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Exogenous arbuscular mycorrhizal fungi increase soil organic carbon and change microbial community in poplar rhizosphere

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Abstract: Arbuscular mycorrhizal fungi (AMF) increase soil organic carbon (SOC) deposition via secretion of glomalin-related soil protein (GRSP) and modulation of plant carbon partition. Two exogenous AMF inocula (*Rhizophagus irregularis* and *Glomus versiforme*) were applied to the roots of *Populus* × *canadensis* seedlings grown in the unsterilized nursery soil. The diversity of fungal and bacterial communities was assessed by the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method, while the accumulation of GRSP and SOC content in 22.5 cm-deep soil was measured. The results indicated that two AMF additions increased root colonization frequency as well as poplar biomass, especially root biomass accumulation. Two AMF applications improved the easily extractable-GRSP, total-GRSP, and SOC accumulation in the rhizosphere of poplar seedlings, limited the fungal community, and exerted no influence on the bacterial community. The effect of *G. versiforme* on GRSP and SOC accumulation was higher than that of *R. irregularis*. The AMF introduced GRSP, and SOC accumulation was highly correlated the limited fungal species richness.

Keywords: mycorrhizal symbiosis; microorganism; soil inoculant; plant root; mycoforestry

Many microorganisms thrive in the soil, especially in the plant rhizosphere (Mendes et al. 2013). The rhizosphere microbiome affects plant growth and development, and greatly contribute to plant production and ecosystem sustainability. Arbuscular mycorrhizal fungi (AMF) form a mutual association with majority of terrestrial plants (Smith and Read 2008). The symbioses are the important components of the terrestrial ecosystem, linking plant roots and soil system via large networks of fungal hyphae (Smith and Read 2008). AMF may affect root exudation, carbohydrate metabolism of the host plant, and influence the diversity of the microbial community in the rhizosphere (Rillig et al. 2006). Studies showed that the microbial community composition in the rhizosphere was different between mycorrhizosphere and non-mycorrhizosphere because AMF stimulates some microbial groups while suppressing others (Marschner and Baumann 2003).

Also, AMF contributes to the soil organic carbon (SOC) stock, mainly depending on the glomalin-related soil protein (GRSP) secreted by AMF (Driver et al. 2005). Glomalin is a poorly characterized fungal glycol-protein and released by AMF into the soil environment after fungal death or during hyphal turnover, and quantified from the soil as GRSP (Driver et al. 2005). Moreover,

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GRSP may be a relatively stable compound which is insoluble in water and resistant to heat degradation, glue-like and may represent a potentially important SOC component (Steinberg and Rillig 2003).

AMF is associated with many *Populus* species, improving their biomass yield, nutrient absorption, and tolerance to adverse environmental conditions (Liu et al. 2016). Nevertheless, the effects of exogenous AMF on the poplar rhizospheric microbial community, GRSP and SOC accumulation are less well-known. In this study, the polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) method was used to detect changes in bacterial and fungal communities induced by application of two relatively efficient AMF species (*Rhizophagus irregularis* and *Glomus versiforme*) in the poplar rhizosphere. GRSP and SOC content changes by AMF were also investigated to analyse the contribution of AMF to SOC storage within 22.5 cm-deep soil.

MATERIAL AND METHODS

Growth medium and plant material. Collection of soil was described at Liu et al. (2016). Soil was air-dried, ground, sieved (≤ 2 mm), homogenized and used as the growth medium.

Cuttings of *Populus* × *canadensis* (*P. nigra* × *P. deltoides*) cv. Neva were prepared as described by Liu et al. (2016). Each cutting was cultivated in one pot (22.5 cm × 22.5 cm, height × diameter) filled with 4 kg of the growth medium.

AMF inoculation. The AMF inoculum, *Rhizophagus irregularis* (Błaszk, Wubet, Renker & Buscot) Walker & Schüßler (BGC BJ09) and *Glomus versiforme* (Karsten) Berch (BGC GD01C), were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences, China. The inoculum comprised spores (50 spores per gram), mycelia, infected root fragments and sand. The inoculation was identical to Liu et al. (2016).

Experimental design and growth condition. Three treatments were: RI – inoculated with *R. irregularis*; GV – inoculated with *G. versiforme*; CK – control. Each treatment had 5 replications.

The experiment was carried out in a greenhouse with 12 h light per day at a temperature range of 25–35°C. Pots were arranged in a completely randomized block design. After the growth period (120 days), the rhizosphere soil from 3 randomly selected replications was collected by shaking roots slightly, and stored at –20°C before analyses.

AM colonization. Staining of roots and calculation of AM colonization was done as described by Liu et al. (2016).

DNA extraction and nest PCR. Soil DNA extraction and determination was also described at Liu et al. (2016). The highly variable V3 region of bacterial 16S rRNA gene was amplified as described by Hu et al. (2013) with GC clamp (40 bp adhere to the 5' end) in primer 341F. The ITS1 region of the fungal 18S rDNA gene was amplified as described by Gardes and Bruns (1993) with GC clamp (40 bp adhere to the 5' end) in primer ITS1-f.

PCR products were analysed by 1.2% (w/v) agarose gel electrophoresis, stained with EB and visualized under UV light. The obtained PCR products were stored at –20°C for DGGE.

DGGE analysis. Nest PCR products were used for the DGGE analysis as described by Zhang et al. (2010); they contained a vertical denaturing gradient of 30% to 60% for fungi and 40% to 70% for bacteria in the 8% (w/v) polyacrylamide (acrylamide:bis-acrylamide = 37.5:1) gel. The 100% denaturing acrylamide was defined as the denaturing acrylamide containing 40% formamide and 7 mol/L urea. DGGE images were digitized and analysed by Quantity One software 4.6.2 (Bio-Rad, Hercules, USA). Based on the number and intensity of bands in DGGE profiles; species richness (S); Simpson index (D); Shannon-Weiner index (H) and Evenness index (E) were calculated as described by Zhang et al. (2010).

$$H = -\Sigma\,(p_i)\,\ln(p_i)$$

$$E = H/\ln S$$

$$D = \Sigma(p_i^2)$$

Where: i – index number for each band present in a DGGE profile; p_i – frequency of the given band i, which is calculated by dividing the number of DGGE profiles that contain the band i by the total number of DGGE profiles considerers.

Determination of GRSP and soil organic carbon content. The easily extractable GRSP (EE-GRSP) and total GRSP (T-GRSP) were extracted according to the method described by Wright and Upadhyaya (1998). The SOC content was determined using the potassium bichromate titrimetric method described by Bao (2000).

Statistical analysis. For statistical analysis, data were checked by a one-sample Kolmogorov-Smirnov test and subjected to one-way ANOVA with 95% confidence limit ($P < 0.05$) using the program package Statistica (version 9.1; StatSoft Inc., Tulsa, USA). Fisher's *LSD* (least significant difference) was performed to evaluate the significance of the difference of the means at

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Table 1. Arbuscular mycorrhizal (AM) colonization frequency, shoot and root dry weight of poplar seedlings

	AM colonization frequency (%)	Shoot DW	Root DW	Colonized root DW
		(g)		
CK	23.7	5.19 ± 0.81	1.24 ± 0.24[b]	0.29 ± 0.06[b]
RI	88.3	7.16 ± 1.29	1.91 ± 0.29[a]	1.69 ± 0.26[a]
GV	87.4	6.88 ± 1.09	1.67 ± 0.07[ab]	1.46 ± 0.06[a]
Inoculation	na	ns	*	*

*P = 0.05; n = 3; ns – not significant; na – not applicable; colonized root DW = root DW × AM colonization frequency. Values with different letters indicate a significant difference (Fisher's *LSD* (least significant difference)-test). CK – control; RI – inoculated with *Rhizophagus irregularis*; GV – inoculated with *Glomus versiforme*; DW – dry weight

a significance level of $P \leq 0.05$. Pearson's correlation coefficients were used to assess the relationships among variables (n = 3).

RESULTS

AM colonization frequency and biomass accumulation. The frequency of AM colonization was 23.7% with control, 88.3% with *R. irregularis* inoculation, and 87.4% with *G. versiforme* inoculation (Table 1).

Application of both AMF did not improve poplar shoot dry weight, but improved root dry weight and the colonized root dry weight, while the effect of *R. irregularis* was stronger than that of *G. versiforme* (Table 1).

Fungal and bacterial community structures analysis. Both *R. irregularis* and *G. versiforme* affected the fungal community (Figures 1 and 2). Inoculation with *R. irregularis* decreased species richness but increased evenness of the fungal community. Inoculation with *G. versiforme* increased the Simpson index but decreased the species richness, Shannon-Wiener index and evenness of fungal community (Figure 2).

Inoculation with *R. irregularis* only increased species richness but had no significant effect on Shannon-Wiener index, Simpson index, and evenness index of the bacterial community. Inoculation with *G. versiforme* did not show any significant influence on the bacterial community (Figure 2).

GRSP and SOC content. Both *R. irregularis* and *G. versiforme* caused increases in EE-GRSP and T-GRSP contents, while *G. versiforme* had a greater effect than *R. irregularis* in EE-GRSP and T-GRSP accumulation (Figure 3a,b).

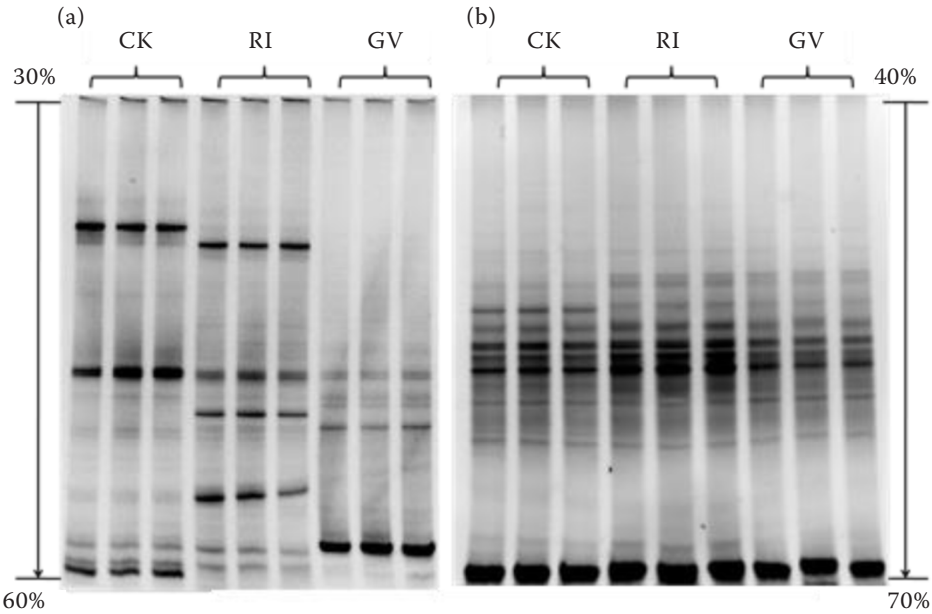

Figure 1. Denaturing gradient gel electrophoresis patterns of (a) fungal and (b) bacterial communities. CK – control; RI – inoculated with *Rhizophagus irregularis*; GV – inoculated with *Glomus versiforme*; 30, 40, 60, 70% were the concentrations of denaturing acrylamide gel

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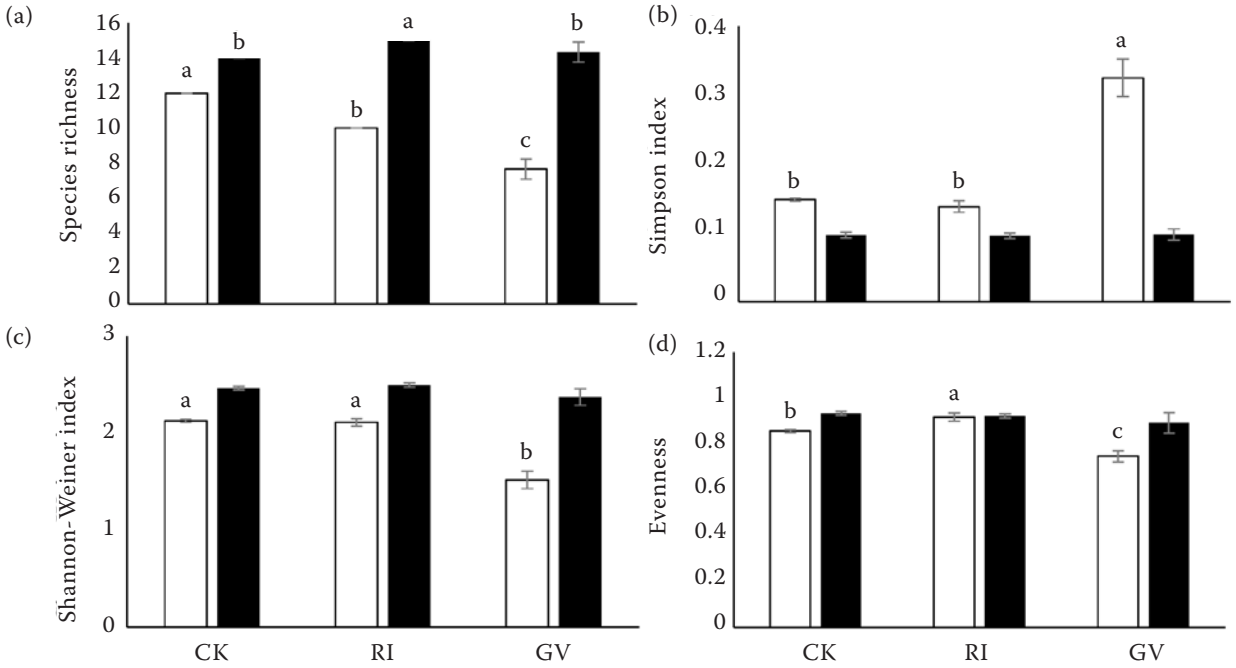

Figure 2. (a) Species richness; (b) Simpson index; (c) Shannon-Weiner index, and (d) Evenness of fungal (white column) and bacterial (black column) community in the poplar rhizosphere. The data were means ± standard deviation (n = 3). Different letters in each column indicate significant differences (Fisher's *LSD* (least significant difference)-test, $P < 0.05$). CK – control; RI – inoculated with *Rhizophagus irregularis*; GV – inoculated with *Glomus versiforme*

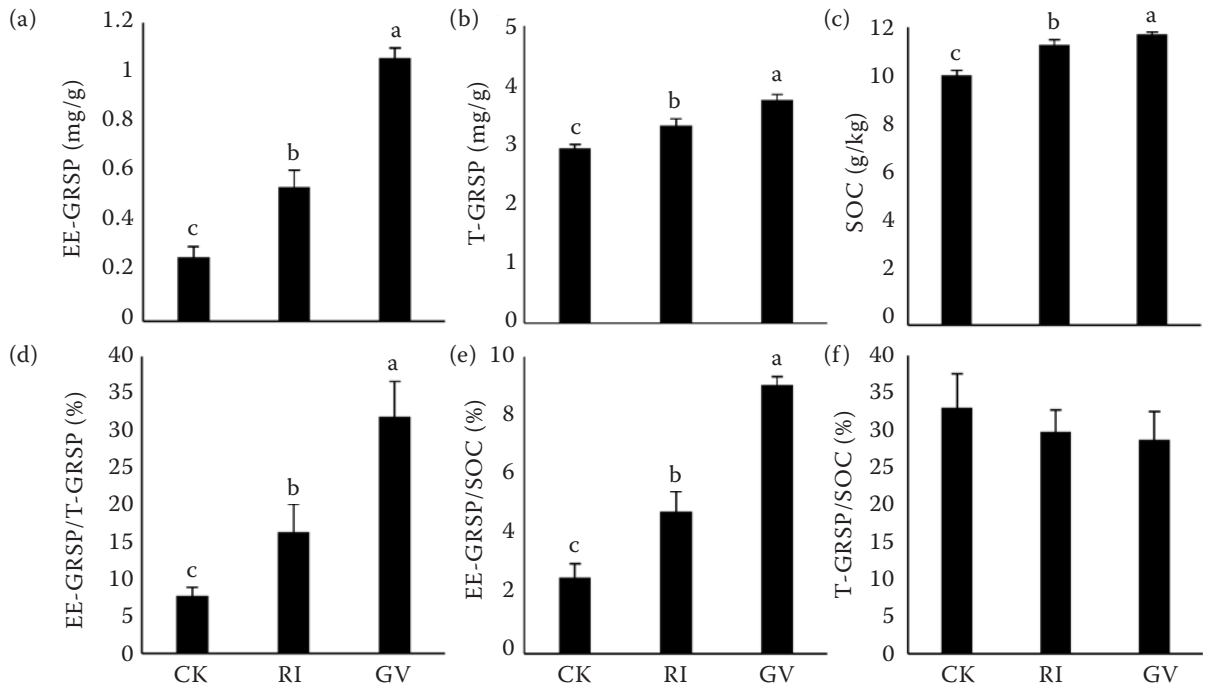

Figure 3. Effect of arbuscular mycorrhizal fungi (AMF) on (a) easily extractable glomalin reactive soil protein (EE-GRSP); (b) total glomalin reactive soil protein (T-GRSP) and (c) soil organic carbon (SOC) content in the poplar rhizosphere, and the ratio between (d) EE-GRSP/T-GRSP; (e) EE-GRSP/SOC and (f) T-GRSP/SOC. The data were means ± standard deviation (n = 3). Different letters in each column indicate significant differences (Fisher's *LSD* (least significant difference)-test, $P < 0.05$). CK – control; RI – inoculated with *Rhizophagus irregularis*; GV – inoculated with *Glomus versiforme*

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Both *R. irregularis* and *G. versiforme* increased the SOC content in the rhizosphere (22.5 cm depth), compared with that in the control treatment (Figure 3c).

EE-GRSP/T-GRSP, EE-GRSP/SOC, and T-GRSP/SOC ratios. Both *R. irregularis* and *G. versiforme* caused a significant increase in the EE-GRSP/T-GRSP and EE-GRSP/SOC ratios, while the effect of *G. versiforme* was greater than that of *R. irregularis* (Figure 3d,e). There was no significant difference between T-GRSP/SOC among treatments (Figure 3f).

Correlation analysis. EE-GRSP and T-GRSP were highly correlated with each other, and they were both positively correlated with SOC (Table 2). The correlation coefficient between T-GRSP and SOC was higher than that between EE-GRSP and SOC. Colonized root dry weight, instead of root dry weight, was positively correlated with T-GRSP and SOC in the rhizosphere, and the correlation coefficient of SOC was higher than that of T-GRSP.

Both root dry weight and colonized root dry weight was positively correlated with bacterial species richness. Only T-GRSP was negatively correlated with bacterial evenness.

EE-GRSP, T-GRSP, and SOC were negatively correlated with fungal species richness and fungal Shannon-Wiener index. EE-GRSP and T-GRSP were positively correlated with fungal Simpson index, and only EE-GRSP was negatively correlated with fungal evenness. Colonized root dry weight was negatively correlated with fungal species richness.

DISCUSSION

AMF colonization of plant roots was the basis for functional symbiosis. Both exogenous AMF increased the colonization frequency from 23.7% to more than 80% (Table 1). This was consistent with the previous study that applies AMF inoculum in soils containing native AMF (Wu et al. 2017). In company with increased colonization, the biomass, especially root dry weight, significantly increased; it may happen due to the introduced AMF that improved mineral nutrients, water absorption and photosynthesis (Liu et al. 2016). However, the application of AMF inoculum did not always improve colonization and plant biomass accumulation in unsterilized soil (Paluch et al. 2013). A further study of the use of AMF inoculum in unsterilized soil should proceed with the consideration that different plant and AMF combinations may generate different effects.

Rhizosphere was defined as a small area surrounding and influenced by plant roots, where large numbers of microorganisms interact with the plant (Mendes et al. 2013). Both exogenous AMF lowered the fungal species richness and Shannon-Wiener index. This may be due to the exogenous AMF increased colonization that strengthened (1) the symbiosis effect on root exudates, which shapes the fungal community in root rhizosphere (Okubo et al. 2016); (2) competition for ecological niches and soil nutrients, and (3) AMF deposition products that contain fungistatic compounds (Smith and Read 2008). The influence on fungal community also varied between *R. irregularis* and *G. versiforme*. This may be attributed to the AMF identity that differentially affected reasons discussed above (Koch et al. 2006), and the specific mechanisms need further research.

For bacteria, the application of two AMF inocula did not modify the bacterial community. This was shown by the study of Marschner and Baumann (2003), in which sterilized quartz-soil mixture was used as a growth substrate, and bacterial community

Table 2. Correlation coefficients among variables involves in GRSP content, SOC, and microbial community

	EE-GRSP	T-GRSP	SOC	FS	FH	FD	FE	BS	BH	BD	BE
EE-GRSP	1	0.96***	0.86**	–0.98***	–0.94***	0.91***	–0.74*	0.17	–0.66	0.03	–0.66
T-GRSP	0.96***	1	0.93***	–0.98***	–0.85**	0.82**	–0.6	0.29	–0.64	0.11	–0.7*
SOC	0.86**	0.93***	1	–0.93***	–0.69*	0.64	–0.37	0.52	–0.43	0.02	–0.6
Shoot DW	0.5	0.48	0.56	–0.48	–0.23	0.19	0.01	0.32	0.12	0.26	–0.03
Root DW	0.46	0.45	0.6	–0.5	–0.19	0.12	0.11	0.7*	0.1	–0.32	–0.21
Colonized root DW	0.66	0.7*	0.87**	–0.73*	–0.39	0.32	–0.04	0.71*	–0.07	–0.14	–0.37

EE-GRSP – easily extractable glomalin reactive soil protein; T-GRSP – total glomalin reactive soil protein; SOC – soil organic carbon; BS – bacterial species richness; BH – bacterial Shannon-Werner index; BD – bacterial Simpson index; BE – bacterial Evenness index; FS – fungal species richness; FH – fungal Shannon-Werner index; FD – fungal Simpson index; FE – fungal Evenness index; DW – dry weight; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

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between two AMF (*Glomus intraradices* and *Glomus mosseae*) inoculated treatment did not differ from each other but differed from a non-mycorrhizal treatment after 6 weeks growth. They concluded that the AMF modified bacterial community change was plant-mediated. As to current study, the unmodified bacterial community in two AMF inoculated treatment may be attributed to the unmodified AMF composition (two common AMF inoculum account for a small portion of growth medium, 5 g/4 kg) and the stable response of poplar to AMF after 120 days (Hartmann et al. 2009).

AMF contributed to the SOC accumulation mainly through secreting GRSP (Singh et al. 2013). Both AMF increased the EE-GRSP and T-GRSP content. This was reported by previous studies (Bedini et al. 2009, Zhang et al. 2016). Although treatment that received *R. irregularis* had higher root DW and colonized root DW than the treatment receiving *G. versiforme*, the latter accumulated more GRSP than the former one (Figure 3). This may occur due to the higher production of mycelium by *G. versiforme* (Zhang et al. 2016), and the accumulation of GRSP replied to hyphal turnover and death of mycelia (Driver et al. 2005). EE-GRSP accumulation was more efficient than T-GRSP and contributed to higher ratios of EE-GRSP/T-GRSP, and EE-GRSP/SOC in AMF inoculum-applied treatments. This may be explained by the experimental time (120 days), and the relatively high amount of former existing AMF mycelium (for T-GRSP accumulation) and SOC in unsterilized soil, which maintained the stable T-GRSP/SOC ratio (Bedini et al. 2009, Zhang et al. 2016).

EE-GRSP and T-GRSP were highly correlated with each other, and with SOC (Table 2). Similar results were observed in other studies (Bedini et al. 2009, Zhang et al. 2016, Gałązka et al. 2017, 2018) and confirmed that the contribution of AMF on SOC depend on the secretion of GRSP. The mycorrhizal colonized root dry weight, instead of root dry weight, was positively correlated with the GRSP accumulation. This resembled the results of Bedini et al. (2009), who indicated that the increased GRSP can be explained by the contribution of the mycorrhizal plant root system, as they found a higher correlation between GRSP and colonized root volume than total root volume. The colonized root dry weight also highly correlates with the SOC content, which supports the opinion that mycorrhizal hyphae together with plant roots contribute highly to SOC (Högberg and Högberg 2002). The negative correlations between fungal species richness and GRSP, SOC and colonized root dry weight indicated that the suppression of fungi by AMF application was a combined effect of AMF-modulated plant root and produced GRSP, but the specific mechanisms need further study. Both root dry weight and colonized root dry weight correlated with bacterial species richness support the idea that the AMF modulated bacterial community via the mediation of a host plant (Marschner and Baumann 2003).

In conclusion, exogenous AMF application could increase the colonization of poplar seedlings roots and the biomass (especially root biomass) accumulation, improve the EE-GRSP, T-GRSP, and SOC accumulation in the rhizosphere, limited the fungal community and exerted no influence on the bacterial community.

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