Effects of Agronomic Practices on Phytoremediation of an Aged PAH-Contaminated Soil

Paul E. Olson, Ana Castro, Mark Joern, Nancy M. DuTeau, Elizabeth Pilon-Smits, and Kenneth F. Reardon* Colorado State University

Phytoremediation offers an ecologically and economically attractive remediation technique for soils contaminated with polycyclic aromatic hydrocarbons (PAHs). In addition to the choice of plant species, agronomic practices may affect the efficiency of PAH phytoremediation. Inorganic nutrient amendments may stimulate plant and microbial growth, and clipping aboveground biomass might stimulate root turnover, which has been associated with increases in soil microbial populations. To assess the influence of fertilization and clipping on PAH dissipation in a nutrient-poor, aged PAH-contaminated soil, a 14-mo phytoremediation study was conducted using perennial ryegrass (Lolium perenne) as a model species. Six soil treatments were performed in replicate: unplanted; unplanted and fertilized; planted; planted and fertilized; planted and clipped; and planted, clipped, and fertilized. Plant growth, soil PAH concentrations, and the concentrations of total and PAH-degrading microorganisms were measured after 7 and 14 mo. Overall, planting (with nearly 80% reduction in total PAHs) and planting + clipping (76% reduction in total PAHs) were the most effective treatments for increased PAH dissipation after 14 mo. Fertilization greatly stimulated plant and total microbial growth, but negatively affected PAH dissipation (29% reduction in total PAHs). Furthermore, unplanted and fertilized soils revealed a similar negative impact (25% reduction) on PAH dissipation after 14 mo. Clipping did not directly affect PAH dissipation, but when combined with fertilization (61% reduction in total PAHs), appeared to mitigate the negative impact of fertilization on PAH dissipation. Therefore, fertilization and clipping may be included in phytoremediation design strategies, as their combined effect stimulates plant growth while not affecting PAH dissipation.

Poly cyclic aromatic hydrocarbons (PAHs) are a group of organic compounds comprised of fused aromatic rings that occur naturally and via human activities (Juhasz and Naidu, 2000; Dietz and Schnoor, 2001). PAHs are widespread soil contaminants and typically are found as mixtures of low (two–four aromatic rings) and high (five or more aromatic rings) molecular weight (MW) chemicals. Low MW PAHs are less hydrophobic (log Kow < 3–5) and are more water soluble and bioavailable than the high MW PAHs, and are thus moderately biodegradable under aerobic conditions (Bossert and Bartha, 1986; Juhasz and Naidu, 2000; Olson et al., 2003). In contrast, high MW PAHs (log Kow > 5) are much less bioavailable and undergo very slow aerobic biodegradation. The biodegradation of these large PAHs by cometabolism is generally more rapid and occurs to a greater extent (Bouchez et al., 1995; 1999; Dietz and Schnoor, 2001; Olson et al., 2003).

Over the last decade, phytoremediation of contaminated soils, sediments, and ground water has emerged as an ecologically and economically sound cleanup technology and gained widespread public acceptance (Dietz and Schnoor, 2001; Olson et al., 2003; Parrish et al., 2005; Pilon-Smits, 2005). Vegetation can affect the fate of soil contaminants in several ways, including sorption to roots, enhanced direct volatilization (Marr et al., 2006), and uptake into the plant. One aspect of phytoremediation involves the biodegradation of organic compounds in the plant rhizosphere, the zone around the plant roots. This rhizodegradation may involve the activities of both the plant and its root microbial flora, and is especially important for the biological remediation of hydrophobic pollutants such as PAHs. The mechanisms and plant-microbe interactions involved in rhizodegradation are still largely unknown, and constitute an important area of study. However, it is well known that vegetation can profoundly affect soil physical-chemical properties. Plant roots disrupt soil aggregates and penetrate micropores, creating air and water channels and exposing soil layers to the activity of soil microorganisms. Also, plants modify the pH of their rhizosphere, and release compounds via excretion and root turnover. These plant-released compounds contain both primary and secondary plant metabolites that affect microbial growth, composition, and activity and that may also act as surfactants for hydrophobic compounds (Juhasz and Naidu, 2000; Kuiper et al., 2001, 2004; Hutchinson et al., 2003; Olson et al., 2003). The potential for PAH degradation by certain soil microbial populations has been dem-

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Abbreviations: MPN, most probable number; MW, molecular weight; PAH, polycyclic aromatic hydrocarbons.
Table 1. Characteristics of the soil used in this study (reprinted from Olson et al., 2007). Details of the methods used are provided in Soltanpour and Workman (1981).

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
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<tbody>
<tr>
<td>pH</td>
<td>6.8</td>
</tr>
<tr>
<td>Organic matter (g/kg)</td>
<td>23</td>
</tr>
<tr>
<td>NO\textsubscript{3}-N (mg/kg)</td>
<td>3.2</td>
</tr>
<tr>
<td>P (mg/kg)</td>
<td>9.3</td>
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<tr>
<td>K (mg/kg)</td>
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<tr>
<td>Zn (mg/kg)</td>
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<tr>
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<tr>
<td>Mn (mg/kg)</td>
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<tr>
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<tr>
<td>Silt (%)</td>
<td>13</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>20</td>
</tr>
<tr>
<td>Texture</td>
<td>Sandy clay loam/Sandy loam</td>
</tr>
</tbody>
</table>

Table 2. Clipping, fertilizing and sampling schedule.

<table>
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<tr>
<th>Fertilization</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
</tr>
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<tbody>
<tr>
<td>Clipping</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N:P:K (20:20:20)</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N (slow-release)</td>
<td>X</td>
<td>X</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td>Sampling</td>
<td>t_0</td>
<td>t_1</td>
<td>t_2</td>
<td></td>
<td></td>
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</tbody>
</table>

This cool-season grass has a widespread geographic distribution that allows for ease of growth and maintenance in a multitude of potential locations. Grasses in general appear to be effective for phytoremediation of organics, probably due to the ability of this plant family to develop highly branched and often deep root systems. It has also been suggested that Poaceae plant species may favor root growth over shoot growth as a strategy to become more PAH tolerant (Huang et al., 2004).

We report here on a 14-mo study aimed at assessing the influence of two agronomic practices—fertilization and clipping—on PAH dissipation in the same aged PAH-contaminated soil used in our previous work. This aged soil, collected from a PAH contaminated site in Alameda, CA, offered an ideal substrate for this study, given that the age of the PAH contaminant is a limiting factor for its dissipation (Allard et al., 2000; Parrish et al., 2005). Perennial ryegrass was used as a model plant, and different combinations of fertilization and clipping were evaluated. Aboveground plant biomass, PAH concentrations, and the abundance of total and PAH-degrading microorganisms were measured.

Materials and Methods

Experimental Conditions

The aged PAH-contaminated soil used for this study was obtained from a vegetated site at the U.S. Coast Guard Housing Area at the Alameda Naval Air Station (NAS) (Alameda, CA, USA), as described previously (Olson et al., 2007). Since the soil was obtained from a vertical excavation (15–180 cm below surface) and was chemically and physically heterogeneous, it was thoroughly homogenized using a Mini-Microenfractionator (H&H Ecosystems, North Bonneville, WA, USA). The blended soil was tested for general soil properties by the Soil, Water, and Plant Testing Laboratory, Colorado State University (Fort Collins, CO, USA). The Microenfractionator uses a large impeller and airflow to intensely mix soil and break aggregates, thus reducing the chemical and physical heterogeneity of the soil as delivered from NAS. The soil characteristics are shown in Table 1. The mixed soil was distributed into 2-L plastic pots to a consistent depth of approximately 20 cm, and plant seeds were sown directly into the soil.

Perennial ryegrass (Lolium perenne) seeds were purchased from Granite Seed Company (Lehi, UT). For each treatment, eight replicate pots filled with PAH-contaminated soil were used. Six treatments were compared: (i) unplanted; (ii) unplanted and fertilized; (iii) planted; (iv) planted and fertilized; (v) planted and clipped; and (vi) planted, fertilized, and clipped. All pots were placed on plastic saucers, and kept in a heated greenhouse in Fort Collins, CO at 22°C under natural light. All pots were watered from the bottom as needed to maintain field capacity (moist, not saturated). The amount of water added to the pots varied throughout the growing season and the course of the 14-mo study.

Several treatments were periodically fertilized during the duration of the experiment (Table 2). Fifty milliliters of Peters Professional 20-20-20 water-soluble fertilizer (Scotts), diluted to manufacturer’s recommendation, were watered into the top of each pot at 0, 2, 4, 6, 8, 10, 12, and 14 mo. In addition, approximately 15...
g of slow-release Scotts Topdress Special (17-3-6) for container plants were broadcast on the soil surface at 0 and 6 mo.

Aboveground dry biomass was measured for all planted treatments as an indication of plant productivity. For clipped treatments (Table 2), plant material was removed utilizing a hand pruner approximately 1 cm above the soil surface at 6, 8, 9, and 11 mo. At the completion of the experiment, all planted material (both clipped and unclipped treatments) was removed in a similar fashion at the soil level. At the time of removal, plant material was placed in brown paper bags, allowed to dry in a plant oven at 40°C, and subsequently weighed.

Soil was sampled at the start of the experiment (November 2000, t0; post-homogenization), after 7 mo (t1; post germination and plant maturation), and after 14 mo (t2; during the second growing season). At t1 and t2, soil samples of approximately 10 g were taken from each pot using a soil core sampler and sieved to remove root material. Sieved samples were stored at −80°C until analysis.

PAH Extraction and Analysis

To extract PAHs, 2 g wet soil was treated with 2 g Na2SO4 and 10 mL acetone, then vortexed for 30 s. Soil samples were then sonicated using a 550 Sonic Dismembrator (Fisher Scientific, Hampton, NH, USA) vibrating at 20 kHz for 2 min (1-s on/off cycles). Samples were analyzed for individual PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene,anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, indol(1,2,3-cd)pyrene, dibenzo(ah)anthracene, and benzo(ghi)perylene) by gas chromatography using an internal standard (deuterated phenanthrene, PHE d10) as a response factor and external standards to quantify concentration as described by Olson and Fletcher (2001). The gas chromatograph (Hewlett-Packard 5890 GC) was equipped with a flame-ionization detector (GC-FID). The column and conditions were described previously (Olson et al., 2007). An additional portion of each sample was dried to determine PAH concentrations as mg/kg dry weight of soil. PAH dissipation was calculated as the overall reduction in extractable PAH levels compared to initial (t0) concentrations.

Microbial Enumeration

Total microbial population size was estimated by quantifying the total DNA in the soil samples, kept at −80°C. DNA was isolated from the soil samples using the Fast DNA SPIN Kit for Soil (Qbiogene Inc., Irvine, CA) following the manufacturer’s protocol. Briefly, soil samples (0.5 g) were mixed with lysis buffer (978 μL sodium phosphate and 122 μL MT buffer). The mixture was homogenized using a mini-beadbeater for 3 min, followed by a centrifugation step of 30 s at 14,000 × g. To the supernatant, 250 μL of protein precipitation solution were added, mixed gently, and the mixture centrifuged for 5 min at 14,000 × g. The supernatant from this step was gently mixed with 1 mL of binding matrix suspension. Six hundred μL of supernatant were transferred to SPIN Filter tubes, and centrifuged for 1 min at 14,000 × g. Subsequently, the samples were washed with salt-ethanol wash solution and centrifuged for 1 min at 14000 × g. To the matrix-bound DNA, 50 μL DNase/pyrogen-free water were added, the tubes gently agitated, and the mixture incubated for 2 min at room temperature. The mixture was centrifuged for 1 min at 14000 × g and the DNA eluted to a new test tube. The DNA concentration of a solution was estimated by measuring the absorbance at 260 and 280 nm in a Beckmann DU 800 UV spectrophotometer.

The PAH-degrading microbial population was enumerated using a modification of the most-probable number (MPN) procedure described by Wienn and Venosa (1996). Soil dilutions in a range of 10−1 to 10−11 were prepared using sterile double-distilled water. Each well of a 96-well plate received 10 μL of a four-PAH solution (10 g/L phenanthrene, 1.0 g/L chrysene, 1.0 g/L benzo(a)pyrene, and 1.0 g/L pyrene in pentane) and the plate was allowed to evaporate. Soil dilutions (20 μL) and 180 μL of Bushnell-Haas Broth medium (1.0 g/L KH2PO4, 1.0 g/L K2HPO4, 1.0 g/L NH4NO3 g/L, 0.2 MgSO4.7H2O, 0.05 g/L FeCl3, 0.02 g/L CaCl2.2H2O) were then added. The 96-well plates were allowed to incubate, covered, at room temperature for 3 wk. PAH degradation resulted in a yellow-brown color, which was visually detected and used to estimate the MPN of PAH-degrading microbes in the soil.

Statistical Analyses

Statistical analyses (Student’s t test, correlation) were performed using JMP-IN software (SAS Institute, 2002).

Results

Plant Growth

To study the effects of two agronomic practices on PAH dissipation, perennial ryegrass was seeded on aged, non-spiked PAH-contaminated soil (Table 1) and subjected to six clipping and/or fertilization treatments: unplanted (U); unplanted and fertilized (U+F); planted (P); planted and fertilized (P+F); planted and clipped (P+C); and planted, clipped, and fertilized (P+C+F) (Table 2). At the second sampling point (t2), 14 mo after planting, perennial ryegrass that was clipped periodically produced 23% more shoot biomass than ryegrass that was not clipped (p < 0.05) (Fig. 1A). Fertilization had a striking effect on plant biomass (65 g shoot DW), leading to a higher biomass production in comparison with unfertilized ryegrass (5 g shoot DW). Similarly, the amount of clipped biomass of the P+C+F treated ryegrass was significantly higher than that of unfertilized clipped (P+C) ryegrass over the course of this study (Fig. 1B). Among the P+F treatments, clipping did not affect final shoot biomass (Fig. 1A). However, the combination of clipping and fertilization appeared to stimulate plant growth rate (Fig. 1B).

PAH Concentrations

Soil PAH concentrations (Table 3) were presented in Fig. 2 and Table 3. The summed concentration of the lower Mw PAHs (two–four rings) in unplanted soils decreased from 599 mg kg−1 soil DW at t0, to 260 mg kg−1 soil DW at t1, and 177 mg kg−1 soil DW at t2 (Fig. 2A). In unplanted soils that were fertilized, this decrease in low Mw PAH concentration was significantly smaller (decrease of approximately 34% at t1 and at t2). Planting with ryegrass, on the other hand, significantly
enhanced low MW PAH dissipation (129 mg kg\(^{-1}\) soil DW at \(t_1\) and 113 mg kg\(^{-1}\) soil DW at \(t_2\)) compared to unplanted soil. Similar results were obtained when the ryegrass was periodically clipped (Fig. 2A). Fertilization of ryegrass-planted soils, however, resulted in significantly less PAH dissipation compared to unplanted or planted soil, similar to fertilized unplanted soil. Interestingly, when the plants were both clipped and fertilized, the clipping appeared to mitigate the negative effect of fertilization on low MW PAH dissipation, especially at \(t_1\) (Fig. 2A).

The initial summed concentration of high MW PAHs (five or more rings) at \(t_0\) was 189 mg kg\(^{-1}\) soil DW (Fig. 2B). Overall, the different treatments yielded patterns of high MW PAH dissipation that were similar to those described above for low MW PAHs, but due to the lower concentrations and therefore higher coefficient of variance, no significant differences were present between the treatments. The total PAH concentration at \(t_0\) was 788 mg kg\(^{-1}\) soil DW (Fig. 2C). The different treatments resulted in similar patterns of PAH dissipation as those described above for low MW PAHs, with similar levels of significance.

When the results for individual PAHs are considered, it can be noted from Table 3 that planted soils contained significantly \((p < 0.05)\) lower concentrations of five of the PAHs at \(t_1\) and for 11 of the PAHs at \(t_2\) compared to unplanted soils. At \(t_1\) and \(t_2\), the most persistent PAHs were phenanthrene (three rings), fluoranthene (four rings), and pyrene (four rings), while the least recalcitrant PAHs was naphthalene (two rings). Dissipation of benzo(k)fluoranthen (B(k)F), a five-ring PAH, was also higher than most, but this may be an artifact of the very low concentration of B(k)F at \(t_1\).

The percentage PAH dissipation was calculated and grouped by ring number to evaluate trends between PAH size and dissipation patterns (Fig. 3). The dissipation of the two-ring PAH naphthalene was high (85–96%) for all treatments, including unplanted soil (Fig. 3A). Still, at \(t_1\), soils subjected to the P, P+C, and P+C+F treatments had significantly lower naphthalene concentrations than unplanted soil (a 12% decrease). At \(t_2\), 95% of the naphthalene had dissipated from all soils. At \(t_1\), the dissipation of three-ring PAHs did not differ significantly among treatments. However, after another 7 mo, a negative impact of fertilization was noted in that the dissipation of three-ring PAHs in fertilized soils (both unplanted and planted, with 55% and 52% dissipation, respectively) was significantly lower than in unplanted, unfertilized soil (79%) (Fig. 3B). All other treatments resulted in no significant differences compared to unplanted soil. At both \(t_1\) and \(t_2\), the dissipation of four-ring PAHs was 15% higher in the P and P+C soils than in unplanted soils, while dissipation of these PAHs in P+C+F soils was higher only at \(t_1\) (Fig. 3D, E). Interestingly, dissipation of four-ring PAHs was significantly lower (16%) for fertilized soils (both unplanted and planted) compared to unplanted soil (Fig. 3C). The same patterns were observed for
the dissipation of five- and six-ring PAHs, except that the inhibitory effect of fertilization was larger at both sampling points (Fig. 3D, E). When total PAH dissipation is considered, trends similar to those described for the three- to six-ringed PAHs were observed (Fig. 3F). However, the only significant differences were at t2, when the two fertilized soils (either unplanted or planted) showed 30% lower PAH dissipation compared to unplanted soil.

### Microbial Populations

At t1, the total DNA concentration was higher in the P+F and P+C+F treated soils than in soils subjected to the other treatments; specifically, it is notable that clipping did not lead to an increase in the total microbial density (Table 4). In contrast, only the P+C treatment resulted in an increased MPN value for PAH-degrading microorganisms at t2 (vs. unclipped ryegrass and vs. unplanted soil), and the MPN of PAH degraders in the P+F treated soils was the lowest of any of the treatments.

### Discussion

#### Vegetation and PAH Dissipation

Vegetation and PAH dissipation studies showed that planting non-spiked, aged, PAH-contaminated soil with perennial ryegrass had a beneficial effect on PAH dissipation. This positive effect of planting may be explained at least in part by the rhizosphere effect: the existence of plant roots that facilitate general microbial growth (Binet et al., 2000; Parrish et al., 2005). In addition, the plant roots may have released compounds that either enhanced PAH bioavailability (e.g., biosurfactants; Déziel et al., 1996; Kumara et al., 2006) or that stimulated microbial PAH degradation (e.g., by gene induction; Pilon-Smits, 2005; Singer et al., 2003), or via cometabolism (Singer et al., 2003). These factors are consistent with the observation that the difference in PAH dissipation between planted and unplanted soils was the largest for the five- and six-ring PAHs, which are the most hydrophobic and most recalcitrant to microbial degradation, often requiring cometabo-
In this context, it is interesting to note that Miya and Firestone (2001) suggested that amendments of root exudates and debris significantly enhanced phenanthrene soil degradation by increasing phenanthrene bioavailability and/or by increasing the number of phenanthrene-degrading microorganisms. Johnsen and Karlson (2005), investigating the PAH degradation capacity by soil microbial populations, concluded that the accumulation of pyrene in soil was a consequence of its low bioavailability rather than the lack of PAH microbial population. In another study, Krutz et al. (2005) found a similar correlation between pyrene degradation and the concentration of pyrene-degrading bacteria. In the current study, the abundance of PAH-degrading microorganisms also increased substantially in planted soils, offering another clue as to the mechanism responsible for the positive effect of planting on PAH dissipation.

In general, the dissipation of the two-ring PAH, naphthalene, was the highest of all the PAHs, as would be expected. Low MW PAHs have a relatively low hydrophobicity and relatively high water solubility, making them moderately biodegradable (Juhasz and Naidu, 2000; Olson et al., 2003). In unplanted, unfertilized soils, there were only small differences in dissipation among the various higher MW PAHs. The positive effect of planting on PAH dissipation, relative to unplanted soil, was visible at both sampling points (i.e., during two subsequent growing seasons). However, most of the PAH dissipation appears to have taken place during the first 7 mo of the experiment (t0–t1), and in this period a relatively high rate of PAH dissipation was noted in the unplanted soil as well. Because of the inherent heterogeneity of PAHs in contaminated soils, the experimental soil was microenfractioned (mixed) to evenly distribute the PAHs in the soil. Therefore, a possible explanation for this phenomenon is that the PAHs removed in this time period represent the bioavailable fraction of the PAHs (due to mixing and/or biological processes), leaving behind the fraction that was more tightly bound to the soil (Scow and Hutson, 1992; Shor et al., 2003). Whether this fraction was essentially (and permanently) non-bioavailable (and therefore not a risk to the environment), or whether over time this PAH fraction would have become available is an interesting question that would require further study.

Certain elements of the design of this study (soil homogenization, watering regimen, greenhouse conditions) that were necessary to isolate the variables of interest (clipping and fertilization) also resulted in different conditions for plants and soil microorganisms than might be found in field applications. An evaluation of these effects in a field environment might thus be of interest.

**Effect of Clipping on PAH Dissipation**

It has been hypothesized that shoot clipping would lead to a higher root decay and turnover, which in turn would promote the growth and activity of soil microbial populations, including those involved in pollutant degradation (Leigh et al., 2002). Moreover,
frequent clipping has been associated with the “overcompensation” process in plants, which could mean a higher plant growth rate (Turner et al., 1993; Villarreal et al., 2006). The latter appears to indeed have been the case in the current study, since biomass production was stimulated by clipping. Although clipping produced a small increase in the abundance of PAH-degrading microorganisms, there was no evidence that clipping promoted PAH dissipation. Direct measurements of the amount and composition of root exudates released as a result of clipping would be of interest; it is possible that either clipping did not stimulate root exudation or that the types of chemicals released from the clipping treatment were not those that are associated with the stimulation of microbial PAH degradation. Another consideration is that after comparing pyrene and phenanthrene mineralization rates in soils ranging from pristine to heavily polluted areas, Johnsen and Karlson (2005) concluded that the PAH degradation capacity by indigenous soil microorganisms is already present in the soils.

**Effect of Fertilization on PAH Dissipation**

Inorganic nutrients such as nitrogen and phosphorus are often reported to limit the bioremediation of PAHs in soils, and it has been observed that fertilization may enhance bioremediation in PAH-contaminated soils (Hutchinson et al., 2003; Davis et al., 2003). Similarly, fertilization with nitrogen, phosphorus, and potassium is known to promote plant growth, as these elements are often limiting. Indeed, in the current study, fertilizing twice with slow-release NPK (17:3:6) and bi-monthly with NPK (20:20:20) had a pronounced positive effect on plant growth over the course of this experiment. The abundance (MPN) of PAH-degrading microorganisms, however, did not respond favorably to fertilization, in contrast to total microbial DNA levels. These findings may have implications for the design of phytoremediation strategies. Increases in plant biomass may represent a reduction of soil erosion and PAH leaching into the surroundings. Furthermore, it likely indicates an increase in root density, which would provide a larger root surface to harbor the soil microbial populations and consequently, the PAH-degrading microorganisms (Hutchinson et al., 2001; Pravecek et al., 2006). The finding that total microbial density was higher in planted and fertilized soils than in unplanted but unfertilized soils, while the abundance of PAH-degrading microorganisms was lower, may indicate that other microbes competed more efficiently for inorganic nutrients (or were more limited by them in their growth), giving the PAH degraders a relative disadvantage under conditions of higher nutrient availability. It is interesting that the total and PAH-degrading microbial population sizes in unplanted soils were both unaffected by the addition of these inorganic nutrients. One possible explanation is the competition for inorganic nutrients between the plant and the soil microbial populations. Other similar studies have reported mixed results in this respect. Hutchinson et al. (2001), using an aged PAH-contaminated soil and similar time frame and fertilization schedule, found that the different fertilization rates did not significantly affect the soil microbial population. Pravecek et al. (2006), studying the microbial bioavailability of pyrene under aerobic and anaerobic conditions, showed that addition of nitrogen and sulfur could not be related to the amount of pyrene metabolized.

Interestingly, the negative effect of fertilization on PAH dissipation seems to be alleviated by plant clipping. As mentioned above, it has been suggested from ecological studies that clipping above-ground biomass increases root turnover, leading to an increase in soil microbial population (see above, Leigh et al., 2002; Villarreal et al., 2006). If this is indeed the case, then clipping and fertilization effectively provide the rhizosphere community with a carbon source in addition to inorganic nutrients. It is feasible that this has a profoundly different effect on the microbial composition than adding inorganic nutrients alone. PAH dissipation may also be specifically stimulated via release of plant compounds that induce microbial operons involved in PAH dissipation (Gilbert and Crowley, 1997; Chen and Atiker, 1999), or by compounds that act as catabolites. In support of the latter, the MPN of PAH-degrading microbes, measured via a PAH degradation activity assay, increased between t1 and t2, when ryegrass was both fertilized and clipped.

If clipping indeed counters the negative effect of fertilization on PAH dissipation, but not its promoting effect on plant growth and its associated benefits, clipping and inorganic fertilizing may be an attractive combination of agronomic practices in PAH phytoremediation.

**Conclusions**

In agreement with previous studies, planting aged PAH-contaminated soil with perennial ryegrass was shown to significantly enhance PAH dissipation. This positive effect of vegetation on PAH dissipation may have been due to the positive effect of root-released compounds on both the abundance of microbial PAH degraders and the bioavailability of the PAHs. As such, these results strengthen the hypothesis that plant-microbe interactions significantly affect PAH bioremediation.

The agronomic practices of clipping and fertilization did not improve PAH dissipation per se, and fertilization alone even appeared to impede this process, perhaps by favoring other microbial growth rather than that of PAH degraders. Interestingly, clipping alone led to an increased population of PAH-degrading microorganisms, and appeared to mitigate the negative effect of fertilization. Since the addition of inorganic nutrients greatly stimulated plant growth, and may be necessary in nutrient-deficient soils, the combination of fertilization and clipping may be an attractive agronomic practice to be incorporated in phytoremediation design strategies.

PAH dissipation appeared to mainly take place in the first 7 mo after planting, rather than in the second 7 mo. This suggests that extrapolations from short-term studies may not provide the most valid results, but also that remediation of the bioavailable fraction of PAHs may be feasible within as little as one growth season. Although fertilization alone was not effective, the overall trends in this study suggest that managing ryegrass by a combination of clipping (mowing) and fertilization at phytoremediation field sites may lead to higher plant productivity, abundant root growth, and higher total and PAH-degrading microbial densities. These factors in turn may ultimately result in greater total PAH dissipation over time frames longer than those evaluated in this study.
Acknowledgments

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References


