

LOW HOST SPECIFICITY OF ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH DOMINANT STEPPE PLANTS IN INNER MONGOLIA

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Abstract. Arbuscular mycorrhizal fungi (AMF) play potentially essential roles in maintaining the diversity and functioning of plant communities in Eurasian steppe representing one of the most important terrestrial ecosystems. However, it remains unclear about the symbiotic relationship between AM fungal communities and the dominant grass species in the steppe ecosystem. We thus examined the species diversity and community composition of AM fungi colonizing three dominant plant species (*Leymus chinensis*, *Stipa grandis* and *Cleistogenes squarrosa*), using field sampling and molecular analyses, in Xilinguole steppe, Inner Mongolia. Our results showed all three plants were well colonized by AM fungi, and 51 operational taxonomic units (OTUs) belonging to six genera with members of *Glomus* dominating in the roots of all the three plants species. By comparison, we found that *L. chinensis* had the most diverse AM fungi within the roots. OTU richness of AM fungi was higher in the roots of *S. grandis* than in the roots of *C. squarrosa* and *L. chinensis*. However, the community composition of AM fungi in three host plant species showed no significant difference. Based on these findings, we concluded that the dominant plant species held diverse AM fungal taxa in their roots, while the host preference did not significantly drive the differences in AM fungal community composition within their roots and resulted in low host specificity. Findings of this study would broaden the concept of host specificity and its implications on plant succession in the largest grassland ecosystem of China.

Keywords: *arbuscular mycorrhizal fungi, diversity, community composition, host specificity, symbiosis*

Introduction

Arbuscular mycorrhizal (AM) symbiosis formed between higher plant roots and the fungi belonging to the phylum *Glomeromycota* is one of the most common mutualistic associations in terrestrial ecosystems (Desai et al., 2016; Stürmer, 2012). In grasslands, the vast majority of plant species are AMF hosts (Bonfante and Genre, 2010; Eom et al., 2000; Harrier, 2001). In these symbiotic associations, plants provide carbon to the fungi to complete their life cycle (Zhang et al., 2016b). In return, AMF benefits their hosts in various ways including enhanced nutrient acquisition and improved protection against biotic and abiotic stresses (Latef et al., 2016; Huang et al., 2017; Chen et al., 2017). Therefore, AM symbionts could play potentially

important roles in plant growth, community diversity and ecosystem stability (Torrecillas et al., 2012).

The host plant strongly influences the AMF diversity and community composition inside the roots (Pivato et al., 2007; López-García et al., 2014; Zheng et al., 2016). However, owing to the wide range of preference for host plants (Krüger et al., 2012), the AMF normally show low host specificity (Lee et al., 2013), and most of the previous studies found that AMF had no host specificity (Clapp et al., 1995; Santos et al., 2006; Smith and Read, 2008). For example, Su et al. (2011) studied the AMF community composition in five plant species of Inner Mongolia and found 18 AMF species belonging to five genera and concluded that most of the AMF had no host specificity, but few species showed a certain degree of the host preference. However, some findings showed that AMF community composition is host-dependent (Lugo and Cabello, 2002; del Mar Alguacil et al., 2018). Bentivenga and Hetric (1992) found host plant had a significant effect on AM fungal sporulation in tallgrass prairie grasses. Li et al. (2010) explored three plant species of hot and arid ecosystem of southwest China and found different AM fungal community composition. Several studies have documented that selectivity between AMF and host plants may be the cause of presence or absence of host specificity (Bever, 2002; Helgason et al., 2002; Zhang et al., 2010). Moreover, Yang et al. (2012) hypothesized that discerning pressure from host plant at different levels (functional groups and taxonomic order) may be a driver for the distribution of AMF. However, the detailed explanation still needs several studies. The previous suppositions are mainly based on morphological investigations and a little molecular work is involved. Therefore, considering the contrasting opinions regarding AMF host specificity and insufficient investigations, further studies are needed to deepen the understanding about the role of AMF in typical steppe. This would broaden the concept of host specificity and its implications on plant succession in the largest grassland ecosystem of China because the low host specificity favours underground communication and nutrient transfer pathways via mycorrhizal networks.

The typical steppe in Inner Mongolia covers more than 20% of total grassland area in China (Xu et al., 2014), and AMF holds a significant value in the success of plant species in the steppe. In this study, we analyzed and compared the diversity and community composition of AMF colonizing the roots of three dominant perennial plant species; *Leymus chinensis* (*L. chinensis*), *Stipa grandis* (*S. grandis*), and *Cleistogenes squarrosa* (*C. squarrosa*). These three plant species are considered long-lived dominant grass species of the typical steppe, Xilinguole (Vandenkoornhuyse et al., 2003). The sheepgrass (*L. chinensis*) is a perennial forage plant and of great significance for grassland productivity and ecosystem (He et al., 2017). While, *S. grandis* is known as needlegrass also has significant nutritional value for the cattle and sheep in this region of Inner Mongolia (Su et al., 2010). The *Cleistogene squarrosa* is the most abundant C₄ perennial bunchgrass species of this region and the importance of *Cleistogene* has been recognized for the development of sustainable grassland system (Liang et al., 2002). Concerning the ecological and economic importance of these three plant species, our goal was to identify the differences in diversity and community composition of AMF in three plant species belonging to the same family (Poaceae) to determine the host specificity. These findings can do a great deal towards a comprehensive understanding of AM fungi associations with roots of perennial plant species and host specificity.

Materials and methods

Study site

The study was carried out in the Inner Mongolia Grassland Ecosystem Research Station (43°38'55.9"N, 116°09'06.3"E), Inner Mongolia Autonomous of China (Fig. 1). This grassland system has semiarid climatic conditions with a short plant growing period ranging from May to September. The average annual precipitation is 343 mm, and the mean annual temperature is 0.3 °C with average monthly temperature ranging from -21.6 °C (January) to 19.0 °C (July). The main soil type in this site is chestnut soil with relatively homogenous physical and chemical properties (Li et al., 2015; Ren et al., 2016).

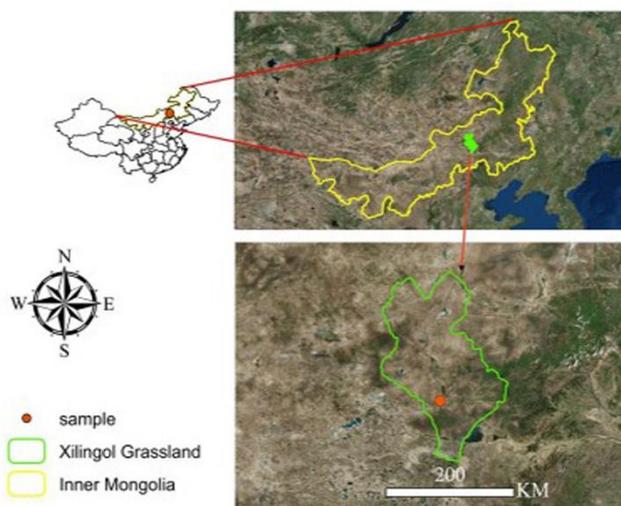


Figure 1. The geographic location of the sampling site. The green line is the boundary of grassland and red dot is the location of sample plot. The map was created with ArcGIS 10.2

Field sampling

The sampling was performed on June 24, 2014, and we randomly selected seven sampling points with a distance of 100 m from each other in a 1 × 1 km area as replicates. So, we collected 21 total plant samples and seven samples per species. To diminish the influence of soil type and geographic factors for each point, we selected closely adjacent *L. chinensis*, *S. grandis*, and *C. squarrosa* species for each sampling point. Corresponding, we obtained the roots and the rhizosphere soil of each plant using the soil drill. All the samples were preserved in zip lock bags and stored at 4 °C.

AMF structure and colonization

AMF colonization or root length colonization is an indicator of fungal growth within plant roots. In this study, the roots of the collected plants were washed carefully with tap water and cut into segments of 1 cm length. For each sample, approximately 100 root segments were randomly chosen and cleared in 10% KOH at 90 °C and stained with 0.05% Trypan blue, and then examined the percentage of AMF colonization using the magnified intersection method at 200× magnification (Nikon-E100) (Mcgonigle et al., 1990).

DNA extraction and sequencing

In our study, the AM fungi within the roots of *L. chinensis*, *S. grandis*, and *C. squarrosa* was identified through PCR amplified 18S-rRNA gene fragments with AMF-specific primers, as partial sequences of small subunit (SSU) genes appeared to be more informative than ITS genes for AM fungi (Redecker et al., 2006). For each sample, the total DNA was extracted from 80 randomly obtained fine roots using MoBio Power Soil[®] DNA Isolation kits (QIAGEN, Valencia, CA, USA). 18S rRNA gene was amplified using a nested PCR protocol. Based on the previous study (Krüger et al., 2009), the chosen primers were AML1/AML2 because they have better coverage and specificity than NS31/AM1, which have been extensively used in recent years (Helgason et al., 1999; Simon et al., 1992). A 10-fold dilution of the DNA was first amplified using the general eukaryotic primers NS1/NS4 (White et al., 1990). In the second phase of nested PCR, the product was amplified using AML1/AML2, and the PCR product was visualized on 1% agarose gel. The expected 800 bp bands were cut out and purified using an AxyPrep DNA Gel Extraction Kit (Axygen, Union City, CA, USA). The purified product was sequenced using the Sanger platform to confirm the presence of AM fungi before proceeding further. Purified DNA was cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and then transformed into *E. coli* DH-5 α (Tiangen, Beijing, China). The resulting DNA sequences were aggregated using BioEdit to perform the subsequent analysis.

Sequence analysis

We compared our clone DNA sequences with the GenBank database on the NCBI website (<http://www.ncbi.nlm.nih.gov/>). The closest match was selected to identify the sequences, and only those recognized as *Glomeromycota* were included for further taxonomic analysis.

All the selected AM fungal sequences were gathered from the previous steps, then we conducted standard operational taxonomic units (OTUs) analysis using the Mothur software (Schloss et al., 2009). The sequences with not less than 97% matched, were grouped into OTU (Helgason et al., 1998). After finishing clustering, we selected one sequence in each OTU as the representative sequence and aligned them together with their closest matched identified AM fungal sequences from the NCBI database; then Mega 6.06 program (Kumar et al., 2007) was utilized to align the sequences. Furthermore, in Mega, Kimura2-parameter model was computed to perform neighbor-joining phylogenetic analysis with 1000 bootstrap replicates to calculate the support value of the tree with *Mortierella polycephala* (ID: X89436) and *Endogone pisiformis* (ID: X58724) as outgroups to root the tree (Tamura et al., 2007; Öpik et al., 2003). To further quantify and compare the fungal community diversity, the Shannon-Wiener index was computed with vegan package (Oksanen et al., 2007) in R (Team, 2013) based on the identified OTUs. The Shannon diversity index was calculated according to the following equation:

$$D = \sum_{i=1}^S p_i \times \ln p_i$$

where S is the total number of species in the sample and p_i is the relative abundance of certain AM fungus species of the sample (Shannon and Weaver, 1949).

Nucleotide sequence accession numbers

A total of 51 representative sequences of the clones detected were submitted to NCBI (National Center for Biotechnology Information) GenBank (<http://www.ncbi.nlm.nih.gov>) with the accession number LS997508-LS997558.

Statistical analysis

The SPSS statistical software was used to compare the AMF colonization rate, species richness, and Shannon diversity indexes among the three species. The data were analyzed using one-way Analysis of Variance method (ANOVA) and significant differences between the different plant species in ANOVA were compared based on Tukey's HSD post-hoc test at $P \leq 0.05$. To gain further insight into the potential relationship between AM fungal communities and their host plant species, NMDS (nonmetric multidimensional scaling) was conducted using PAST (Paleontological Statistics) version 3.21.

Results

AMF colonization

The results showed that all the plant species were well colonized by AMF. *S. grandis* showed an average of 92% colonization, whereas *C. squarrosa* and *L. chinensis* showed average colonization rates of 87% and 90%, respectively (Fig. 2). One-way analysis of variance (ANOVA) indicated that there were no significant differences as shown by Tukey's HSD test ($F(2,18) = 0.054$) in the AMF colonization among the three dominant species.

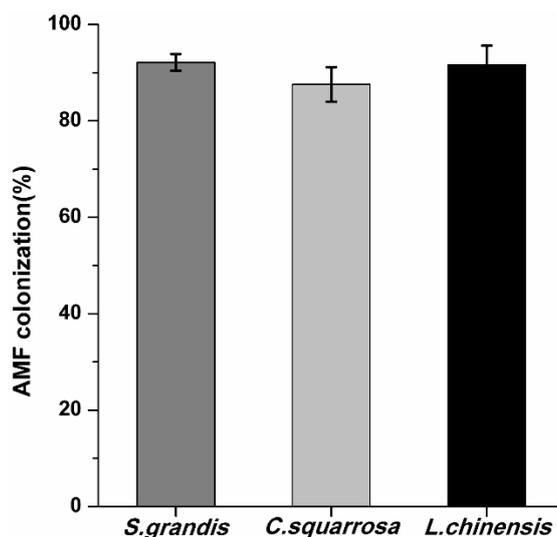


Figure 2. AMF colonization. AM fungal colonization (%) observed in three plant species. Data are means \pm SE

Identification and phylogenetic analysis of AM fungi

A total of 750 clones were sequenced, and among them, 587 non-chimeric sequences were identified as AMF and clustered into 51 OTUs at 97% sequence similarity. These

phylotypes or sequences types classified into six genera including *Rhizophagus*, *Glomus*, *Diversispora*, *Claroideoglomus*, *Paraglomus*, and *Ambispora* (Fig. 3). Of all these AM fungal phylotypes, 19 belonged to *Rhizophagus* (*Rhizophagus* 01-19), 24 to *Glomus* (*Glomus* 01-24), one from each *Diversispora* (*Diversispora* 01) and *Claroideoglomus* (*Claroideoglomus* 01), four from *Paraglomus* (*Paraglomus* 01-04), and *Ambispora* (*Ambispora* 01-02) group contains two phylotypes.

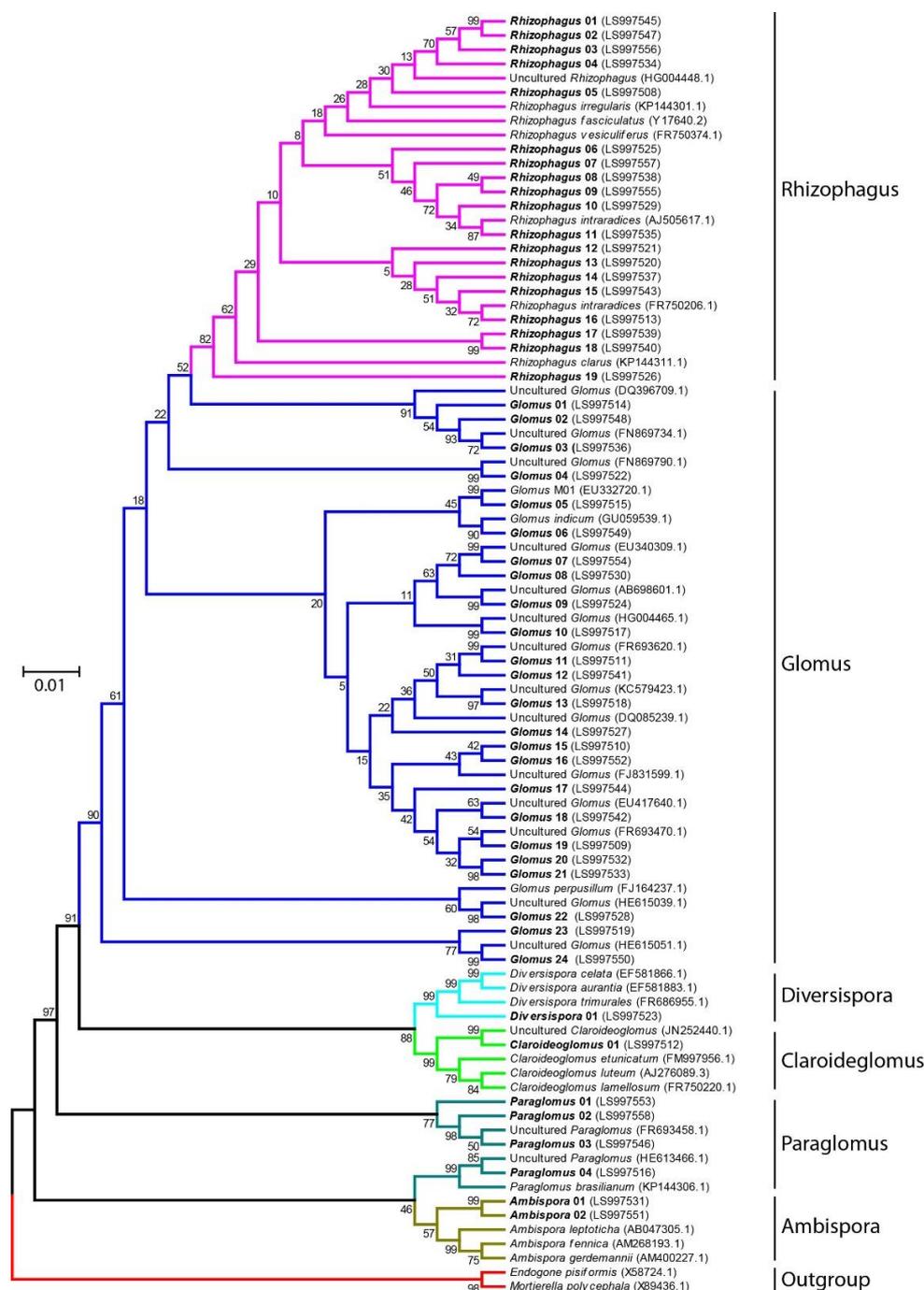


Figure 3. Phylogenetic tree. The neighbor-joining phylogenetic tree was constructed based on 18S rDNA sequences of the 51 OTUs (bold), and the reference sequences were downloaded from NCBI (their corresponding numbers are shown in the parentheses). The bootstrap values by 1000 replications were calculated. The scale bar represents the sequence divergence at 1%

In terms of OTUs, *Rhizophagus*-05 and *Glomus*-15 were the most abundant in all three species, with a relative abundance of 27% and 11% respectively, whereas *Glomus*-19 was found only in *S. grandis* and *C. squarrosa* and had a relative abundance of 13% (Table 1).

Table 1. Number of clones detected for each AM fungi phylotype and relative richness. The most closely related sequences with their accession number from the <http://www.ncbi.nlm.nih.gov> are given

OTUs identified	SG	CS	LC	Accession	Identity (%)
<i>Rhizophagus</i> 01 (LS997545)	0	0	1	HG004448.1	0.99
<i>Rhizophagus</i> 02 (LS997547)	0	0	1	FR693470.1	0.99
<i>Rhizophagus</i> 03 (LS997556)	0	1	0	FJ831599.1	0.99
<i>Rhizophagus</i> 04 (LS997534)	1	0	1	FR693620.1	0.99
<i>Rhizophagus</i> 05 (LS997508)	40	54	62	JN252440.1	0.99
<i>Rhizophagus</i> 06 (LS997525)	5	0	0	FR750206.1	0.99
<i>Rhizophagus</i> 07 (LS997557)	1	0	0	DQ396709.1	0.99
<i>Rhizophagus</i> 08 (LS997538)	2	0	0	EU332720.1	0.99
<i>Rhizophagus</i> 09 (LS997555)	0	0	1	HE613466.1	0.99
<i>Rhizophagus</i> 10 (LS997529)	0	2	1	HG004465.1	0.99
<i>Rhizophagus</i> 11 (LS997535)	0	2	0	KC579423.1	0.99
<i>Rhizophagus</i> 12 (LS997521)	4	0	7	EU340309.1	0.96
<i>Rhizophagus</i> 13 (LS997520)	10	0	3	FR750206.1	0.99
<i>Rhizophagus</i> 14 (LS997537)	1	1	0	FR750206.1	0.99
<i>Rhizophagus</i> 15 (LS997543)	0	1	0	FN869790.1	0.99
<i>Rhizophagus</i> 16 (LS997513)	5	15	1	FR686955.1	0.95
<i>Rhizophagus</i> 17 (LS997539)	0	1	0	AB698601.1	0.99
<i>Rhizophagus</i> 18 (LS997540)	1	0	0	FR750374.1	0.98
<i>Rhizophagus</i> 19 (LS997526)	2	1	1	Y17640.2	0.98
<i>Glomus</i> 01 (LS997514)	2	17	0	DQ085239.1	0.98
<i>Glomus</i> 02 (LS997548)	1	0	0	HE615039.1	0.98
<i>Glomus</i> 03 (LS997536)	2	0	0	Y17640.2	0.97
<i>Glomus</i> 04 (LS997522)	1	0	10	EU340309.1	0.99
<i>Glomus</i> 05 (LS997515)	12	6	0	AM268193.1	0.88
<i>Glomus</i> 06 (LS997549)	1	0	0	FR693470.1	0.96
<i>Glomus</i> 07 (LS997554)	0	0	1	FR693470.1	0.98
<i>Glomus</i> 08 (LS997530)	3	0	0	HG004448.1	0.98
<i>Glomus</i> 09 (LS997524)	1	0	6	AJ505617.1	0.97
<i>Glomus</i> 10 (LS997517)	3	0	14	FN869790.1	0.98
<i>Glomus</i> 11 (LS997511)	3	23	5	FR750206.1	0.96
<i>Glomus</i> 12 (LS997541)	0	1	0	Y17640.2	0.97
<i>Glomus</i> 13 (LS997518)	7	3	6	Y17640.2	0.97
<i>Glomus</i> 14 (LS997527)	2	0	1	Y17640.2	0.87
<i>Glomus</i> 15 (LS997510)	20	23	22	FR693620.1	0.97
<i>Glomus</i> 16 (LS997552)	0	1	0	EU417640.1	0.98
<i>Glomus</i> 17 (LS997544)	1	0	0	FR750206.1	0.96
<i>Glomus</i> 18 (LS997542)	0	1	0	FR693470.1	0.97
<i>Glomus</i> 19 (LS997509)	27	48	0	HG004448.1	0.97

<i>Glomus</i> 20 (LS997532)	0	2	0	FR693458.1	0.97
<i>Glomus</i> 21 (LS997533)	2	0	0	HG004448.1	0.96
<i>Glomus</i> 22 (LS997528)	3	0	0	DQ396709.1	0.95
<i>Glomus</i> 23 (LS997519)	1	0	13	GU059539.1	0.97
<i>Glomus</i> 24 (LS997550)	0	0	1	HE615051.1	0.97
<i>Diversispora</i> 01 (LS997523)	0	0	8	AM268193.1	0.87
<i>Claroideoglomus</i> 01 (LS997512)	0	0	30	FJ831599.1	0.98
<i>Paraglomus</i> 01 (LS997553)	1	0	0	FR693458.1	0.96
<i>Paraglomus</i> 02 (LS997558)	0	0	1	EU340309.1	0.98
<i>Paraglomus</i> 03 (LS997546)	1	0	0	Y17640.2	0.97
<i>Paraglomus</i> 04 (LS997516)	0	17	0	HG004448.1	0.96
<i>Ambispora</i> 01 (LS997531)	1	0	2	AJ505617.1	0.97
<i>Ambispora</i> 02 (LS997551)	0	0	1	FR693458.1	0.96
Total clones	167	220	200		
Total richness	32	20	25		

AMF diversity inside roots

The most dominant genera were *Glomus* and *Rhizophagus*, accounting for 50% and 39% of the total clones, respectively, whereas, the *Ambispora* and *Diversispora* were the least frequent genera, making up only 0.6% and 1% of the total clones, respectively (Fig. 4). At the genus level, all six AM fungal genera were found in *L. chinensis* whereas only four and three genera were found in *S. grandis* and *C. squarrosa*, respectively (Fig. 4).

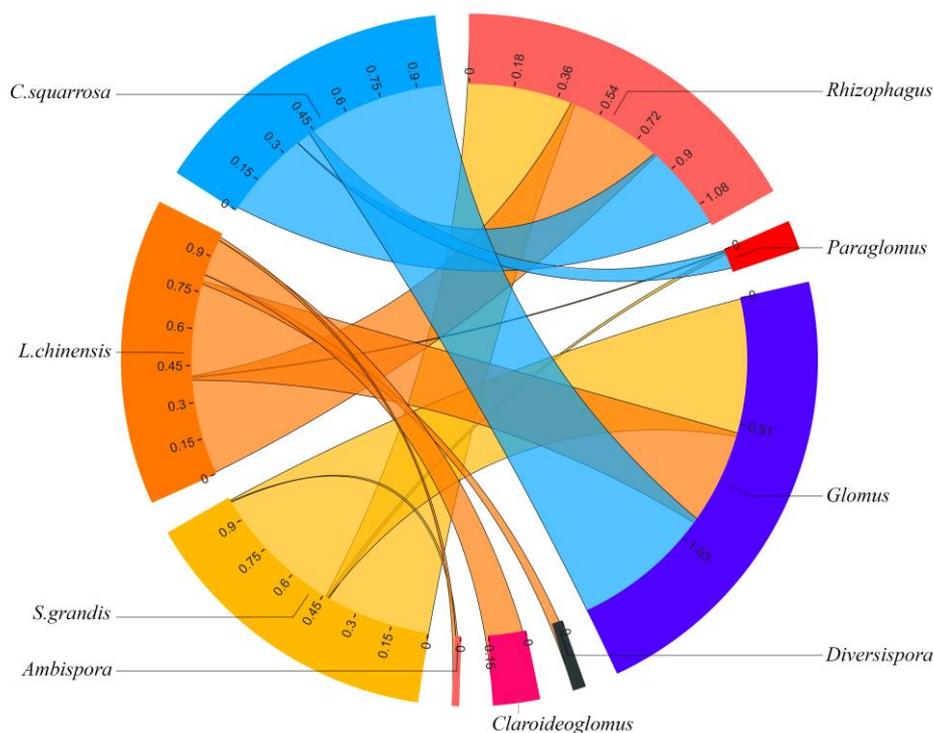


Figure 4. Chord diagram showing the relative abundance of AMF. The relative abundance of AMF genera detected in the roots of the three plant species

Likewise, the detected OTUs (identified genus) were not evenly distributed among the three plant species. Among the 587 sequence types classified as AM fungi, 167 were from *S. grandis*, 220 were from *C. squarrosa*, and 200 were from *L. chinensis*; however, regarding the OTUs identified data (Table 1), *S. grandis* appeared to possess the most AMF phylotypes, i.e., 32, whereas, *C. squarrosa* and *L. chinensis* possessed 20 and 25 AMF phylotypes, respectively.

Concerning species richness, *S. grandis* also showed the higher richness of the AMF OTUs, followed by *C. squarrosa* and *L. chinensis* (Fig. 5). Analysis of variance showed that there were statistically significant differences found in species richness ($p < 0.05$). Therefore, we concluded that *S. grandis* had significantly more diversified AM fungal phylotypes than *L. Chinensis* and *C. squarrosa*. Notably, some OTUs were observed for limited hosts. *Rhizophagus* 06, 07, 08, and 18, *Glomus* 02, 03, 06, 08, 17, 21, and 22, *Paraglomus* 01 and 03 were found in *S. grandis*, *Rhizophagus* 03, 11, 15, and 17, *Glomus* 12, 16, 18, 20, and *Paraglomus* 04 were observed in *C. squarrosa*, and in *L. chinensis*, *Rhizophagus* 01, 02, 09, *Glomus* 07, 24, *Diversispora* 01, *Claroideoglomus* 01, *Paraglomus* 02, and *Ambispora* 02 were found (Table 1).

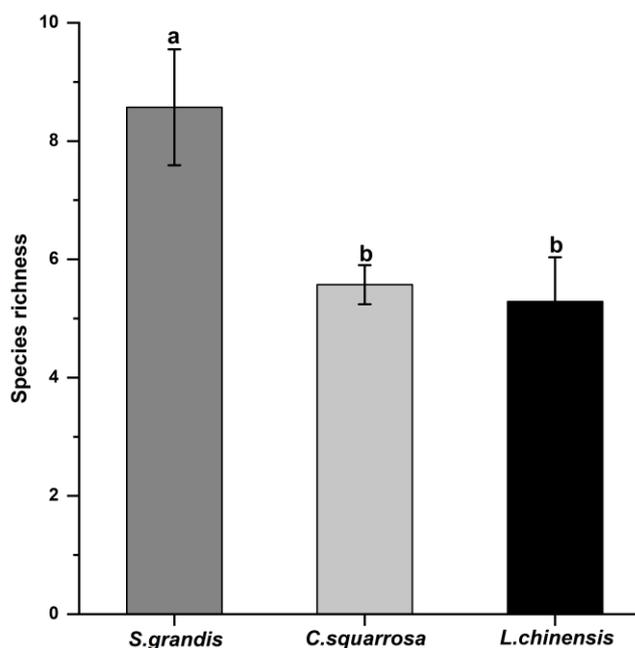


Figure 5. AMF richness. AMF richness in three different plant species. The analysis of variance revealed that *S. grandis* deviated significantly from the other two plant species and showed more species richness. Data are means \pm SE. Identical letters denote non-significant differences, whereas different letters indicate significant differences, as shown by Tukey's HSD test at $P < 0.05$

Furthermore, the Shannon-Wiener index used to indicate species diversity in a community. As our results showed, *S. grandis*, *C. squarrosa* and *L. chinensis* exhibited an average value of Shannon diversity index 1.62, 1.30, and 1.08 respectively, and non-significant differences were found ($p = 0.17$) (Fig. 6).

The number of OTUs specific to their host plant was 13 for *S. grandis* and 9 for both *C. squarrosa* and *L. chinensis* (Fig. 7). The OTUs data set thus demonstrated the

uniqueness of AMF-plant combinations of dominant plant species found in Xilinguole steppe regions.

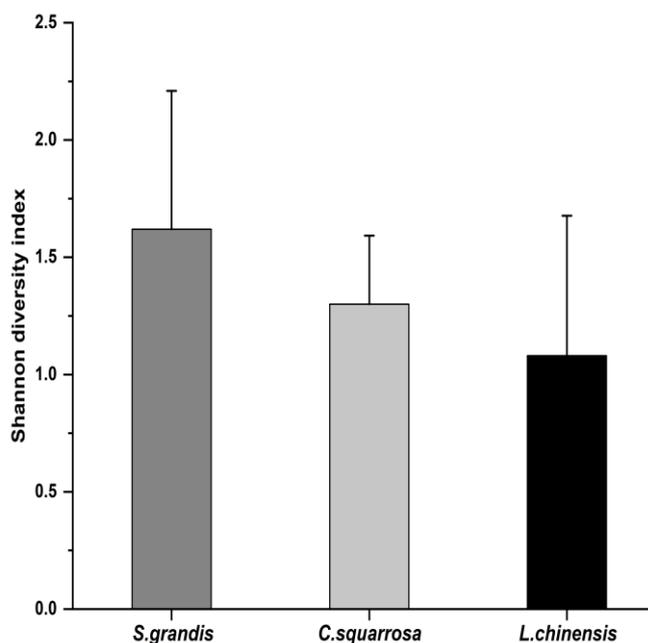


Figure 6. Shannon-Wiener index. The Shannon's diversity index (mean ± SE), and the analysis of variance revealed a non-significant difference in *S. grandis*, *C. squarrosa*, and *L. chinensis*

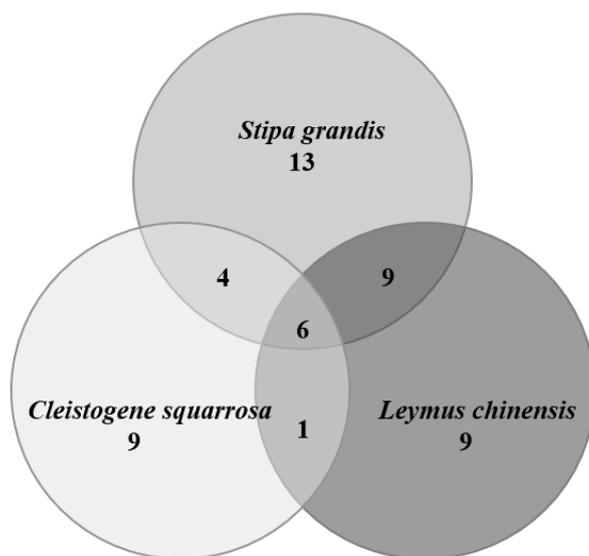


Figure 7. Venn diagram showing the number of shared and unique OTUs among the host plants

Moreover, the NMDS analysis indicated that the AMF community components living with one plant species did not tightly group according to their host plant species (Fig. 8). It implies that at both the genus and OTU levels, AMF showed a host preference character to a certain extent; however, the host plant species may not be solely the determinant for shaping the overall AM fungal community within roots.

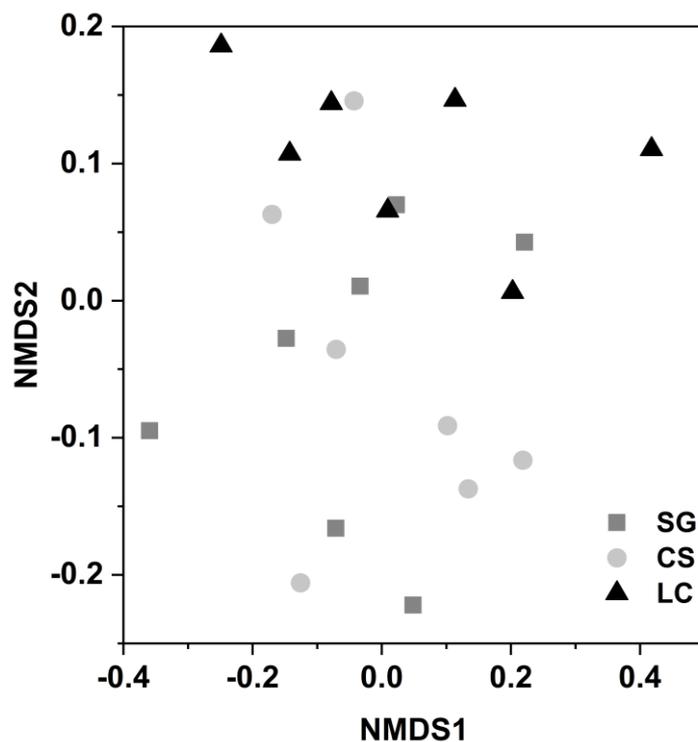


Figure 8. NMDS analysis. Non-metric multidimensional scaling (NMDS) of the community composition of arbuscular mycorrhizal fungi in the three plant species from seven different sites (stress = 0.189). Gray square boxes correspond to SG (*S. grandis*), light gray circles to CS (*C. squarrosa*), and black triangles to LC (*L. chinensis*)

Discussion

AMF colonization

In this study, AM fungal colonization in different plant species was high (85%) at the beginning of the growing season, which is in line with the distinct colonization rates studied previously among different co-occurring plants (Li et al., 2010; Lugo et al., 2003; Su et al., 2011). However, the rate of colonization was non-significant among the three species, which is consistent with Su et al. (2011). Studies on AM fungal colonization in Inner Mongolia, tallgrass prairie grasses and six locations on the European coast showed similar results in early growth seasons, and AMF colonization was the highest in this period (Bentivenga and Hetrick, 1992; Rodríguez-Echeverría et al., 2008; Su et al., 2011). This is because during the early growing season, the plants grow fast with high metabolic activity and high level of nutrient exchange between host plants and fungi, leading to a higher percentage of AM colonization (Baslam et al., 2011; Bentivenga and Hetrick, 1992; Muthukumar and Udaiyan, 2002; Wang et al., 2015; Su et al., 2011). The structures of AMF, including vesicles and arbuscules are essential sites for nutrient storage and their exchange between the host plants and fungi (Müller et al., 2017; Berruti et al., 2016). Moreover, genus *Glomus* was found to be the most abundant, suggesting that the AMF species belonging to this genus can produce more hyphal segments and spores that can colonize onto plant roots extensively (Zhao et al., 2017). Consistent with this, all three plant species, in our study, were dominated by *Glomus* showing the highest percentage of AMF colonization.

AM fungal diversity and community composition

We found that the number of sequence types/OTUs detected were 51, inferring the rich AMF diversity in this typical steppe. The species richness of AMF in the roots of *S. grandis*, *C. squarrosa*, and *L. chinensis* was 32, 20, and 25, respectively. Moreover, the diversity indexes also indicated the high diversity in all three plant species, especially in *S. grandis*. These findings are supported by the previous studies showing that the plant species in the field conditions held higher AMF diversity in their roots (Öpik et al., 2006; Torrecillas et al., 2012), and perennial plant species of semiarid region hosted the higher AM fungal diversity in their roots (Alguacil et al., 2012). Moreover, the AM fungal community in the current research exhibited that 50% of the total OTUs were contributed from the genus *Glomus*, suggesting it as the most abundant and wide spread genus among the three plant species. These results supported the previous reports that widely distributed AMF had higher abundance in the local environment but with low host specificity (Husband et al., 2002; Öpik et al., 2006; Wirsal, 2004). Moreover, it is also documented that AMF diversity increases with the increase in plant diversity (Alguacil et al., 2011; Torrecillas et al., 2012) because different plant species dominate the typical steppe of Inner Mongolia during the growing season (Su et al., 2011). This explanation is in agreement with our results that *S. grandis* showed a greater extent of AM fungal diversity when compared to *L. chinensis* and *C. squarrosa*, as *S. grandis* also held the highest AMF colonization, which may be attributed to the colonization of more diversified AMF taxa. Alguacil et al. (2011) also concluded that the non-metric multidimensional scaling plot revealed that AM fungal communities of all three plants overlapped, suggesting low host specificity of AMF for these species. This might be because all the species belong to the same family *poaceae*. This is also in line with previous finding stating the absence of host specificity in *poaceae* (Torrecillas et al., 2012). Another explanation could be that the composition of host plant does not effectively influence AM fungal communities. There are also some environmental factors that have substantial effects on the community composition of AMF such as soil properties including structure (Lekberg et al., 2007), fertility (Egerton-Warburton et al., 2007; del Mar Alguacil et al., 2010), moisture content (Wolfe et al., 2007), disturbance (Rodríguez-Echeverría and Freitas, 2006), and climatic factors including temperature and precipitation (Treseder, 2013). Widespread study in Tibetan alpine steppe stated that different plants at the same site had no significant difference in AMF community composition in their rhizosphere soil (Zhang et al., 2016a). In most cases, the AMF community associated with the respective host is not so unique, and there is the probability that a subset of that AMF community is also connected with many other host plants, and it has been reported that a limited number of fungal species associated with globally 90% of plant families, resulting in lower host specificity of AMF (Natasha Teutsch and Hawkes, 2010). In contrast, there are also mounting evidences indicating that co-occurring plant species held apparently distinct AM fungal communities within their roots, including plants in tropical forests (Husband et al., 2002), permanent grasslands (Vandenkoornhuyse et al., 2003), oak-woodlands (Douhan et al., 2005), semiarid coastal dunes (Martínez-García and Pugnaire, 2011), high mountainous meadows (Sýkorová et al., 2007), and farmland (Paul et al., 2013).

Despite the overall community composition similarity, in terms of genus and OTUs, some AMF were still found to be specific to their host plants in our study (*Table 1*). Likely, Lugo and Cabello (2012) found that some AMF species were limited to *B. subarista* and *P. stuckertii*, whereas the *Glomus* species was only associated with *P.*

stuckertii. Thus, the effects of host plant on AM fungal community composition are still controversial and needs further research to disentangle the reality.

Conclusion

In summary, the overall higher rate of mycorrhizal colonization in the roots of three dominant species of Xilinguole typical grassland reflects the strong association and mutual relationship of plant-AMF. Moreover, there were significant differences in species richness of AMF among the three perennial plant species. However, there were no significant differences in community composition of AMF; some genera exposed a little host preference, wherein *Glomus* was the most dominant in reflecting the actual community composition. These findings can be of vital importance in studying the success of plant species in their ecosystems based on the integrity of their symbiotic relationships with AMF.

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