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Arbuscular mycorrhizal phytoremediation of soils contaminated with phenanthrene and pyrene

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ABSTRACT

An available remediation technique — arbuscular mycorrhizal phytoremediation (AMPR) — is further proposed for soils contaminated with phenanthrene and pyrene as representative polycyclic aromatic hydrocarbons (PAHs) utilizing a greenhouse pot experiment. The initial concentrations of phenanthrene and/or pyrene in soils were 103 mg kg^{-1} and 74 mg kg^{-1}, respectively. The host plant was alfalfa (*Medicago sativa* L.), and the experimental arbuscular mycorrhizal fungi (AMF) were *Glomus mosseae* and *G. etunicatum*. More than 98.6% and 88.1% of phenanthrene and pyrene were degraded after 70 days in soils with AMPR. Use of multiple mycorrhizal species significantly promoted degradation of PAHs in soils. The co-contaminant (pyrene) present clearly inhibited the degradation of a single PAH (phenanthrene) in soil. Mycorrhizal colonization caused increased accumulation of PAHs in plant roots but a decrease in shoot. However, plant uptake contributed negligibly to PAH dissipation in AMPR, and plant accumulated PAHs amounted to less than 3.24% of total PAH degradation in mycorrhizal soils. In contrast, the optimized microbiota in mycorrhizal association was responsible for PAH degradation in AMPR. The high rate of PAH dissipation in mycorrhizal soils, the evident promotion of PAH degradation by AM colonization, and the healthy plant growth suggest encouraging opportunities for AMPR of PAH-contaminated soils.

1. Introduction

Soil is a primary natural resource for humans. However, organic pollutants frequently find their way into soils as a result of air deposition, sewage irrigation, and industrial accidents. Soil contamination by organics poses a great threat worldwide to agricultural food quality, the food chain, and eventually to human health. Polycyclic aromatic hydrocarbons (PAHs) are by-products of the incomplete combustion or pyrolysis of organic materials, and are of the main pollutants of concerns in the environment due to their recalcitrance and strong mutagenic/carcinogenic properties [1,2]. The hydrophobic characteristics and persistence of PAHs result in their accumulation and enrichment in soils [3–6]. They are widespread and present at high concentrations of hundreds of mg kg^{-1} in soils of many countries [7,8]. Hence, immediate action is required to remediate such contaminated sites at risk.

Phytoremediation is recognized as one of the most cost-effective, reliable, and promising technology for decontamination of polluted soils [6,9]. The enhanced degradation of organic pollutants is due to plant-stimulated microbial degradation in the rhizosphere [10]. The surface area of root–soil contact and rhizosphere microbial activity are major limiting factors in the phytoremediation process, and are theoretically met by mycorrhizal associations.

Plant–microbe symbioses are ubiquitous in the environment. As the most widespread type of mycorrhizae, arbuscular mycorrhizae (AM) are starting to receive attention in the context of phytoremediation of organic pollutants [10–13]. Recent studies have shown that AM have positive effects on plant establishment and survival in contaminated soils [13,14], suggesting the potential of AM in phytoremediation schemes. To our knowledge, Binet et al. [15] and Joner et al. [10] first reported the effects of inoculating herbaceous plants with AM on degradation of organics in soils. Positive effects of AM inoculation were subsequently observed on degradation of PAHs including phenanthrene, anthracene, chrysene, dibenz(a,h)anthracene, pyrene, and benzopyrene, and the mycorrhiza-associated microbiota was considered to be responsible for the PAH reduction in soil [10,16–18]. Enhanced dissipation of other organic contaminants in soil by the presence of AM has also been reported in literature [19].

However, there are about 170 species of AM and more than 1000 suspected organic pollutants [20]. Only a very limited number of AM and organics have been investigated. More experimental data on arbuscular mycorrhizal phytoremediation (AMPR) are urgently needed to support this technique. In addition, there are still important challenges to be considered in AMPR. Many results reported so

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far are generally concerned with only a single pollutant and/or a single AM. This is not the case in the natural environment. The effects of co-existing AM on phytoremediation of soil containing multiple pollutants require further study. Variation of the microbial community and enzyme in mycorrhizal soils is not well defined. As a result, the mechanisms involved in AMPR still need to be further evaluated and more experimental data are required. Moreover, although plant uptake of organics is negligible in the removal of organics by phytoremediation [3,6], information about PAH distribution in plants is essential to guide the screening of plants used in phytoremediation and to ensure the security of agricultural products grown on contaminated sites. However, little experimental data are hitherto available on the impacts of AM on plant uptake of organics such as PAHs in soil.

Thus the objectives of this work were to investigate the effects of co-existing AM on AMPR for soils with single/multiple PAH pollutants,and the impacts of AM on plant uptake and accumulation of PAHs in soils. The mechanisms of AMPR for PAH-contaminated soils were elucidated.

2. Materials and methods

2.1. Solutes

Phenanthrene and pyrene (purity >98%) were obtained from Aldrich Chemical Co. The molecular weight (M_w, g mol^{-1}), solubility in water at 25 °C (S_w, mg L^{-1}), and log K_{ow} (where K_{ow} denotes the octanol–water partition coefficient) of phenanthrene and pyrene are 178.23 and 202.26 g mol^{-1}, 1.18 and 0.12 mg L^{-1}, 4.46 and 4.88, respectively [21].

2.2. Greenhouse pot experiment

Soil samples were collected from the A (0–20 cm) horizon in Nanjing, China, with a pH of 6.02, 2.41% soil organic matter, and originally free of PAHs. The soil type is Typic Paleudalfs. The clay, silt, and sand contents of the tested soil were 24.7%, 61.9%, and 13.4%, respectively. Soil samples were air-dried and sieved through a 2-mm mesh.

Original inoculum of the AM fungi *Glomus mosseae* (AMF1; BGC GD01A) and *G. etunicatum* (AMF2; BGC HUN02C) was propagated in pot culture on sorghum for 10 weeks in a zeolite-sand mixture in a greenhouse. Then inoculum, a mixture of spores, mycelium, sand and root fragments, was air-dried and sieved (<2 mm).

The plant growth medium was a 3:1 (w/w) mixture of soil and sand (sieved through a 2-mm mesh). The soil mix (henceforth referred to as soil) was sterilized by γ-radiation (10 kGy, 10 MeV γ-rays) to inactivate the native AM fungi. Soil samples were then spiked with a solution of phenanthrene and/or pyrene in acetone (10% of soil to be spiked). After the acetone had evaporated, the spiked soils were progressively mixed with clean soil and homogenized. To ensure homogeneity of the treatments, soils were then sieved again through a 2-mm mesh [22]. The final concentrations of phenanthrene and/or pyrene in treated soils, chosen according to the general concentrations observed in contaminated soil, were 103 mg kg^{-1} and 74 mg kg^{-1} (on a dry weight basis), respectively. The treated soils were then packed into pots (350 g dry weight soil per pot). Mycorrhizal pots were inoculated with 20 g AMF1 and/or AMF2. The non-mycorrhizal controls received an equivalent amount of γ-radiation-sterilized inoculum to provide similar conditions, except for the absence of the active mycorrhizal fungus. All pots were equilibrated in a glass greenhouse to 50% water-holding capacity.

Pre-germinated seeds of alfalfa (*Medicago sativa* L.) were sown in each pot. The seedlings grew in greenhouse at 25–30 °C during daytime and at 20–25 °C during night, and were thinned 7–10 days after emergence, to leave six plants per pot, giving spiked soils with phenanthrene and/or pyrene (a) without AM inoculation, (b) with AMF1 inoculation, (c) with AMF2 inoculation, and (d) with AMF1 and AMF2 inoculation. Each treatment was replicated in triplicate, and the treated pots were arranged randomly in the greenhouse and re-randomized every 4 days. Soils and plants were destructively sampled after 30, 45, 60 and 70 days since sowing. Plant shoots and roots separated from soils were washed with distilled water and then dried with filter paper.

2.3. Sorption experiment

The same pot cultivation system was used to obtain alfalfa roots for sorption experiment without AMF inoculation. The sterilized soils packed in pots were un-spiked and free of PAHs. Roots of alfalfa were collected 60 days after cultivation. A modified glass bead compartment cultivation system was utilized to obtain AM hyphae according to Chen et al. [23] and Gao et al. [20].

A batch experiment was conducted to determine PAH sorption by roots and hyphae according to literatures [20,24]. We mixed 20 ml PAH solution in water containing 0.05% NaN_3 with roots or AM hyphae in 30-ml glass centrifuge tubes sealed with screw caps. The tubes were shaken in the dark for 24 h at 250 rpm on a gyratory shaker to reach the equilibrium state. An aliquot of supernatant was removed and analyzed for PAH concentrations. All equilibrium concentrations of PAH measured in solution were below their aqueous solubilities.

2.4. Determination of root colonization

Root colonization by AM was determined according to Phillips and Hayman [25] and Huang et al. [19]. A 1-g subsample of fresh roots was randomly taken and cut into approximately 1-cm pieces to estimate the proportion of total root length colonized by AM. Root segments were immersed in 10% (v/v) KOH for 10 min at 90 °C in a water bath, rinsed with water, and then stained with 0.1% trypan blue for 3–5 min at 90 °C in a water bath. Mycorrhizal colonization was determined by the grid line intersect method [19,25]. Briefly, the stained root segments were arranged lengthwise on a thin layer of PVA mounted on a microscope slide. A hairline graticule inserted into the eyepiece of a compound microscope acted as a line of intersection with the roots. Fungal structures at each intersection were calculated by observation at 200× magnification.

2.5. PAH analysis of soil, plant, and water samples

The soil from pots was carefully collected, homogenized, and passed through a 20-mesh standard sieve. Sample preparation included mixing the sample with anhydrous Na_2SO_4 to remove moisture and ultrasonicating in 10 ml dichloromethane for 1 h, followed by centrifugation at 3000 × *g* for 20 min. Then, 3 ml of supernatant were filtered through a 2-g silica gel column with a 12 ml 1:1 (v/v) elution of hexane and dichloromethane. The solvent fractions were then evaporated off, and exchanged for methanol, to a final volume of 2 ml [8,24]. The samples were then rotary-evaporated and exchanged for methanol to a final volume of 2 ml for HPLC analysis. Recoveries of known amounts of phenanthrene and pyrene through the complete analytical process amounted to 91.3–95.6% with a relative standard deviation (RSD) of less than 3.1%.

Plant samples were freezing-dried, ground, and homogenized, and extracted by ultrasonication for 1 h in a 1:1 (v/v) solution of acetone and hexane. The solvent was then decanted, collected, and replenished. This process was repeated three times. The solvents were then evaporated and exchanged for 2 ml hexane, followed

Table 1
Arbuscular mycorrhizal colonization (%) of alfalfa (*Medicago sativa* L.) exposed to PAH(s) in soil.

AMF type	PAHs in soils	Time (days)		
		45	60	70
AMF1	Phenanthrene	56.1 (6.73)a	55.7 (11.7)ab	60.5 (4.95)b
AMF2	Phenanthrene	–	–	76.5 (15.5)a
AMF1 + AMF2	Phenanthrene	47.5 (8.09)a	63.3 (9.07)a	70.5 (7.78)a
AMF1	Pyrene	–	–	66.0 (9.90)ab
AMF1 + AMF2	Pyrene	–	–	54.0 (14.1)bc
AMF1	Phenanthrene + pyrene	26.4 (7.56)b	48.3 (17.8)ab	52.5 (2.12)c
AMF2	Phenanthrene + pyrene	–	–	61.0 (5.66)ab
AMF1 + AMF2	Phenanthrene + pyrene	30.3 (9.99)b	52.3 (4.16)b	41.0 (14.3)d

Data in brackets are standard deviations (SD). AMF means arbuscular mycorrhiza. (–) Not measured. Values in the same columns followed by the same letter are not significantly different ($P < 0.05$).

by filtration through a 2-g silica gel column with 12 ml 1:1 (v/v) elution of hexane and dichloromethane. Samples were then evaporated and exchanged for methanol to a final volume of 2 ml for HPLC analysis [20]. The average recoveries obtained by spiking plant samples with phenanthrene and pyrene were 103% ($n = 5$, RSD < 3.3%) and 90% ($n = 5$, RSD < 4.1%) for the entire procedure.

3-ml aliquots of solution collected from sorption experiment were mixed with 7 ml of methanol (HPLC grade). The mixture was filtered through a 0.22-mm filter, and the PAH concentration was determined by HPLC [24].

The treated soil and plant extracts as well as the water samples were analyzed for the parent contaminant using an HPLC fitted with a Φ4.6 mm × 250 mm reverse-phase C_{18} column, with methanol as the mobile phase, at a flow rate of 1 ml min^{-1}. Chromatography was performed at 30 °C. Phenanthrene and pyrene were detected at 245 and 234 nm, and their detection limits were 44.1 and 50.2 pg, respectively.

2.6. Statistical analysis

The significance of treatments was tested using two-way analysis of variance using the SPSS version 13.0 software package ($P < 0.05$). All data and figures were processed using the SPSS (SPSS 13.0) and Microsoft Excel software packages.

3. Results and discussion

3.1. Colonization by AM

Joner et al. [10] reported that colonization by *G. mosseae* of clover and ryegrass in soils spiked with anthracene, chrysene, and dibenz(a,h)anthracene was 20–40% and 0.5–5.0%, respectively . Here, colonization by the two tested AM species, *G. mosseae* (AMF1) and/or *G. etunicatum* (AMF2), of alfalfa in soils contaminated with phenanthrene and/or pyrene ranged from 26.4% to 76.5% after 45–70 days (Table 1). Since the soils in our greenhouse experiments were sterilized, the colonization of roots by soil indigenous fungi was undetectable. Compared with phenanthrene-spiked soil, soil co-contamination by phenanthrene and pyrene generally reduced mycorrhizal colonization. For instance, the respective colonization of AMF1 on alfalfa in phenanthrene-spiked soils was 56.1%, 55.7%, and 60.5% after 45, 60, and 70 days. By contrast, the corresponding colonization in soils co-contaminated with phenanthrene and pyrene was 13.2–52.9% lower. This indicates enhanced toxicity to mycorrhizae with the addition of the co-contaminant in the soil environment.

3.2. AM enhanced degradation of PAHs in soils

Enhanced degradation of phenanthrene and pyrene was observed in vegetated soils with AM inoculation versus non-AM controls (CK) after 30–70 days (Fig. 1). The presence of AM enhanced the rhizospheric effects on tested PAHs. As shown, relative to CK, the concentrations of phenanthrene and pyrene in mycorrhizal soils were 10.0–63.5% and 23.4–47.0% lower, respectively, and more than 98.6% of phenanthrene and 88.1% of pyrene had been degraded in mycorrhizal soils after 70 days.

The enhancement of PAH degradation was more significant when multiple mycorrhizal species were present in alfalfa-planted soils. As seen in Fig. 1, the residual concentrations of phenanthrene in soils with AMF1 after 30, 45, 60, and 70 days were 3.85, 1.78, 1.41, and 1.35 mg kg^{-1}, respectively, whereas concentrations in soils with both AMF1 and AMF2 were 15.6–35.6% lower. Similarly, concentrations of pyrene in soils with both mycorrhizal species (AMF1 and AMF2) were 8.60–21.5% lower than in soils with AMF1 alone. Thus, the coexisting mycorrhizal species promoted dissipation of PAHs in the rhizosphere.

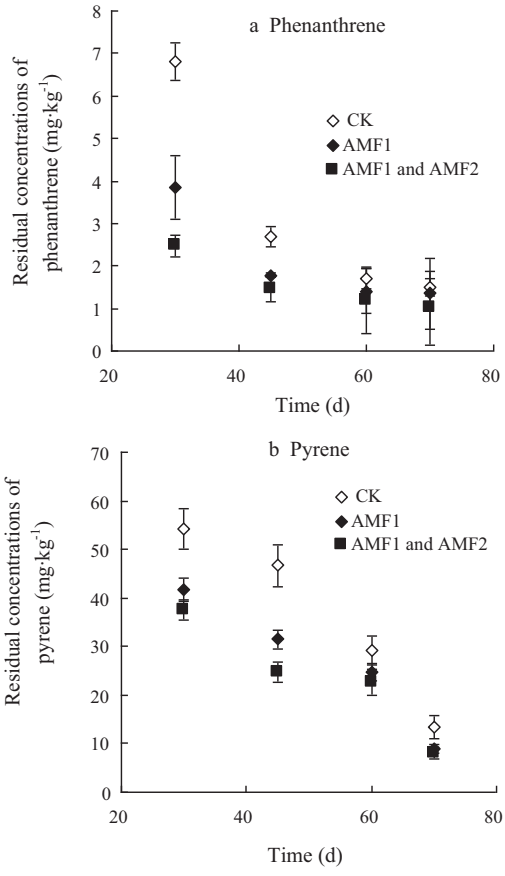

Fig. 1. Residual concentrations of phenanthrene (a) and pyrene (b) in various treated soils as a function of time. Error bars are standard deviations (SD).

The most significant promotion of PAH degradation in soils containing the tested AM was observed at 30 and 45 days, whereas residual PAH concentrations had changed little by 70 days (see Fig. 1). Clearly, the presence of AM reduced the degradation time of the tested compounds. As reported by Gao et al. [26], the availabilities of persistent organic pollutants (POPs) in soils decrease over time, and their available portions including desorbing and non-desorbing fractions that can be taken up by plants and/or soil-inhabiting animals are most readily biodegradable and dissipate rapidly in soils. In contrast, bound residues of POPs in soils are generally recalcitrant [1]. In the first stage, the available fractions of PAHs in the rhizosphere degraded rapidly, and enhanced microbiota activity resulting from mycorrhizal associations led to the positive phytoremediation effects of AM. However, the available portions of PAHs were reduced over time, and the bound residues remained in the soils. Thus, PAHs became harder to degrade in soils, and little difference was observed between AMF and non-AMF treatments at 70 days.

Recently, the use of mycorrhizae to enhance phytoremediation of organic pollutants has received more attention due to their potential effects on plant establishment in contaminated soils [10,11]. Joner et al. [27] found that the ectomycorrhizal fungus *Suillus bovinus* forming a mycelium in soil in close contact with hydrophobic pollutants impeded rather than promoted PAH degradation in the soil. By contrast, positive effects of AM inoculation were reported on degradation of phenanthrene, anthracene, chrysene, pyrene, dibenz(a,h)anthracene, and benzopyrene, and the mycorrhiza-associated microbiota was considered to be responsible for the observed PAH reduction in the soil [10,16–18]. This is in agreement with results of the present study.

Residual concentrations of pyrene for all treatments were much higher than those of phenanthrene. Degradation rates (D; %), i.e., the amount degraded relative to the initial amount in the soil, were also calculated. D values of phenanthrene in soils with AM colonization were 96.3–99.0% after 30–70 days, which is 12.1–120% higher than those of pyrene. This indicates that PAHs with higher molecular weights and more benzene rings (pyrene) are more recalcitrant and difficult to degrade in soils. This result is also supported by other studies [3,7].

Environmental contamination often leads to the presence of mixtures of organic contaminants in soils. For instance, industrial enterprises such as coking plants have resulted in the simultaneous accumulation over decades of PAHs at high concentrations in surrounding soils. Here, we investigated the degradation of phenanthrene in AM soils with a co-contaminant (pyrene). As shown in Fig. 2, the presence of pyrene in soil significantly inhibited dissipation of phenanthrene in the rhizosphere. The residual concentrations of phenanthrene in single phenanthrene-spiked soils were 0.94–3.33 mg kg^{-1}, which is ~34.6% lower than in soils with pyrene as co-contaminant, indicating that co-contamination inhibits degradation of a single chemical in the soil environment.

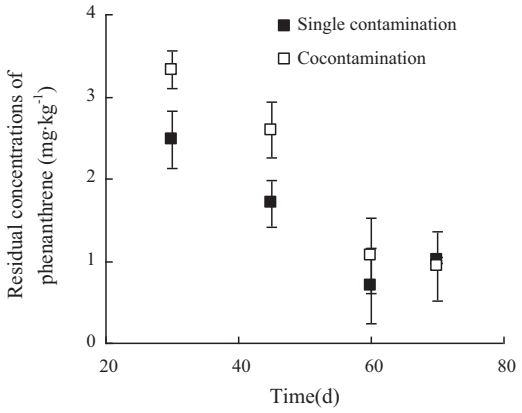

Fig. 2. Residual concentrations of phenanthrene in soils contaminated by single phenanthrene or phenanthrene with cocontaminant of pyrene. Error bars are standard deviations (SD).

PAHs are considered a serious health risk at very low concentrations; thus, even a small remediation enhancement by AM is potentially important. The high rate of phenanthrene and pyrene dissipation in mycorrhizal soils, the evident promotion of phenanthrene and pyrene degradation by AM colonization, and the healthy plant growth observed in this study all suggest potentially encouraging opportunities for arbuscular mycorrhizal phytoremediation (AMPR) of PAH-contaminated soils.

3.3. Mechanisms of enhanced degradation of PAHs in soils with AM

3.3.1. Plant uptake of PAHs

As shown in Table 2 and Fig. 3, alfalfa clearly took up and accumulated PAHs from spiked soils. Root concentrations of phenanthrene and pyrene were always significantly higher than in shoots, irrespective of inoculation with AMF1, AMF2, or both, indicating restricted translocation of the tested PAHs into the plant body. Arbuscular mycorrhizal inoculation significantly impacted alfalfa uptake of the tested PAHs from soils. As shown in Fig. 3, inoculation with *G. mosseae* and/or *G. etunicatum* clearly caused a decrease in PAH concentrations in shoots. The respective concentrations of phenanthrene in shoot from soils inoculated with AMF1 and both AMFs after 45, 60, and 70 days were 18.3–31.6% and 33.1–43.1% lower than those in non-mycorrhizal control treatments. In contrast to shoots, AM-inoculated roots consistently accumulated more phenanthrene than non-mycorrhizal roots. Root concentrations of phenanthrene in plants grown for 70 days in phenanthrene-spiked soils with AM inoculation were ~40.2% higher than those of non-mycorrhizal controls (see Table 2). Recently, Wu et al. [28] investigated the uptake of DDT by alfalfa

Table 2
Concentrations of phenanthrene and pyrene in plant roots and shoots for various treatments after 70 days.

AMF	PAHs in soils	Plant concentrations of phenanthrene (mg kg^{-1})		Plant concentrations of pyrene (mg kg^{-1})	
		Shoot	Root	Shoot	Root
No AMF	Phenanthrene	0.73 (0.05)c	1.27 (0.23)c	–	–
AMF1	Phenanthrene	0.61 (0.14)cd	1.78 (0.73)bc	–	–
AMF2	Phenanthrene	0.59 (0.03)d	1.33 (0.20)c	–	–
AMF1 + AMF2	Phenanthrene	0.59 (0.08)d	1.63 (0.04)b	–	–
AMF1	Phenanthrene + pyrene	1.29 (0.08)a	3.05 (0.16)a	0.47 (0.12)a	16.26 (7.30)a
AMF2	Phenanthrene + pyrene	1.21 (0.23)a	3.86 (1.36)ab	0.53 (0.18)a	11.54 (4.73)ab
AMF1 + AMF2	Phenanthrene + pyrene	0.85 (0.09)bc	3.66 (1.05)a	0.58 (0.11)a	8.87 (4.83)b

Data in brackets are standard deviations (SD). AMF means arbuscular mycorrhiza. (–) Not measured. Values in the same columns followed by the same letter are not significantly different ($P < 0.05$).

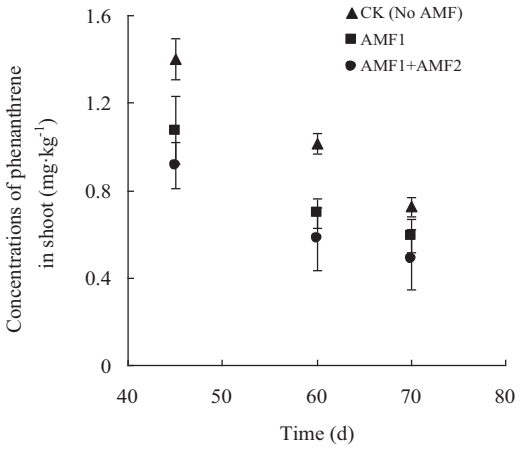

Fig. 3. Concentrations of phenanthrene in shoots of alfalfa grown in phenanthrene-contaminated soils as a function of uptake time.

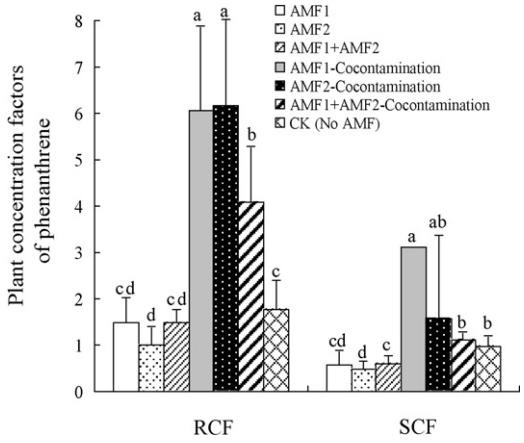

Fig. 5. Plant concentration factors of phenanthrene for various treatments. RCF and SCF are root concentration factor and shoot concentration factor, respectively. Error bars are standard deviations (SD). Values above columns for RCF or SCF followed by the same letter are not significantly different ($P < 0.05$).

(*Medicago sativa* L.) after AM inoculation, and found that mycorrhizal colonization led to increased accumulation of DDT in roots but a decrease in shoots. The present results agree with these findings, and highlight the importance of AM inoculation to plant uptake of organic contaminants.

Enhanced PAH uptake by alfalfa roots in the presence of AM could be attributed to increased PAH partitioning in roots by AM colonization. Recent studies have shown that hydroponic organic contaminants in soils enter plants primarily via a passive process. This transport process can be treated as a series of contaminant partitions, comprising partitions from soil to soil pore water, from soil water to plant root, and from xylem water to shoot [8,20,29]. The partitioning of organic chemicals between water and root is the first and predominant step in determining the process of uptake of organic compounds by plants. Extension of mycorrizal hyphae greatly increases the surface area of root–soil contact, and the length of hyphae could be several orders of magnitude greater than that of plant roots [11,13]. The partitioning of phenanthrene between plant root and water as well as hyphae and water was examined, and the results are shown in Fig. 4. Regression was performed between the amounts of phenanthrene absorbed by roots or hyphae and its equilibrium concentration in solution. The partition isotherms by the roots and hyphae were significantly linear ($P < 0.05$). The partition coefficient (K_{rt}, L/kg) of phenanthrene between root or hypha and water is expressed as:

$$K_{rt} = \frac{Q_{eq}}{C_w}$$

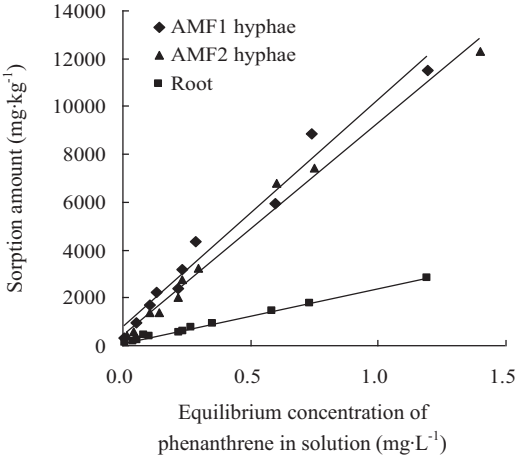

Fig. 4. Sorption of phenanthrene by root and arbuscular mycorrhizal hyphae.

where Q_{eq} denotes the amount of phenanthrene absorbed by the root or hypha (mg kg^{-1}), and C_w is the equilibrium concentration of phenanthrene in the aqueous phase (mg L^{-1}) [29]. K_{rt} values obtained by linear regression of the absorption data were 2275 L/kg for roots, 9499 L/kg for AMF1 hyphae, and 8862 L/kg for AMF2 hyphae. Clearly, the partitioning of phenanthrene between hyphae and water was much stronger than that between root and water. This indicates that mycorrhizal colonization significantly enhanced partitioning of PAH between root and soil pore water, resulting in increased root concentrations after AM inoculation.

The presence of the co-contaminant (pyrene) significantly influenced uptake of phenanthrene by mycorrhizal plants. As shown in Table 2, concentrations of phenanthrene in mycorrhizal roots and shoots were clearly much higher in phenanthrene and pyrene-spiked versus single phenanthrene-spiked soils. In our previous work, plant concentrations of PAHs generally increased as PAH concentrations in the soil environment increased [3]. This is also supported by results of other studies [5]. As shown in Fig. 2, the presence of a co-contaminant (pyrene) led to higher residual concentrations of phenanthrene in soils, and consequently resulted in higher concentrations of phenanthrene in the plant body.

The values for SCF and RCF for uptake of phenanthrene by alfalfa with AM inoculation are provided in Fig. 5. RCFs for all treatments were 1.48–6.16, which is generally much higher than the corresponding SCFs (0.49–3.11). The presence of a co-contaminant (pyrene) significantly enhanced RCF and SCF values for alfalfa uptake of phenanthrene from spiked soils. For instance, RCFs for phenanthrene-spiked soils with AMF1, AMF2, and both were 1.49, 0.99, and 1.49, respectively, whereas RCFs for phenanthrene and pyrene-spiked soils with these AMF(s) were 6.05, 6.16, and 4.10, respectively. A similar trend was found for SCFs of phenanthrene uptake by alfalfa.

The contribution of plant accumulation of PAHs to the dissipation of PAHs from soils was calculated (Table 3). Although plants definitely took up PAHs, the accumulated amounts of phenanthrene and pyrene in plants were less than 1.12% and 3.24% of their total degradation in soils, indicating the minor contribution of plant accumulation to the AMPR. In theory, a portion of these PAHs after plant uptake from the soil environment may have been metabolized in the plants during the experiment. Plant metabolism of PAHs has been reported [2,4]; however, for PAHs of higher molecular weight and with more benzene rings, like phenanthene and pyrene, only a very small portion is metabolized in plants. Phytovolatilization has been shown to be significant for hydrophilic volatile organic

Table 3
Contribution of plant accumulation of phenanthrene and pyrene to their degradation in various treated soils. The initial concentrations of phenanthrene and pyrene in soils were 103 mg kg^{-1} and 74 mg kg^{-1}, respectively.

Treatments	Phenanthrene			Pyrene		
	D (mg pot^{-1})	P (μg pot^{-1})	T (%)	D (mg pot^{-1})	P (μg pot^{-1})	T (%)
AMF1	36.0	3.31	0.92	23.5	7.63	3.24
AMF2	35.8	4.01	1.12	21.9	6.14	2.79
AMF1 + AMF2	35.7	2.95	0.83	18.1	4.42	2.45

D – dissipation amounts of phenanthrene or pyrene in soils. P – plant accumulated amounts of phenanthrene or pyrene. T – contributions of plant accumulation to the total dissipation of PAHs in soils (%).

chemicals such as MTBE [30]. For hydrophobic organic chemicals, such as phenanthrene and pyrene, with generally low H values, their uptake by plants could be described as partitioning between the soil aqueous solution and the plant roots [29]. The translocation of these compounds from root to shoot is known to be highly restricted [3]. Therefore, phytovolatilization of phenanthrene and pyrene should be negligible. On the whole, plant uptake and accumulation were a minor contribution to the dissipation of the tested PAHs in the AMPR process.

3.3.2. AM promoted microbial biodegradation of PAHs in the rhizosphere

The loss of phenanthrene and pyrene from soils with mycorrhizal and non-mycorrhizal plants consists of abiotic dissipation (e.g., leaching, sorption to greenhouse pots, photo-oxidation, and volatilization), microbial biodegradation, and plant uptake and accumulation. Abiotic dissipation has been shown to be a minor pathway for loss of PAHs from vegetated soils [6], and there should be little difference in abiotic dissipation between mycorrhizal and non-mycorrhizal soils. Plant accumulation accounted for less than 1.12% and 3.24% of the total dissipation of phenanthrene and pyrene from mycorrhizal soils, respectively, and in theory, even less for the enhanced degradation of PAHs in mycorrhizal soils. As such, the dissipation of test PAHs in soils with non-mycorrhizal plants (CK treatment) resulted dominantly from the microbial biodegradation in rhizosphere, and the enhanced loss of the tested PAHs in mycorrhizal versus non-mycorrhizal soils should predominantly come from mycorrhiza-promoted microbial biodegradation. This suggestion is supported by the microbes found in soils. As seen in Table 4, the respective amounts of bacteria, fungi, and actinomycetes in soils with AMF1 or AMF2 were 9.85–48.2%, 270.1–328.5%, and 46.1–91.8% higher than those in non-mycorrhizal control soils, suggesting that colonization by AM significantly promoted microbial production, and that the mycorrhiza-associated microbiota are responsible for the observed reductions in PAH concentrations in the presence of mycorrhiza.

On the other hand, mycorrhizal colonization may also alter the soil microbial (1) activity and (2) community. The former is supported by several observations on the enhanced activities of enzymes mainly generated by microbial exudation in mycorrhizal soils. In our previous work, levels of polyphenol oxidase and catalase in soils vegetated with clover (*Trifolium repens* L.) were significantly increased by inoculation with *G. mosseae* and *G. etunicatum* [17]. Similarly, enhanced peroxidase activity in mycorrhizal soil is well documented [31]. The latter effect is related to mycorrhiza-influenced root exudation and formation of an extraradical mycelium. The microbial community is closely correlated to root exudations. Mycorrhizal colonization is reported to modify root exudations both qualitatively and quantitatively, resulting in changes in the microbial community [10]. Extraradical hyphae length was several orders of magnitudes greater than that of plant roots [13]. Formation of a hyphosphere clearly modifies the soil environment, including soil microbial structure. Recently, we observed increased quantities of bacteria, fungi, and actinomycetes in both rhizospheric and hyphospheric soils with AM, but microbial communities differed significantly between the rhizosphere and hyphosphere [11].

Table 4
Numbers of microorganisms in various treated soils.

Soil treatments	Numbers of microorganisms		
	Bacteria ($\times 10^7$)	Fungus ($\times 10^5$)	Actinomycetes ($\times 10^6$)
CK	7.31 (0.68)b	3.48 (0.79)c	2.93 (0.90)b
AMF1	10.83 (0.89)a	12.90 (0.76)b	4.28 (1.00)ab
AMF2	8.03 (1.76)ab	14.91 (0.42)a	5.62 (1.70)a

Data in brackets are standard deviations (SD). AMF means arbuscular mycorrhiza. CK means no-AMF inoculation control. Values in the same columns followed by the same letter are not significantly different ($P < 0.05$).

4. Conclusions

An effective remediation technique — AMPR — was proposed for soils contaminated with PAHs. Multiple mycorrhizal species were proved to be more effective to PAH degradation in soil. The co-contaminant (pyrene) clearly inhibited degradation of a single PAH (phenanthrene) in soil. Uptake of PAHs by mycorrhizal plants involved in AMPR was extensively reported. Mycorrhizal colonization caused an increase in PAH accumulation in roots but a decrease in shoots. However, plant uptake contributed negligibly to PAH dissipation in AMPR. By contrast, the effective microbiota in the mycorrhizal association was suggested to be responsible for the reductions in PAH concentrations in AMPR.

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