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Short Communication

Glomalin-related soil protein enhances the availability of polycyclic aromatic hydrocarbons in soil

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ABSTRACT

This is the first observation of glomalin-related soil protein (GRSP)-enhanced availability of phenanthrene as a representative polycyclic aromatic hydrocarbon (PAH) in soils based on an *n*-butanol extraction procedure and a desorption batch assay. The butanol-extractable amount of phenanthrene increased ~204.4% in soils with GRSP added at 0–25 mg/kg. Phenanthrene desorption in soils increased monotonically with the increase of added GRSP concentration (0–50 mg/L) in solution. GRSP addition led to reduced soil organic matter in soil solids and increased concentrations of dissolved organic matter in solution, which dominates the mechanism of GRSP-enhanced phenanthrene availability. The results provide insight into the role of GRSP in soil PAH availability, and will be beneficial for risk assessment and remediation strategies at PAH-contaminated sites.

Polycyclic aromatic hydrocarbon (PAHs) with mutagenic/carcinogenic properties have been found at concentrations of hundreds of mg/kg in urban and agricultural soils, and may be absorbed by plants (De Nicola et al., 2015). Since plants form the basis of human and animal food chains, soil PAH contamination confers major risks to human and ecological health. Knowledge of PAH availability in soil is of crucial importance for risk assessment and remediation of contaminated sites.

In recent decades, research has revealed that natural organic matter (NOM), as an important soil constituent, may extensively influence the availability of PAHs in soil (Sun et al., 2012). Among NOM, glomalin is produced on the hyphae of arbuscular mycorrhizal fungi (AMF), and its concentrations in soils are up to four times as great as humic acid concentrations (Lozano et al., 2016; Schindler et al., 2007). Glomalin is operationally defined with the accepted protocol involving a harsh extraction of soil by autoclaving in a sodium citrate buffer (Aguilera et al., 2011; Lovelock et al., 2004). Since the link between glomalin and various protein fractions in soil is not yet clearly defined, researchers now use the broader term 'glomalin-related soil protein' (GRSP), reserving the term 'glomalin' for the purified protein or gene product (Chern et al., 2007; Rillig, 2004). Recent studies using synchrotron-based X-ray absorption near-edge structure (XANES) spectroscopy, pyrolysis field-ionization mass spectrometry (Py-FIMS) and proteomics techniques revealed that GRSP contains not only large amounts of soil-related heat-stable proteins but proteins of non-mycorrhizal origin (Gillespie et al., 2011). GRSP can accumulate in soil to several g/kg (Driver et al., 2005; Gil-Cardeza et al., 2014; Malekzadeh et al., 2016; Spohn and Giani, 2010), and accounts for 52% of total C in an organic soil (Gillespie et al., 2011). Unfortunately, it is unclear how GRSP influences the PAH availability in the soil environment.

Therefore, this study examined the impact of GRSP on the availability of phenanthrene as a representative PAH in soils. Phenanthrene was selected because it is commonly found in contaminated soils and has been examined in many environmental studies (Ling et al., 2009). The molecular weight, solubility in water at 25 °C, and log-transformed octanol–water partition coefficient ($\log K_{ow}$) of phenanthrene are 178.23 g/mol, 1.18 mg/L, and 4.45, respectively (Yaws, 1999). Experiments were conducted with three zonal soils (Table 1). The phenanthrene availability in soils with added GRSP was evaluated using a mild solvent-extraction procedure with *n*-butanol and a desorption batch experiment. The total GRSP was extracted from a subsoil in Nanjing, China by autoclaving for 60 min at 121 °C in a 50 mmol/L sodium citrate buffer (pH 8.0), as described by Wright and Upadhyaya (1998). The

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Table 1
Some properties of test soils.

Soil type	pH value	location	Soil organic carbon content (f_{oc}; g/kg)	Soil texture		
				Clay (%)	Sand (%)	Silt (%)
Kandiudult	4.64	Yingtan, China	3.55	32.7	42.5	24.8
TypicPaleudalf	7.25	Nanjing, China	6.28	33.6	51.9	14.5
Mollisols	6.05	Harbin, China	13.99	37.3	45.2	17.5

GRSP comprises 29.64% C, 4.299% H, 6.977% N, 27.43% O, 0.523% S, 3.20% Fe, and 53.02% ash.

The *n*-butanol extraction procedure revealed that GRSP addition enhanced the availability of phenanthrene in soils (Fig. 1). *n*-Butanol is a most appropriate extraction solvent for predicting the bioavailability of PAHs to organisms (Liste and Alexander, 2002). Sterilized phenanthrene-contaminated soils with added GRSP were cultivated for 60 days using batch microcosms, and the addition of GRSP at 5–25 mg/kg significantly increased the butanol-extractable amounts of phenanthrene in a concentration-dependent manner; higher GRSP concentrations resulted in more extractable phenanthrene in the soils (Fig. 1). The concentrations of extractable phenanthrene in soils with 25 mg/kg GRSP were 52.2–204.4% higher than those in controls without added GRSP. Therefore, GRSP addition significantly enhances soil PAH availability.

Phenanthrene desorption may also be used to indicate its availability in soil (Ling et al., 2009). The phenanthrene-spiked soils were aged for 0–60 days, and then a batch desorption experiment was conducted. Clearly, phenanthrene desorption increased monotonically with the GRSP concentrations at 0–50 mg/L (Fig. 2). The amount of phenanthrene desorption with GRSP at 50 mg/L was 46.8–190.8% greater than that without GRSP in soils aged for 60 days. Soils with added GRSP showed significant aging effects (Nowak et al., 2011). Phenanthrene desorption decreased markedly with aging time from 0 to 60 days, irrespective of the addition of GRSP (Fig. 2), which may be attributable to the desorbed phases of phenanthrene changing from being loosely bound to more strongly bound forms with increasing aging time (Nowak et al., 2011). Despite this PAH redistribution, the added GRSP still increased phenanthrene desorption, which further supported the GRSP-enhanced phenanthrene availability in soils.

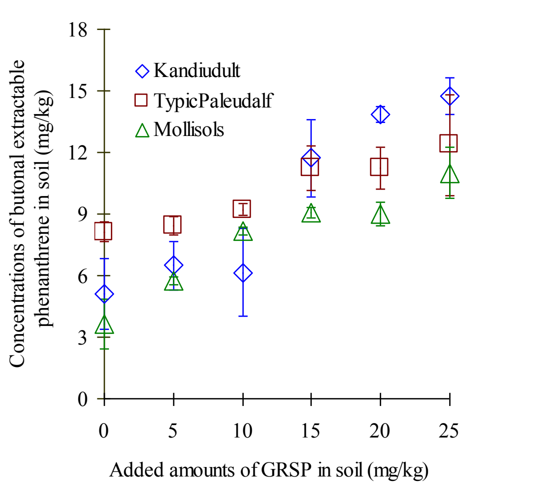

Fig. 1. Concentration of *n*-butanol-extractable phenanthrene as a function of the amount of added glomalin-related soil protein (GRSP) in sterilized soils after a 60-day incubation. Each amber glass microcosm was filled with 20 g phenanthrene-spiked soils. GRSP solution was added to give soil concentrations of 0–25 mg/kg. A 0.2% NaN_3 solution was also added to each microcosm. The soil water content was adjusted to 30% of the soil water-holding capacity. After incubation at 25 °C for 60 days, the soils were sampled and freeze dried, and polycyclic aromatic hydrocarbons (PAHs) were extracted with *n*-butanol solvent. Three replicates were prepared for each treatment. Error bars are the standard deviations (SD).

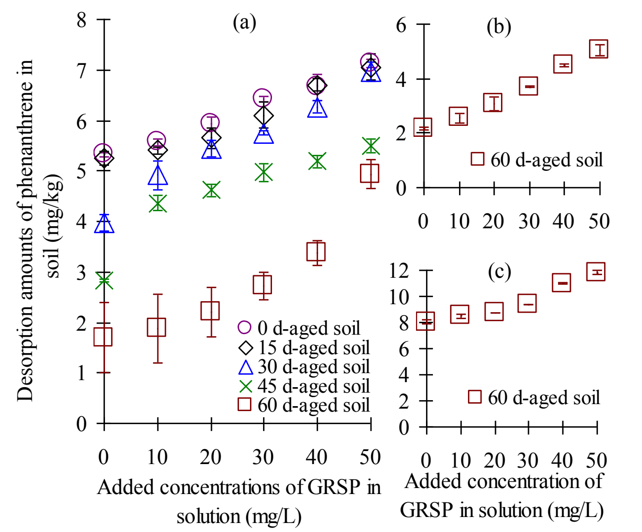

Fig. 2. Desorption of phenanthrene from TypicPaleudalf (a), Mollisols (b) and Kandiudult (c) by glomalin-related soil protein (GRSP). Error bars are standard deviations (SD). First, 25 mL of GRSP (0–50 mg/L) solution with 0.05% NaN_3 was added to 30-mL glass centrifuge tubes containing phenanthrene-contaminated soils in which the compound had been aged for 0–60 days. The tubes were shaken in the dark at 200 r/min on a gyratory shaker for 24 h. The solution and soil were separated by centrifugation. An aliquot of supernatant was removed, analyzed for phenanthrene concentration, and then the phenanthrene desorption was calculated. Each treatment was conducted in triplicate. All phenanthrene equilibrium concentrations in solution were below its aqueous solubility. Losses, such as to photochemical decomposition, volatilization, or sorption to the tubes, were found to be negligible.

Soil organic matter (SOM) is the predominant pool for PAHs in soil (Chiou et al., 1998). Since the soils were NaN_3-sterilized during the availability experiments, GRSP-enhanced phenanthrene availability is associated with chemical reactions rather than microbial processes, and the GRSP-increased release of SOM into solution may be a dominant mechanism. The observed increase of dissolved organic matter (DOM) concentration in solution (C_{doc}) with GRSP addition supported this notion (Fig. 3). The C_{doc} increased with the GRSP concentration increment in the phenanthrene desorption system. This means that a portion of SOM is released from solids and becomes DOM in solution with GRSP addition, and addition of more GRSP resulted in higher C_{doc} and phenanthrene availability (Fig. 2a). The GRSP-enhanced phenanthrene desorption (ΔD; mg/kg) was obtained as follows:

$$\Delta D = D_{GRSP} - D_o \quad (1)$$

where D_{GRSP} and D_o (mg/kg) are the respective amounts of desorbed phenanthrene from soils with and without added GRSP. The GRSP-increased C_{doc} in solution (ΔC_{doc}; mg DOC/L) was calculated as: