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Arbuscular mycorrhizal fungal hyphae contribute to the uptake of polycyclic aromatic hydrocarbons by plant roots

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ABSTRACT

The arbuscular mycorrhizal (AM) hyphae–mediated uptake of polycyclic aromatic hydrocarbons (PAHs) by the roots of ryegrass (*Lolium multiflorum* Lam.) was investigated using three-compartment systems. *Glomus mosseae* and *Glomus etunicatum* were chosen, and fluorene and phenanthrene were used as representative PAHs. When roots were grown in un-spiked soils, AM hyphae extended into PAH-spiked soil and clearly absorbed and transported PAHs to roots, resulting in high concentrations of fluorene and phenanthrene in roots. This was further confirmed by the batch equilibration experiment, which revealed that the partition coefficients (K_d) of tested PAHs by mycorrhizal hyphae were 270–356% greater than those by roots, suggesting the great potential of hyphae to absorb PAHs. Because of fluorene's lower molecular weight and higher water solubility, its translocation by hyphae was greater than that of phenanthrene. These results provide new perspectives on the AM hyphae–mediated uptake by plants of organic contaminants from soil.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), which are persistent organic contaminants in soils, are listed by the US Environmental Protection Agency as priority pollutants and are of concern worldwide because of their carcinogenic and/or mutagenic properties (Joner and Leyval, 2003; Wild et al., 2005). High concentrations of PAHs have been found in soils all over the world (Gao et al., 2006, 2008; Sung et al., 2001). Given that plants form the basis of human and animal food webs, daily consumption of PAH-contaminated plants could potentially increase human and animal exposure to hazardous substances (Collins et al., 2006; Gao and Collins, 2009). A better understanding of plant uptake of PAHs from soils is therefore essential to protecting human and ecological health wherever exposure to contaminated soils occurs.

There has been considerable interest in the past decades in understanding the uptake and accumulation of PAHs by plants (Simonich and Hites, 1994; Wild and Jones, 1992; Zhu et al., 2007). Plants can be exposed to PAHs in different ways. Foliage uptake of atmospheric PAHs occurs via the deposition of particle-bound compounds and the retention of vapor-phase PAHs on waxy leaf cuticles (Howsam et al., 2001; Kipopoulou et al., 1999). The rate and extent of plant root uptake depends on the physiochemical properties of PAHs, soil characteristics, and plant species and physiology (Chiou et al., 2001; Collins et al., 2006; Gao and Ling, 2006; Zhu and Gao, 2004). In a previous study, we observed that root concentrations and root concentration factors of phenanthrene and pyrene were significantly positively correlated with root lipid content (Gao and Zhu, 2004). Other studies have also shown the effects of plant composition on root uptake of lipophilic contaminants from soils (Chiou et al., 2001; Li et al., 2005; Simonich and Hites, 1994).

Plant–arbuscular mycorrhizal fungi (AMF) symbioses are ubiquitous in the environment. Arbuscular mycorrhizae (AM) have positive effects on plant establishment and survival in contaminated soils by increasing nutrient uptake, improving drought tolerance, and potentially protecting roots from plant pathogens (Hart and Trevors, 2005; Hildebrandt et al., 2007; Khan et al., 2000; Liao et al., 2003). The impacts of AMF inoculation on the uptake of organic pollutants by plants have recently been reported. Huang et al. (2007) and Wu et al. (2008) found that AM colonization led to increased accumulation of DDT and atrazine in roots but a decrease in shoots of maize (*Zea mays* L.) and alfalfa (*Medicago sativa* L.). Previously, we observed that colonization by *Glomus mosseae* and *Glomus etunicatum* caused an increase in PAH accumulation in roots but a decrease in shoots of alfalfa (Cheng et al., 2008; Debiane et al., 2009). These findings highlight the importance of AM inoculation to plant uptake of organic contaminants. However, the mechanisms involved in mycorrhizal root uptake of organic chemicals from soil environments are still not fully understood.

AM colonization results in the formation of an abundance of extraradical hyphae. These hyphae can be 5–50 m in length per

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gram of soil, which is several orders of magnitude longer than plant roots (Khan et al., 2000). With their small diameters (2–15 μm), AM hyphae have access to sites, such as fine soil pores, that are unavailable to plant roots. AM hyphae can concentrate and transfer heavy metals from soil to plant bodies (Burkert and Robson, 1994; Chen et al., 2003). However, very little information is available on the uptake of organic contaminants in soils or soil fine pores by AM hyphae and their translocation to plant roots. The contributions of AM hyphae to root uptake of organics – including PAHs – is still under investigation.

Thus, the aim of the present study was to investigate the effects of AM hyphae on the uptake of fluorene and phenanthrene, as representative PAHs, from soils by roots of ryegrass (*Lolium multiflorum* Lam.) using three-compartment systems. Results of this work provide insight into the mechanisms of mycorrhizal root uptake of organic contaminants in soils and can aid in the assessment of PAH-related risks at contaminated sites.

2. Methods

2.1. Chemicals

Fluorene and phenanthrene with a purity >98% were provided by Aldrich Chemical Co. The molecular weight (M_w, g/mol), solubility in water at 25 ℃ (S_w, mg/L), and logK_{ow} (where K_{ow} denotes the octanol–water partition coefficient) of fluorene and phenanthrene are 166.22 and 178.23 g/mol, 1.90 and 1.18 mg/L, 4.18 and 4.46, respectively (Yaws, 1999).

2.2. Mycorrhizal inoculum

Original inoculum of the AM fungi *G. mosseae* (AMF1; BGC GD01A) and *G. etunicatum* (AMF2; BGC HUN02C) was kindly provided by the Institute of Plant Nutrition and Fertilizers, Beijing Academy of Agronomy and Forestry. The inoculum 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). For the extraction of spores and sporocarps, 20 mL of inoculum were treated by the wet sieving and decanting method. The resulting material was centrifuged with 80% saccharose (Huang et al., 2007). Quantification was carried out in 9-cm-diameter Petri dishes with gridlines of 1 cm per side under a stereoscopic microscope at ×50 magnification. The inoculum contained about 382 and 6420 spores per 20 mL for AMF1 and AMF2, respectively.

2.3. Host plants

Ryegrass has been proven to be effective to remediate contaminated soils by organic chemicals (Gao and Zhu, 2004; Joner et al., 2001). Seeds of ryegrass (*L. multiflorum* Lam.) were surface sterilized in a 10% (v/v) solution of hydrogen peroxide, rinsed with sterile distilled water, dipped in a solution of 3 mmol/L calcium nitrate for 4 h in the dark, pregerminated on moist filter paper for 48 h, and were then ready for sowing.

2.4. Soil treatments

A typical zonal soil (Typic Paleudalf) of East China previously free of PAHs was collected from the A (0–20 cm) horizon in Nanjing, China; it had a pH of 6.02, 14.3% soil organic carbon content (f_{oc}), 24.7% clay, 13.4% sand, and 61.9% silt. Soil samples were air-dried and sieved through 2-mm mesh. The soil samples were sterilized by γ-radiation (10 kGy, 10 MeV γ-rays) to inactivate the native AM fungi. Samples were then spiked with a mixture of high-purity fluorene and phenanthrene in acetone. After the acetone evaporated, the spiked soil samples sieved again several times to homogenize the soil (Wang and Jones, 1994). The final concentrations of fluorene and phenanthrene in the treated soils, chosen according to the general concentrations observed in contaminated soil, were 79.52 and 72.35 mg/kg (on a dry weight basis), respectively.

2.5. Experiment design

2.5.1. Greenhouse experiments

Three-compartment systems were used in a greenhouse (Fig. 1). Each system was divided by metal mesh into three parts (A, B, and C) from top to bottom. Compartments A and B were separated by a 1-mm mesh through which roots could pass. Compartments B and C were separated by a 30-μm mesh through which roots could not pass but AM hyphae could. Compartment C was filled with the spiked soils, whereas compartments A and B were packed with un-spiked clean soils. AMFs were inoculated into the compartment B soils; thus, mycelia were born in compartment B, and mycorrhizal hyphae passed through the 30-μm mesh and extended into compartment C. Non-mycorrhizal treatments received the same amount of sterilized inoculum in B compartment.

Pregerminated ryegrass seeds were sown in compartment A of each system. The seedlings were thinned 7–10 days after emergence, leaving eight ryegrass plants per pot. Each treatment was repeated in triplicate, and the treated pots were located randomly in the greenhouse at 25–30 °C during daytime and at 20–25 °C during night and moved every 4 days. Soils and plants were destructively sampled 30, 45, 60, and 70 days after sowing. Plant shoots and roots separated from soils were washed with distilled water. Fresh plant material and soil samples were stored at −20 °C for PAH detection.

2.5.2. Sorption experiments

The compartment cultivation system used to obtain ryegrass roots without AMF inoculation is shown in Fig. 1. The sterilized soils packed in the systems were un-spiked and free of PAHs. Roots of ryegrass were collected from compartments A and B 60 days

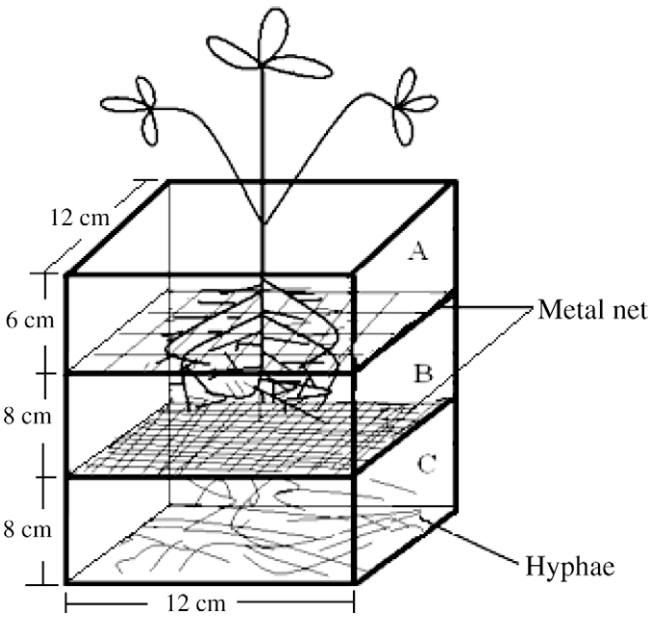

Fig. 1. Three-compartment cultivation system (compartments are numbered A, B and C).