils. Both root debris–amended and exudate-amended soil then maintained larger phenanthrene degrader populations than in control soils later in the experiment after much of the phenanthrene had been utilized. Thus, root amendments had a greater impact over time on phenanthrene degraders than heterotrophs resulting in selective maintenance of degrader populations in amended soils compared with controls.

While a number of studies have shown that plants enhance biodegradation of a variety of soil and water contaminants (Anderson et al., 1993; Flathman and Lanza, 1998; Schnoor et al., 1995; Aprill and Sims, 1990; and others), the mechanism(s) involved remain poorly understood. Application of plant-enhanced bioremediation on a large scale as an effective and reliable remediation option requires a reasonable understanding of how plant roots interact with the microbial community of degraders responsible for contaminant biotransformation in soil. The current study assesses the impact of raw root debris and water-soluble root exudates on phenanthrene biodegradation rates and degrader community dynamics with the intent to elucidate how plants impact contaminant biodegradation in petroleum-impacted soils.

Plants supply soil and root-associated microbes with soluble exudates that increase microbial numbers and activity (Curl and Truelove, 1986). Certainly one way roots could influence the activity of microorganisms responsible for degrading soil contaminants is through root exudation. One study investigated hybrid poplar-enhanced atrazine biodegradation in soil microcosms and found that the addition of root exudates caused a mineralization enhancement of 23% compared with acetate-fed controls (Burken and Schnoor, 1996). Parathion mineralization was also stimulated in soils amended with root exudates, but greater stimulation resulted from the presence of a growing bean plant (*Phaseolus vulgaris* L.) (Hsu and Bartha, 1979). Root exudates potentially supply microbes with C, N, P, and/or other micronutrients required for growth of contaminant degraders. Root exudates may also supply primary C substrates for organisms that carry out cometabolic biotransformation (Haby and Crowley, 1996; Donnelly et al., 1994). However, 2,4-dichlorophenoxyacetic acid mineralization was inhibited in a soil continuously supplied with a synthetic root exudate solution compared with unamended soil, indicating that exudates can be preferentially utilized for growth over a contaminant (Kunc and Rybárová, 1989) or serve as competitive inhibitors.

Another way that plants potentially influence contaminant biodegradation is through the introduction of decomposing root material. Decaying root debris contributes significantly to increased soil C availability in rhizosphere soil and its specific role in plant-enhanced bioremediation is unknown. Increased microbial biomass and activity are characteristic of the commonly observed *rhizosphere effect* in planted soils independent of contaminant biodegradation (Curl and Truelove, 1986). Increased atrazine mineralization was observed in the presence of decomposing corn roots following shoot harvest and was attributed to increased microbial biomass (Seibert et al., 1981). The addition of ground hybrid poplar roots produced a 165% increase in atrazine mineralization (Burken and Schnoor, 1996), but the effect was primarily attributed to the fact that hybrid poplar root tissues contain enzymes that have the capability to dehalogenate and degrade atrazine (Schnoor et al., 1995). Compounds that are structurally analogous to select contaminants occur in plant cell wall components, senesced root tissues, and secondary root decomposition products, potentially stimulating appropriate enzymatic pathways for degradation. For example, n-alkanes in plant waxes (Reynhardt and Riederer, 1994) and phenanthrene derivatives such as retene and nudol (Bhandari et al., 1985) are aliphatic and aromatic hydrocarbons that occur naturally in plant materials. Whether or not these compounds are present in rhizo-

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Abbreviations: MPN, most probable number; PAH, polycyclic aromatic hydrocarbon; TOC, total organic carbon; TSB, trypticase soy broth; HPLC, high performance liquid chromatography; GC-FID, gas chromatography–flame ionization detector.

sphere soil in quantities sufficient to prime specific biodegradation activities or to select for degradation capacity is not known.

Few studies have investigated polycyclic aromatic hydrocarbon (PAH) biodegradation in rhizosphere or root exudate–amended soils. Increased pyrene mineralization was observed in an alfalfa (*Medicago sativa* L.) rhizosphere soil amended with organic acids compared with water-irrigated rhizosphere and bulk soils (Reilley et al., 1996). No significant mineralization enhancement was detected between water-irrigated rhizosphere and bulk soils. Pyrene biodegradation enhancement in rhizosphere soil was dependent on a combined 15 μM succinic acid and 10 μM formic acid amendment. We have selected phenanthrene as our model PAH in the current study.

Adsorption of PAHs to plant roots may occur resulting in an apparent biodegradation enhancement in root-amended and rhizosphere soils. The affinity of PAHs to root material depends on plant species and the PAH in question. Partitioning of PAHs from soil to roots is possible, but the very high octanol–water coefficients and low solubilities of most PAHs suggest that roots would have to be in very close proximity to soil adsorption sites before transfer to the root surface could be made (Schwab et al., 1998). A negligible amount of benzo[a]pyrene (0.12%) was taken up by roots from contaminated soil planted with tall fescue (Banks et al., 1999), while <0.03% of anthracene and pyrene soil contaminants accumulated in alfalfa, fescue (*Festuca arundinacea* Schreb), sudangrass [*Sorghum vulgare* L.], and switchgrass (*Panicum virgatum* L.) plant biomass (Reilley et al., 1996).

Enrichment of microbial degraders in rhizosphere soil has been previously reported in a number of different studies. Microorganisms capable of utilizing chlorobenzoate either as a sole C source or via cometabolism were present in numbers 40 times higher in soils planted with ryegrass (*Lolium multiforum* Lam. × *Lolium perenne* L.) than in nonrhizosphere soils (Haby and Crowley, 1996). Despite the reported inhibition of 2,4-D (2,4-dichlorophenoxyacetic acid) mineralization, the proportion of contaminant degraders was 16.7% larger in soil supplied with synthetic root exudate solution compared with unamended soil (Kunc and Rybárová, 1989). Soils planted with alfalfa and alpine bluegrass (*Poa alpina* L.) were significantly enriched with microbial populations that utilized a mixture of organic chemicals including phenanthrene (Nichols et al., 1997). In earlier work we documented selective enrichment of phenanthrene degraders as well as a less-diverse degrader population in soil planted with slender oat (*Avena barbata* Pott ex Link) compared with unplanted controls (Miya and Firestone, 2000).

In the current study we partition the impact of slender oat roots into root exudates and root debris components and assess the role of each component by analyzing amended and unamended soil over time. We compare phenanthrene biodegradation rates in root exudate-amended, root debris–amended, exudate + debris–amended, and an unamended control soil to determine which root component(s) are responsible for enhanced phenanthrene biodegradation in slender oat rhizosphere soil. We also quantify the effects of each component on numbers of heterotrophic bacteria and phenanthrene degraders. By determining which root component(s) are responsible for enhanced phenanthrene biodegradation and phenanthrene degrader enrichment in slender oat rhizosphere soil, we hope to provide insight into the mechanisms responsible for plant-enhanced bioremediation of PAHs in soil.

MATERIALS AND METHODS

Soil

Soil was obtained in April 1999 from a northern California Bay Area oil refinery. Upon sampling, the soil was taken immediately to the laboratory and sieved through a 6.35-mm (0.25-inch) mesh, removing the larger rocks, and stored at 4°C until phenanthrene addition. Chemical characteristics on the <2-mm fraction have already been described previously (Miya and Firestone, 2000).

Avena Root and Root Exudate Collection

Slender oat plants were grown in minirhizotrons (Jaeger et al., 1999) filled with soil collected from the University of California Hopland Research and Extension Center. Plants were watered every other day and fertilized with half-strength Peters Professional All-Purpose Plant Food (Grace Sierra Horticultural Products Co., Milpitas, CA) once a week. The roots were harvested after 8 wk in a growth chamber with a 14-h photoperiod at approximately 450 μmol photons/m^2-s and 15 to 20°C. Roots were separated from the soil by three consecutive washes in deionized water. Collected roots were then stored at 4°C in a sealed plastic bag. Roots were cut into 1- to 2-cm sections before being added to phenanthrene impacted soil for root debris–amended and root debris + exudate–amended treatments. Root samples (n = 5) were dried at 60°C for 24 h, and ground using a ball and mill apparatus for 24 h in preparation for C and N analyses using an Automated Nitrogen Carbon Analyzer–Isotope Ratio Mass Spectrometer (Europa Scientific, Crewe, UK). Root P was determined using a modified Kjeldahl digestion on the ground root material (Parkinson and Allen, 1975) and measured using a Thermo Jarrell Ash IRIS HR inductively coupled radial plasma–optical emission spectrophotometer (Thermo Jarrell Ash Corp., Franklin, MA).

Sterile oat root exudates were collected from hydroponically grown plants. Seeds were surface-sterilized after removal of the seed husk in 50% ethanol for 1 min followed by immersion into a 10% bleach solution for 5 min and finally rinsed in sterile deionized water for 1 min. Seeds were then placed in petri plates with wetted filter paper (Whatman no. 42) and allowed to germinate for 5 d under incandescent light. Sprouted seeds were then placed into previously autoclaved 25-mL Erlenmeyer flasks containing 5 mL of 0.22-μm filter-sterilized (Corning, Corning, NY) half-strength Hoagland's solution. Each plant shoot was allowed to grow through a bored foam stopper (no. 1 septa corer size hole) at the top of each flask while water-soluble root exudates were collected in the bottom for 14 d under incandescent light. The exudate solution was then filter-sterilized through an Acrodisc 0.2-μm sterile syringe filter (Gelman Sciences, Ann Arbor, MI) in a laminar flow hood and stored at 4°C in solvent-rinsed and autoclaved amber bottles until use. The amino acid content of the root exudates was estimated using a meso-diaminopimelic acid assay (Daniels et al., 1994) while a phenol–sulfuric acid method (Ashwell 1966; Dubois et al., 1956) was used to estimate carbo-

hydrate concentration. Total organic carbon (TOC) in the root exudate solution was analyzed using a 1010 Wet Oxidation TOC analyzer (OI Analytical, College Station, TX). Both dissolved organic nitrogen (DON) content after Kjeldahl digestion and nitrate content in the exudate solution was analyzed on a LACHAT (LACHAT Instruments, Milwaukee, WI).

Phenanthrene Addition, Extraction, and Measurement

Phenanthrene addition, extraction, and measurement have been previously described (Miya and Firestone, 2000) and are summarized here. Soil was spread into a stainless steel tray and phenanthrene dissolved in hexane was then aseptically added (100 mg/kg final) using a solvent-rinsed 10-mL glass syringe equipped with a 0.2-μm syringe filter and a 25 gauge 1.6-cm (5/8-inch) needle. Soil samples were mixed using a stainless steel spatula and then spread into another stainless steel tray for 60 min in a laminar flow hood to ensure hexane volatilization. A total of 15 kg of phenanthrene-spiked soil was prepared for this experiment. Half of the phenanthrene-spiked soil was then amended with 2.4 g/kg (oven-dried root mass/oven-dried soil mass) of slender oat root debris/250 g soil by spreading and mixing in a stainless steel tray. The root density employed was designed to mimic densities previously observed after 20 d of growth in minirhizotron experiments conducted in our laboratory (data unpublished) and is similar to that typically observed in the field.

Approximately 400 g of phenanthrene-spiked soil was then placed into each of 18, 150-mm diam. Pyrex glass funnels (Corning, Corning, NY) with 0.5 g of glass wool placed on the bottom. Eighteen additional funnels were each filled with 400 g of root debris–amended and phenanthrene-spiked soil. All soils were then watered to field capacity and maintained at the Oxford Tract greenhouse facility (Univ. of California, Berkeley). After 24 h, nine of the root debris–amended funnels were also amended with four 1-mL aliquots of root exudate solution becoming root debris + root exudate treated, as well as nine of the soil-only funnels, becoming the root exudate treatments. Funnels were then watered or amended with root exudates every other day. At t = 2, 4, 6, 8, 10, 13, 14, 16, and 20 d, one funnel from each treatment (unamended, root debris–amended, exudate-amended, root debris + exudate–amended) was taken to the laboratory for analysis. Triplicate soil samples were collected from each funnel using a no. 9 septa corer (1.5 cm diam. by 6 cm height).

The design of each funnel was intended to preserve as much of the experimental design that was implemented in earlier whole-plant experiments. Each rhizosphere treatment funnel in previous experiments was planted with four slender oat plants. Rhizosphere samples were collected from three of the four planted locations (one sample per plant), immediately beneath the slender oat shoot to the bottom of the funnel using a no. 9 septa corer. In the current experiment, all exudate-amended funnels were amended with the exudate solution at precisely the same four locations every other day (1 mL of exudate solution at each location), in our best attempt to imitate four actively exuding plants at these locations. Each funnel was marked with four pieces of tape equidistant from each other on the funnel rim. One mL of exudate solution was applied to each of the four specific soil locations [2.54 cm (1 inch) from the funnel–soil edge toward the center from each of the four pieces of tape] every other day. Soil samples were obtained from three of these four treated soil locations and extended to the bottom of the funnel using the no. 9 septa corer in an attempt to harvest similar volumes and encompass comparable variability. The standard deviation of the three treatment replicates per time point represented the variability associated with uneven distribution of exudates and/or root debris between sampling locations. Preservation of the experimental design implemented in earlier whole-plant experiments allowed for a valid comparison with the current study.

The phenanthrene extraction procedure was modified from USEPA method 3550. Triplicate samples from each time point were extracted with methylene chloride and phenanthrene was quantified using GC-FID (Hewlett-Packard, Avondale, PA). One solvent blank per time point was carried out to ensure glassware cleanliness and solvent purity while calibration checks of known concentration were run intermittently at every 10th sample.

Phenanthrene biodegradation rates were calculated using two methods. Maximum linear biodegradation rates (mg/kg per day) were obtained by conducting linear regression analysis on selected data points from each treatment over 6 to 8 d, during which phenanthrene was most rapidly degraded. First order phenanthrene biodegradation constants (k) were also obtained for each treatment using nonlinear regression analysis fitting all data points to the following equation:

$$C_t = C_o \exp(-kt)$$

where C_t is the concentration of phenanthrene (mg/kg) at time t (d), C_o is the initial phenanthrene concentration (mg/kg), and k (1/d) is the first order phenanthrene biodegradation constant.

Most Probable Number Assay

A phenanthrene-degrader MPN assay was modified from the method described by Wrenn and Venosa (1996) and previously described (Miya and Firestone, 2000). For heterotrophic population estimates, 400 μL of 10% strength Trypticase Soy Broth (TSB) was used as the growth medium. Treatment to control soil ratios were calculated at individual time points for both phenanthrene degraders and heterotrophic populations.

Chemicals

Phenanthrene used in the following experiments was >96% pure [high performance liquid chromatography (HPLC)] from Sigma Chemical Co. (St. Louis, MO) while optima grade hexane and methylene chloride stabilized with amylene was purchased from Fisher Scientific (Fair Lawn, NJ). All glassware and materials in contact with petroleum hydrocarbons were solvent rinsed with certified ACS methanol (Fisher Scientific, Fair Lawn, NJ) before use.

Statistical Analyses

Statistically significant differences ($P < 0.05$) between unamended, root debris–amended, exudate-amended, and root debris + root exudate–amended soil phenanthrene concentrations and population sizes at individual time points was determined using a series of two-tailed student t-tests. Significant differences between phenanthrene biodegradation rates and first-order biodegradation rate constants between treatments were determined using an analysis of covariance.

RESULTS

Table 1 presents the characteristics of the slender oat root debris and root exudates. The exudate amendments added 0.54 mg C/kg soil and 1.7 mg N/kg soil every other day. These values correspond to total additions

Table 1. Slender oat root debris and root exudate characteristics.

Characteristic	Value ± SD	Units
Roots		
C	35.1 ± 0.5	%
N	1.15 ± 0.26	%
C/N ratio	30.5	–
P	0.36 ± 0.05	%
Root exudates		
Total organic C (TOC)	53.8 ± 1.6	g/L
Total N	169 ± 7.5	g/L
Carbohydrates	18.4 ± 2.5	g/L†
Amino acids	14.2 ± 3.7	g/L‡

† Glucose equivalent carbohydrates.
‡ Meso-diaminopimelic acid equivalent amino acids.

of 4.9 mg C/kg soil and 15.2 mg N/kg soil over the course of the 20-d experiment. The residual half-strength Hoagland's solution from the hydroponic solution contained a significant amount of inorganic N. The amount of C added as root exudate over the course of the 20-d experiment is comparable to the amount of labile C detected in available C determinations of rhizosphere soil from slender oat–planted field soils (V. Eviner, personal communication, 2001). The amount of C and N added in the form of root debris at the beginning of the experiment was 842 mg C/kg soil and 27.6 mg N/kg soil, respectively. In preceding experiments analyzing intact rhizospheres (data not shown) we found comparable root densities (2.40 ± 0.21 g root/kg soil) after 20 d of plant growth.

Table 2 is a summary of phenanthrene biodegradation rates expressed as the maximum linear biodegradation rate (mg/kg per day) and first-order biodegradation rate constant (k). Phenanthrene biodegradation rates from a fully functioning slender oat rhizosphere soil and an unplanted bulk soil from earlier experiments are included in Table 2 for comparison (Miya and Firestone, 2000). In earlier whole-plant studies we used the same incubation chambers and location, plant species, and soil from the same site.

Based on the maximum linear biodegradation rates, only the combined debris + exudate–amended soil differed significantly from the control. However, when rate constants are analyzed using the first-order biodegradation model, both the exudate-amended soil and the combined debris + exudate–amended soil degraded phenanthrene significantly faster than the control. Further analysis of first-order biodegradation rate constants showed that the combined debris + exudate–amended soil degraded phenanthrene significantly faster than soil amended with only debris.

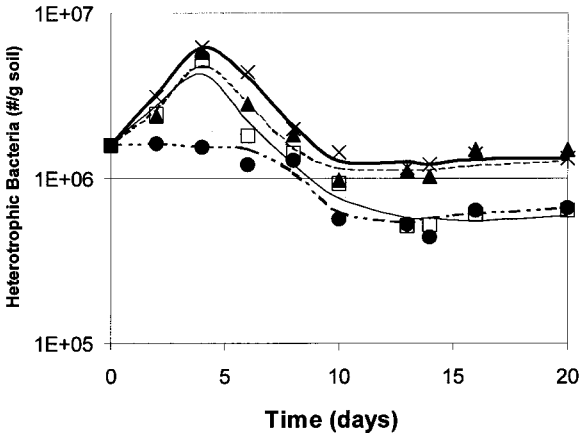

Fig. 1. Numbers of heterotrophic bacteria over time in control (*circle*), root debris–amended (*square*), exudate-amended (*triangle*), and combined debris + exudate–amended soils (X). LSD = 1.18×10^6.

Figure 1 shows heterotrophic population numbers with time after the addition of root material. All plant-derived amendments resulted in heterotrophic population increases during the first 4 d, while heterotrophic numbers in the control soil remained stable. The numbers of heterotrophic bacteria in all amended soils then decreased after Day 4; numbers in exudate-amended and the combined debris + exudate–amended soils decreased to levels comparable to the initial heterotrophic population size, but remained significantly higher than control and debris-amended soils ($P < 0.05$) for the duration of the experiment.

Figure 2 shows phenanthrene degrader populations and corresponding soil phenanthrene concentrations plotted over time. Phenanthrene degraders in all treatments increased rapidly during the first 6 d from an initial population size of 6.83×10^4. The degrader population in the control soil then decreased to a significantly lower stable level than degrader populations in amended soils. Phenanthrene biodegradation was most rapid in the combined debris + exudate–amended soil (Table 2).

Comparison of populations in amended soils with those in control soil reveals the impact of an amendment on heterotrophic and degrader populations. Thus, we used ratios of bacterial numbers in amended and control soils to exhibit the effects of root amendments on the dynamics

Table 2. Summary of biodegradation rates, rate constants, coefficients of determination (r^2), and the amount of time necessary for at least 75% of the added phenanthrene to be degraded in each treatment.

Treatment	Linear regression		Nonlinear regression		Time until ≥75% phenanthrene biodegraded
	Biodeg rate†	r^2	Rate constant k†	r^2	
	mg/kg per day		day^{-1}		days
Control	6.23A	0.70	0.112a	0.92	15
Root debris	9.07AB	0.81	0.133ab	0.94	12
Exudate	9.11AB	0.79	0.150bc	0.91	10
Debris + exudate	12.93B	0.90	0.187c	0.91	9
Rhizosphere‡	15.52	0.97	0.250	0.86	8
Bulk soil‡	10.68	0.98	0.116	0.91	15

† Different letters indicate differences ($P < 0.05$) between treatments.
‡ Data calculated from results presented in Miya and Firestone, 2000.

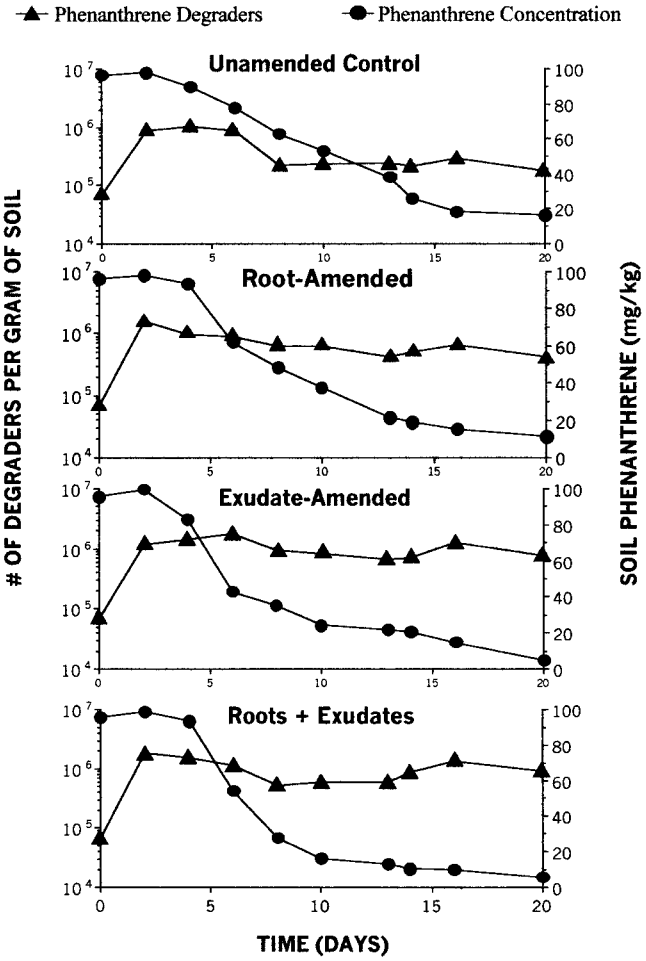

Fig. 2. Phenanthrene degrading bacteria (LSD = 4.21 × 10^5) and corresponding soil phenanthrene concentrations over time.

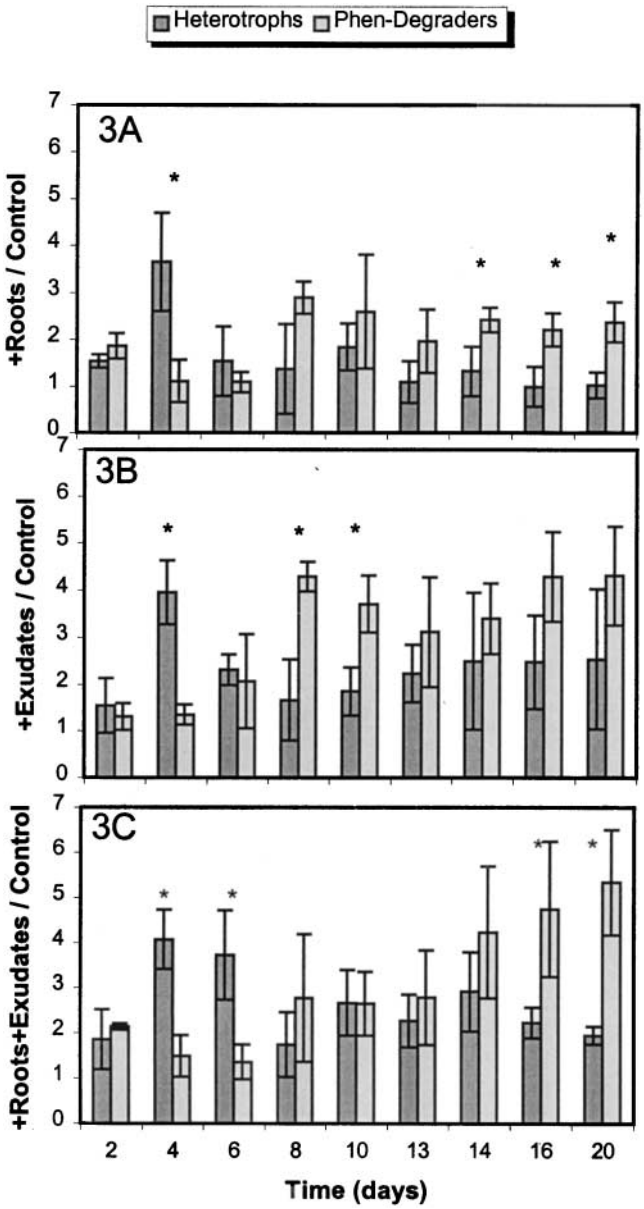

Fig. 3. Ratios of populations in amended and control soils over time for heterotrophic and phenanthrene-degrader communities in (*A*) debris-amended, (*B*) exudate-amended, and (*C*) debris + exudate–amended soils. *$P < 0.05$.

of heterotrophs and phenanthrene degraders. Figures 3A, 3B, and 3C show the population ratios of amended to control soils over time. Within 4 d of root amendment addition, heterotrophic numbers increased significantly compared with controls, while phenanthrene degraders remained stable. After prolonged exposure to the root amendments, phenanthrene degraders became enriched compared with control soils. The largest effects are seen in the combined debris + exudate–amended to control population ratios (Fig. 3C).

DISCUSSION

Phenanthrene Biodegradation

The impacts of the debris and exudate amendments on phenanthrene biodegradation rates were roughly additive (Table 2). The combination of slender oat debris and exudates produced the strongest biodegradation enhancement. However, the combined treatment did not produce phenanthrene biodegradation rates that were as large as previously observed in actively growing slender oat rhizosphere soil. Direct comparisons between the current experiment and planted microcosms from the earlier study are problematic. Sterile, hydroponic collection of the root exudates may have removed or altered exudate components compared with the quantity and composition of slender oat root exudates that are produced as roots grow in soil. Many effects of an intact rhizosphere are not included in root material amendments, including spatial and temporal variations in water content, exudation patterns, and soil pH. However, our goal was to isolate the impacts of individual rhizosphere components on phenanthrene biodegradation. Based on the rates of phenanthrene disappearance across treatments, the exudate addition had the strongest individual influence on biodegradation. Both treatments receiving root exudates had significantly lower phenanthrene concentrations by the end of the experiment compared with the unamended control; soil amended with only root debris did not (Fig. 2). Given these results, potential

exudate-driven petroleum biodegradation enhancement mechanisms warrant further discussion.

Root exudates may enhance contaminant biodegradation rates by providing essential nutrients such as N, P, or labile C that increase the population size and activity of heterotrophs and phenanthrene degraders. Dissolved organic and inorganic N in the root exudate solution provides available forms of N that are potentially limiting in petroleum-contaminated soil.

Root exudates may also increase the bioavailability of phenanthrene resulting in enhanced biodegradation rates. Interactions between PAHs and dissolved organic compounds present in exudates may enhance the aqueous phase concentration and/or desorption of hydrophobic contaminants (Kordel et al., 1997). Potential biosurfactants such as saponin have been detected in *Avena* sp. (Nisius, 1988). Significantly lower phenanthrene concentrations in exudate-amended soils at the end of the 20-d experiment (Fig. 2) is consistent with increased phenanthrene bioavailability. However, these results provide only indirect evidence for this hypothesis.

Root exudates could also serve as primary substrates for cometabolic transformation of phenanthrene. While cometabolism of PAHs has been reported in a few studies (Keck et al., 1989; Bouchez et al., 1995; Tiehm and Frizsche, 1995) biotransformation required other PAHs such as naphthalene, fluorene, and anthracene as primary substrates. Although rhizosphere-enhanced cometabolic biodegradation has yet to be demonstrated, numerous studies investigating chlorinated organic biotransformation in rhizosphere soil have proposed such a mechanism (Brigmon et al., 1998; Gilbert and Crowley, 1997; Haby and Crowley, 1996; Donnelly et al., 1994; and others). Results from our investigations with phenanthrene do not appear to support enhancement of cometabolic biodegradation as a viable mechanism. Phenanthrene degrader populations (Fig. 2) increase significantly in response to phenanthrene addition, indicating that they are using the substrate as a source of C or energy. Earlier experiments (Miya and Firestone, 2000) with fully functioning rhizosphere systems were consistent with the degrader community dynamics observed in the current study. Contaminant cometabolism did not appear to be a significant mechanism for plant-enhanced phenanthrene biodegradation in our system.

Slender oat root debris amendments did not enhance phenanthrene biodegradation compared with control soils. Although the debris amendment contained more total C and N than the exudate additions, the C and N added as root debris was less labile and apparently less available to degrader organisms. The discrete nature of root debris in combination with the high octanol–water coefficient and a low water solubility of phenanthrene may physically limit the impact of debris on phenanthrene degradation. As a net result, the debris amendment in our soil microcosms had less of an impact on phenanthrene biodegradation than did the addition of soluble exudate material.

Can we conclude that the impacts we detected using individual rhizosphere components realistically simulated an intact plant–soil system? Our experimental design was carefully constructed to simulate root inputs into the soil. However, flawless simulation of root debris and exudate inputs from an intact root system is virtually impossible. We added root debris at densities comparable with those present in our intact microcosms (four plants per microcosm). We added exudates from hydroponically grown slender oat plants every other day, roughly comparable with exudate inputs from 5.6 plants per soil microcosm over the same time period. Although a perfect rhizosphere simulation was unlikely, our results do indicate that the impact of slender oat exudates was larger than root debris on phenanthrene biodegradation in rhizosphere soil.

Microbial Community Dynamics

All plant-derived amendments produced rapid increases in heterotrophic populations during the first 4 d (Fig. 1) followed by comparatively rapid reductions during the subsequent 4 d. While the decrease in heterotrophic numbers suggests depletion of available C, decreases occurred even in the soils receiving additional exudate amendments every 2 d. Another explanation for the reduction in heterotrophic numbers over time could be protozoal grazing of bacteria triggered by the initial bacterial population increase (Clarholm, 1985). Increased bacterial numbers in slender oat rhizosphere soil have been shown to stimulate protozoal numbers (Jaeger, unpublished data, 2001) potentially resulting in rapid population fluctuations similar to the patterns observed in our amended soils.

Heterotrophs most likely use the more labile C and N components present in root debris during the initial phases of root decomposition (Berg, 1984; McClaugherty et al., 1984). Heterotrophic numbers in soils amended with root debris declined to levels similar to unamended controls for the final week of the experiment (Fig. 1). Higher heterotrophic numbers in root exudate and exudate + debris–amended soils throughout the experiment may be supported by the continuous supply of exudate amendments.

Phenanthrene degrader populations increased significantly between 0 and 4 d (Fig. 2). However, degrader populations in amended soils did not differ significantly from the control, indicating that the phenanthrene degrader population increase resulted primarily from the phenanthrene addition and not the root-derived amendments. The impact of phenanthrene on the degrader community is much larger than the impact of root-derived amendments. It is not until after 8 d that enrichment of phenanthrene degraders by root-derived amendments begins to appear in exudate-amended soils (Fig. 3B). This is also in agreement with our previous study in which selective enrichment of contaminant degraders in phenanthrene-contaminated slender oat rhizosphere soil occurred only during the mature phases of plant growth (Miya and Firestone, 2000). The presence of C and N sources in root-derived amendments appears to maintain higher phenanthrene degrader populations after a contamination event.

Maintenance of larger contaminant degrader popula-

tions during the later part of the experiment in root-amended soil (Fig. 3A) suggests that recalcitrant components of the root debris may be responsible. Root components in this category could include suberized cell walls, cutin, lignin, waxes, and tannins. Some of these components are structurally and chemically analogous to compounds commonly found in petroleum hydrocarbon mixtures. The presence of phenanthrene analogs in root debris may have helped maintain degraders during the later part of the experiment ($t > 10$ d) after most of the phenanthrene was consumed.

The exudate-amended soil also shows maintenance of phenanthrene degraders compared with nonamended soils after 8 d (Fig. 3B). This amendment also appears to help maintain phenanthrene degrader populations compared with control soil in which degrader numbers decrease to a level significantly lower than in amended soil (Fig. 2). The magnitude of heterotrophic and phenanthrene degrader response to exudates is greater than the corresponding responses in root debris–amended soil potentially due to higher C and N quality and availability in root exudates compared with root debris. Thus, both bacterial populations and phenanthrene biodegradation show the greatest response to exudate amendments.

Since we observed that phenanthrene biodegradation rates were roughly additive in the combined debris + exudate treatment, it is not surprising to see similar patterns of heterotrophic and degrader populations in the combined treatment. The trends observed in the combined treatment (Fig. 3C) are roughly a composite of root debris–amended soil (Fig. 3A) and exudate-amended soil (Fig. 3B). Thus, selective maintenance of phenanthrene degraders appears to be due to both decaying root debris as well as water-soluble root exudates. However, maintenance of phenanthrene degraders does not appear to be a long-term rhizosphere characteristic. A temporary shift sustaining degraders in rhizosphere soil is consistent with the results in Miya and Firestone (2000) as well as other whole-plant experiments that have been conducted in our laboratory for up to 80 d after planting.

Significantly higher average contaminant degrader populations were detected in amended soils (Fig. 2), as were faster phenanthrene biodegradation rates (Table 2). A larger number of phenanthrene-degrading microbes would potentially increase the frequency of phenanthrene encounter by degraders (Holden and Firestone, 1997). However, it is important to note that phenanthrene degrader enrichment observed in root debris–amended soil (14–20 d), in exudate-amended soil (8–10 d), and in the combined treatment (16–20 d) (Fig. 3A–3C) did not correspond temporally with maximum rates of phenanthrene biodegradation (Table 2). In general, >75% of the phenanthrene had already been degraded before degrader enrichment was detected. This observation is consistent with previous studies indicating that, although enrichment of degraders does occur in rhizosphere soil, the phenomenon may not be necessary for enhanced biodegradation (Kunc and Rybárová, 1989; Miya and Firestone, 2000).

Soils amended with root exudates supported significantly faster contaminant biodegradation rates compared with unamended controls as microbes either utilized the labile C and N constituents to increase population size and activity and/or organic components within root exudates increased phenanthrene bioavailability. However, the impact of the contaminant itself on the degrader community was larger than any of the root-derived amendments tested. Potential contaminant analogs found in decaying root debris as well as labile C and N in continuously supplied root exudates appear to contribute to a relatively long-term selective maintenance of contaminant degraders in rhizosphere soil. These rhizosphere components maintained degrader populations after most of the contaminant has been consumed; this resulted in the apparent degrader population enrichment.

Our results indicate that root exudates stimulate rates of phenanthrene biodegradation in slender oat rhizosphere soil. Root exudates and decomposing root debris combine to maintain larger populations of phenanthrene degraders in rhizosphere soil.

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