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Glomalin-related soil protein influences the accumulation of polycyclic aromatic hydrocarbons by plant roots

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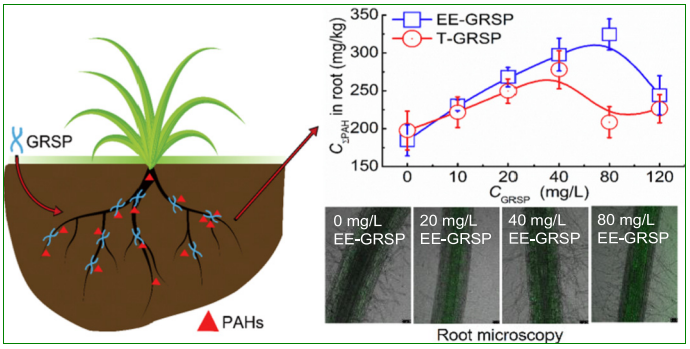
HIGHLIGHTS

- GRSP enhanced root PAH accumulation in a GRSP-concentration-dependent manner.
- The PAHs in root were fractionated into weekly adsorbed, strongly adsorbed and absorbed fractions.
- The adsorbed PAH fraction contributed overwhelmingly to total PAH content in roots.
- GRSP-induced changes in root PAH accumulation were mainly ascribed to GRSP-affected PAH sorption.

GRAPHICAL ABSTRACT



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ABSTRACT

Studies have demonstrated that the inoculation of soil with arbuscular mycorrhizal fungi (AMF) enhances the content of glomalin-related soil protein (GRSP), which in turn elevates the availability of polycyclic aromatic hydrocarbons (PAHs) in soil. However, few studies have examined the influence of GRSP on PAH accumulation by plants and their tissues. Understanding of this issue would provide new perspectives on the role of GRSP in PAH uptake by plants at contaminated sites. This investigation was the first observational study of the GRSP-influenced PAH accumulation in roots of ryegrass (*Lolium multiflorum* Lam.). GRSP (0–120 mg/L) enhanced the root PAH accumulation in a GRSP-concentration-dependent manner, based on the observed root concentrations and root concentration factors (RCFs). The greatest enhancement of ΣPAH accumulation appeared at 40 mg/L of the total GRSP (T-GRSP) and 80 mg/L of the easily extracted GRSP (EE-GRSP), respectively. The weakly and strongly adsorbed fractions accounted for 88.8–94.4%, while the absorbed fraction contributed no >11.2% of total PAH accumulation in roots. The capacity of PAH adsorption on roots was enlarged in the presence of GRSP (0–120 mg/L). As the adsorbed fraction dominated the total PAH contents in roots overwhelmingly, the GRSP-induced changes in root PAH accumulation were ascribed to GRSP-affected PAH sorption by roots.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) with strong mutagenic and carcinogenic properties are widely detected in soils and waters (Ling and Gao, 2004; Timoney and Lee, 2011; Rajtor and Piotrowska-Seget, 2016; Azah et al., 2017). In last several decades, anthropogenic activities, such as vehicular emissions and industrial combustion of fossil fuels mainly contribute to the release of PAHs into the environment. Understanding of PAH accumulation by plants should be taken into specific consideration, given that they may potentially accumulate in plants and subsequently enter into food chains (Harvey et al., 2002; Gao and

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Ling, 2006; Navarro et al., 2009), posing serious threats to human health.

Many researches have indicated that arbuscular mycorrhizal fungi (AMF) association may influence the plant uptake of organic pollutants including PAHs in soil (Rabie, 2005; Wu et al., 2011; Rajtor and Piotrowska-Seget, 2016). However, the mechanisms involved are poorly understood. Glomalin-related soil protein (GRSP) is mainly produced by AMF hyphae and is commonly quantified in soils as the total GRSP (T-GRSP) and easily extracted GRSP (EE-GRSP), as described by Schindler et al. (2007). GRSP has been found in multiple ecosystems, including agricultural lands, grasslands, forest, deserts, and non-cultivated soils (Treseder and Turner, 2007). GRSP is considered to be a stable form of organic matter, and GRSP concentrations in soil amounted to 2–15 mg/g, which contributed to 5%–10% of total soil organic carbon (SOC) (Lovelock et al., 2004; Rillig et al., 2006; Lozano et al., 2016). Most of the available information concerning GRSP is related to its role in the formation of soil aggregates (Rillig, 2004; Wu et al., 2012; Singh et al., 2016). In the past years, GRSP has been investigated for its role in sequestration of potentially toxic elements in soils (Cornejo et al., 2008; Vodnik et al., 2008; Aguilera et al., 2011). However, the scant research available on the effects of GRSP on chemical and biological processes of organic pollutants in soils makes it imperative for further investigation.

GRSP is a putative homolog of a heat shock protein that stabilizes soil aggregates and increases the hydrophobicity of soil particles (Gadkar and Rillig, 2006; Kohler et al., 2010; Malekzadeh et al., 2016), which in theory can affect the fate of PAHs with hydrophobic properties in soil. Recent studies have demonstrated that inoculation of alfalfa (*Medicago sativa* L.) with *Glomus etunicatum* and *Glomus lamellosum* in PAH-contaminated soils enhanced the soil GRSP contents (Bedini et al., 2009; Gao et al., 2017b). T-GRSP and EE-GRSP can promote the release of PAHs from soil solids into solution and elevate PAH availability in soil (Gao et al., 2017a). This would theoretically favor the binding of PAHs to roots. However, to the best of our knowledge, little research has been undertaken to examine the influence of GRSP on PAH accumulation by plants and their tissues.

To this end, this study was designed to examine the impact of GRSP on PAH accumulation in roots. First, the fate of PAHs within water-ryegrass root systems in the presence of GRSP was investigated. Root accumulated PAHs were then fractionated into weakly adsorbed, strongly adsorbed and absorbed fractions. The relationship of GRSP-induced changes in root PAH accumulation with GRSP-affected PAH adsorption was revealed in this paper, and the underlying mechanism of PAH accumulation by plant roots after GRSP exposure was clarified. Results of this investigation would helpful in understanding the mechanism of AMF-influenced plant uptake of PAHs at contaminated sites.

2. Materials and methods

2.1. Chemicals

Naphthalene, acenaphthene, phenanthrene, fluoranthene and pyrene with a purity > 98% were supplied by Aldrich Chemical Co. The five PAHs were used for all dissolution/extraction and plant uptake experiments. The general properties of the test PAHs are shown in Table S1 in Supplementary Information (SI).

GRSP was extracted from a Typic Paleudalf surface soil in Nanjing, China, using reported methods (Wright and Upadhyaya, 1998; Rillig and Steinberg, 2002; Lovelock et al., 2004). T-GRSP was extracted exhaustively from 1.0 g soil with 8 mL 50 mM sodium citrate (pH 8.0) at 121 °C for 60 min in the autoclave. The supernatant was collected by centrifugation at 5000*g* for 15 min. The extraction process was repeated 3–5 times until the solution was almost colorless. Whereas EE-GRSP resulted from single extractions of soil at a neutral pH (7.0), shorter autoclaving time (30 min) and at a lower molarity of the extraction sodium citrate buffer (20 mM). The GRSP extracts were then precipitated by titrating to a pH of 2.1 with 0.1 M HCl and centrifuged at 5000*g* for 10 min after incubation in ice for 1 h. The solid material was completely re-dissolved in 0.1 M NaOH, and the samples transferred to hydrated dialysis tubing (≤3500 Da) and dialyzed against deionized water for 60 h with constant mixing. Purified GRSP was freeze-dried and kept in a desiccator. Stock solutions of GRSP were prepared by reconstituting an appropriate amount of GRSP in a base solution, followed by dilution to the required concentration. The pH was adjusted to 7.0 with HCl (0.1 M) before use. The main element contents in GRSP were determined by Elemental Analyzer. The above dried-GRSP was also used for the determination of the structural and functional properties of GRSP. The general properties of GRSP are given in SI.

2.2. Hydroponic cultivation experiment

Ryegrass (*Lolium multiflorum* Lam.) was selected to uptake of PAHs from hydroponic culture as a function of GRSP content. The seeds were disinfected in 75% ethyl alcohol solution for 20 min, followed by washing with distilled water. Then the seeds were soaked in water for 24 h. The seeds were germinated in vermiculite followed by culture in brown glass jars containing half-strength Hoagland solution. After growing for one week, the very similar plants were transferred to experimental settings that contained 250 mL GRSP solution with PAHs. The concentrations of T-GRSP and EE-GRSP were 0, 10, 20, 40, 80 and 120 mg/L. The initial PAH concentrations are listed in Table S1, and they are under water solubility, less than saturation concentration. The lower parts of the stems were loosely bound with a Teflon tape. The plants were put into glass jars through drill holes in the caps, and the roots were just immersed below the surface of the nutrient solution. The mouths of the glass jars were covered with aluminum foil to minimize the vaporization of PAHs. A spiked, no-GRSP control treatment with ryegrass plants was also included. Five replicates of each treatment were performed. The experiments were conducted in a small greenhouse programmed for a 16 h light and 8 h dark cycle. The day and night temperatures were maintained at 25 ± 2 °C and 20 ± 2 °C, respectively.

2.3. Sorption experiments

Batch experiments were conducted to determine PAHs partitioning between root and water as a function of GRSP contents (Gao et al., 2008b). Triplicate root samples (50 mg) were placed into 30-mL glass centrifuge tubes shield with Teflon-lined screw caps. A 20 mL PAHs solution containing 0.05% NaN_3 and a given concentration of GRSP was added. Controls without roots were prepared in the same way to account for the possible loss of PAHs other than through sorption by the roots. The mixture was agitated in the dark at 200 rpm on a gyratory shaker for 24 h at 25 °C. After equilibration, samples were centrifuged at 3000 rpm for 15 min, with the supernatants being separated for PAHs and GRSP analysis in aqueous solutions. The amounts of PAHs and GRSP sorbed by ryegrass roots were calculated.

2.4. PAH analysis of solution and plant samples

After six days of hydroponic cultivation, the whole ryegrasses were taken out from the exposure solutions. The plant samples were rinsed with deionized water, wiped with the tissue paper. The ryegrass seedlings were separated into roots and shoots for the subsequent analysis. PAHs adsorbed on the root surface are difficult be removed by water rinsing. While organic solvent, such as methanol, preferred in PAH removal on such surfaces (Jiao et al., 2007). The roots were stored in glass bottles for further analysis.

A 3 mL aliquot of the finalized solution was mixed with 7 mL methanol (HPLC grade), filtered through a 0.22-μm filter unit, and analyzed using high-performance liquid chromatography (HPLC) (Shimadzu