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Short Communication

Inoculation with arbuscular mycorrhizal fungi increases glomalin-related soil protein content and PAH removal in soils planted with *Medicago sativa* L.

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A B S T R A C T

This was the first observation of the enhancement of glomalin-related soil protein (GRSP) content by the arbuscular mycorrhizal fungi (AMF) inoculation of soils contaminated with pyrene as a representative polycyclic aromatic hydrocarbon (PAH), and GRSP content was significantly positively correlated with PAH removal in mycorrhizal soils. Both total GRSP and easily extractable GRSP promoted the release of pyrene from soil solids into solution, consequently assisting the microbial biodegradation of pyrene in soil. The results provide insight into the role of GRSP in AMF-aided rhizoremediation and will be beneficial for improving remediation efficiency at PAH-contaminated sites.

Soil contamination by PAHs remains a major issue worldwide, as PAHs have highly mutagenic and carcinogenic properties and are commonly found in soil at high concentrations in many countries (Calonne et al., 2014). Rhizoremediation has been recognized as one of the most cost-effective, reliable, and promising technologies for decontaminating PAH-polluted soils (Gao et al., 2011a). Rhizosphere microbial activity is a major limiting factor in the rhizoremediation process, which is theoretically improved by mycorrhizal associations.

AMF are mutualistic symbionts living in association with the roots of the majority of land plants (Cheng et al., 2012). The large network of AMF hyphae, spreading from mycorrhizal roots into the surrounding soil, improves the nutritional status of the rhizosphere, enhancing the microorganism activity (Rosier et al., 2006), thereby promoting PAH biodegradation and rhizoremediation. During AMF-aided rhizoremediation, glomalin is produced and enters into soil as AMF hyphae and spores decompose (Violi et al., 2008). The carbon concentration of glomalin, measured operationally in soil as GRSP, can be 2–25 times that of humic acid and even as much as 25% of the total soil carbon content (Rillig et al., 2006). Unfortunately, the response of GRSP to AMF inoculation in PAH-contaminated soil remains to be verified, and little information has been available on the relationship between GRSP and PAH dissipation, limiting understanding of the mechanisms and processes of AMF-aided rhizoremediation.

Therefore, in this study we examined the GRSP response to AMF inoculation and the correlation between GRSP and PAH removal in contaminated soils planted with alfalfa (*Medicago sativa* L.). *Glomus etunicatum* (Ge) and *Glomus lamellosum* (Gla) were chosen, and pyrene was used as a representative PAH. The molecular weight, solubility in water at 25 °C, and log-transformed octanol-water partition coefficient of pyrene are 202.26 g/mol, 0.12 mg/L, and 4.88, respectively (Yaws, 1999). A typical zonal surface soil (Typic Paleudalf) previously free of PAHs was collected from Nanjing, China. The soil had a pH of 6.02 and was composed of 14.3% soil organic carbon content, 24.7% clay, 13.4% sand, and 61.9% silt. Soil samples were artificially contaminated with pyrene and aged for 60 days (Gao et al., 2011a). The final pyrene concentration in soils was 43 mg/kg. Mesocosm experiments were conducted with five replicates, and pre-germinated alfalfa seeds were sown in soils

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inoculated with Ge or Gla. Soils and plants were destructively sampled 25–80 days after sowing, and soil pyrene was detected by high-performance liquid chromatography (HPLC) with a fluorescence detector following solvent extraction (Gao et al., 2011b).

Although PAH toxicity to mycorrhizas has been reported, with reduced root colonization (Calonne et al., 2014), we observed high colonization rates of alfalfa roots by Gla and Ge in soils with pyrene; these rates ranged from 11% to 56% in 25–80 days, and they were all higher than 50% after 60 days (Fig. 1a). Vesicles for nutrient storage emerged and were directly observed on root surfaces (Fig. 1b and c). Members of the genus *Glomus* often sporulate in roots, and vesicles may therefore also function as reproductive units (Harper et al., 2017). The total numbers of spores in soil were estimated following the method of Bedini et al. (2009), and they increased monotonically 25–80 days post-sowing for Gla- and Ge-inoculated soils with PAH (Fig. 1d). Soil hyphal density varied from 138 to 351 cm/g and from 142 to 343 cm/g after 25–80 days in soils with Ge and Gla, respectively (Fig. 1e); moreover, extending the cultivation time enlarged hyphal density. The abundant spores and hyphae suggested the possibility of GRSP production in PAH-contaminated soils.

Circumstantial evidence has indicated an active role of AMF in GRSP production (Rillig and Steinberg, 2002). Our study provides the first observation of the GRSP response to AMF inoculation in soils contaminated with organic pollutants. GRSP was extracted from treated soils as total GRSP (T-GRSP) and easily extractable GRSP (EE-GRSP), as described by Bedini et al. (2007). The contents of both T-GRSP and EE-GRSP in alfalfa-vegetated soils with pyrene and inoculated with AMF generally increased from 25 to 80 days and varied between 980 and 1567 μg/g for T-GRSP and between 357 and 453 μg/g for EE-GRSP, respectively, which were dramatically higher than the GRSP content in control soils without AMF inoculation (Fig. 2a, c). This supports the role of AMF in GRSP production, irrespective of PAH contamination. Another novel finding is that T-GRSP and EE-GRSP contents were significantly positively correlated with spore numbers and hyphal density in PAH-polluted soils (Fig. 2c and d). This further supports the issue that GRSP is produced and released into soil as AMF hyphae and spores decompose (Preger et al., 2007).

Pyrene has been documented to be relatively recalcitrant in soils compared with smaller PAHs (Gao et al., 2011b). We observed the highly successful AMF-aided rhizoremediation of soils with pyrene. On the whole, the loss of pyrene in mycorrhizal soils increased over the sampling period, with 38.3–68.9% and 39.4–71.3% of pyrene degraded in Ge and Gla mycorrhizal soils, respectively (Fig. 3a). Interestingly, we observed for the first time that T-GRSP and EE-GRSP contents were significantly positively correlated with PAH removal in mycorrhizal soils (Fig. 3b and c), indicating the significant role of GRSP in the degradation of soil PAHs.

GRSP may change the physiochemical and biological properties of soil (Kohler et al., 2010), thereby directly or indirectly facilitating PAH degradation in soil. As previously documented, microbial biodegradation was the dominant contribution to the dissipation of PAHs in AMF-aided rhizoremediation, whereas the plant accumulation and abiotic dissipation of PAHs were negligible (Gao et al., 2011a). Thus, the microbial activity and PAH release in mycorrhizal soils are the two major limiting factors in the AMF-aided

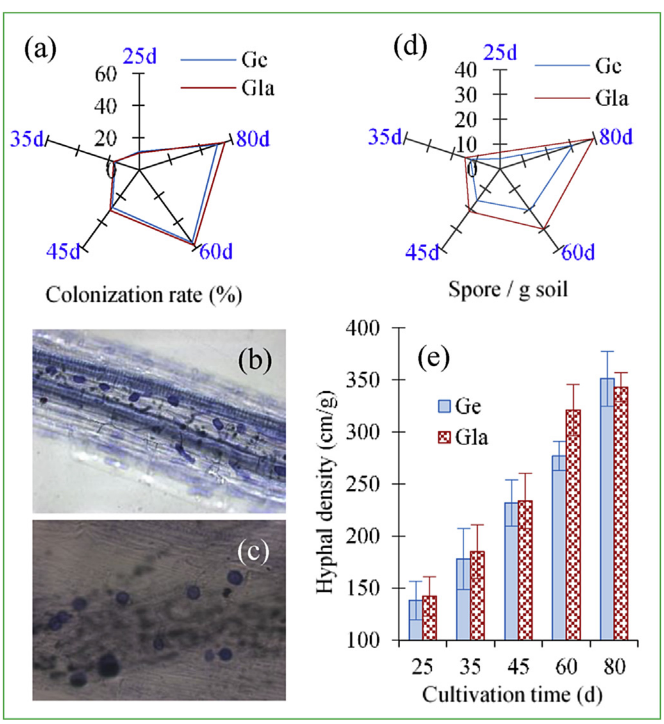

Fig. 1. Colonization of alfalfa by *Glomus etunicatum* (Ge) and *Glomus lamellosum* (Gla) in soils contaminated with pyrene, 25–80 days post-sowing. (a) Mycorrhizal colonization rates of alfalfa roots. (b) and (c) are vesicles of Ge and Gla, respectively, on root surfaces at Day 60. (d) Is the total numbers of spores in soil. (e) Hyphal density of Ge and Gla in soils contaminated with pyrene, determined per the method of Bedini et al. (2009). Experiments were conducted with five replicates. Error bars represent standard deviations.

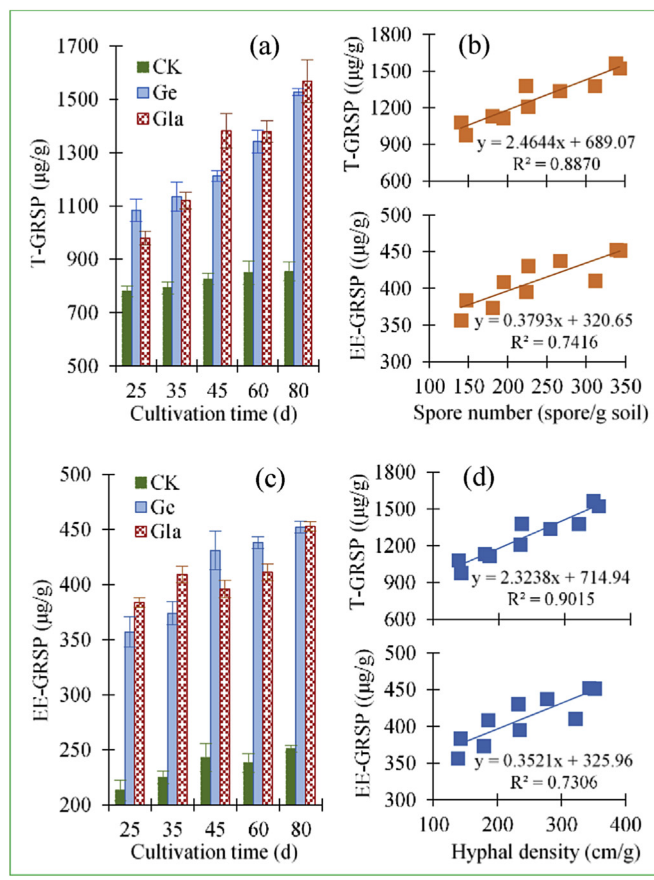

Fig. 2. (a) Total glomalin-related soil protein (T-GRSP) and (b) easily extractable GRSP (EE-GRSP) content in mycorrhizal soils with Ge and Gla 25–80 days post-sowing. (c) and (d) are correlations of GRSP content with spore amount and hyphal density, respectively. CK is the control soil without arbuscular mycorrhizal fungi inoculation. Experiments were conducted with five replicates. Error bars represent standard deviations.