

What drives differences in root-associated arbuscular mycorrhizal fungal community among plant populations?

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LETTER TO THE EDITOR

Elymus sibiricus populations drive the community of root-associated arbuscular mycorrhizal fungi in a monoculture agroecosystem

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Dear Editor,

Arbuscular mycorrhizal fungi (AMF) belonging to phylum Glomeromycota, form symbiotic associations with roots of over 80% of land plant species in terrestrial ecosystems ([van der Heijden et al., 2015](#)). A growing body of evidence shows that symbiotic AMF are important for plant growth and health ([van der Heijden et al., 2015](#)). A plant root is commonly associated with a complex AM fungal community in terrestrial ecosystems ([Pivato et al., 2007](#); [Davison et al., 2015](#); [Mao et al., 2015](#)). The diversity and composition of AMF in the roots of several model plants ([Pivato et al., 2007](#)) and crops ([Mao et al., 2015](#)) has been widely studied. The host plant is a key factor affecting the root-associated AMF community (RAMFC), and studies found that the effects of host plants on RAMFC could be operating on the levels of different species ([Pivato et al., 2007](#)), cultivars ([Mao et al., 2015](#)) and plant genotype ([An et al., 2010](#)). *Elymus sibiricus* is one of the most commonly distributed cool-season perennial grasses found in temperate regions and is becoming increasingly important as a forage grass to providing good quality forage for livestock ([Chen and Jia, 2002](#)). Some studies had been focused on the symbiotic association between forage plants in *Elymus* and AMF ([Liu et al., 2012](#); [Chu et al., 2016](#)), and the presence of AMF in roots appears likely to improve the resistance of *E. nutans* to cold stress ([Chu et al., 2016](#)) and increase

the biomass of this grass species (Yang et al., 2018). The present study was conducted to investigate, using sequencing technology, the potential host effects of *E. sibiricus* populations on the diversity of their RAMFC.

Details of the accession numbers, and if the seeds originated from wild populations or from cultivars, are found in the [Table S1 in Supporting Information](#). Four *E. sibiricus* accessions were from Inner Mongolia, China (M), and three of them were from wild populations and one of a cultivar; four accessions, all from cultivars, were from the Qinghai and Sichuan provinces, China (C); three accessions, all from wild populations, were from Kazakhstan (K); and three accessions, all from wild populations, were from Russia (R). Seeds of these 14 accessions were sown in 14 experimental plots (each plot: 1 m × 1 m, 9 individual plants) at the Yuzhong campus of Lanzhou University (103°34' E, 35°34' N, altitude 1720 m) in 2012. The climate information for this present study was presented in [Table S2 in Supporting Information](#). The sampling processes, and the measurements of soil properties and mycorrhizal colonization were supplied in the [Supporting Information](#).

DNA of plant roots was extracted from 0.1 g root material using a plant DNA kit (Tiangen, Beijing) according to the manufacturer's instructions. AMF sequences were amplified using the small subunit (SSU) rRNA gene primer, and the AMV4.5NF-AMDGR primer pair (van Geel et al., 2014). The process of PCR and sequencing, and the analysis of sequencing data are detailed in the [Supporting Information](#). The DNA sequences used in this study have been deposited in Sequence Read Archive (SRA) of NCBI database under accession numbers SRX6973663 to SRX6973676. Community richness and diversity was determined with the Chao1 and Shannon indices respectively. Principal coordinates analysis (PCoA) of RAMFC based on the operational taxonomic units (OTUs) level was performed using Bray-Curtis distances through the R-package Vegan, and these statistically significant differences were obtained through the Multi Response Permutation Procedure (MRPP) based on the Bray-Curtis dissimilarities. Redundancy analysis (RDA) between relative abundance of RAMFC genera and rhizosphere soil properties were performed by CANOCO for Windows 4.5.

The differences of colonization and alpha diversity in roots, and rhizosphere soil properties among the four different populations were tested using a one-way analysis of variance through

SPSS 22.0 (SPSS, Chicago, IL, USA). Fishers Least Significant Differences (LSD) test was used to determine whether differences between means were statistically significant. In all tests, a P -value of < 0.05 was considered to be statistically significant.

There were no significant ($P>0.05$) differences of soil properties among all soil samples (Table S3 in Supporting Information). However, there was a significantly ($F=7.681$, $P=0.006$) lower total root colonization rate for plants of the four populations from M compared to the three from R, three from K, and two from C populations which showed no significant differences (Figure 1A). A total of 523,183 sequences were detected from all root samples, and these sequences were assigned into 69 AMF OTUs with 5 main genera through the Neighbor-Joining (NJ) phylogenetic tree (Figure S1). The most dominant genera in RAMFC of R, K, C and M *E. sibiricus* populations were *Claroideoglomus*, *Rhizophagus*, *Glomus* and *Rhizophagus*, respectively (Figure 1B).

The richness and diversity of AMF in rhizosphere soil were significantly ($P=0.000$) higher than those in the RAMFC of *E. sibiricus*, studied by the Shannon and Chao1 tests (Figure 1CD). The diversity of the RAMFC of the R populations was significantly ($P<0.05$) higher than those of the C and M populations, while the diversity of RAMFC of the K and C populations was significantly ($P<0.05$) higher than those of the M populations (Figure 1D). The results of PCoA showed that the composition of RAMFC was significantly ($P=0.032$) different among the four populations through MRPP analysis (Figure 1E).

In this study, no significant relationships between the diversity and richness of RAMFC and soil properties were observed (Table S4 in Supporting Information). The first and second axis of RDA explained 48.5% and 27.3% of the variance, respectively (Figure 1F), and the *Glomus* and *Claroideoglomus* abundance was positively related to soil organic carbon (SOC), total N (TN), the ratio of available N to available P (AP), ammonium N (AN), total P (TP) and available N, while *Rhizophagus* and *Paraglomus* abundance was positively related to the content of potassium (AK), pH and the ratio of SOC to TN, and *Funneliformis* abundance was positively related to AP (Figure 1F). The best structural equation modeling (SEM) model ($\chi^2=9.986$, $Df=8$, $P=0.266$, $NFI=0.801$, $RMSEA=0.246$) explained 62.0% and 72.1% variations of in diversity and richness of RAMFC, respectively, and the increase in the diversity of RAMFC was correlated with increasing soil AP

and decreasing soil SOC, while the decrease in the richness of RAMFC was correlated with increasing soil AP and nitrate N (NN), and decreasing soil SOC and TN ([Figure S2 in Supporting Information](#)).

The present study showed that different *E. sibiricus* populations were dominated by different AM fungal taxa at the genus level. Our study also indicated that the host preferences and host selectivity were present among different populations.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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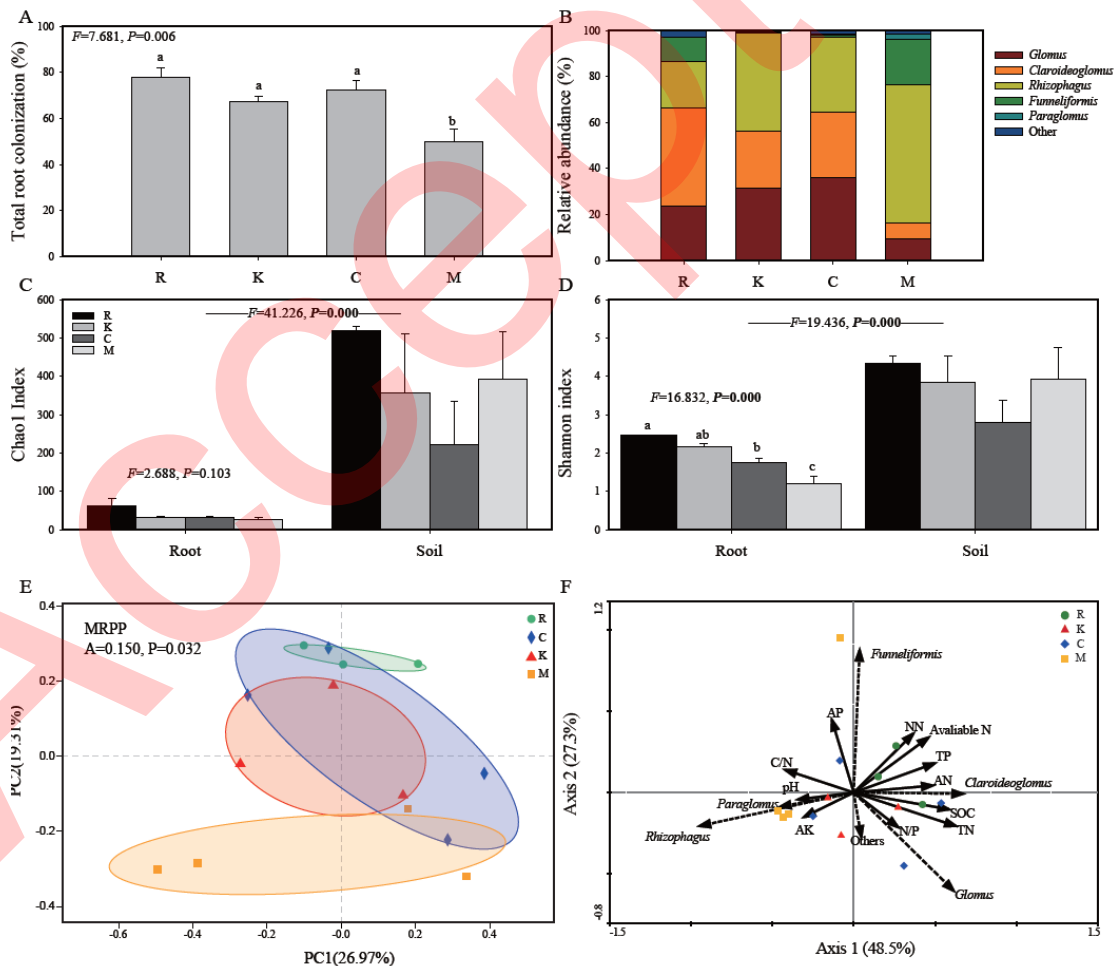


Figure 1 Total root colonization in roots (A), relative abundance of five main AM fungal genera in roots (B), Chao (C) and Shannon (D) index of root-associated and rhizosphere soil AM fungal community, principal coordinates analysis (PCoA) of root-associated AM fungal

communities at AM fungal operational taxonomic units (OTUs) level (E), and Redundancy analysis (RDA) of relative abundance of root-associated AM fungal phyla (F) of *E. sibiricus* populations from the four different countries or regions and soil properties. R=Russian, n=3; K= Kazakhstan, n=3; C=Qinghai and Sichuan in China, n=4; M=Inner Mongolia, China, n=4. The values were means \pm standard error. a, b and c indicate significant difference at 0.05 level. Soil factors indicated include AP (Available P), SOC (Soil Organic Carbon), AK (Available potassium), N/P (Available N: Available P).

SUPPORTING INFORMATION

Supplementary Methods

Table S1 The information of *Elymus sibiricus* accessions used in this study.

Table S2 The characteristics of sampling sites.

Table S3 Rhizosphere soil properties of *E. sibiricus* populations from different countries or regions

Table S4 Spearman correlations of soil properties and root-associated AM fungal diversity.

Figure S1 Neighbor-Joining (NJ) tree showing AM fungal operational taxonomic units (OTUs) detected in roots of different *E. sibiricus* populations from different countries or regions

Figure S2 The results of final structural equation modelling (SEM) showing the causal relationships among different *E. sibiricus* populations from the four different countries or regions