

# PAH dissipation in spiked soil: Impacts of bioavailability, microbial activity, and trees

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## Abstract

While trees have demonstrated potential in phytoremediation of several organic contaminants, little is known regarding their ability to impact the common soil contaminant PAHs. Several species of native North American trees were planted in soil artificially contaminated with three PAHs. Plant biomass, PAH dissipation, and microbial mineralization were monitored over the course of one year and environmental conditions were allowed to follow typical seasonal patterns. PAH dissipation and mineralization were not affected by planting. Extensive and rapid loss of PAHs was observed and attributed to high bioavailability and microbial activity in all treatments. The rate of this loss may have masked any significant planting effects. Anthracene was found to be more recalcitrant than pyrene or phenanthrene. Parallel soil aging studies indicated that sequestration to soil components was minimal. Contrary to common inferences in literature, amendment with decaying fine roots inhibited PAH degradation by the soil microbial community. Seasonal variation in environmental factors and rhizosphere dynamics may have also reduced or negated the effect of planting and should be taken into account in future phytoremediation trials. The unique root traits of trees may pose a challenge to traditional thought regarding PAH dissipation in the rhizosphere of plants.

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## 1. Introduction

Polycyclic aromatic hydrocarbon (PAH) contamination is commonly found on Superfund sites and on those designated as Resource Conservation and Recovery Act (RCRA) sites or brownfields. Since PAHs are known to produce negative health effects, efficient and predictable remediation strategies are needed to reduce the risk of exposure. Phytoremediation is emerging as a potentially cost-effective technology; plants have often been shown to reduce the level of PAHs in soil (Davis et al., 2002).

Dissipation of PAHs from soil is often attributed to microorganisms living in the rhizosphere associated with plant roots. Microbial communities have been documented

to be larger and more active in planted versus unplanted soil, and the rhizosphere is often enriched in organisms capable of hydrocarbon degradation (Binet et al., 2000). Root exudates are frequently implicated in explaining these phenomena (Yoshitomi and Shann, 2001). Although less well studied, fine root mortality can also affect microbial degradation of PAHs by providing readily available nutrients (Olson et al., 2003). The utilization of root-derived carbon as a sole source of energy by PAH and other contaminant degraders is often used to support the hypothesis that microbial degradation of organic contaminants should be enhanced in the rhizosphere (Yoshitomi and Shann, 2001; Leigh et al., 2002; Rentz et al., 2004).

Plants (and plant types) are known to vary widely with respect to root parameters such as morphology, root exudation (Grayston et al., 1996), fine root turnover (Gill and Jackson, 2000), root decomposition (Van der Krift et al., 2002), and associated microbial communities (Smalla

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et al., 2001). If the dominant mechanism of PAH dissipation in planted soil is associated with the activity of rhizosphere microbial communities, then it would be expected that remediation potential would also vary across plant species and life-history types. Among studies that have evaluated multiple species within a given experiment, some have found species-specific and life-history differences with respect to enhancement of PAH dissipation (Liste and Alexander, 2000) while others have not (Chen et al., 2003).

Grasses and annual herbs have been the primary focus of experiments evaluating the potential of plants to remediate PAH contaminated soils (Davis et al., 2002), but the predictive power of these studies is compromised by the brief durations and constant environmental conditions often employed. Trees have received very little attention with regard to PAHs, although their perennial life-history and extensive root systems suggest they may be desirable for use in phytoremediation. It has also been speculated that trees may have greater rates of rhizodeposition (Grayston et al., 1996) including fine root turnover (Gill and Jackson, 2000) and exudation. Hybrid poplar trees have demonstrated the ability to remediate soil and groundwater contaminated with trichloroethylene (Newman et al., 1999) and trinitrotoluene (Thompson et al., 1998) and poplars and other species are frequently planted by land managers on a variety of contaminated soils. However, few studies, have directly examined the ability of trees to impact PAH dissipation. Olson et al. (2001) used forensic evidence to suggest that red mulberry trees may have enhanced PAH dissipation in a historically contaminated sludge basin. Accelerated dissipation from pyrene-spiked soil has been shown with pine seedlings (Liste and Alexander, 2000) and with species of tropical trees (Tang et al., 2004), but the species in these studies are limited in potential use by their restricted habitat ranges.

The work presented here was designed to address the paucity of information available on the use of perennial, woody plants in phytoremediation of PAH contaminated soils. In this year-long study, broad-ranging North American trees were grown in soil uniformly spiked with three model PAHs, and the changes in soil contamination and availability were regularly monitored. In order to mimic a more realistic setting, the temperature and light environment was allowed to fluctuate with ambient conditions. Replicate pots of trees were destructively sampled in the fall (three months after planting) and again after an entire year (summer). At each sampling time, tree biomass was measured, the soils were destructively analyzed for PAHs, and the ability of the endogenous microbial community to mineralize freshly added pyrene was determined in serum bottle studies.

## 2. Materials and methods

### 2.1. Soil properties and preparation

Shredded topsoil (Hafner and Sons, Cincinnati, OH) was acquired in bulk for use in this study. The Agricultural

Analytical Services Laboratory of Pennsylvania State University determined soil properties. Particle size distribution (19.1%, 53.2%, 27.7% sand, silt, and clay) identified the soil as a silty clay loam. The percent organic matter was 1.8% and the pH 7.7. Cation exchange capacity (CEC) was 17.3 cmolc kg<sup>-1</sup>. Nutrient levels were determined to be the following: nitrate nitrogen (29.8 mg kg<sup>-1</sup>), ammonium nitrogen (2.5 mg kg<sup>-1</sup>), phosphate (52.5 mg kg<sup>-1</sup>), calcium (24.2 cmolc kg<sup>-1</sup>) and potassium (0.2 cmolc kg<sup>-1</sup>).

The above soil was amended with PAHs in a manner that was optimized for uniformity of contamination. The procedure mixed clean bulk soil with PAH-spiked soil to bring the final concentration of each test PAH (anthracene, phenanthrene and pyrene; purchased from Acros Organics and Aldrich, 98+% purity) to 300 mg kg<sup>-1</sup>. Although many studies have utilized lower levels of PAHs in spiked soil (EX-100 mg kg<sup>-1</sup>) and only tested a single PAH (Liste and Alexander, 2000; Chekol et al., 2002; Chen et al., 2003; Genney et al., 2004), we used higher contaminant loads and multiple PAHs to better approximate PAH burdens reported in the field and to examine PAH specific dissipation and/or planting effects. Further, it was thought that this level of contamination would result in greater PAH sequestration in soil and allow for a longer experimental duration than would lower spiking levels. The PAH-spiked soil was prepared (in batches) as follows: 20.25 g of each PAH was dissolved in acetone (3 l) and added to 5 kg of sieved (2 mm) soil. This slurry was mixed thoroughly and allowed to evaporate overnight in a fume hood. Two of these 5 kg amounts were combined and considered one batch. Each batch was then mixed for 1 h on a horizontal tumbler, removed, and separated into ten 1 kg aliquots. For each 171 pot, 1 kg of spiked soil was added to 12.5 kg 'clean' soil (sieved to 15 mm) and mixed thoroughly with a spade. Uncontaminated control pots consisted of soil handled the same (including amendment with acetone), but without the addition of PAHs. This method of spiking has been shown to have minimal effects on soil microorganisms in comparison with other methods (Brinch et al., 2002).

### 2.2. Tree remediation study

Five tree species were selected to represent broad habitat ranges. Seedlings or cuttings were obtained from the following nurseries in the spring of 2003: red mulberry seedlings and black willow cuttings—Silva Native Nursery (Glen Rock, PA), rooted hybrid poplar cuttings—Hramor Nursery (Manistee, MI), sycamore seedlings—Duncan Nursery and Seed Company (Newburgh, IN), and black locust seedlings—Kentucky Division of Forestry (Frankfort). All seedlings or cuttings were approximately one year old, with the exception of red mulberry, which were two years old.

Immediately after the contaminated and control pots were prepared, they were arranged in a randomized block design in a greenhouse. All pots were kept dry for one month

after spiking in order to reduce microbial activity and allow for initial aging and sorption of PAHs to soil matrices to take place. After the aging period pots of contaminated soil were planted with a single seedling. Black willow, red mulberry, and sycamore trees were replicated ten times. Five replicates were used for hybrid poplar and black locust. Ten pots of contaminated soil were left unplanted. Each tree species was also planted in uncontaminated soil (three replicate pots/species). In light of possible negative effects of spiking procedures and dry soil conditions on microbial communities, 50 ml of a fresh soil inoculum was added to each pot after the trees were planted in order to ensure the presence of an active and representative soil microbial community. The inoculum was prepared by blending 25 g (fresh wt.) of soil from a forested nature preserve (Burnet Woods, Cincinnati, OH) with 500 ml of distilled water for 30 s. Several batches of extract were blended, combined, and filtered to 53  $\mu\text{m}$  before application.

All pots were watered as needed in 500 ml doses. Within each treatment (contaminated or uncontaminated), all pots of a given species (or all unplanted pots) were watered equally. During normal watering activities individual replicates were occasionally observed to be more moist than others, in which case those pots would not be watered. All pots were rearranged every two weeks for the first 8 months and every month for the next four. Throughout the course of the study, greenhouse temperatures were allowed to follow ambient conditions through the use of ventilation fans, glass pane removal, and shade cloth. Air temperatures ranged from  $-4$  to  $39$   $^{\circ}\text{C}$  and soil temperatures from  $1$  to  $29$   $^{\circ}\text{C}$ . No supplemental lighting was provided and soils were not fertilized. During warmer months, pesticide was applied to all trees to control the growth of mites. After the first growing season, all trees dropped their leaves and went through a period of dormancy, and all but two trees broke dormancy after the winter.

### 2.3. Tree study sampling

Five replicate pots of contaminated treatments were destructively sampled 3 and 12 months after initiation of the study (black locust and hybrid poplar were only sampled at 12 months). In planted pots, sampling involved carefully separating the entire tree from the soil. After removing the tree, any roots remaining in the soil were collected. The soil from both planted and unplanted pots was then sieved to 4.75 mm and thoroughly mixed. Approximately 100 g of soil from each pot was then set aside for use in PAH extraction and analysis or for mineralization studies (described below). For determination of growth in contaminated and uncontaminated soil, plant measurements (height, number of leaves, number of nodes) were taken before destructive sampling. Separate shoot and root dry weights were determined for each tree after sampling. Prior to oven drying, roots were washed, and segregated into coarse and fine roots ( $<1$  mm).

### 2.4. Microbial mineralization in tree study soil

Within one week of sampling, serum bottle radiorespirometry studies (Knaebel and Vestal, 1988) were begun to evaluate the ability of the microbial community to degrade PAHs. For each pot, approximately 50 g of soil (sampled as described above and stored at room temperature) was homogenized with a mortar and pestle. Five g (fresh wt.) was then placed into a serum bottle, amended with  $^{14}\text{C}$ -pyrene (Sigma, 4, 5, 9, 10— $^{14}\text{C}$ ) in acetone and 1 ml of sterile DI water. Soils sampled at 3 months received 40 000 dpm while 12 month samples received 26 000. Each bottle was vortexed and maintained at room temperature in the dark. Evolved  $^{14}\text{CO}_2$  resulting from  $^{14}\text{C}$ -pyrene mineralization was captured on base-saturated wicks and measured by liquid scintillation (Packard 2200 CA Tri Carb<sup>®</sup> liquid scintillation analyzer). Wicks were removed, replaced, and counted up to 60 days. Soil from the 12 month sampling was also used to test the effect of fine root mortality and decay on pyrene mineralization. One additional replicate from each red mulberry pot was prepared as above and amended with 0.02 g (fresh wt.) of fine root tissue ( $<1$  mm in diameter) collected from each red mulberry tree just after destructive harvesting. Amendment with excised fine roots may closely mimic natural fine root turnover since little translocation of nutrients is thought to occur prior to natural fine root death (Gordon and Jackson, 2000).

### 2.5. Extraction and analysis of tree study soil

Soil samples were stored at  $-20$   $^{\circ}\text{C}$  until extracted. After thawing, samples ( $\sim 50$  g) were further homogenized with a mortar and pestle and 5 g (fresh wt.) of soil from each sample was placed in 50 ml solvent resistant Nalgene centrifuge tubes and shaken with 15 ml of acetone for 24 h on a horizontal shaker (200 rpm) at room temperature. This extraction method was chosen based on its simplicity, support in the literature (Schwab et al., 1999), and superior performance when compared to variations of this method (including duration, volume, and solvent) and accelerated solvent extraction (ASE) performed on soil spiked and aged in the tree study (data not shown). Tubes were then centrifuged, decanted, and the supernatant stored in glass vials at  $4$   $^{\circ}\text{C}$  until analysis. Extracts were quantified using a Shimadzu GC-14A gas chromatograph equipped with a DB-5 capillary column (30 m length, 0.25 mm ID, 0.25  $\mu\text{m}$  film thickness) and flame ionization detector. GC conditions were as follows: injector temperature,  $300$   $^{\circ}\text{C}$ , starting oven temperature,  $180$   $^{\circ}\text{C}$ ; ramp  $10$   $^{\circ}\text{C min}^{-1}$  to  $240$  (no hold);  $15$   $^{\circ}\text{C min}^{-1}$  to  $285$  (no hold);  $20$   $^{\circ}\text{C min}^{-1}$  to  $325$  (hold 3 min), detector temperature,  $360$   $^{\circ}\text{C}$ . Helium was used as the carrier gas at a flow rate of  $1.5$   $\text{ml min}^{-1}$  and a split ratio of 4:1 was used. Standards were prepared from the same PAH stock chemicals that were used to spike the soil. A minimum of three standards were used to prepare standard curves, ranging from  $5$   $\text{mg ml}^{-1}$  to

250. Standards and extracts were amended with 1-bromo-2-methyl naphthalene as an internal standard, which was then used in PAH quantification.

### 2.6. Soil aging study

It is well documented that organic contaminants can become sequestered into the matrix of a soil (Hatzinger and Alexander, 1995). This process is referred to as “aging” and is generally a function of time. To determine if aging was a factor in the tree study, a separate investigation of PAH extractability was conducted. Five gram (fresh wt., sieved to 2 mm) of the same soil used in the tree study was placed in 30 ml glass vials. The vials were divided into three treatments: non-sterile/inoculated, sterilized/inoculated, and sterilized/not-inoculated. Each vial in the two sterilized treatments was autoclaved three times (on consecutive days) for 1 h at 121 °C. All vials were spiked with a PAH–acetone solution to bring the soil to the same final (PAH) concentration as used in the pot study. Vials were mixed by gently shaking and the caps were loosened in order to allow the acetone to volatilize. Over the next week, each vial was vortexed twice to ensure uniformity. Non-sterile and sterile–inoculated treatments were then given 1.5 ml of sterile distilled water and 50 µl of the soil microbial inoculum (described previously). The remaining sterilized vials were amended with 1.55 ml of sterile distilled water, bringing the moisture content of all vials to 70% of field capacity. Caps were tightened, sealed with parafilm, and stored at room temperature in the dark for the duration of the experiment.

Approximately 2, 4, 7, and 12 months after spiking, three replicates of each treatment were first extracted with a mild solvent (1-butanol) to estimate bioavailability, followed by a second, more exhaustive extractant (methylene chloride) to determine total PAH remaining (Kelsey et al., 1997). To begin an extraction, a 15 ml volume of butanol was added to the vial and then vortexed for 120 s. The resulting slurry was decanted into Nalgene centrifuge tubes. The original vials were rinsed with 15 ml of butanol, which was added to the centrifuge tube. Each tube was then centrifuged for ten min and the supernatant was collected and stored at 4 °C until analysis. Methylene chloride (30 ml) was added to the soil pellet and the tube was shaken on a horizontal shaker at 200 rpm for 24 h. The tube was centrifuged again and the supernatant collected and stored as before. To allow comparison between this study and the

pot study, an acetone extraction (as described above) was also performed on a separate set of replicate tubes sampled after 12 months (Table 1).

### 2.7. Data analysis

Tests of significance were evaluated with SYSTAT® 10.2 software. The quantity of PAHs remaining in the soil was compared within and across treatments and sampling times using ANOVA models and post-hoc Tukey tests. For each sampling time, differences in <sup>14</sup>C-pyrene mineralization among treatments were evaluated using one-way ANOVA for individual time points and across all time points with a repeated measures ANOVA. Correlations among PAH dissipation, mineralization, and plant biomass were investigated with linear regression.

## 3. Results and discussion

PAHs were rapidly lost from both planted and unplanted soils in this study. Three months after planting, 8–10% of the initial PAH concentration was recovered across treatments and only 1–3% remained 12 months after planting. Analysis of variance across the entire data set (Table 2), indicates that dissipation varied significantly with the PAH being measured and with the passage of time (Fig. 1). PAH specific dissipation in soil is expected based on differences in structure and physico-chemical properties among individual PAHs. PAH loss was not, however,

Table 2  
ANOVA of PAH levels at both sampling times in the tree study

Source	Sum-of-squares	df	Mean-square	F-ratio	p
PAH	13 685	2	6842	13.43	0.000007
TIME	9963	1	9962	19.55	0.00002
PLANTING	991	3	330	0.65	0.58
TIME * PAH	5108	2	2554	5.01	0.008
PLANTING * PAH	705	6	117	0.23	0.96
PLANTING * TIME	99	3	33	0.07	0.97
Error	47892	94	509		

Dependent variable: PAH recovery.

$n = 111$  multiple  $R = 0.63$  squared multiple  $R = 0.40$ .

Only the four planting treatments (black willow, red mulberry, sycamore, and unplanted) harvested at both sampling dates were included in the model. Planting treatments remained unresolved when analyzed within the 3- or 12-month data exclusively.

Table 1  
PAH recovery from soil in the aging study after 12 months

Soil treatment	Butanol	Methylene chloride	Total (butanol + methylene chloride)	Acetone
Non-sterile/inoculated	87 ± 19	41 ± 5	128 ± 21	65 ± 2
Sterilized/inoculated	162 ± 67	44 ± 13	207 ± 80	227 ± 79
Sterilized/not inoculated	925 ± 31	160 ± 15	1085 ± 27	1069 ± 33

Values represent the mean (± SE) mg kg<sup>-1</sup> of total PAH removed by sequentially applied butanol and methylene chloride and by the single step acetone extraction used in the tree study,  $n = 3$ .

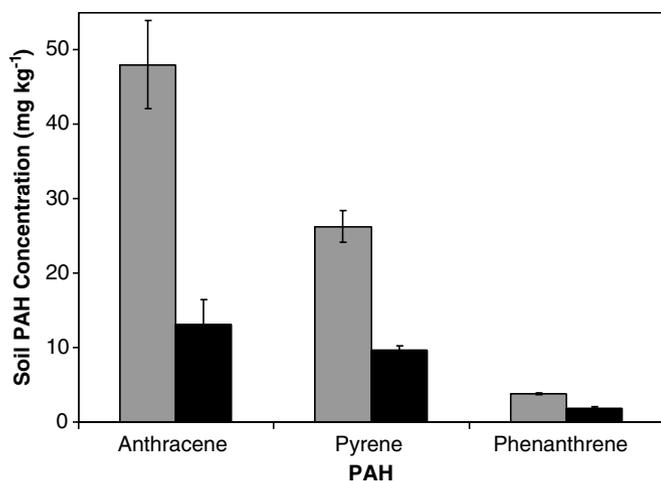


Fig. 1. PAH levels remaining in the soil of planted and unplanted (tree study) pots after 3 (gray) and 12 months (black). Bars represent means calculated from only the four treatments sampled at both sampling times. At 3 months, the amount of PAH remaining in the soil varied between compounds (anthracene > pyrene > phenanthrene,  $p < 0.05$ ), but after a year all were found at the same concentration in the soil. Independent analysis of all the treatments sampled at 12 months showed that levels of anthracene and pyrene remained statistically equivalent but were both significantly higher than phenanthrene ( $p < 0.05$ ). Soil sampled from each pot (within one week of setup and before planting) initially contained  $276 \pm 8$ ,  $305 \pm 6$ , and  $309 \pm 6$  mg kg<sup>-1</sup> of anthracene, pyrene, and phenanthrene respectively (mean  $\pm$  SE).

increased or decreased by planting the soil—regardless of the species involved.

Pyrene mineralization was also not affected by planting. These results were not anticipated when the study was initiated. After a year of experimentation the question was not simply (as planned), “*how do perennial, woody species impact soil PAH levels over extended time?*”, but rather “*what accounts for the near complete and rapid loss of PAHs from substantially contaminated soils?*”.

The most obvious explanations for the observed decrease in PAHs include those associated with soil processing—occurring during the initial spiking or the later extractions. The possibility of a miscalculation in the initial spiking step was eliminated by analysis of soil cores taken from each pot during the period between setup and planting. The levels found in these archived cores were as intended, essentially all equivalent to 300 mg kg<sup>-1</sup> of each PAH (see Fig. 1 caption). The low standard deviation across pots, instead, confirms the high degree of uniformity resulting from the labor-intensive spiking procedure.

The observed loss of PAHs over time was also not an artifact of the procedure used to extract the soil or a function of soil aging. There was some concern that the efficiency of PAH extraction would change as the compounds aged and sequestered into the soil matrix, but—as can be seen in the results of the aging study (Fig. 2)—that was not the case. Extraction consistently recovered all of the PAHs added to non-inoculated sterile soils while inoculated sterile and non-sterile soils showed consistent declines

in recovery over time ( $p < 0.05$ ). This was not due to changes in the physical structure of the soil because of autoclaving. Although it is known that sterilization can alter soil properties (Trevors, 1996), there were no significant differences in total PAH recovery between non-sterile and sterilized–inoculated treatments, suggesting that any changes due to sterilization had little impact on PAH fate in the soil and that the sterile treatment may be used to reflect the contribution of abiotic processes to PAH dissipation in non-sterile soils.

As in the tree study, the overall extent of PAH loss from non-sterile soils in the aging study was clearly compound-dependent; phenanthrene was consistently lost more rapidly and completely than either anthracene or pyrene. This similar pattern between the two studies was not seen in the magnitude of loss, which was greater in the tree study, especially in the first few months. In the tree study, 9% of the total PAHs remained in soils 3 months after planting (4 months after spiking) (Fig. 1), while 31% and 40% of total PAHs remained in the biologically active soils in the aging study after the same aging period (Fig. 2). The potted soils were subject to greater variation in moisture content, temperature, and light conditions, and open to ambient air. These factors likely contributed to any differences between the two studies.

Butanol extraction has been used elsewhere to estimate PAH availability in soil (Kelsey et al., 1997). Here, butanol extraction of soil aged for 12 months (Table 1) removed 85%, 78%, and 68% the total PAHs remaining (respectively) in the sterile, sterile/inoculated, and non-sterile/inoculated treatments. This high level of availability combined with the demonstrated high efficiency of the extraction procedures, suggest that the dissipation of PAHs in non-sterile soils of the aging study and (by analogy in the potted soil of the tree study) must be a result of biotic processes as opposed to abiotic sorption to soil constituents. The level of soil organic matter, a factor positively correlated with abiotic sequestration of PAHs in soil (Chung and Alexander, 2002), was low in our soil (1.8%) and provides further reason to discount abiotic sorption as a significant factor in the PAH dissipation found.

It is likely that microbial degradation of PAHs is largely responsible for the PAH dissipation in both studies conducted here. In the aging study, the presence or absence of microbes was the only significant factor affecting PAH dissipation. Although planting was an additional variable in the tree study, it did not affect the ability of soil microbial communities to mineralize pyrene (see Fig. 4 caption). Microbial communities present in soil sampled from all PAH-spiked pots (planted and unplanted) were clearly capable of completely degrading pyrene. PAH metabolites were not observed in the GC chromatograms of any soil extracts, further indicating that complete mineralization of PAHs also likely occurred in these experimental systems. A rapid shift of microbial activity in response to the presence of PAHs may explain the high rate of PAH dissipation and could also serve to minimize the amount available for

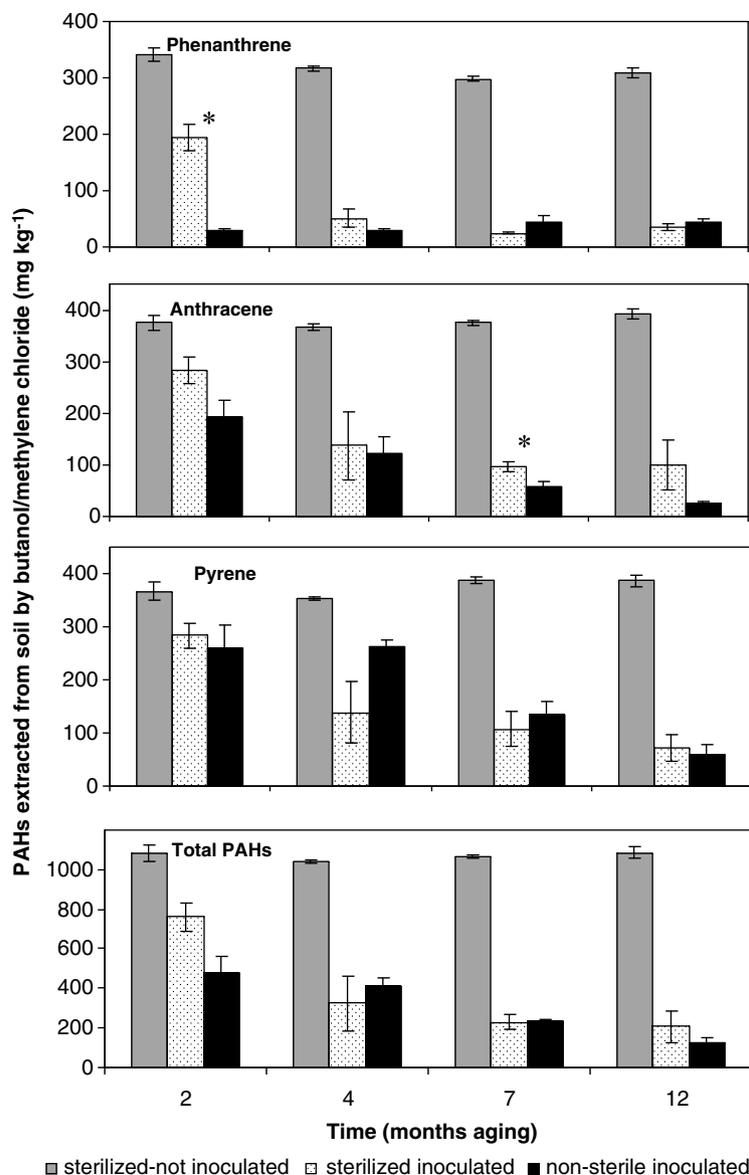


Fig. 2. Total and individual PAHs extracted from aging study soils over time by butanol and methylene chloride extractions. PAH recovery in sterilized (not inoculated) treatments was consistent across sampling times with one exception, a small but statistically significant difference occurred between phenanthrene levels recovered at 2 and 7 months ( $p < 0.05$ ). Within a given sampling time, extractable PAHs in non-sterile and sterile inoculated treatments generally did not differ (exceptions noted by \*,  $p < 0.05$ ).

abiotic sequestration in soil. Previous work has shown that PAH sequestration in soil is inversely related to the initial degradation rate of PAHs by microbes (Nam and Alexander, 2001).

The use of weathered contaminated soils from field sites may overcome problems associated with high bioavailability and microbial activity in spiked soil studies. However, field soils must often be homogenized to gain sufficient statistical power and this leads to priming effects that also result in artificially high bioavailability and microbial activity that can obscure treatment effects (Joner et al., 2004). For studies such as ours, in which the volume of soil required is quite high, the scale of collection, mixing, and exposure associated with generating a field soil is such that

use of spiked soils more feasible. It is advised that future studies utilizing either spiked or field contaminated soils be subjected to aging periods of at least one year prior to planting.

The previously mentioned lack of a planting effect on PAH dissipation was not due to the overall health of the trees. Although significant differences in tree biomass occurred between species, they did not vary for the same species grown in PAH or clean soil (data not shown). Among other factors, tree species differed significantly with respect to root biomass (Fig. 3), a factor that would be expected to contribute to PAH dissipation. Despite this, no differences in PAH dissipation occurred. Neither PAH dissipation nor pyrene mineralization were significantly

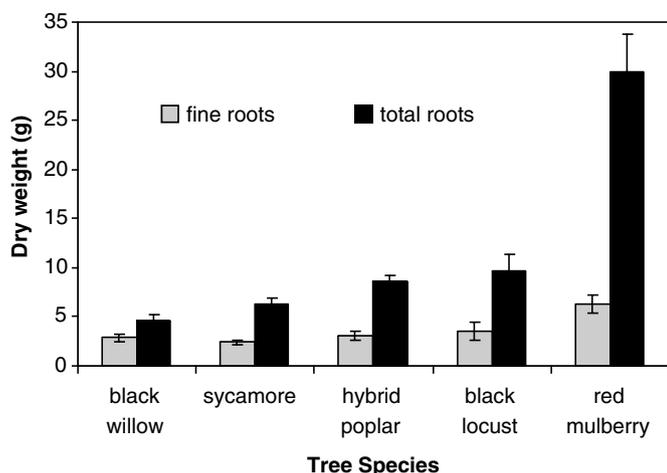


Fig. 3. Tree root weight after final harvest (12 months). Red mulberry trees had significantly more total roots than each of the other species and more fine roots than black willow, sycamore, and hybrid poplar ( $p < 0.05$ ). Root to shoot ratios for experimental trees were (from left to right above)  $0.35 \pm 0.03$ ,  $0.49 \pm 0.07$ ,  $0.51 \pm 0.18$ ,  $0.73 \pm 0.27$ , and  $1.02 \pm 0.43$ .

related to any measure of plant biomass. This further implies that factors other than planting, such as bioavailability and bulk microbial degradation, had a greater impact on PAH dissipation in this study.

The absence of a planting effect is not unique to this study. Despite strong lines of evidence supporting the ability of red mulberry trees to enhance dissipation of PAHs in one industrial sludge (Olson et al., 2001), planted treatments of red mulberry failed to reduce PAH levels more than unplanted treatments on another industrial sludge (Qiu and Loehr, 2002). Ryegrass (Binet et al., 2000), tall fescue, and switchgrass (Chen et al., 2003) have been reported to have beneficial effects on PAH dissipation in soil by some authors while other studies have shown no effect (Chekol et al., 2002) or even reduced dissipation (Olexa et al., 2000) in treatments planted with these species. Similarly, three species of pine tree seedlings appeared to foster dissipation of pyrene in one experiment (Liste and Alexander, 2000) while a fourth species inhibited mineralization of another 4-ring PAH, fluorene, in a separate study (Genney et al., 2004). Multiple factors are likely to be responsible for the lack of planting effects in this study and for the diversity of outcomes in separate studies utilizing the same plant species. Variation in soil properties is one important factor. Several studies have revealed different effects on PAH dissipation or PAH degraders when comparing planted treatments in different soils within the same experiment (Binet et al., 2000; Olexa et al., 2000; Chekol et al., 2002). Thus, our results are not likely representative of outcomes in other soils.

In the tree study conducted here, the magnitude of PAH loss (by microbial action) may simply have masked any subtle effects of planting and/or individual species. Additionally, trees may by their very nature pose problems for the demonstration of organic phytoremediation by rhizo-

sphere degradation. Most treatability and mechanistic studies have utilized herbaceous annuals and grasses. When compared to these plant types, tree roots are structurally and functionally different, particularly with regard to their perennial and woody nature and relationships with ectomycorrhizal fungi (Linderman, 1988). Unique root dynamics associated with these traits, including exudation and root turnover, could lead to relatively more complex rhizosphere dynamics and important effects on PAH degradation in the rhizosphere.

At least one important aspect of tree root input to the rhizosphere inhibited degradation in the experimental system used for this study. Final  $^{14}\text{C}$ -pyrene mineralization by the soil microbial community was reduced by 10% due to the addition and subsequent decay of excised red mulberry fine roots (difference not significant,  $p < 0.25$  in paired  $t$ -tests for day 20–65 [Fig. 4]). These results contradict previous research suggesting that fine root turnover may have contributed to reduced PAH concentrations in contaminated sludge in the root zone of mulberry trees (Olson et al., 2001). Other research regarding impacts of root-derived carbon on PAH degradation is similarly conflicted. In contrast to studies demonstrating positive effects of root exudates from annuals or herbaceous plants on PAH degradation (Yoshitomi and Shann, 2001), recent studies of trees, including hybrid poplar, red mulberry, and willow, indicate that root exudates and/or root extracts can actually inhibit the phenanthrene degrading activity (Rentz et al., 2004) and PAH catabolic gene expression (Kamath et al., 2004) of individual bacterial degraders. While the authors suggested this inhibition may be overcome by the increased number of bacteria in the rhizosphere of plants, it is not known whether this actually occurs, whether the effects scale up to the level of a microbial community, or what other factors may control this

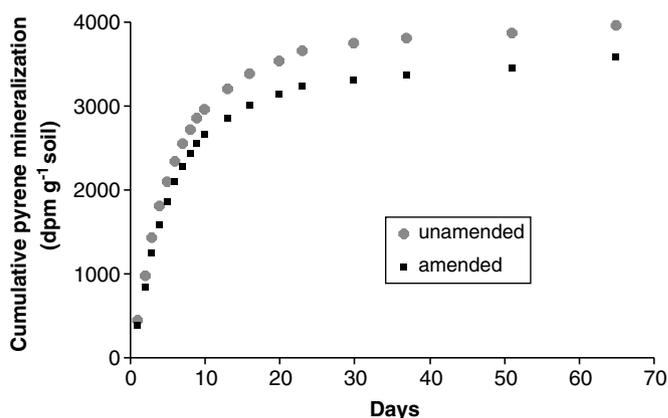


Fig. 4. Effect of excised red mulberry fine roots on pyrene mineralization in soil. Pairs of amended and unamended soil samples were taken from replicate pots planted with red mulberry trees ( $n = 5$ ). Final cumulative mineralization of all planted and unplanted treatments ranged from 48% to 55% of the initial added dpm at 3 months and from 57% to 64% at 12 months. Planted and unplanted treatments did not differ with respect to the ability of the soil microbial community to degrade pyrene.

outcome. Qiu et al. (2004) showed that mineralization of benzo[a]pyrene in soil collected from the root zone of mulberry trees was inhibited by a plant phenolic known to be released from mulberry roots and previously suggested to enhance organic contaminant degradation due to its ability to support the growth of PCB degraders (Leigh et al., 2002).

In light of these confounding results and the complex nature of tree root dynamics, traditional views of rhizosphere effects on PAH dissipation may be oversimplified; particularly so with respect to trees. Differences in the quantity and quality of nutrients released by root exudation and root mortality (Olson et al., 2003) likely lead to variable and potentially opposing effects on microbial PAH degradation, which could reduce or neutralize the net effect of planting on PAH degradation. The balance between these spatially and temporally variable root processes and related microbial responses may determine if soil contaminants are degraded or not. The impact of such effects would increase in studies such as ours, which use perennial, woody species and/or allow treatments to experience daily and seasonal changes in environmental conditions over the course of a year or longer. Fine root growth and mortality are known to be affected by temperature and follow distinct seasonal patterns (Tierney et al., 2003). Since exudation is highest at growing root tips (Grayston et al., 1996), exudation should also vary in the same manner. Soil microbial populations in planted ecosystems also show seasonal variation (Smalla et al., 2001). Although not measured in our study, seasonal patterns of fine root growth and mortality have been reported for one year old red mulberry trees (Leigh et al., 2002). Therefore, seasonal variation in environmental factors and related changes in rhizosphere dynamics may have played a role in our ability to statistically determine the fate of PAHs in planted treatments.

#### 4. Conclusions

Rapid and extensive loss of PAHs was observed regardless of the presence or absence of trees. Microbial degradation was considered to be the dominant mechanism of PAH loss, as bioavailability and extractability remained high throughout parallel soil aging experiments. The effects of trees on PAH degradation may be more pronounced in soils with lower bioavailability or with less active microbial communities. The inhibitory effect of decaying roots on microbial mineralization of pyrene suggests that the overall effect of trees on PAH dissipation may have been further masked by the complex root dynamics of woody perennials and the seasonal component of the study.

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