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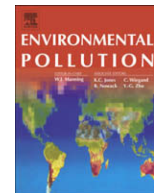

Phenanthrene uptake by *Medicago sativa* L. under the influence of an arbuscular mycorrhizal fungus

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Colonization by an arbuscular mycorrhizal fungus promoted root uptake and decreased shoot uptake of phenanthrene by Medicago sativa L.

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ABSTRACT

Phenanthrene uptake by *Medicago sativa* L. was investigated under the influence of an arbuscular mycorrhizal fungus. Inoculation of lucerne with the arbuscular mycorrhizal fungus *Glomus etunicatum* L. resulted in higher phenanthrene accumulation in the roots and lower accumulation in the shoots compared to non-mycorrhizal controls. Studies on sorption and desorption of phenanthrene by roots and characterization of heterogeneity of mycorrhizal and non-mycorrhizal roots using solid-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) demonstrated that increased aromatic components due to mycorrhizal inoculation resulted in enhanced phenanthrene uptake by the roots but lower translocation to the shoots. Direct visualization using two-photon excitation microscopy (TPEM) revealed higher phenanthrene accumulation in epidermal cells of roots and lower transport into the root interior and stem in mycorrhizal plants than in non-mycorrhizal controls. These results provide some insight into the mechanisms by which arbuscular mycorrhizal inoculation may influence the uptake of organic contaminants by plants.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are recalcitrant by-products of the incomplete combustion or pyrolysis of organic materials and they have strong mutagenic or carcinogenic properties (Joner et al., 2001). They have been found to be widespread in soils worldwide (Wang et al., 2007; Daly et al., 2007). Degradation is an important process for PAHs in soil and has been investigated in numerous studies. In addition, PAHs are lipophilic and may accumulate in vegetation. As a result, there has been considerable interest in the uptake of PAHs by plants during the last few decades (Wild and Jones, 1992; Wild et al., 2005).

Plant uptake of organic compounds is an important process when considering the risks associated with land contamination, the role of vegetation in the global cycling of persistent organic pollutants, and the potential for industrial discharges to contaminate the human food chain (Binet et al., 2000a; Fryer and Collins, 2003; Li et al., 2005). Root uptake and subsequent translocation into various plant parts through the transpiration process are an important pathway for the uptake of organic chemicals by plants (Hsu et al., 1990; Burken and Schnoor, 1997; Newman et al., 1997; Sicbaldi et al., 1997) except for the process of plant contamination following aerial deposition (Böhme et al., 1999; McLachlan, 1999; Smith and Jones, 2000). It is therefore important to understand the transport and fate of soil organic pollutants in soil–plant systems.

Mycorrhizas are the most widespread mutualistic associations between microorganisms and higher plants, occurring in 80–90% of all seed-bearing plant species in ecosystems throughout the world (Harrison, 1997; Smith and Read, 1997). The extraradical mycelium of the fungus forms an active continuum from soil to plant that is able to take up, translocate and transfer nutrients to the host plant (Smith et al., 2004). Despite the recognized effects of arbuscular mycorrhizal fungi on the mineral nutrition of plants and their sequestration and transport of heavy metals (Colpaert and Vandenkoornhuyse, 2001), very limited information is available on their effects on the uptake and translocation of organic contaminants by plants. A positive contribution of arbuscular mycorrhizal (AM) inoculation to PAH tolerance of plants has been demonstrated

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in previous studies (Leyval and Binet, 1998; Binet et al., 2000b; Joner and Leyval, 2001; Verdin et al., 2006) and the PAH anthracene has been detected in lipid bodies of root cells and fungal hyphae (Verdin et al., 2006), indicating the uptake and storage of the pollutant by plant roots and the mycorrhizal fungus. However, it is still unclear how AM fungi influence the uptake of PAHs by plant roots as well as their translocation to plant shoots, although our previous studies have demonstrated that mycorrhizal inoculation has a significant stimulatory effect on root uptake of organic contaminants (e.g. atrazine and DDT) but their translocation to shoots is limited by AM inoculation (Huang et al., 2007; Wu et al., 2008). Furthermore, there is virtually no information available on the mechanisms involved in the influence of AM fungi on plant uptake and translocation of organic pollutants.

The aim of the present study was to investigate the effects of arbuscular mycorrhizal colonization on the uptake of phenanthrene by lucerne (alfalfa). Sorption and desorption behaviour of phenanthrene on roots was compared between mycorrhizal and non-mycorrhizal roots. ^{13}C NMR analysis was employed to characterize the composition of mycorrhizal roots in order to elucidate the mechanisms involved. In addition, a two-photon excitation microscopy (TPEM) technique was used to observe phenanthrene accumulation in different growth zones in the living plant roots that enabled direct *in situ* visualization of the uptake of phenanthrene.

2. Materials and methods

2.1. Soil preparation and experimental design

A loamy soil (pH in water 7.32, Olsen P 4.5 mg kg^{-1}, cation exchange capacity (CEC) 25 cmol kg^{-1}, organic matter (OM) content 3.11%, dissolved organic carbon (DOC) 335.8 mg kg^{-1}, clay 23%, silt 35%, sand 32% and an initial phenanthrene concentration 0.15 mg kg^{-1}) was air-dried, ground and passed through a 2-mm nylon sieve. The growth medium (henceforth referred to as soil) was a 1:1 (w/w) mixture of acid-washed sand (1–2 mm) and soil, which was sterilized by γ-radiation (10 kGy, 10 MeV γ rays) and received mineral nutrients at rates of 30 mg P (KH_2PO_4), 60 mg N (NH_4NO_3), and 67 mg K (K_2SO_4) kg^{-1} soil. The soil was then artificially spiked with phenanthrene (Sigma Chemical Co., purity of 96%) in acetone. When the acetone was evaporated the spiked soils were mixed with uncontaminated soil (about 90% (w/w) of total soil) to produce initial concentrations of 0, 2.5, 5.0 and 10.0 mg kg^{-1} in soil and thoroughly mixed and preconditioned by incubation for four weeks at room temperature and in the dark. The concentrations of phenanthrene in the spiked soils after incubation were Soxhlet extracted, cleaned with a column packed with silica gel and measured by high performance liquid chromatography (HPLC), and were determined to be 0.37, 2.44, 4.05 and 9.46 mg kg^{-1}, respectively.

2.2. Pot experiment

Each pot received 650 g of treated soil. Mycorrhizal treatments received 50 g inoculum (*Glomus etunicatum*, BGC USA01) and each pot contained about 22,000 spores. The non-mycorrhizal treatments were set up using an equivalent amount of sterilized inoculum together with an aqueous filtrate (sieving through a 20 μm nylon mesh) of non-sterilized soil to provide a similar microflora except for the absence of the mycorrhizal fungus. All the treatments were set up in triplicate. Five uniform seedlings of *Medicago sativa* L. were planted in each pot immediately after germination on moist filter paper overnight. The upper 2–5 mm of each pot was covered with non-spiked sterilized soil to minimize the loss of phenanthrene by evaporation. All pots were lined with polyethylene to prevent cross-contamination and loss of water. The pots were positioned randomly and re-randomized every two days. Pots were kept in a controlled environment growth chamber at a light intensity of 250 μmol m^{-2} s^{-1} provided by supplementary illumination (14-h photoperiod) with a 25 °C/20 °C day/night temperature regime and a relative humidity of 70%. Distilled water was added as required to maintain soil moisture content at 70% of water holding capacity by regular weighing. Nitrogen (as NH_4NO_3) was added to each pot 30 and 45 d after sowing to provide a total of 120 mg N per pot. Plant shoots and roots were harvested separately after growth for 60 d. Root samples were carefully washed with tap water to remove any adhering soil particles. The shoot and root samples were then rinsed thoroughly with distilled water, blotted carefully with tissue paper and freeze-dried. Mycorrhizal colonization of roots was determined by the grid line intersect method (Giovannetti and Mosse, 1980).

2.3. Phenanthrene analysis

Freeze-dried plant tissues were submitted to Soxhlet extraction with dichloromethane and acetone (1:1, v/v) for 24 h and then cleaned with Florisil columns with 15 mL elution of hexane and dichloromethane (1:1, v/v) after they were chopped with pruning shears and ground up using a mortar and pestle. Samples were exchanged with methanol and analyzed with a reverse-phase HPLC (C_{18} column, 4.6 mm × 25 cm, Agilent Technologies, Wilmington, USA) with UV detector. The mobile phase was methanol:water (90:10) with a flow rate of 1 mL min^{-1}. The average recovery was obtained by spiking control ground plant materials with phenanthrene and was 97.5% ($n = 5$, RSD $< 4.05\%$) for the entire procedure.

2.4. Experiments on root sorption and desorption kinetics

Experiments on phenanthrene adsorption by intact roots were carried out in triplicate by mixing around 8 mg of fresh root material from plants grown for two months in non-spiked soils, either mycorrhizal or non-mycorrhizal, with 30 mL of a mixture of 0.01 mol L^{-1} $CaCl_2$ and 0.1 g L^{-1} NaN_3 solution containing 0.4 mg L^{-1} of phenanthrene in 50 mL glass centrifuge tubes which were then sealed with Teflon-lined screw caps. After the suspensions were shaken end-over-end for predefined time intervals of 10, 20, 30, 60, 90, 120, 240, 360, 1440, and 2880 min at room temperature (21 ± 2 °C), the tubes were centrifuged for 20 min and phenanthrene in the supernatants was determined by HPLC. The amount of phenanthrene sorbed on the plant material was calculated from the difference between the initial and final concentrations and expressed on a dry plant weight basis. The loss of phenanthrene during the procedure was found to be negligible.

Desorption experiments were performed in sequential decant-refill steps following the completion of equilibration sorption experiments. At the end of the sorption experiments, 10 mL of the supernatant solution were withdrawn and the remaining solution was again brought to 30 mL by addition of 10 mL background desorption solution, and then the suspension was shaken for a period of time identical to that used for the adsorption experiments. After the appropriate time of incubation the bottle was centrifuged at 2000 × *g* for 20 min and phenanthrene in the supernatant was determined by HPLC. The amount of phenanthrene remaining in lucerne was calculated as the difference between the initial amount sorbed and the final amount desorbed.

2.5. Sequential extraction

Mycorrhizal and non-mycorrhizal roots harvested from soil contaminated with phenanthrene were subjected to stepwise desorption by a three-step sequential extraction procedure modified from Chen et al. (2007). 0.4-g aliquots of fresh root sample and 20 mL of 0.01 mol L^{-1} $CaCl_2$ were placed in 50 mL glass centrifuge tubes with Teflon-lined screw caps, shaken for 2 h, and the bulk solutions were centrifuged. The $CaCl_2$ fraction represented phenanthrene weakly adsorbed on root surfaces. The fraction representing the phenanthrene strongly adsorbed on the root surfaces was then extracted by shaking the roots with 20 mL methanol for 2 h. The CH_3OH solution was purified using the same method as used for plant tissues. Following the CH_3OH extraction, the root tissues were freeze-dried and pulverized and were then subjected to Soxhlet extraction and cleanup by the same procedure used for the plant tissue extraction. Finally, the extracts were concentrated under nitrogen. Phenanthrene extracted using Soxhlet extraction was the fraction accumulated within the roots.

2.6. Two-photon excitation microscopy (TPEM)

Fresh lucerne roots harvested after plant growth for two months in contaminated soil at 9.46 mg kg^{-1} were rinsed thoroughly with distilled water, sliced and then used to visualize the uptake of phenanthrene into the living roots. A Bio-Rad MRC1024 laser confocal scanning system was used with a Spectrophysics Titanium–Sapphire laser and a Nikon Eclipse E800 inverted microscope. Images were collected and processed using Bio-Rad Lasersharp 2000 imaging software. Plant-specific autofluorescence excitation and emission profiles were determined when excited at 350 nm using TPEM.

2.7. Solid-state ^{13}C NMR analysis

Non-mycorrhizal and mycorrhizal roots were subjected to ^{13}C NMR analysis to obtain their chemical group distribution. Solid-state ^{13}C NMR data were acquired using cross-polarization and magic angle spinning (CPMAS) on a 300-MHz NMR spectrometer (Varian, San Francisco, USA). Spectra were acquired at a frequency of 75 MHz for ^{13}C, MAS spinning rate of 13 kHz, contact time of 2 ms, and 1 s recycle delay. The number of scans ranged from 5000 to 10,000 per sample. Run time was dictated by the percentage of organic carbon in the sample. The spectrum was quantified by integrating the regions as follows: paraffinic carbons (0–50 ppm); substituted aliphatic carbons including alcohols, amines, carbohydrates, ethers, and methoxyl and acetal carbon (50–109 ppm); aromatic carbons (109–163 ppm); carboxyl carbons (163–190 ppm); and carbonyl carbons (190–220 ppm) (Chen et al., 2005). Relative standard deviations (RSD) of the data for the non-mycorrhizal

Table 1
Mean shoot and root dry matter yield (freeze-dried basis), proportion of root length colonized by the AM fungus and total amount of phenanthrene in shoots and roots after cultivation of lucerne with or without mycorrhizal inoculation in soil containing different levels of added phenanthrene (mean ± SE, $n = 3$).

Phenanthrene concentration (mg kg^{-1})	Mycorrhizal status	Shoot weight (g)	Root weight (g)	Shoot/root ratio	Mycorrhizal colonization (%)	Total amount of phenanthrene (μg)	
						Shoots	Roots
0.37	Non-mycorrhizal	0.96 ± 0.22	0.42 ± 0.02	2.3	0.0	0.33 ± 0.02	0.54 ± 0.04
	Mycorrhizal	0.76 ± 0.02	0.32 ± 0.04	2.4	69.9 ± 1.0	0.19 ± 0.02	0.72 ± 0.05
2.44	Non-mycorrhizal	0.90 ± 0.04	0.40 ± 0.02	2.3	0.0	0.35 ± 0.01	0.92 ± 0.05
	Mycorrhizal	0.71 ± 0.06	0.32 ± 0.04	2.2	69.4 ± 2.7	0.21 ± 0.07	1.97 ± 0.09
4.05	Non-mycorrhizal	0.81 ± 0.04	0.44 ± 0.01	1.8	0.0	0.57 ± 0.06	1.96 ± 0.04
	Mycorrhizal	0.77 ± 0.09	0.42 ± 0.03	1.8	56.1 ± 3.2	0.42 ± 0.04	2.63 ± 0.16
9.46	Non-mycorrhizal	0.81 ± 0.10	0.44 ± 0.01	1.8	0.0	0.81 ± 0.04	3.27 ± 0.18
	Mycorrhizal	0.82 ± 0.11	0.44 ± 0.02	1.9	52.7 ± 0.2	0.67 ± 0.07	3.67 ± 0.18
Significance of:							
Phenanthrene level		n.s.	*		***	***	***
Mycorrhizal status		***	n.s.		***	***	***
Phenanthrene level × mycorrhizal status		*	**		***	n.s.	***

***Significant effect at $p < 0.001$; **Significant effect at $p < 0.01$; *Significant effect at $p < 0.05$; n.s., not significant.

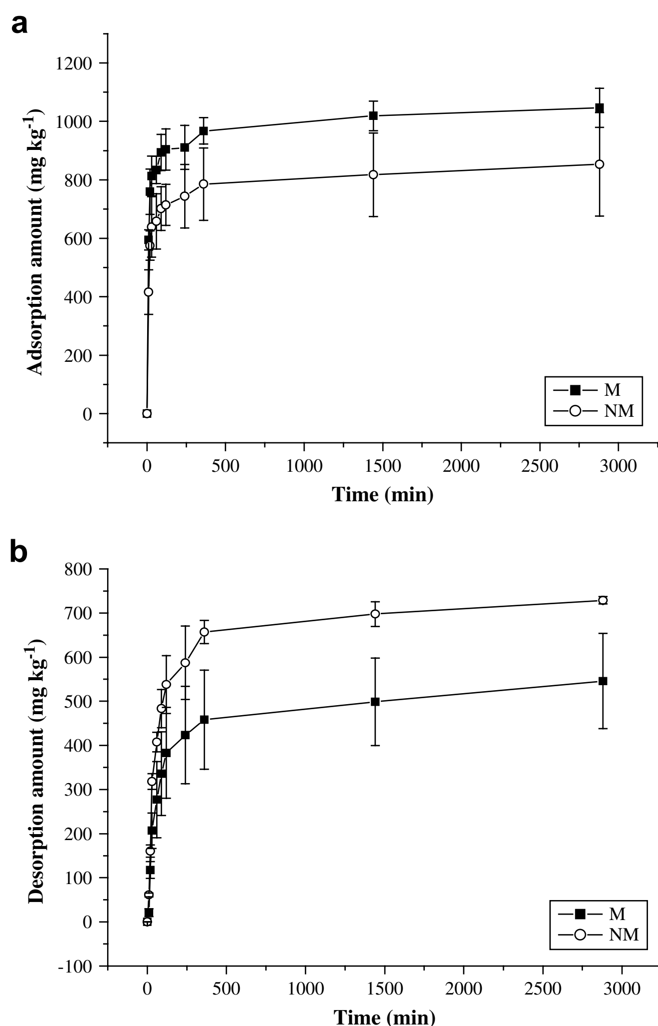

Fig. 1. Sorption (a) and desorption (b) kinetics of phenanthrene by fresh lucerne roots. NM, non-mycorrhizal; M, mycorrhizal. Data are means of three replicates and are expressed on a dry matter basis.

treatment were obtained by the analysis of three repeated root samples and the RSD values were 0.20% and 0.46% for the aromatic (109–163 ppm) and the aliphatic carbons (0–109 ppm), respectively.

2.8. Data analysis

Data were tested by two-way analysis of variance using the SPSS version 10.0 software package. Means and standard errors were calculated from three replicates.

3. Results and discussion

3.1. Mycorrhizal root colonization and plant biomass

Mycorrhizal root colonization and dry biomass of lucerne after growth for 60 d are presented in Table 1. Roots of uninoculated plants remained uncolonized. Mycorrhizal colonization of the roots of inoculated plants decreased from 69.9% to 52.7% with increasing phenanthrene concentration. Mycorrhizal colonization did not significantly affect the dry weight of lucerne roots ($p > 0.05$) but decreased the shoot dry weight ($p < 0.001$). Phenanthrene concentration had a negative effect only on root dry weight ($p < 0.05$) in non-mycorrhizal plants but increased both root ($p < 0.01$) and shoot ($p < 0.05$) dry weights in mycorrhizal plants and this may be ascribed to the contribution of mycorrhizal colonization to the utilization of phenanthrene as a carbon source by the roots (Corgié et al., 2006). Further analysis demonstrates that mycorrhizal

Table 2
Phenanthrene in two fractions sequentially extracted by methanol and Soxhlet extraction from non-mycorrhizal and mycorrhizal roots. Concentrations are expressed on a dry matter basis.

Initial phenanthrene concentration (mg kg^{-1})	Mycorrhizal status	Methanol extraction (%)	Soxhlet extraction (%)
0.37	Non-mycorrhizal	3.23	96.77
	Mycorrhizal	2.32	97.68
2.44	Non-mycorrhizal	2.65	97.34
	Mycorrhizal	1.76	98.24
4.05	Non-mycorrhizal	1.78	98.22
	Mycorrhizal	0.80	99.20
9.46	Non-mycorrhizal	1.18	98.82
	Mycorrhizal	0.60	99.40

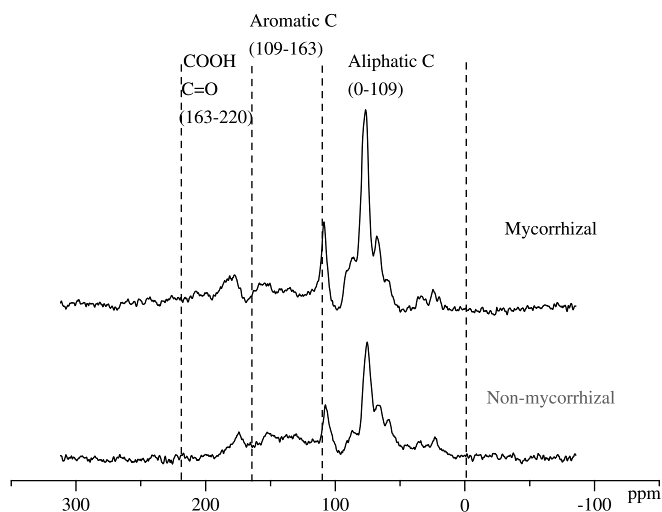

Fig. 2. CPMAS ^{13}C NMR spectra of mycorrhizal (a) and non-mycorrhizal (b) root fractions.

colonization made no difference to shoot/root ratio and shoot yield decreased more with increasing phenanthrene level than did root yield irrespective of inoculation, indicating that the shoots are more sensitive to increasing phenanthrene application level than are the roots.

3.2. *Phenanthrene accumulation in plant tissues*

Phenanthrene accumulation in both shoots and roots increased with increasing concentration of phenanthrene in the soil (Table 1). Roots accumulated much more phenanthrene than did shoots. Phenanthrene accumulation in roots was significantly elevated by mycorrhizal inoculation ($p < 0.001$). In contrast, mycorrhizal plants showed lower shoot phenanthrene accumulation than non-mycorrhizal controls ($p < 0.001$). These findings agree with our previous work on atrazine (Huang et al., 2007) and DDT (Wu et al., 2008) and highlight the importance of AM inoculation in plant uptake and transportation of organic pollutants.

3.3. *Sorption and desorption kinetics experiments on roots*

Adsorption onto the root surface is an important process for the transfer of hydrophobic pollutants into plant tissues and we therefore initially assumed that differences in sorption and desorption characteristics might be a key factor affecting the differences in phenanthrene accumulation between mycorrhizal and non-mycorrhizal lucerne. Sorption of phenanthrene by fresh lucerne roots was observed with rapid sorption occurring during the first 90 min (Fig. 1) followed by a period of slow sorption and equilibrium was attained within 6 h. The biphasic pattern of absorption was similar to that described in previous reports on atrazine uptake by excised soybean and corn roots (Darmstadt et al., 1984; Moody et al., 1970; Price and Balke, 1982; Nedumpara, 1996). Root absorption of solutes may be partitioned into apoplastic (the volume external to cell membranes) and symplastic components. A previous study has demonstrated that a short rinse with cold absorption solution is effective in removing atrazine from the apoplastic space (Price and Balke, 1983). In the present experiment the amounts of phenanthrene accumulated in tissues could be washed easily from roots and this is strong evidence of apoplastic uptake. Fast adsorption and desorption kinetics also suggest that the uptake of phenanthrene by the roots was via the apoplastic pathway which occurred readily with loose binding. A significant effect of mycorrhizal status was observed on phenanthrene sorption by roots ($p < 0.05$). Mycorrhizal roots consistently exhibited higher phenanthrene adsorption than did non-mycorrhizal roots, and phenanthrene desorption was greater from non-mycorrhizal than from mycorrhizal roots. We further conducted stepwise extraction of the roots after harvest from phenanthrene contaminated soil using $CaCl_2$, methanol and Soxhlet extraction, which were considered to represent the fractions weakly and strongly adsorbed on the root surface and accumulated by roots, respectively. The $CaCl_2$ extractable fraction was too small to be detected. The fraction percentage extracted by methanol was generally higher in non-mycorrhizal roots than mycorrhizal roots. However, the results obtained by Soxhlet extraction showed the opposite trend, with higher percentages for mycorrhizal than for non-mycorrhizal roots (Table 2). These results confirm that the sorption of phenanthrene was higher and its sequestration was tighter on mycorrhizal than on non-mycorrhizal roots. The hyphae of the fungus have a radius of approximately 1.5 μm and a large surface area (Abou El Seoud, 2008), therefore direct hyphal uptake and sequestration by the extraradical mycelium may be involved in this process, resulting in the increased sorption and decreased desorption observed in the case of mycorrhizal roots as observed by Verdin et al. (2006) for the accumulation of anthracene in fungal hyphae in an *in vitro* experiment. Research has established that AM fungi can also promote the volume and total absorption area of the root system (Wu and Xia, 2006), thus leading to an increase in phenanthrene sorption on mycorrhizal roots. Another possible explanation for the higher adsorption and lower desorption of phenanthrene in mycorrhizal roots is that the structure and composition of roots were changed. This was examined in detail by the ^{13}C NMR spectral analysis as shown below.

3.4. *^{13}C NMR spectral analysis*

Changes in the structure and composition of roots resulting from AM inoculation may have contributed to the differences in uptake of phenanthrene by roots. Mycorrhizal and non-mycorrhizal roots were therefore subjected to ^{13}C NMR measurement to characterize the functional groups present.

The ^{13}C CPMAS NMR spectra of different fractions and C-containing functional group contents of mycorrhizal and non-mycorrhizal roots are presented in Fig. 2 and Table 3. Differences in carbon-type distributions existed between mycorrhizal and

Table 3
Integration results of solid-state ^{13}C NMR spectra distribution of C chemical shift, ppm (%).[a]

Mycorrhizal status	Distribution of C chemical shift (ppm) (%)							Aliphatic C (%)	Aromatic C (%)
	0–45	45–65	65–95	95–108	108–145	145–160	160–220		
Mycorrhizal	5.2	18.7	46.6	16.1	4.3	3.5	6.2	86.6	7.8
Non-mycorrhizal	12.3	23.0	40.3	14.0	2.2	1.9	6.2	89.6	4.1

[a] Aliphatic C: total aliphatic carbon region (0–109 ppm). Aromatic C: total aromatic carbon region (109–163 ppm).

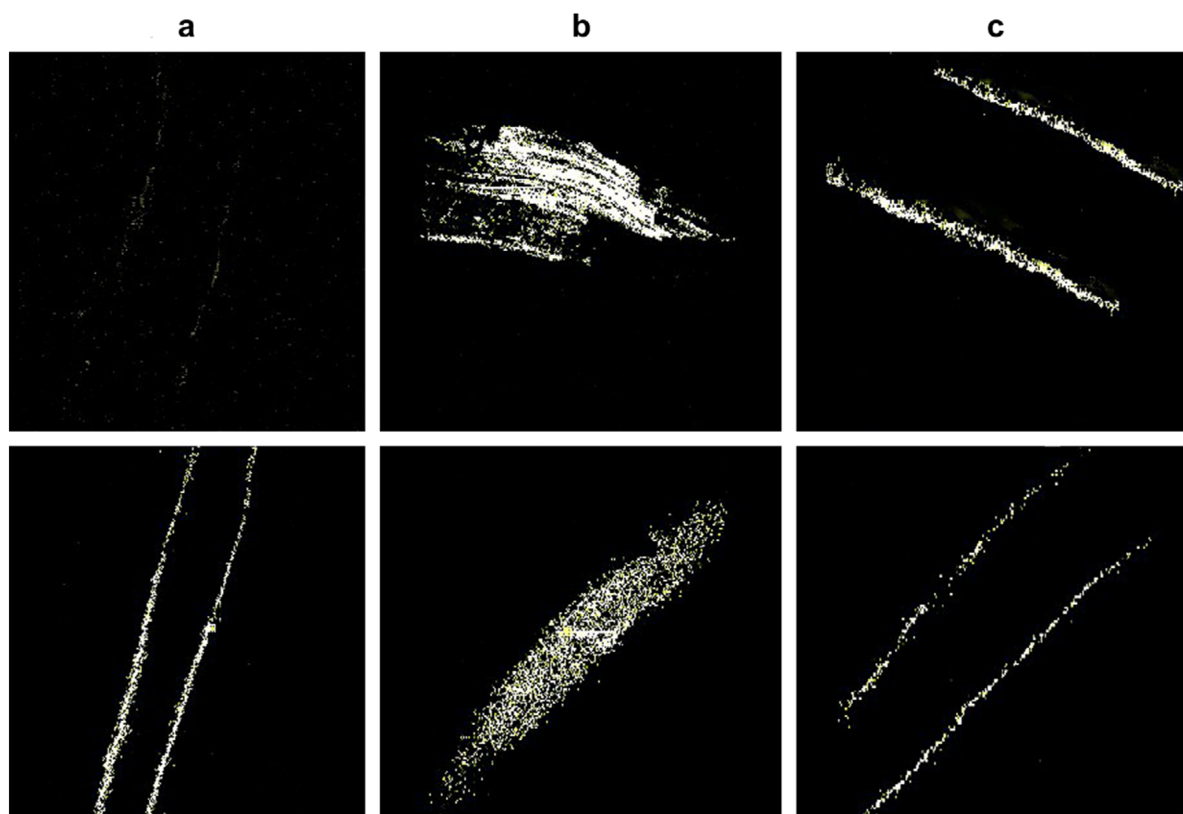

Fig. 3. Autofluorescence of mycorrhizal (lower) and non-mycorrhizal roots (upper); a, b, and c represent zones of elongation, root hairs and branching of roots.

non-mycorrhizal roots. The levels of aromatic C content were 4.1% in non-mycorrhizal roots and 7.8% in mycorrhizal roots. As far as we know, effects of arbuscular mycorrhiza on root composition and surface characteristics have not been investigated. The ^{13}C NMR spectral results obtained in the present study suggest that mycorrhizal colonization changes root surface and composition characteristics and this has important implications for the uptake of phenanthrene by plant roots because the association between hydrophobic pollutants and root components determines the surface sorption and transfer of hydrophobic pollutants into plant tissues. Numerous investigations have indicated that sorption of hydrophobic pollutants is regulated mainly by aromatic domains of organic matter (Chin et al., 1997; Chiou et al., 1998) although some other studies suggest a negligible contribution of aliphatic C (Salloum et al., 2002; Lin et al., 2007). Mycorrhizal roots had higher aromatic C and therefore their affinity for phenanthrene was higher than that of non-mycorrhizal roots with lower aromatic C, which is consistent with the observation of higher adsorption and lower desorption of phenanthrene by mycorrhizal roots. Furthermore, strong interaction between phenanthrene and mycorrhizal roots results in high sequestration of phenanthrene in roots and limits its translocation to plant shoots. We therefore observed higher phenanthrene accumulation in mycorrhizal roots and lower accumulation in the shoots of mycorrhizal plants compared to the non-mycorrhizal controls.

3.5. TPEM analysis

Two-photon excitation microscopy (TPEM) is a technique that enables direct *in situ* visualization of the movement of compounds that autofluoresce inside living plant tissues and has been pioneered by Wild et al. to track the uptake and movement of PAH inside living plant leaves (Wild et al., 2004) and roots (Wild et al., 2005). To further improve our understanding on the influence of mycorrhizal inoculation on plant phenanthrene uptake and translocation, we employed this technique to observe differences between mycorrhizal and non-mycorrhizal lucerne roots in the localization of phenanthrene. Very little fluorescence was observed scattered in the control plants without phenanthrene treatment and there was no significant difference between mycorrhizal and non-mycorrhizal roots. As illustrated in Fig. 3, for the plant roots with phenanthrene uptake, phenanthrene was localized in the epidermal cells along the elongation zone (a), root hair zone (b) and branching zone (c). Comparison between the upper and lower parts of Fig. 3 shows that the intensity of fluorescence was much higher in the elongation zone of the mycorrhizal than the non-mycorrhizal roots but was lower in the root hair zone and branching zone in the mycorrhizal roots compared to the non-mycorrhizal controls. These observations clearly indicate that phenanthrene uptake in the elongation zone was greater in mycorrhizal roots than in non-mycorrhizal controls. The longitudinal movement of phenanthrene towards the shoots, however, was more inhibited inside mycorrhizal roots. These observations are in broad agreement with the results obtained for phenanthrene concentrations in roots and shoots of mycorrhizal and non-mycorrhizal lucerne.

4. Conclusions

We observed that phenanthrene accumulation was higher in roots and lower in shoots of mycorrhizal lucerne compared to non-mycorrhizal control plants and this was further validated by the direct visualization of reduced movement of phenanthrene towards the shoots in mycorrhizal plants using a TPEM technique.

This study provides a fundamental insight into the mechanisms involved in the uptake of organic contaminants by mycorrhizal plants. Arbuscular mycorrhizal inoculation resulted in changes in chemical composition and therefore influenced the sorption and uptake of phenanthrene by roots as well as its translocation within the plants.

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