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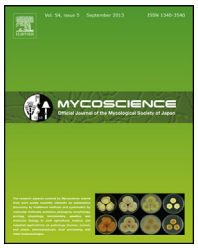

Full paper

# Effect of plant species on communities of arbuscular mycorrhizal fungi in the Mongolian steppe

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ABSTRACT

Communities of arbuscular mycorrhizal (AM) fungi were investigated in *Stipa krylovii*, *Leymus chinensis* (Poaceae), *Allium bidentatum* (Liliaceae), and *Astragalus brevifolius* (Fabaceae) in the Mongolian steppe to examine the effect of plant species on the communities in this study. The AM fungal communities were examined by molecular analysis based on the partial sequences of a small subunit of the ribosomal RNA gene. The sequences obtained were divided into 23 phylotypes by the sequence similarity >98%. Many of the AM fungal phylotypes included AM fungi previously detected in high-altitude regions in the Tibet and Loes plateaus, which suggested that these AM fungi may have wide distribution with stressful conditions of aridity and coldness. Among the 23 phylotypes, 12 phylotypes were found in all four plants, and 87.4% of the all obtained sequences were affiliated into these 12 types. For the distribution of the AM fungal phylotypes, overlapping of the phylotypes among the four plant species were significantly higher than that simulated by random chance. These results suggested that AM fungal communities were less diversified among the examined plant species.



## 1. Introduction

The arbuscular mycorrhiza (AM) is a ubiquitous symbiosis between terrestrial plants and fungi in the phylum Glomeromycota (Smith and Read 2008). In this symbiosis, AM fungi provide the host plant with soil nutrients, particularly phosphate (Jayachandran and Shetty 2003). Furthermore, alleviations of plant disease and abiotic stresses on host plants are known effects of AM symbiosis (Rabie 1998; Ruiz-Lozano et al. 2001). AM fungi usually have broad host ranges, although some preferences or functional diversifications between plants and AM fungi have been suggested (Helgason et al. 2002; Vandenkoornhuyse et al. 2003). Differential responses of AM fungi depending on the host plant could affect plant community composition (van der Hejiden et al. 1998; van der Hejiden et al. 2006). Therefore, AM fungal community can be a determinant of plant community. Meanwhile, many studies showed that AM fungal communities are diversified among different plant species (Sanders and Fitter 1992; Bever et al. 1996; Vandenkoornhuyse et al. 2002, 2003). Thus, it is also considered that plant community also could be a determinant of the AM fungal community.

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The Mongolian steppe, including mountain forest steppe and desert steppe, is a part of the Eurasian steppe that extends from Hungary in the west to northeastern China in the east. It covers approximately 70% of Mongolia with an annual precipitation of 100–300 mm (Jigjidsuren and Johnson 2003). The steppe zone is mainly used as a rangeland for livestock. After privatization of livestock farming due to the democratization of Mongolia in 1990, overgrazing by livestock became a problem in steppe vegetation. Consequently, changes of floristic composition caused by overgrazing have been reported (Li 1989; Nakamura et al. 1998, 2000).

The most plant families in the steppe vegetation, such as Poaceae, Asteraceae, Fabaceae, and Liliaceae, form the arbuscular mycorrhiza (Wang and Qiu 2006). The effect of grazing on AM fungal colonization and communities has been examined in some steppe vegetations (Tian et al. 2009; Ba et al. 2012). These studies examined AM fungal communities based on the number of spores collected from soil samples. The spore numbers can be used as quantitative values, but they do not usually reflect the AM fungal colonization because of differences in sporulation depending on the AM fungal species.

In the Mongolian steppe, the AM fungal community in *Stipa krylovii* Roshev. (Poaceae) was examined by molecular analysis (Goomaral et al. 2013). This plant is one of the dominant plant species in steppe vegetation and is known as a preferred feed for livestock (Wallis de Vries et al. 1996). However, the AM fungal diversity in the environment may have been underestimated because of the single plant species examined. We hypothesized in this study that AM fungal communities may be diversified among the different plant species in the Mongolian steppe and that increasing the number of plant species examined may detect more AM fungal taxa in the environment. Understanding the AM fungal community in the environment could provide further insight into the restoration of degraded vegetation due to overgrazing. Accordingly, AM fungal communities in *Leymus chinensis* (Trin.) Tzvel. (Poaceae), *Allium bidentatum* Fisch. ex Prokh. (Liliaceae), *Astragalus brevifolius* Ledeb. (Fabaceae), and *S. krylovii* (Poaceae) in the Mongolian steppe were examined in this study.

## 2. Materials and methods

### 2.1. Sampling

Sampling was conducted at Hustai National Park in the steppe zone of Mongolia at the end of July 2011. The mean temperatures in January and July, and the mean annual precipitation were −20.5 °C, 19.0 °C, and 222.5 mm, respectively (averaged for the period of 1996–2010). The steppe vegetation is covered with herbaceous plants with some shrubs. We selected four dominant plant species in the area, *S. krylovii*, *L. chinensis*, *Al. bidentatum*, and *As. brevifolius*, to investigate the AM fungal communities. Eight sampling plots of 1 × 1 $m^2$ containing all four plant species were established in the area (N47°29′20″, E105°46′17″ – N47°42′09″, E106°02′01″; Fig. 1, Table 1). In each plot, soil core samples (5 cm in diameter and 10 cm in depth) containing plant roots were collected under the shoot of each plant species examined. One soil core sample was collected for one plant species in each sampling plot. Accordingly, 32 soil core samples were collected in total.

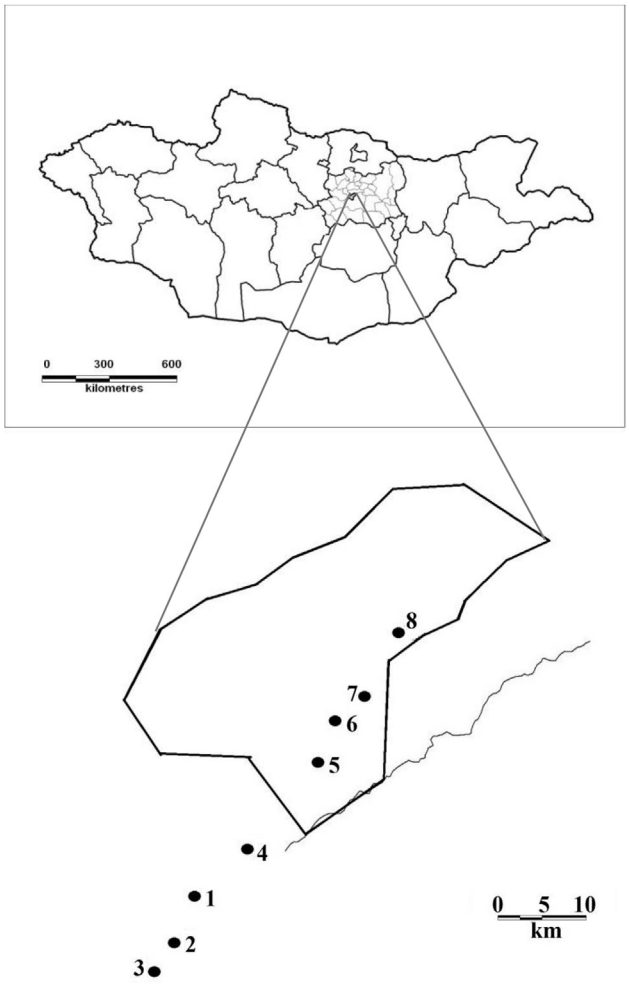


**Fig. 1 – Location of the sampling plots in Hustai National Park, Mongolia.**

### 2.2. Soil chemical analysis

The four soil cores in each sampling plot, one each for the four plant species, were combined and mixed to make one soil sample. Soil pH (soil:water, 1:2.5 v/v) and available phosphorus (Truog-P; Truog 1930) were measured for the samples.

**Table 1 – Locations of sampling plots.**

| Plot | Latitude | Longitude | Altitude (m) |
|---|---|---|---|
| 1 | N 47°29′20.3″ | E 105°46′17.7″ | 1205 |
| 2 | N 47°26′59.5″ | E 105°44′41.7″ | 1142 |
| 3 | N 47°25′56.2″ | E 105°43′40.9″ | 1137 |
| 4 | N 47°32′09.4″ | E 105°50′20.6″ | 1135 |
| 5 | N 47°36′08.1″ | E 105°56′00.9″ | 1151 |
| 6 | N 47°38′06.2″ | E 105°57′16.6″ | 1209 |
| 7 | N 47°39′05.5″ | E 105°58′47.9″ | 1205 |
| 8 | N 47°42′09.0″ | E 106°02′01.5″ | 1239 |

Total carbon and nitrogen was also analyzed using an Elementarvario EL CHNS analyzer (Elementar, Hanau, Germany).

### 2.3. Molecular analysis

The roots connected to the shoot of the collected plants were washed with tap water to remove the attached soil debris. Fine roots (ca. 200 mg fresh weight) were randomly selected from each of the washed root sample, and DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. The partial fungal small subunit of ribosomal RNA gene (SSU rDNA) was amplified by polymerase chain reaction (PCR) from the extracted DNA using the AM fungal-specific primers AML1 and AML2 (Lee et al. 2008) by Takara Ex Taq Hot Start Version (Takara Bio, Otsu, Japan). The reaction mixture contained 1.0 μl of the extracted template DNA diluted at 1:10, 0.15 units of Taq polymerase, 0.15 μM of each primer, 2.4 μM of each dNTP, and 3.0 μl of the supplied PCR buffer in a total volume of 30 μl. The PCR program performed on a PC-818S Program Temp Control System (Astec, Fukuoka, Japan) were as follows: an initial denaturation step at 94 °C for 2 min followed by 35 cycles at 94 °C for 30 s, 50 °C for 30 s, 72 °C for 1 min and a final elongation step at 72 °C for 5 min.

The PCR products were cloned into pGEM-T Easy Vectors (Promega, Madison, WI, USA). For each PCR product, at least 20 clones were randomly chosen, and plasmid DNA was extracted using MagExtractor Plasmids (Toyobo, Tokyo, Japan). The DNA inserts were sequenced on a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator v3.1 Cycle Sequencing Kit using T7 and SP6 promoter primers as sequencing primers.

For all of the obtained sequences, multiple sequence alignment was performed using ClustalX 2.0.12 (Larkin et al. 2007). Neighbor-joining analysis (Saitou & Nei 1987) was performed for the aligned data set using ClustalX with bootstrap analysis of 1000 replications (Felsenstein 1985). The AM fungal phylotypes were defined on the basis of sequence similarities, and tree topology. The rarefaction curve was computed for each sample by plotting the number of AM fungal phylotypes detected against the number of sequences using the Analytic Rarefaction 1.3 software (Hooland 2003). For each sample, additional clones were sequenced until the rarefaction curves tended to plateau.

Some representative DNA sequences selected from each AM fungal phylotype to maintain the tree topology were deposited into the DNA Data Bank of Japan database with the accession numbers AB749460 to AB749535. The selected sequences were subjected to BLAST searches (Altschul et al. 1997), and similar sequence data were downloaded from the GenBank database. For the set of sequenced and downloaded data, multiple sequence alignments were performed as described above. For phylogenetic analysis, the maximum likelihood (ML) method was applied using PhyML 3.0 (Guindon et al. 2010). The best-fit ML tree was inferred under the GTR model. To examine statistical support for the obtained tree topology, bootstrap analysis was performed with 1000 replications. The tree obtained was drawn using Treeview software (Page 1996).

### 2.4. Statistical analysis

We applied EcoSim (Entsminger 2012), a null modeling software for community ecology, to examine the overlap of AM fungal phylotypes among the four plant species examined, *S. krylovii*, *L. chinensis*, *Al. bidentatum*, and *As. brevifolius*. After calculation of the detection ratio of each AM fungal phylotypes (number of the samples with the relevant AM fungal phylotype/number of the samples examined) in each examined plant species, we applied the niche overlap module of EcoSim for the question, "Do the four plants differ in the AM fungal phylotypes colonized?" The patterns of AM fungal overlap among the four host plants was compared with statistical randomization of the original data with 1000 iterations to examine whether the observed level of the overlap is different from that expected due to random chance.

## 3. Results

### 3.1. Soil chemical properties

The soil pH ranged from 7.6 to 7.9 (average, 7.8; Table 2). The available soil phosphorus ranged from 38.7 to 80.9 mg $kg^{-1}$ (average, 56.3 mg $kg^{-1}$; Table 2). The average total soil carbon and nitrogen were 17.3 and 2.17 g $kg^{-1}$, respectively (Table 2).

### 3.2. Molecular analysis

After cloning the PCR products, 18–56 clones were sequenced for each sample until the rarefaction curves tended to plateau. Accordingly, a total of 904 partial sequences of SSU rDNA of AM fungi were obtained from 30 samples (eight samples each of *S. krylovii* and *Al. bidentatum* and seven samples each of *L. chinensis* and *As. brevifolius*). The phylotypes were determined to have the sequence similarity >98% with at least two sequences. Consequently, 899 sequences were divided into 23 phylotypes (Fig. 2, Table S1). Among the 23 phylotypes, 12 phylotypes were found in all four plants, and 87.4% (790/904) of the AMF sequences were affiliated to these 12 phylotypes. Among them, two phylotypes (Glo17 and Glo22) were in *Glomus* group B, one (Div1) was in *Diversispora*, and the others were in *Glomus* group A. The number of phylotypes detected was different depending on the plant species examined, i.e.,

**Table 2 – Soil chemical properties of the examined plots in Hustai National Park in Mongolia.**

| Plots | pH | Available P (mg $kg^{-1}$) | Total C (g $kg^{-1}$) | Total N (g $kg^{-1}$) |
|---|---|---|---|---|
| 1 | 7.9 | 61.1 | 20.0 | 2.27 |
| 2 | 7.9 | 46.5 | 20.3 | 2.50 |
| 3 | 7.8 | 80.9 | 15.4 | 2.03 |
| 4 | 7.8 | 55.3 | 19.1 | 2.33 |
| 5 | 7.6 | 44.5 | 15.5 | 2.00 |
| 6 | 7.8 | 38.7 | 12.3 | 1.53 |
| 7 | 7.7 | 52.4 | 16.7 | 2.27 |
| 8 | 7.6 | 70.7 | 18.7 | 2.43 |
| Average | 7.8 | 56.3 | 17.3 | 2.17 |

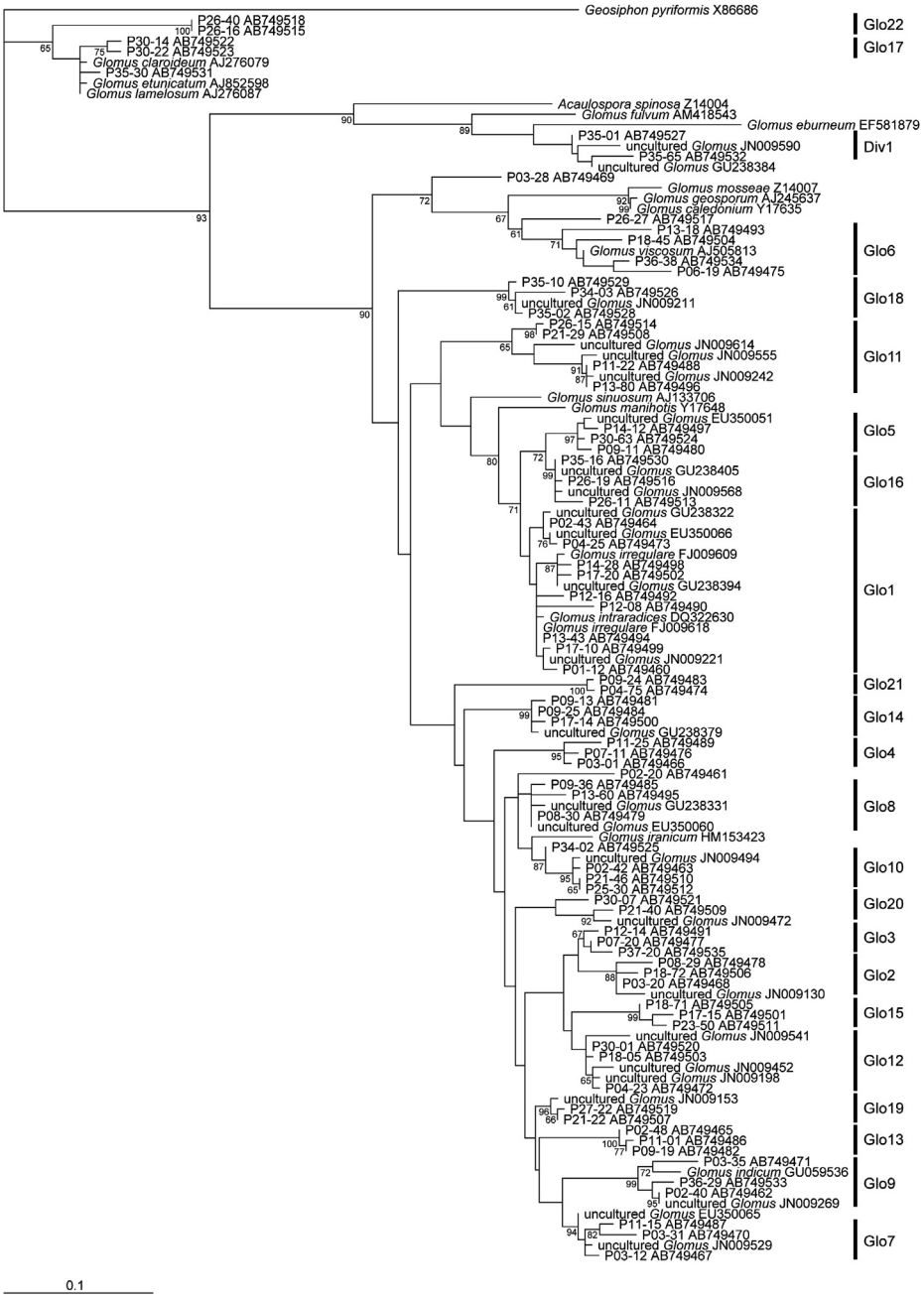


**Fig. 2 – Maximum likelihood phylogenetic tree based on partial sequences of the small subunit of nuclear ribosomal RNA gene of arbuscular mycorrhizal fungi obtained from *Stipa krylovii*, *Leymus chinensis* (Poaceae), *Allium bidentatum* (Liliaceae), and *Astragalus brevifolius* (Fabaceae) in steppe vegetation in Mongolia and the GenBank database. For the sequences obtained in this study, representative sequences of the phylotypes (Glo1–22 and Div1) and four independent sequences were analyzed. The tree is rooted to *Geosiphon pyriformis* (X86686) in Glomeromycota. The sequence numbers are related to the sample and clone numbers. Bootstrap values exceeding 60% (1000 replications) are shown. The scale is shown so that evolutionary distances can be inferred. Accession numbers are given for all sequences.**

15 types in *S. krylovii* and *Al. bidentatum*, 18 in *As. brevifolius*, and 20 in *L. chinensis*. In each phylotype, some representative sequences were selected to maintain the tree topology, and ML analysis was performed with similar sequences downloaded from the GenBank database. ML analysis resulted in one ML tree (ln $L = -4522.96777$; Fig. 2). The five independent sequences (P02-20, P03-28, P26-27, P34-02, and P35-30) that did not have similar sequences were also present in the phylogenetic tree. Some phylotypes included identified AM fungal sequences. The most frequent AM fungal phylotype was Glo1, which included *G. intraradices* Schenck and Smith and *G. irregulare* Błaszkowski, Wubet, Renker & Buscot. In fact, 30.1% (272/904) of the detected AM fungi belonged to Glo1 (Table S1). The dominance of this AM fungal group in the Mongolian steppe was also observed in *S. krylovii* in Uvurkhangai, Mongolia (Goomaral et al. 2013). Only two other

phylotypes included the identified AM fungal sequences. Glo6 included *G. viscosum* Nicolson, and Glo9 included *G. indicum* Błaszkowski, Wubet & Buscot. Interestingly, many of the phylotypes (Glo1, Glo2, Glo7, Glo8, Glo9, Glo10, Glo11, Glo12, Glo14, Glo16, Glo18, Glo19, Glo20, and Glo23) included AM fungi previously detected in high-altitude regions in the Tibet and Loes plateaus (2400–4500 m in altitude) (Liu et al. 2009, 2011, 2012).

### 3.3. *Statistical analysis*

The observed overlap of AM fungal phylotypes among the examined plants were relatively high in all pairs (Table 3), highest between *Al. bidentatum* and *As. brevifolius* (0.937) and lowest between *S. krylovii* and *L. chinensis* (0.878). The observed niche overlap index 0.905 was significantly higher than the means of simulated indices 0.521 due to 1000 simulations ($P < 0.0001$).

## 4. Discussion

The AM fungal communities in roots of four herbaceous plant species, *S. krylovii*, *L. chinensis*, *Al. bidentatum*, and *As. brevifolius*, were examined in Mongolian steppe in this study. In total, 23 AM fungal phylotypes were detected, that is 20 types in *Glomus* group A, two types in *Glomus* group B, and one type in *Diversispora*. For the molecular detection of AM fungi, various primers have been developed in some regions of rDNA. Among them, majority of natural AM fungal data have been obtained by NS31-AM1 for SSU rDNA, and many data is available in Genbank database (Öpik et al. 2010). The primer set AML1-AML2 used in this study is the revised one for NS31-AM1, which could have higher adaptation for diverse AM fungi (Lee et al. 2008). Therefore, the dominance of *Glomus* group A fungi is considered to be a reflection of the actual AMF communities, however it is still probable that further AM fungi may be detected by using other primer sets, or by analysis on spore communities.

Among the 23 phylotypes, 12 phylotypes were found in all four plants, and 87.4% (790/904) of the all obtained AMF sequences were affiliated to these 12 phylotypes. Meanwhile, only three phylotypes were detected in one plant species. This result suggested that dominant AM fungi were shared by the four host plants. Furthermore, the significantly higher overlap of AM fungal phylotypes among the examined plant species was shown, which also suggested that AM fungal communities were less diversified among them. Because of the low reliability of the quantitativeness of PCR products, we did not use the actual number of obtained AM fungal sequences as quantitative values for the analysis. Instead, we used binary data ("1" or "0") to indicate the presence or absence of each phylotype in each sample. Because the qualitative values could underestimate the differences of AM fungal communities, it is probable that preference on some AM fungi may be different among the examined plants in the environment.

Mongolian steppe is a vast land, while small soil cores from the very limited area was only examined in this study. Thus, more extensive and intensive study would reveal more diverse AM fungal communities even in the same plant species. Meanwhile, many of the AM fungal phylotypes determined in this study included AM fungi previously detected in high-altitude regions in the Tibet and Loes plateaus, which suggested that these AM fungi may have wide distribution with stressful conditions of aridity and coldness.

*Stipa krylovii*, the most preferred plant species by livestock, is selectively decreased by grazing in usual, and restoration to increase this plant is required in some overgrazed areas. The common AM fungi among the different plant species suggested that AM fungal colonization of *S. krylovii* from other surrounding plant species could be expected in such vegetation restoration.

**Table 3 – Observed pairwise niche overlaps of arbuscular mycorrhizal fungal phylotypes among the four plant species, *Stipa krylovii*, *Leymus chinensis*, *Allium bidentatum*, and *Astragalus brevifolius*.**

| | *L. chinensis* | *Al. bidentatum* | *As. brevifolius* |
|---|---|---|---|
| *S. krylovii* | 0.878 | 0.897 | 0.930 |
| *L. chinensis* | | 0.896 | 0.894 |
| *Al. bidentatum* | | | 0.937 |

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.myc.2012.12.005.

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