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Phenanthrene and pyrene uptake by arbuscular mycorrhizal maize and their dissipation in soil

F.Y. Wu[a,1], X.Z. Yu[a,1], S.C. Wu[a], X.G. Lin[b,c], M.H. Wong[a,c,*]

[a] Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China
[b] State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, East Beijing Road 71, Nanjing 210008, PR China
[c] Joint Open Laboratory of Soil and the Environment, Hong Kong Baptist University and Institute of Soil Science, Chinese Academy of Sciences, PR China

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) commonly found in soils can be degraded in rhizosphere, but may also be taken up by plants. The effects of arbuscular mycorrhizal (AM) fungi on uptake of phenanthrene (PHE) and pyrene (PYR) in maize and on their dissipation in soil were investigated using the three-compartmentalized rhizoboxes. Inoculation of *Glomus mosseae* significantly ($p < 0.01$) increased PHE and PYR concentrations in maize roots and significantly ($p < 0.05$) enhanced PYR translocation from roots to stems in the soil treatments of the PHE + PYR spiked-soils added into the central compartment of the rhizoboxes. There was a significant ($p < 0.05$) dissipation gradient of PHE and PYR observed away from the maize roots, with the highest dissipation rates recorded in rhizosphere zone in the central compartments of the rhizoboxes, followed by near rhizosphere zone and bulk soil zone in the outer compartments. However, *G. mosseae* only exerted minimal impacts on dissipation of PHE and PYR in the rhizosphere. The present study suggested that the hyphae and extraradical mycelium of AM fungi could play important roles in the uptake and translocation of PHE and PYR in plants. The present results indicated that there is a potential for the use of AM fungi and plant for remediating PAHs contaminated soils.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in soils and sediments, mainly as a result of incomplete combustion of fossil fuel, waste and other organic materials [1]. Many PAHs and their epoxides, especially those with high molecular weight and having more than three fused benzene rings, are highly toxic, mutagenic and/or carcinogenic to humans [2,3]. Bioremediation of PAH-contaminated soils has received increasing public concern. Phytoremediation, using plants and their associated micro biota for the *in situ* clean-up of contaminated soils, has been recognized as a cost-effective method for the removal of organic pollutants from soils for a number of years [4]. PAHs can be degraded in the rhizosphere [5–8], but may also be taken up by plants [9,10]. Plant-promoted biodegradation of PAHs in rhizosphere was the predominant contribution to their dissipation, while leaching, plant uptake, abiotic degradation, mineralization to CO_2, and irreversible sorption were insignificant in the overall mass balance of the organic pollutants [5,9].

The rhizosphere is a layer of soil immediately surrounding plant roots where physical, chemical and biological parameters are strongly modified by the root activities [11], and thus is distinguished from the bulk soil by a depletion of immobile nutrients (NH_4^+, $H_2PO_4^-$/HPO_4^{-2}, and micronutrients) and an elevated microbial activity [12]. The qualitative or quantitative differences in soil characteristics between rhizosphere soil and bulk soil [13,14] could influence the process of dissipation of PAHs in the rhizosphere. Based on meta-analysis, Ma et al. [7] indicated that there were significant rhizosphere effects on PAH dissipation in a variety of soil–plant systems. Elevated degradation of organic pollutants including PAHs in the rhizosphere when compared with bulk soil was revealed [15,16]. Biodegradation of PHE in soils was a function of distance to roots and decreased from 76 to 42% within 9 mm from the roots [17]. In previous studies, bulk soil and rhizosphere soil were commonly separated by gentle crushing and shaking, followed by vigorous rubbing and shaking of the root systems, in order to investigate the PAHs dissipation gradient in soil along plant roots [16,18]. Using this method is very likely mixing soil samples at different distances along plant roots. Yanai et al. [14] found that there were measured and modeled differences in nutrient concentrations between rhizosphere and bulk soil separated by this hand shaking method. In the present study, a compartmentalized rhizobox

* Corresponding author at: Croucher Institute for Environmental Sciences, Department of Biology, Hong Kong Baptist University, 224 Waterloo Road, Kowloon Tong, Hong Kong, China. Tel.: +852 3411 7746; fax: +852 3411 7743.
E-mail address: mhwong@hkbu.edu.hk (M.H. Wong).
[1] The two authors contributed equally to this article.

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was used to separate bulk soil and rhizosphere soil to investigate gradient dissipation of PHE and PYR in soils.

Arbuscular mycorrhiza (AM) is inhabited in the rhizosphere of approximately 80% of all terrestrial plant species, and enhances their host plants by exploiting mineral nutrients beyond the rhizosphere [19]. AM fungi could also colonize plants survived in PAHs field-contaminated soils [20], indicating that the symbioses may be crucial for plant establishment on contaminated sites. AM fungi could play important roles on the dissipation of PAHs in soils [16,21–23]. Limited studies showed that AM fungi could also affect uptake and the translocation of PAHs in plants [24,25]. However, there is very limited information available on the mechanisms involved.

The major objectives of the present study were to investigate: (1) the effects of AM colonization on the uptake and distribution of PHE and PYR in maize (*Zea mays* L.); (2) the contribution of AM fungi to PHE and PYR biodegration in the presence of maize; and (3) the gradient dissipation of PHE and PYR in a radial direction away from maize root.

2. Materials and methods

2.1. Preparation of PHE and PYR spiked-soils

The sandy loam soil (clay 4.4%, silt 19.7%, sand 75.9%) used in the present experiment was collected from Loi Tung Village in the New Territories Hong Kong. The soil was characterized by an undetectable level of PAHs, 1.26% organic matter, and a pH of 6.20 [26]. After the soil was air-dried and sieved (2 mm mesh), appropriate mixtures of PHE and PYR (obtained from SIGMA Chemical Co. with purities higher than 98 and 96%, respectively) were spiked into the soils to achieve a level of 50 + 50 mg kg^{-1} PHE + PYR. The PHE + PYR spiked-soils were then kept in the dark at about 20 °C for 2 years, so did the unspiked soils. At the start of the experiment, the initial concentrations of PHE and PYR in the aged soils were 12.0 ± 0.81 and 7.4 ± 0.72 mg kg^{-1}, respectively.

2.2. Experimental system

Rhizobox systems, which permit the spatial separation of root and hypha growing zones in the soil, were used in the present study and contained three compartments each, a central compartment for root and hyphal growth and two outer compartments for hypha growth only (Supplementary data). A nylon net (mesh size, 30 μm) was used, which allowed hypha and not the roots of maize to pass to adjacent compartment [27]. Eight treatments (three replicates each) were performed with four soil treatments: (1) clean soils in all the three compartments (0-0-0); (2) PHE + PYR spiked-soils in the central compartment only (0-P-0); (3) PHE + PYR spiked-soils in the two outer compartments only (P-0-P); (4) PHE + PYR spiked-soils in all the three compartments (P-P-P); and two inoculation treatments: with or without *Glomus mosseae*. The amount of soil used was 375 g and 550 g in the central compartment and in each of the two outer compartments, respectively. Mineral nutrients were added uniformly at rates of 162 mg kg^{-1} N (Urea), 126 mg K kg^{-1} and 50 mg P kg^{-1} (K_2HPO_4) soil in all treatments.

AM fungal inocula (*G. mosseae*, 20 g) were mixed with the prepared soils in the central compartment of the rhizobox, which were purchased from Biorize Sarl Co., France and consisted of spores, colonized root fragments and external mycelium. Sterilized inocula (121 °C, 2 h, 20 g) plus mycorrhizal fungal-free filtrate (20 mL) from the inocula suspension were added to the soils serving as non-mycorrhizal treatments (in order to provide a similar microflora except for the AM fungi). Seeds of maize obtained from Nanjing Agriculture University, China were surface sterilized in 10% hydrogen peroxide for 10 min. After germination on moist filter paper placed in Petri-dishes, two maize seedlings were transplanted to the central compartment. All the rhizoboxes were arranged randomly in a greenhouse, with temperature control (25–30 °C) and supplemented with additional illumination (with a light intensity of 250 μmol m^{-2} s^{-1}). The soils were adjusted regularly to 70% field water capacity with deionized water. In order to minimize the transportation of PHE + PYR in soils due to water flow, deionized water was only added into the outer compartments in the soil treatments of 0-P-0 and only into the central compartments in the soil treatments of P-0-P.

After a growth period of 60 d, maize was divided into three parts: stem, leave and root. Partial fresh roots were reserved for determining AM colonization rates. Stems, leaves and roots were freeze-dried, weighed, ground and prepared for PHE and PYR analyses. In order to investigate the dissipation gradients of PHE and PYR away from the roots, soils in the central compartments (rhizosphere zone, S1) and in the outer two compartments at 0–1 cm (near rhizosphere zone, S2) and 4–5 cm (bulk soil zone, S3) intervals to the nylon net were collected, air-dried and reserved for analyzing residual PHE and PYR.

2.3. AM colonization

Sub-samples of the reserved roots were clarified in 10% KOH (w/w) at 90 °C for 1 h, rinsed three times, bleached with fresh alkaline H_2O_2 solution (30 mL 10% H_2O_2 + 3 mL of NH_4OH + 567 mL deionized water) for 20–60 min, acidified with 1% HCl (1–4 min) and then stained with 0.05% Trypan Blue (modified method of Phillips and Hayman [28]). Mycorrhiza colonization rate of maize roots was measured with a line-intersect method [29].

2.4. Chemical analyses

For the analysis of PHE and PYR in soil, root and shoot, the corresponding samples were extracted according to USEPA Standard Method 3540C. All the samples were extracted for 18 h with acetone and dichloromethane (1:1, 80 mL) in Soxhlet systems. Florisil column was used for purifying the concentrated extracts (USEPA Standard Method 3620B). The eluant was evaporated to less than 2 mL prior to analysis. PHE and PYR concentrations were analyzed using a GC–MS (Agilent GC 6890N with 5390 Mass Selective detector) based on the USEPA Standard Method 8270C. The dissolved organic carbon (DOC) of fresh soil samples was extracted with deionized water (soil:water = 1:5), and analyzed with a total organic carbon (TOC) analyzer (Shimadzu TOC-Vcph, Japan).

Using the certified reference material (CRM104-100, soil matrix, bought from Resource Technology Corporation, US) and spiked plant samples as references, the recoveries of PHE and PYR were 91.4 and 91.1% for soil samples, and 90.4 and 88.2% for plant samples, respectively. The limit of detection defined as the standard deviation from the mean blank ($n = 3$) ranged from 5 to 9 μg kg^{-1}. Solvent blanks and duplicate samples were also analyzed. The variation coefficient of PAHs concentrations between duplicate samples was less than 10%.

2.5. Statistical analyses

Means of data were compared using Student–Newman–Keul's multiple comparison tests at the 5% probability level. To investigate the effects of PHE and PYR in the central compartments, PHE and PYR in the outer compartments and mycorrhizal colonization on maize biomass, accumulation of PHE and PYR in maize and DOC content of soil, two-way analyses of variance were used. All the statistical analyses were carried out using SPSS 11.0.0.

Table 1
Arbuscular mycorrhizal infection rates in roots of maize inoculated with *Glomus mossese* grown in rhizoboxes for 60 d (mean, $n = 3$).

Soil treatments	Mycorrhiza infection rate (%)	
	Noninoculation	Inoculation
0-0-0	7.2 aB	61.2 aA
P-0-P	8.4 aB	58.5 aA
0-P-0	9.4 aB	63.1 aA
P-P-P	10.8 aB	55.8 aA

Notes: 0-0-0 indicates clean soils in all the three compartments, 0-P-0 indicates PHE + PYR spiked-soils in the central compartment only, P-0-P indicates PHE + PYR spiked-soils in the two outer compartments only, P-P-P indicates PHE + PYR spiked-soils in all the three compartments.
Different small letters within the same column indicated significant ($p < 0.05$) difference between different soil treatments, while different capital letters within the same row indicated significant ($p < 0.05$) difference between different inoculation treatments.

3. Results

3.1. AM colonization in maize roots

Table 1 shows the AM colonization rates of maize roots in the compartmentalized rhizoboxes after a growth period of 60 d. Mycorrhizal colonization was observed not only in mycorrhizae inoculated treatments, but also in uninoculated treatments. AM colonization rates of the maize roots inoculated with *G. mosseae* (55.8–63.1%) were significantly ($p < 0.05$) higher than those uninoculated (7.2–10.8%). Spiked PHE and PYE in soils had little effects on AM colonization rates in the maize roots, regardless of the PHE + PYR spiked-soils added into the central compartment or the two outer compartments.

3.2. Influence of G. mosseae and PHE and PYR in soils on maize growth

The growth responses of maize leaves, stems and roots (dry weight basis) are shown in Fig. 1. Inoculation with *G. mosseae* had positive effects on the growth of maize, especially in the soil treatments of the PHE + PYR spiked-soils added into the central compartment (0-P-0 and P-P-P). AM colonization significantly ($p < 0.05$) increased the biomass of leaf and stem of maize in the soil treatments of 0-P-0 and the biomass of maize root in the soil treatments of P-P-P. In addition, spiked PHE and PYE in soils had negative effects on the growth of maize. When not inoculated with *G. mosseae*, the biomass of maize root in the soil treatments of P-P-P was significantly ($p < 0.05$) lower than that in the soil treatments of 0-0-0.

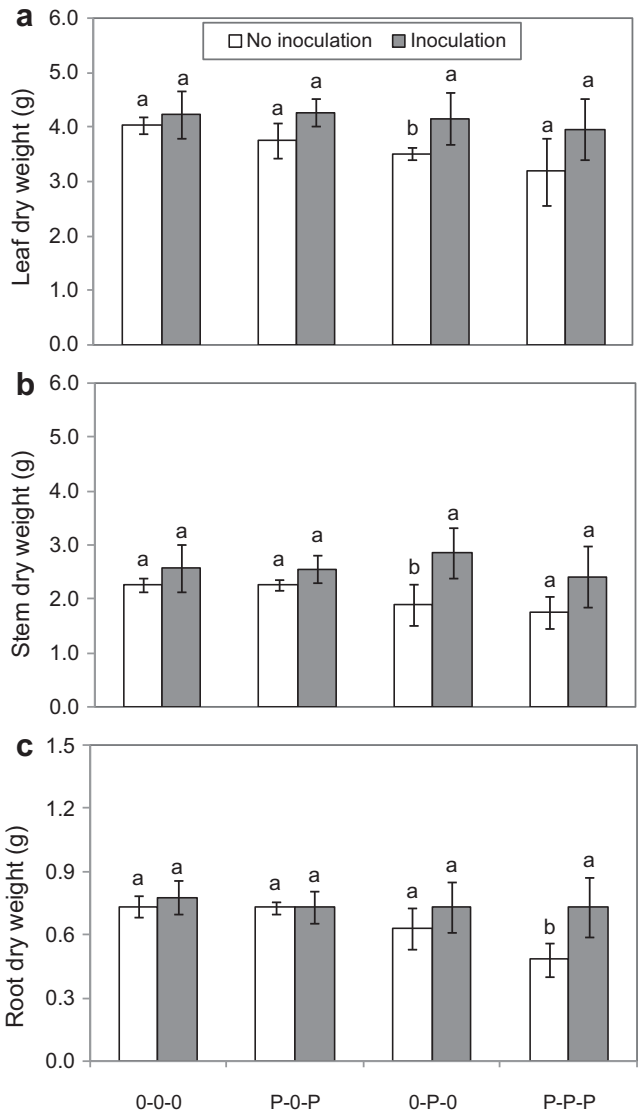

Fig. 1. Dry weight of leaf (a), stem (b) and root (c) of maize inoculated with *Glomus mossese* and no inoculated grown in rhizoboxes containing (1) 0-0-0: clean soils in all the three compartments; (2) 0-P-0: PHE + PYR spiked-soils in the central compartment only; (3) P-0-P: PHE + PYR spiked-soils in the two outer compartments only; or (4) P-P-P: PHE + PYR spiked-soils in all the three compartments for 60 d. Bars with the same letter are not significantly ($p < 0.05$) different between nonmycorrhizal and mycorrhizal plants according to the Student–Newman–Keul's multiple comparison (SNK) test (mean ± SD, $n = 3$).

3.3. Uptake of PHE and PYR in maize

The concentrations of PHE and PYR in the maize are shown in Table 2. Regardless of the soil treatments and inoculation treatments, the maize roots accumulated the highest concentrations of PHE and PYR and stems accumulated the least. In addition, significantly ($p < 0.05$) higher concentrations of PHE were accumulated in the maize roots in all the treatments than PYR, while the concentrations of PHE in the leaves and stems were comparable to PYR. PHE and PYR were not detected in the roots when three compartments of the rhizoboxes contained clean soil (0-0-0), while low concentrations of PHE and PYR were detected in the stems and leaves. The addition of PHE + PYR spiked-soils into the central compartment significantly ($p < 0.05$) increased uptake of PHE and PYR in the leaves, stems and roots, while the addition of PHE + PYR spiked-soils into the two outer compartments exhibited insignificant ($p > 0.05$) effects on the uptake of PHE and PYR in the leaves, stems and roots, except the uptake of PHE and PYR in the maize roots in the soil treatments of 0-P-0. Mycorrhizal colonization significantly ($p < 0.01$) enhanced PHE and PYR concentrations in roots and PYR in stems in the soil treatments of 0-P-0 and P-P-P. The concentrations of PHE and PYR in maize roots inoculated with *G. mosseae* were 1.5–1.9 and 1.6–2.2 times of those without inoculation, respectively.

3.4. DOC concentrations in soils in the central and outer two compartments

DOC concentrations in soils in the central compartments (S1) were significantly ($p < 0.05$) higher than those in the outer two compartments (S2 and S3), except in the soil treatments of P-P-P with inoculation of *G. mosseae* (Table 3). There was no apparent difference in DOC concentrations between S2 and S3, with the exception of the soil treatments of 0-0-0 without inoculation of AM fungi.

Table 2
Phenanthrene (PHE) and pyrene (PYR) concentrations in maize inoculated with *Glomus mossese* grown in rhizoboxes for 60 d (mean ± SD, $n = 3$).

Soil treatments	Leaf		Stem		Root	
	Noninoculation	Inoculation	Noninoculation	Inoculation	Noninoculation	Inoculation
PHE concentrations in maize (mg kg^{-1})						
0-0-0	0.04 ± 0.004 a	0.04 ± 0.003 a	0.02 ± 0.001 a	0.02 ± 0.001 a	ND	ND
P-0-P	0.06 ± 0.01 a	0.07 ± 0.01 a	0.04 ± 0.01 a	0.05 ± 0.01 a	1.77 ± 0.24 b	3.00 ± 0.89 a
0-P-0	0.27 ± 0.01 a	0.20 ± 0.05 b	0.12 ± 0.01 a	0.10 ± 0.03 a	3.87 ± 0.73 b	5.84 ± 0.77 a
P-P-P	0.30 ± 0.02 a	0.29 ± 0.03 a	0.10 ± 0.02 a	0.13 ± 0.01 a	5.24 ± 0.74 b	9.99 ± 1.63 a
PYR concentrations in maize (mg kg^{-1})						
0-0-0	0.01 ± 0.001 a	0.02 ± 0.002 a	0.02 ± 0.001 a	0.01 ± 0.001 a	ND	ND
P-0-P	0.03 ± 0.01 a	0.04 ± 0.01 a	0.02 ± 0.002 a	0.02 ± 0.002 a	0.49 ± 0.08 b	0.81 ± 0.28 a
0-P-0	0.11 ± 0.01 a	0.10 ± 0.01 a	0.05 ± 0.006 b	0.09 ± 0.004 a	1.08 ± 0.11 b	2.44 ± 0.33 a
P-P-P	0.20 ± 0.02 a	0.22 ± 0.05 a	0.05 ± 0.007 b	0.13 ± 0.013 a	1.33 ± 0.19 b	2.66 ± 0.66 a

Notes: 0-0-0 indicates clean soils in all the three compartments, 0-P-0 indicates PHE + PYR spiked-soils in the central compartment only, P-0-P indicates PHE + PYR spiked-soils in the two outer compartments only, P-P-P indicates PHE + PYR spiked-soils in all the three compartments.
Different letters within the same row indicated significant ($p < 0.05$) difference between different inoculation treatments.

Table 3
DOC concentrations in soils in central and outer compartments of rhizoboxes with growing maize inoculated with *Glomus mosseae* for 60 d (mean ± SD, $n = 3$).

Soil treatments	Inoculation treatments	DOC concentration (mg kg^{-1})		
		Central (SI)	Outer (0–1 cm, S2)	Outer (4–5 cm, S2)
0-	No inoculation	179 ± 7.63 bA	148 ± 1.33 aB	131 ± 5.81 aC
0-	Inoculation	190 ± 6.33 aA	153 ± 4.26 aB	143 ± 14.0 aB
0-	No inoculation	183 ± 14.6 aA	157 ± 20.0 aB	140 ± 28.0 aB
0-	Inoculation	183 ± 12.0 aA	146 ± 5.51 aB	148 ± 3.37 aB
P-	No inoculation	156 ± 14.5 bA	141 ± 12.6 aAb	134 ± 12.1 aB
P-	Inoculation	184 ± 12.4 aA	119 ± 17.5 aB	140 ± 12.8 aB
P-	No inoculation	150 ± 7.71 aA	128 ± 13.2 aB	146 ± 10.0 aAB
P-	Inoculation	155 ± 16.3 aA	150 ± 22.1 aA	155 ± 3.83 aA

Notes: 0-0-0 indicates clean soils in all the three compartments, 0-P-0 indicates PHE + PYR spiked-soils in the central compartment only, P-0-P indicates PHE + PYR spiked-soils in the two outer compartments only, P-P-P indicates PHE + PYR spiked-soils in all the three compartments.
Different small letters within the same column indicated significant ($p < 0.05$) difference between different inoculation treatments, while different capital letters within the same row indicated significant ($p < 0.05$) difference among soil samples collected from different zones at the same treatments.

Inoculation with *G. mosseae* significantly ($p < 0.05$) increased DOC concentrations in S1 in the soil treatments of 0-0-0 and 0-P-0 compared with those without inoculation. By contrast, inoculation with *G. mosseae* had insignificant ($p > 0.05$) impacts on DOC content in the outer two compartments (S2 and S3).

3.5. Dissipation of PHE and PYR in soils in the central and outer compartments

Table 4 shows the residual concentrations of PHE and PYR and dissipation rates of the two PAHs in soils in the central and outer compartments of the rhizoboxes. As expected, the levels of PHE and PYR recorded in S1, S2 and S3 in the soil treatments of 0-0-0 were undetectable. However, low concentrations of PHE and PYR were detected in S1 in the soil treatments of P-0-P and in S2 in the soil treatments of 0-P-0 and both concentrations were apparently higher than the initial concentrations. In the soil treatments of the PHE + PYR spiked-soils added into three compartments (P-P-P), there was a significant ($p < 0.05$) increasing gradient of residual PHE and PYR concentrations along the radial direction of the maize roots, with the lowest concentrations of PHE and PYR obtained in S1 in the central compartments, followed by S2 and then S3 in the outer compartments (Table 4). Similar results were also found in the soil treatments of P-0-P. In parallel, dissipation rates of PHE and PYR along roots were exhibited in the order of S1 > S2 > S3 (Table 4). There was no obvious difference in dissipation rates between PHE and PYR. In addition, AM colonization had insignificant ($p > 0.05$) impacts on residual concentrations and dissipation rates of PHE and PYR in soils, except that inoculation with *G. mosseae* significantly ($p < 0.05$) decreased concentrations of PHE in S2 in the soil treatments of 0-P-0.

4. Discussion

In the present study, the compartmentalized rhizoboxes were used to investigate effects of AM fungi on the uptake and distribution of PHE and PYR in maize and the spatial dissipation of PHE and PYR in the rhizosphere of the maize. There was an obvious positive mycorrhizal growth effect when the central compartments contained the PHE + PYR spiked-soils (Fig. 1), indicating that *G. mosseae* could alleviate adverse effects on maize yield caused by PHE and PYR in soils. One of the reasons for the enhanced growth of maize was due to the colonization of AM fungi which could improve the nutrient status of the host plants [19]. Cairney and Meharg [20] also found that AM fungi could play beneficial roles on plants growing on PAHs field-contaminated soils. Such 'growth-benefit' effect was one of the most important reasons when considering involvement of AM fungi in the context of phytoremediation [16,21].

In the soil treatments of 0-P-0 and P-P-P, inoculation with *G. mosseae* resulted in significantly ($p < 0.01$) higher concentrations of PHE and PYR in maize roots than non-mycorrhizal treatments (Table 2). This is in line with other results whereby *Glomus etunicatum* significantly increased PHE accumulation in roots of *Medicago sativa* [24]. These findings also agree with previous work on the effects of AM fungi on other organic pollutants including atrazine [30] and DDT [31]. This is mainly due to mycorrhizal colonization affecting (1) rhizosphere properties which could in turn influence PAHs bioavailability, or (2) root surface properties that influence PAHs accumulation through adsorption.

Rhizosphere is an extremely active area which contains exudates from root. Readily available carbon derived from root exudation often leads to the elevation of DOC content in rhizosphere soil [32]. It has been shown that AM fungi could mediate the secretion of root exudates [33,34]. In the present study, it was con-

Table 4
Residual concentrations of phenanthrene (PHE) and pyrene (PYR) in soils in central and outer compartments of rhizoboxes with growing maize inoculated with *Glomus mosseae* for 60 d (mean ± SD, $n = 3$).

Soil treatments	Inoculation treatments	PHE in soils (mg kg^{-1})			Dissipation rates of PHE (%)		
		Central (S1)	Outer (0–1 cm, S2)	Outer (4–5 cm, S2)	Central (S1)	Outer (0–1 cm, S2)	Outer (4–5 cm, S2)
0-0-0	No inoculation	ND	ND	ND	–	–	–
	Inoculation	ND	ND	ND	–	–	–
P-0-P	No inoculation	0.15 ± 0.02 aC	3.47 ± 0.30 aB	9.63 ± 0.97 aA	–	71.2 ± 2.52 aA	20.1 ± 8.07 aB
	Inoculation	0.19 ± 0.03 aC	3.17 ± 0.13 aB	8.58 ± 0.80 aA	–	73.7 ± 1.03 aA	28.8 ± 6.60 aB
0-P-0	No inoculation	2.55 ± 0.11 aA	0.19 ± 0.03 aB	ND	78.8 ± 0.90 a	–	–
	Inoculation	2.17 ± 0.35 aA	0.09 ± 0.02 bB	ND	82.0 ± 2.94 a	–	–
P-P-P	No inoculation	2.22 ± 0.11 aC	4.03 ± 0.13 aB	7.32 ± 0.18 aA	81.6 ± 0.92 aA	66.5 ± 1.07 aB	39.3 ± 1.46 aC
	Inoculation	2.12 ± 0.17 aC	4.09 ± 0.10 aB	7.30 ± 0.36 aA	82.4 ± 1.43 aA	66.1 ± 0.84 aB	39.4 ± 2.94 aC
Soil treatments	Inoculation treatments	PYR in soils (mg kg^{-1})			Dissipation rates of PYR (%)		
		Central (SI)	Outer (0–1 cm, S2)	Outer (4–5 cm, S2)	Central (SI)	Outer (0–1 cm, S2)	Outer (4–5 cm, S2)
0-0-0	No inoculation	ND	ND	ND	–	–	–
	Inoculation	ND	ND	ND	–	–	–
P-0-P	No inoculation	0.12 ± 0.03 aC	1.41 ± 0.23 aB	6.07 ± 0.89 aA	–	79.7 ± 3.99 aA	17.0 ± 1.34 aB
	Inoculation	0.10 ± 0.02 aC	1.57 ± 0.15 aB	5.60 ± 0.12 aA	–	79.1 ± 1.50 aA	25.9 ± 1.61 aB
0-P-0	No inoculation	1.47 ± 0.11 aA	0.12 ± 0.04 aB	ND	80.1 ± l.53 a	–	–
	Inoculation	1.35 ± 0.05 aA	0.09 ± 0.02 aB	ND	81.7 ± 0.63 a	–	–
P-P-P	No inoculation	1.35 ± 0.07 aB	1.49 ± 0.16 aB	5.52 ± 0.17 aA	81.8 ± 0.70 aA	79.7 ± 2.41 aA	24.4 ± 1.13 aB
	Inoculation	1.26 ± 0.10 aC	1.57 ± 0.12 aB	5.57 ± 0.06 aA	83.0 ± l.36 aA	79.5 ± 1.38 aB	24.3 ± 0.90 aC

Notes: 0-0-0 indicates clean soils in all the three compartments, 0-P-0 indicates PHE + PYR spiked-soils in the central compartment only, P-0-P indicates PHE + PYR spiked-soils in the two outer compartments only, P-P-P indicates PHE + PYR spiked-soils in all the three compartments.
Different small letters within the same column indicated significant ($p < 0.05$) difference between different inoculation treatments, while different capital letters within the same row indicated significant ($p < 0.05$) difference among soil samples collected from different zones at the same treatments. ND indicates that is not detectable;– no calculation.

firmed that *G. mosseae* played an important role in increasing DOC concentrations in the soil treatment of 0-P-0. Bengtsson and Zerhouni [35] showed that DOC could increase PAHs bioavailability. In laboratory batch assays, Ling et al. [36] also found that the bioavailability of PHE and PYR increased with elevating concentrations of citric and oxalic acid (primarily originated from root exudation) added in soil. This indicated that *G. mosseae* could increase PHE and PYR accumulation in the maize roots through increasing the secretion of root exudates.

It has been shown that the root surface of mycorrhizal and non-mycorrhizal plants is different. Yao et al. [42] demonstrated that AM colonization induced more fine roots and less coarse roots. Wu and Xia [37] observed that AM fungi could promote the volume and total absorption area of the root system. Wu et al. [24] indicated that mycorrhizal roots consistently exhibited higher PHE adsorption than did non-mycorrhizal roots, although PHE adsorption onto roots only represented 0.6–3.23% of the total amount extracted from the roots growing in PHE-spiked soils. Therefore, although there was no direct evidence of any changes in root morphology and surface properties of the maize in the present study, it was suggested that the positive mycorrhizal growth of roots (Fig. 1) could increase PHE and PYR sorption on mycorrhizal root. On the other hand, there must be a high density of hyphae in the narrow central compartments of the rhizosphere for the mycorrhizal colonization rates in roots up to 63.1%. Dong et al. [38] found that the hyphal length density ranged 3.3–4.8 m g^{-1} in the rhizosphere soils of white clover and ryegrass colonized by *G. mosseae*. The hyphae of AM fungi have a radius of approximately 1.5 μm and a large surface area [39]. According to Harley [40], on an equal weight basis, the surface area of hyphae is 100 times higher than the surface area of the root. Therefore, adsorption by the extraradical mycelium of *G. mosseae* may also be involved in the significantly higher concentrations of PHE and PYR in mycorrhizal roots of maize.

PHE and PYR were detected in the leaf, stem, and root in the soil treatments of 0-0-0. PHE and PYR in the leaf and stem may be translocated from the maize roots and/or directly from air, for PAHs could be taken up by plants via foliar uptake of compounds which have volatilized from soil surface [41]. The only source of PHE and PYR in the roots in the soil treatment of P-0-P was from the PHE and PYR-spiked soils in the outer two compartments of the rhizoboxes, which may be drawn into the rhizosphere zone by transpiration stream and water flow [43]. Residual low concentrations of PHE (0.15–0.19 mg kg^{-1}) and PYE (0.10–0.12 mg kg^{-1}) after harvest were recorded in soils in the central compartments which previously contained undetectable PAHs in the present study. Liste and Alexander [44] also noted that plant water uptake induced a mass flow of PHE and PYR towards the roots. Regardless of the soil treatments and inoculation treatments, the maize roots accumulated significantly ($p < 0.05$) higher concentrations of PHE than PYR. This was mainly due to that the initial PHE concentrations detected in the spiked soils were significantly higher than PYR (12.0 ± 0.81 vs 7.4 ± 0.72 mg kg^{-1}). In addition, the present study showed that *G. mosseae* significantly ($p < 0.01$) increased PHE and PYR concentrations in roots in the soil treatments of P-0-P (Table 2). This indicated that direct hyphal uptake may be involved in the process, because extraradical mycelium of AM fungi could access (30 μm nylon net) the outer compartments, but not maize roots. It has been shown that AM fungi could accumulate PAHs into lipid bodies of mycelium and the lipid bodies containing PAH could move along hyphae [25,45]. The present study also showed that *G. mosseae* significantly ($p < 0.01$) increased PYR translocation from root to stem of maize (Table 2). By contrast, Wu et al. [24] found that colonization of *G. etunicatum* resulted in significantly lower PHE accumulation in the shoots of *M. sativa*. Generally, there is a certain degree of specificity in the interactions between AM fungi and the host plants [55,56]. The effects of AM fungi on uptake and translocation of PAHs in plants need further investigation.

A significant ($p < 0.05$) dissipation gradient of PHE and PYR was observed away from the maize roots, with the highest dissipation rates in S1 in the central compartments, followed by S2 and S3 in the outer compartments (Table 4). Similar results were also observed by Joner and Leyval [16] and Gao et al. [18]. This may have resulted from significantly ($p < 0.05$) higher DOC concentrations in S1 than in S2 and S3 in the outer compartments (Table 3).

DOC derived from root exudates commonly stimulated microbial activity and modified the microbial populations in rhizosphere [46]. Both the microbial biomass and the number of PAH decomposers were greater in the rhizosphere of ryegrass and clover than in the bulk soil [47,48]. Corgie et al. [49] also found that bacterial populations were a function of the distance to roots and PAH added into the soils.

In the compartmentalized rhizoboxes, mycorrhizal maize played insignificant ($p > 0.05$) roles on the dissipation of PHE and PYR in S1, S2 and S3 when compared with non-mycorrhizal maize (Table 4). Other results also showed that there was no impact of AM fungi on PAHs dissipation observed in the rhizosphere [49,50]. This could be explained by the fact that only a small volume of soil (375 g) was placed in the narrow central compartments, which contained a higher density of maize roots. The dissipation of PAHs from soil was drastically affected by root activities such as degradation, absorption and adsorption [51,52], which might have concealed the impacts of *G. mosseae* on the dissipation of PHE and PYR. In addition, although *G. mosseae* significantly increased uptake of PHE and PYR in the maize roots, the levels taken up by roots only contributed to a small portion (0.16% and 0.07%) of total PHE and PYR in soils and therefore played a minor role on the overall dissipation of PHE and PYR in the rhizosphere. By contrast, other studies demonstrated elevated degradation of PAHs in the presence of AM fungi [16,53]. Joner et al. [54] even found that the ectomycorrhizal fungus *Suillus bovines* impeded rather than promoted PAH degradation. The mechanisms of AM fungi on the dissipation of PAHs in the rhizosphere need further research.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2011.01.024.

References

[1] J. Fu, B.X. Mai, G.Y. Sheng, G. Zhang, X.M. Wang, P.A. Peng, X.M. Xiao, R. Ran, F.Z. Cheng, X.Z. Peng, Z.S. Wang, U.W. Tang, Persistent organic pollutants in environment of the Pearl River Delta, China: an overview, Chemosphere 52 (2003) 1411–1422.
[2] R. Goldman, L. Enewold, E. Pellizzari, J.B. Beach, E.D. Bowman, S.S. Krishnan, P.G. Shields, Smoking increase carcinogenic polycyclic aromatic hydrocarbons in human lung tissue, Cancer Res. 61 (2001) 6367–6371.
[3] S.K. Samanta, O.V. Singh, R.K. Jain, Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation, Trends Biotechnol. 20 (2002) 243–248.
[4] A.C. Dietz, J.L. Schnoor, Advances in phytoremediation, Environ. Health. Perspect. 109 (2001) 163–168.
[5] S.A. Cheema, M.I. Khan, C.F. Shen, X.J. Tang, M. Farooq, L. Chen, C.K. Zhang, Y.X. Chen, Degradation of phenanthrene and pyrene in spiked soils by single and combined plants cultivation, J. Hazard. Mater. 177 (2010) 384–389.
[6] S.H. Lee, W.S. Lee, C.H. Lee, J.G. Kim, Degradation of phenanthrene and pyrene in rhizosphere of grasses and legumes, J. Hazard. Mater. 153 (2008) 892–898.
[7] B. Ma, Y. He, H.H. Chen, J.M. Xu, Z. Rengel, Dissipation of polycyclic aromatic hydrocarbons (PAHs) in the rhizosphere: synthesis through meta-analysis, Environ. Pollut. 158 (2010) 855–861.
[8] S.Y. Xu, Y.X. Chen, W.X. Wu, K.X. Wang, Q. Lin, X.Q. Liang, Enhanced dissipation of phenanthrene and pyrene in spiked soils by combined plants cultivation, Sci. Total Environ. 363 (2006) 206–215.
[9] Y.Z. Gao, L.Z. Zhu, Plant uptake, accumulation and translocation of phenanthrene and pyrene in soils, Chemosphere 55 (2004) 1169–1178.
[10] E. Wild, J. Dent, G.O. Thomas, K.C. Jones, Direct observation of organic contaminant uptake, storage, and metabolism within plant roots, Environ. Sci. Technol. 39 (2005) 3695–3702.
[11] R. Pinton, Z. Varanini, P. Nannipieri, The Rhizosphere: Biochemistry and Organic Substances at the Soil–Plant Interface, CRC/Taylor & Francis, London, 2007.
[12] E.A. Curl, B. Truelove, The Rhizosphere, Springer-Verlag, Berlin, 1986.
[13] D. Vetterlein, R. Jahn, Gradients in soil solution composition between bulk soil and rhizosphere – in situ measurement with changing soil water content, Plant Soil 258 (2004) 307–327.
[14] R.D. Yanai, H. Majdi, B.B. Park, Measured and modelled differences in nutrient concentrations between rhizosphere and bulk soil in a Norway spruce stand, Plant Soil 257 (2003) 133–142.
[15] P. Binet, J.M. Portal, C. Leyval, Dissipation of 3-6-ring polycyclic aromatic hydrocarbons in the rhizosphere of ryegrass, Soil Biol. Biochem. 32 (2000) 2011–2017.
[16] E.J. Joner, C. Leyval, Rhizosphere gradients of polycyclic aromatic hydrocarbon (PAH) dissipation in two industrial soils and the impact of arbuscular mycorrhiza, Environ. Sci. Technol. 37 (2003) 2371–2375.
[17] S.C. Corgie, T. Beguiristain, C. Leyval, Differential composition of bacterial communities as influenced by phenanthrene and dibenzo[a,h]anthracene in the rhizosphere of ryegrass (*Lolium perenne* L.), Biodegradation 17 (2006) 511–521.
[18] Y. Gao, X.Z. Yu, S.C. Wu, K.C. Cheung, N.F.Y. Tam, P.Y. Qian, M.H. Wong, Interactions of rice (*Oryza sativa* L.) and PAH-degrading bacteria (*Acinetobacter* sp.) on enhanced dissipation of spiked phenanthrene and pyrene in waterlogged soil, Sci. Total Environ. 372 (2006) 1–11.
[19] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, 3rd ed., Academic Press, London, 2008.
[20] J.W.G. Cairney, A.A. Meharg, Influences of anthropogenic pollution on mycorrhizal fungal communities, Environ. Pollut. 106 (1999) 169–182.
[21] E.J. Joner, A. Johansen, A.P. Loibner, M.A. Dela Cruz, O.H.J. Szolar, J.M. Portal, C. Leyval, Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil, Environ. Sci. Technol. 35 (2001) 2773–2777.
[22] A. Volante, G. Lingua, P. Cesaro, A. Cresta, M. Puppo, L. Ariati, G. Berta, Influence of three species of arbuscular mycorrhizal fungi on the persistence of aromatic hydrocarbons in contaminated substrates, Mycorrhiza 16 (2005) 43–50.
[23] N.Y. Wu, S.Z. Zhang, H.L. Huang, P. Christie, Enhanced dissipation of phenanthrene in spiked soil by arbuscular mycorrhizal alfalfa combined with a non-ionic surfactant amendment, Sci. Total Environ. 394 (2008) 230–236.
[24] N.Y. Wu, H.L. Huang, S.Z. Zhang, Y.G. Zhu, P. Christie, Y. Zhang, Phenanthrene uptake by *Medicago sativa* L. under the influence of an arbuscular mycorrhizal fungus, Environ. Pollut. 157 (2009) 1613–1618.
[25] A. Verdin, A.L.H. Sahraoui, J. Fontaine, A. Grandmougin-Ferjani, R. Durand, Effects of anthracene on development of an arbuscular mycorrhizal fungus and contribution of the symbiotic association to pollutant dissipation, Mycorrhiza 16 (2006) 397–405.
[26] J.H.C. Wan, M.H. Wong, Effects of earthworm activity and P-solubilizing bacteria on P availability in soil, J. Plant Nutr. Soil Sci. 167 (2004) 209–213.
[27] S.K. Kothari, H. Marschner, E. George, Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize, New Phytol. 116 (1990) 303–311.
[28] J.M. Phillips, D.S. Hayman, Improved procedures for clearing and staining parasitic and vesicular – arbuscular mycorrhizal fungi for rapid assessment of infection, Trans. Brit. Mycol. Soc. 55 (1970) 158–160.
[29] T.P. McGonigle, M.H. Miller, D.G. Evans, G.L. Fairchild, J.A. Swan, A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi, New Phytol. 115 (1990) 495–501.
[30] H.L. Huang, S.Z. Zhang, X.Q. Shan, B.D. Chen, Y.G. Zhu, J.N.B. Bell, Effect of arbuscular mycorrhizal fungus (*Glomus caledonium*) on the accumulation and metabolism of atrazine in maize (*Zea mays* L.) and atrazine dissipation in soil, Environ. Pollut. 146 (2007) 452–457.
[31] N.Y. Wu, S.Z. Zhang, H.L. Huang, X.Q. Shan, P. Christie, Y.S. Wang, DDT uptake by arbuscular mycorrhizal alfalfa and depletion in soil as influenced by soil application of a non-ionic surfactant, Environ. Pollut. 151 (2008) 569–575.
[32] Z.G. Cardon, J.L. Whitbeck, The Rhizosphere: An Ecological Perspective, Elsevier Academic Press, Amsterdam/Boston, USA, 2007.
[33] L. Lioussanne, M. Jolicoeur, M. St-Arnaud, Role of the modification in root exudation induced by arbuscular mycorrhizal colonization on the intraradical growth of *Phytophthora nicotianae* in tomato, Mycorrhiza 19 (2009) 443–448.
[34] P. Marschner, D.E. Crowley, R.M. Higashi, Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.), Plant Soil 189 (1997) 11–20.
[35] G. Bengtsson, P. Zerhouni, Effects of carbon substrate enrichment and DOC concentration on biodegradation of PAH in soil, J. Appl. Microbiol. 94 (2003) 608–617.
[36] W.T. Ling, L.L. Ren, Y.Z. Gao, X.Z. Zhu, B.Q. Sun, Impact of low-molecular-weight organic acids on the availability of phenanthrene and pyrene in soil, Soil Biol. Biochem. 41 (2009) 2187–2195.
[37] Q.S. Wu, R.X. Xia, Effects of arbuscular mycorrhizal fungi on leaf solutes and root absorption areas of trifoliate orange seedlings under water stress conditions, Front. Forest. China 3 (2006) 312–317.
[38] Y. Dong, Y.G. Zhu, F.A. Smith, Y.S. Wang, B.D. Chen, Arbuscular mycorrhiza enhanced arsenic resistance of both white clover (*Trifolium repens* Linn.) and ryegrass (*Lolium perenne* L.) plants in an arsenic-contaminated soil, Environ. Pollut. 155 (2008) 174–181.
[39] I.I.A. Abou El Seoud, Phosphorus efficiency of tagetes plant inoculated with two arbuscular mycorrhizal fungi strains, Aust. J. Basic Appl. Sci. 2 (2008) 234–242.
[40] J.L. Harley, The significance of mycorrhiza, Mycol. Res. 92 (1989) 129–139.

[41] A.M. Kipopoulou, E. Manoli, C. Samara, Bioconcentration of PAHs in vegetables grown in an industrial area, Environ. Pollut. 106 (1999) 369–380.
[42] Q. Yao, L.R. Wang, H.H. Zhu, J.Z. Chen, Effect of arbuscular mycorrhizal fungal inoculation on root system architecture of trifoliate orange (*Poncirus trifoliata* L Raf.) Seedlings, Sci. Hortic. 121 (2009) 458–461.
[43] P.J. Harvey, B.F. Campanella, P.M.L. Castro, H. Harms, E. Lichtfouse, A.R. Schaeffner, S. Smrcek, D. Werck-Reichhart, Phytoremediation of polyaromatic hydrocarbons, anilines and phenols, Environ. Sci. Pollut. Res. 9 (2002) 29–47.
[44] H.H. Liste, M. Alexander, Accumulation of phenanthrene and pyrene in rhizosphere soil, Chemosphere 40 (2000) 11–14.
[45] M.L. Gaspar, M.N. Cabello, M.C. Cazau, R.J. Pollero, Effect of phenanthrene and *Rhodotorula glutinis* on arbuscular mycorrhizal fungus colonization of maize roots, Mycorrhiza 12 (2002) 55–59.
[46] Y. Waisel, A. Eshel, U. Kafkafi, Plant Roots: The Hidden Half, 3rd ed., Marcel Dekker, NY, 2002.
[47] D.L. Johnson, D.R. Anderson, S.P. McGrath, Soil microbial response during the phytoremediation of a PAH contaminated soil, Soil Biol. Biochem. 37 (2005) 2334–2336.
[48] P.E. Olson, A. Castro, M. Joern, N.M. DuTeau, E. Pilon-Smits, K.F. Reardon, Effects of agronomic practices on phytoremediation of an aged PAH-contaminated soil, J. Environ. Qual. 37 (2008) 1439–1446.
[49] S.C. Corgie, F. Fons, T. Beguiristain, C. Leyval, Biodegradation of phenanthrene, spatial distribution of bacterial populations and dioxygenase expression in the mycorrhizosphere of *Lolium perenne* inoculated with *Glomus mosseae*, Mycorrhiza 16 (2006) 207–212.
[50] P. Binet, J.M. Portal, C. Leyval, Fate of polycyclic aromatic hydrocarbons (PAH) in the rhizosphere and mycorrhizosphere of ryegrass, Plant Soil 227 (2000) 207–213.
[51] X.C. Jiao, F.L. Xu, R. Dawson, S.H. Chen, S. Tao, Adsorption and absorption of polycyclic aromatic hydrocarbons to rice roots, Environ. Pollut. 148 (2007) 230–235.
[52] J.A. Rentz, P.J.J. Alvarez, J.L. Schnoor, Benzo[a]pyrene co-metabolism in the presence of plant root extracts and exudates: Implications for phytoremediation, Environ. Pollut. 136 (2005) 477–484.
[53] S.L. Liu, Y.M. Luo, Z.H. Cao, L.H. Wu, K.Q. Ding, P. Christie, Degradation of benzo[a]pyrene in soil with arbuscular mycorrhizal alfalfa, Environ. Geochem. Hlth. 26 (2004) 285–293.
[54] E.J. Joner, C. Leyval, J.V. Colpaert, Ectomycorrhizas impede phytoremediation of polycyclic aromatic hydrocarbons (PAHs) both within and beyond the rhizosphere, Environ. Pollut. 142 (2006) 34–38.
[55] Y.G. Zhu, A.S. Laidlaw, P. Christie, M.E.R. Hammond, The specificity of arbuscular mycorrhizal fungi in perennial ryegrass-white clover pasture, Agr. Ecosyst. Environ. 77 (2000) 211–218.
[56] T. Helgason, J.W. Merryweather, J. Denison, P. Wilson, J.P.W. Young, Fitter AH Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland, J. Eco. 90 (2002) 371–384.