Available online at www.sciencedirect.com

MYCOSCIENCE

ISSN 1340-3540 (print), 1618-2545 (online)

journal homepage: www.elsevier.com/locate/myc

Full paper

Communities of arbuscular mycorrhizal fungi in *Stipa krylovii* (Poaceae) in the Mongolian steppe

Altansukh Goomaral [a], *Koji Iwase* [b], *Jamsran Undarmaa* [c], *Teruyuki Matsumoto* [d], *Masahide Yamato* [d,*]

[a] *The United Graduate School of Agriculture Sciences, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan*
[b] *Department of Natural and Environmental Science, Teikyo University of Science, 2525 Yatsusawa, Uenohara 409-0193, Japan*
[c] *Center for Ecosystem Study, Mongolian State University of Agriculture, P. O. Box-61, Ulaanbaatar 53, Mongolia*
[d] *Fungus/Mushroom Resource and Research Center, Faculty of Agriculture, Tottori University, 4-101 Koyama-Minami, Tottori 680-8553, Japan*

ARTICLE INFO

Article history:
Received 24 February 2012
Received in revised form
23 May 2012
Accepted 5 July 2012
Available online 20 December 2012

Keywords:
Glomus intraradices
Glomus irregulare
Grazing
Principal component analysis (PCA)
Redundancy analysis (RDA)

ABSTRACT

We examined arbuscular mycorrhizal (AM) fungi colonizing the roots of *Stipa krylovii*, a grass species dominating the grasslands of the steppe zone in Hustai and Uvurkhangai in Mongolia. The AM fungal communities of the collected *S. krylovii* roots were examined by molecular analysis based on the partial sequences of a small subunit of ribosomal RNA gene as well as AM fungal colonization rates. Almost all AM fungi detected were in *Glomus*-group A, and were divided into 10 phylotypes. Among them, one phylotype forming a clade with *G. intraradices* and *G. irregulare* was the most dominant. Furthermore, it was also found that most of the phylotypes include AM fungi previously detected in high altitude regions in the Eurasian Continent. Significant correlations were found among soil total N, total plant biomass and AM fungal colonization ratio, which suggested that higher plant biomass may be required for the proliferation of AM fungi in the environment. Meanwhile, redundancy analysis on AM fungal distribution and environmental variables suggested that the effect of plant biomass and most soil chemical properties on the AM fungal communities were not significant.

1. Introduction

In Mongolia, the steppe zone, including mountain forest steppe and desert steppe, covers around 70% of the total land area with annual precipitation of 100–300 mm (Ulziikhutag 1989). The vast area is mainly used as rangeland, where degradation caused by overgrazing has become a serious problem. The vegetation of these ecosystems mainly consists of various herbaceous plant species belonging to families such as the Poaceae, Asteraceae, Liliaceae, Fabaceae, Chenopodiaceae, etc. Most of these plant families are known to have symbioses with arbuscular mycorrhizal (AM) fungi (Wang and Qiu 2006).

AM is the mutualistic symbiotic association between terrestrial plants and fungi of the phylum Glomeromycota (Schüßler et al. 2001). It is well known that AM fungi are important to their host plants in promoting the acquisition of soil nutrients, especially phosphate (Koide 1991). In addition,

* *Corresponding author*. Tel./fax: +81 857 31 5388.
E-mail address: m_yamato@muses.tottori-u.ac.jp (M. Yamato).
1340-3540/$ – see front matter © 2012 The Mycological Society of Japan. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.myc.2012.09.006

it has been suggested that AM fungi could affect water relations of host plants (Augé 2001) and plant community structures (van der Heijden et al. 1998a, b; Börstler et al. 2006).

AM fungal communities in steppe vegetation have been examined in various regions. Tian et al. (2009a) examined AM fungi in the degraded steppe of Inner Mongolia in China. They examined AM fungal colonization of four indicator plant species, *Leymus chinensis* (Trin.) Tzvelev, *Agropyron michnoi* Roshev., *Artemisia frigida* Willd., and *Potentilla acaulis* L., in three grassland sites with different degradation levels: undegraded, moderately degraded, and severely degraded. In their examination, the degradation did not induce any decline in AM colonization; whereas, AM fungal spore numbers and species diversity were distinctly lower in the degraded environment. Jirout et al. (2009) suggested that severe grazing induces a reduction of AM fungal root colonization following their examination of pastures in South Bohemia, Czech Republic. Ba et al. (2012) examined the relationship between the diversity of AM fungi and grazing in a meadow steppe in Jilin Province, China, in which AM fungal diversity was lower under intense grazing pressures. In these studies, spores collected from soil samples were used to evaluate the communities or species diversity of AM fungi. Countable spores have often been used for quantitative evaluations of AM fungi, but they often do not reflect AM fungal colonization in roots because of the different sporulating abilities depending on AM fungal species (Morton et al. 1995).

With the development of AM fungi-specific primers for polymerase chain reactions (PCR) (Helgason et al. 1998; Lee et al. 2008), AM fungi in roots can be directly evaluated by molecular analysis. In order to examine the effect of environmental differences on the community of AM fungi, comparisons should be made within the same plant species because plant species can be determinants of the AM fungal community. Among the herbaceous plant species in Mongolian steppe, *Stipa* (Poaceae) is one of the dominant plant genera, and is also known to be the preferred feed for livestock (Jigjidsuren and Johnson 2003). Among *Stipa* species, *S. krylovii* Roshev. is the most dominant (Ulziikhutag 1989). In this study, we examined AM fungal communities in *S. krylovii* in two different regions, Uvurkhangai and Hustai, in order to examine the effect of environmental factors, including the degrees of grazing, on the community of AM fungi as well as to identify the AM fungi colonizing this plant species.

2. Materials and methods

2.1. Sampling

Sampling was conducted in two regions, Bayan-Undur subprovince in Uvurkhangai province and Hustai National Park in the steppe zone of Mongolia at the end of July 2010 (Fig. 1). The mean temperature in January and June, and mean annual precipitation at Bayan-Undur meteorological station in Uvurkhangai were −19.3 °C, 15.8 °C, and 149.6 mm, respectively (average 1996–2010). Those at Hustai National Park were −20.5 °C, 19.1 °C, and 222.5 mm (average 1999–2010). In both areas, sampling sites were selected in grasslands with various degrees of degradation due to grazing.

In each sampling, we established a sampling plot 10 × 10 m, in which five soil cores, 5 cm diameter and 10 cm depth, containing roots of *S. krylovii* were randomly collected, and mixed to make one sample. In the sampling plot, all plant shoots were collected from three sub-plots of 1 × 1 m and separated into poaceous plants and others. After drying at 80 °C for 24 h, the dry weight of aboveground plant biomass was measured. The ratio of Poaceae to the total aboveground plant biomass was calculated and used as an index of the degradation caused by grazing, because poaceous plants are preferentially grazed by livestock. Of a total of 24 samples collected, 16 were from Uvurkhangai and 8 from Hustai. Region, longitude, latitude, and altitude of the sampling plots are shown in Table 1.

2.2. Soil chemical properties

The soil pH (H_2O) at 1:2.5 of soil to water ratio and available phosphorus (Truog-P) of the soil samples were measured. Total carbon and total nitrogen was also analyzed using an Elementarvario EL CHNS analyzer (Elementar, Hanau, Germany).

2.3. Root colonization by AM fungi

The fine roots of *S. krylovii* isolated from the soil samples were cut into fragments of approximately 1 cm in length, and subsamples of approximately 20 mg fresh weight were used to determine the colonization rate of AM fungi. The roots were cleared with 10% KOH and stained with 0.05% trypan blue. The AM fungal colonization rate was analyzed by the gridline intersection method with at least 100 intersections (Brundrett et al. 1996).

2.4. Molecular analysis

For the molecular identification of AM fungi, approximately 200 mg of fresh fine root fragments were used. Total DNA was extracted from each root sample using a DNeasy Plant Mini Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions. Partial fungal SSU rDNA was amplified by polymerase chain reactions (PCR) from the extracted DNA using AM-fungal specific primers AML1 and AML2 (Lee et al. 2008) in Takara Ex Taq Hot Start Version (Takara Bio, Otsu, Japan). The reaction mixture for PCR contained 1.0 μl of the extracted DNA solution 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 30 μl of the total amount. The PCR program performed on a PC-818S Program Temperature Control System (Astec, Fukuoka, Japan) comprised an initial denaturation step at 94 °C for 2 min, following a step of 35 cycles at 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min with a final elongation step at 72 °C for 5 min.

The PCR products were cloned into pGEM-T Easy Vector Systems I (Promega, Madison, WI, USA). For each PCR product, at least 16 clones were randomly chosen, and plasmid DNA was extracted using MagExtractor Plasmid (Toyobo, Osaka, Japan). The DNA inserts were sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit using T7 and SP6 promoter primers as sequencing primers on a 3130 Genetic Analyzer (Applied Biosystems, Tokyo, Japan). The DNA

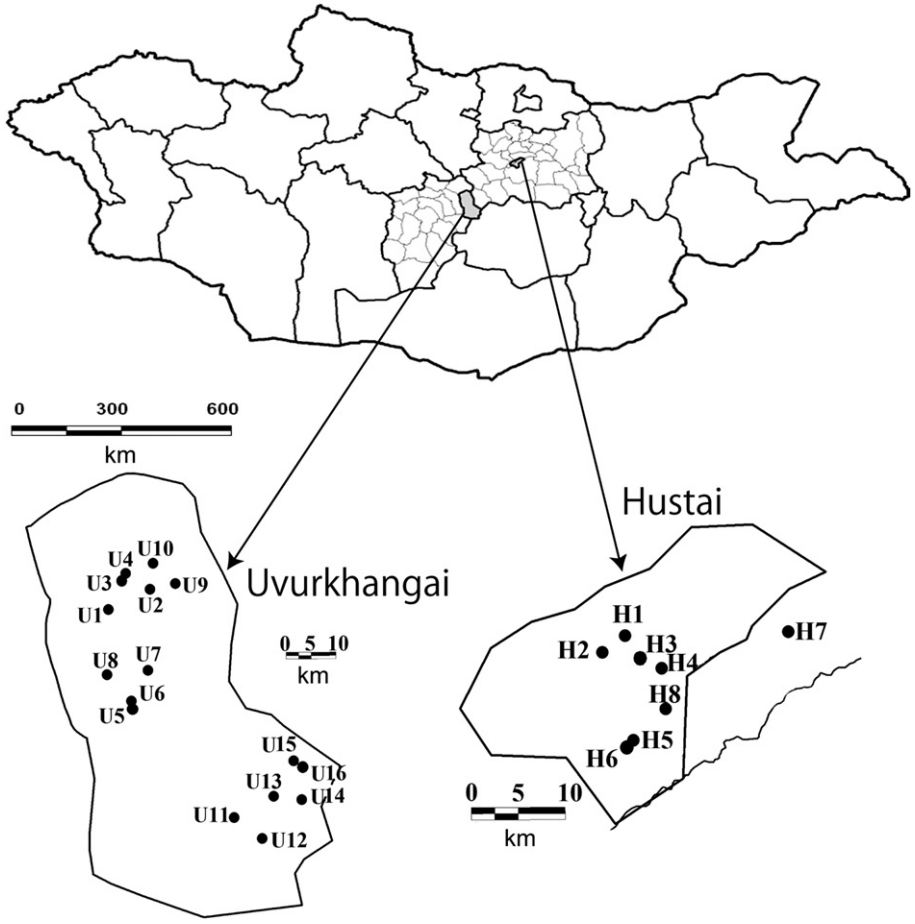

Fig. 1 – Location of the sampling sites in Uvurkhangai and Hustai in Mongolia.

sequences obtained were used in BLAST searches (Altschul et al. 1997), and those not identified as Glomeromycota fungi were excluded from further analyses.

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

Some representative DNA sequences were selected from each AM fungal phylotype to maintain tree topology. The selected sequences were deposited in the DNA Data Bank of Japan (DDBJ) database with accession numbers AB698561–AB698622. Partial sequences between the annealing sites of primers NS31 (Simon et al. 1992) and AM1 (Helgason et al. 1998) for the selected ones were subjected to BLAST searches, and similar sequence data were downloaded from the GenBank database. For the set of sequenced and downloaded data, multiple sequence alignment was carried out as described above. The maximum likelihood (ML) method was applied for the phylogenetic analysis using PhyML 3.0 (Guindon et al. 2010). The best-fit ML tree was inferred under the GTR model. To check the statistical support for the tree topology obtained, bootstrap analysis was performed with 1000 replicates. The tree obtained in the analysis was drawn using Treeview software (Page 1996).

2.5. *Statistical analysis*

The correlations among the soil chemical properties, plant biomass values, AM fungal colonization rates, and number of AM fungal phylotypes were examined using Pearson's coefficient tests.

For the soil chemical properties and plant biomass values, effect of the sampling region, Uvurkhangai and Hustai, were evaluated by analyses of the means with Student's *t*-test or Welch's *t*-test.

In order to infer the relationship between the AM fungal community and environmental factors, multivariate analyses were applied using CANOCO 4.5 (ter Braak and Smilauer 2002). For the data table of response variables, i.e. the distribution of AM fungal phylotypes, the presence or absence of each phylotype was scored as so-called dummy variables "1" or "0" in

Table 1 – Locations of the sampling plots.

Plot	Region	Longitude	Latitude	Altitude (m)
U1	Uvurkhangai	E 104° 06′ 16.5″	N 46° 42′ 23.3″	1730
U2	Uvurkhangai	E 104° 07′ 39.4″	N 46° 42′ 31.6″	1781
U3	Uvurkhangai	E 104° 06′ 09.0″	N 46° 43′ 03.9″	1702
U4	Uvurkhangai	E 104° 06′ 03.7″	N 46° 43′ 15.4″	1697
U5	Uvurkhangai	E 104° 06′ 05.1″	N 46° 31′ 43.3″	1640
U6	Uvurkhangai	E 104° 06′ 01.5″	N 46° 31′ 58.4″	1638
U7	Uvurkhangai	E 104° 07′ 27.4″	N 46° 34′ 10.2″	1632
U8	Uvurkhangai	E 104° 04′ 09.8″	N 46° 34′ 05.8″	1670
U9	Uvurkhangai	E 104° 09′ 54.6″	N 46° 43′ 06.2″	1765
U10	Uvurkhangai	E 104° 08′ 13.0″	N 46° 43′ 59.2″	1717
U11	Uvurkhangai	E 104° 29′ 06.3″	N 46° 16′ 52.2″	1546
U12	Uvurkhangai	E 104° 29′ 58.0″	N 46° 16′ 05.8″	1540
U13	Uvurkhangai	E 104° 35′ 40.6″	N 46° 20′ 56.3″	1516
U14	Uvurkhangai	E 104° 36′ 31.1″	N 46° 20′ 44.0″	1524
U15	Uvurkhangai	E 104° 36′ 18.3″	N 46° 22′ 51.3″	1525
U16	Uvurkhangai	E 104° 36′ 26.6″	N 46° 22′ 36.5″	1524
H1	Hustai	E 105° 54′ 48.6″	N 47° 43′ 13.7″	1395
H2	Hustai	E 105° 53′ 58.9″	N 47° 41′ 38.5″	1403
H3	Hustai	E 105° 54′ 56.2″	N 47° 41′ 45.1″	1356
H4	Hustai	E 105° 57′ 03.6″	N 47° 41′ 27.2″	1292
H5	Hustai	E 105° 57′ 04.9″	N 47° 37′ 17.3″	1192
H6	Hustai	E 105° 56′ 28.1″	N 47° 37′ 05.0″	1191
H7	Hustai	E 106° 10′ 26.2″	N 47° 43′ 49.6″	1202
H8	Hustai	E 105° 59′ 39.3″	N 47° 38′ 44.1″	1210

Table 2 – Plant shoot biomass in the examined plots.

Plot	Total biomass ($g\ m^{-2}$)	Poaceous biomass ($g\ m^{-2}$)	Ratio of poaceous biomass (%)
U1	63.9	26.4	41.3
U2	28.7	10.7	37.3
U3	78.7	2.5	3.2
U4	97.9	4.1	4.2
U5	27.4	14.9	54.4
U6	26.2	14.1	53.8
U7	34.2	9.9	28.9
U8	58.9	9.7	16.5
U9	42.6	1.4	3.3
U10	46.8	25.5	54.5
U11	26.5	8.2	30.9
U12	33.5	13.7	40.9
U13	57.8	0.9	1.6
U14	22.5	2.2	9.8
U15	29.9	11.9	39.8
U16	37.7	13.1	34.7
H1	103.2	81.0	78.5
H2	100.0	2.9	2.9
H3	97.9	71.8	73.3
H4	52.1	16.4	31.5
H5	27.2	1.3	4.8
H6	69.5	16.8	24.2
H7	71.3	35.9	50.4
H8	66.7	29.0	43.5

each sample. For the data on explanatory (environmental) variables, sampling regions, soil chemical properties, and plant biomass values were applied.

Detrended correspondence analysis (DCA) was applied for the response variables data to estimate the heterogeneity through the length of the community composition gradients in species turnover units. The DCA was performed with detrending by segments. After confirming the length of the composition gradients on the first DCA axis, principal component analysis (PCA) was performed to infer the relationship between AM fungal distribution and the environmental variables. PCA was performed with scaling on inter-species correlations with division by standard deviation and centering by species. A diagram of the results was drawn using CanoDraw.

To explain the effects of environmental variables, redundancy analysis (RDA) was applied with scaling on inter-species correlations with division by standard deviation and centering by species. Monte Carlo permutation tests with 999 unrestricted permutations were then performed to manually selected environmental variables.

3. Results

3.1. *Vegetation analysis*

The above ground biomass of total and poaceous plants is shown in Table 2. The ratios of poaceous biomass in Uvurkhangai varied from 1.6 to 54.5% with an average of 28.4%, and those in Hustai varied from 2.9 to 78.5% with an average of 38.6%. We used these values as indexes of grazing degrees. Total plant biomass was significantly higher in Hustai than in Uvurkhangai ($P < 0.01$).

3.2. *Soil chemical properties and AM fungal colonization in roots*

The soil chemical properties of each sample are shown in Table S1. The soil pH was ranged from 6.4 to 8.7 with average of 7.3. The available soil P ranged from 12.5 to 77.2 mg kg^{-1} with average of 28.1 mg kg^{-1}. The average total soil C from Hustai samples was 26.3 g kg^{-1}, which was significantly higher than that from Uvurkhangai samples, 11.5 g kg^{-1} ($P < 0.01$). The total soil C was significantly correlated to the total plant biomass ($R = 0.580$, $P < 0.005$) and poaceous biomass ($R = 0.585$, $P < 0.005$). The average total soil N from Hustai samples was 2.46 g kg^{-1}, which was significantly higher than that from Uvurkhangai samples, 1.40 g kg^{-1} ($P < 0.05$). The total soil N was significantly correlated to the total biomass (Fig. 2a; $R = 0.695$, $P < 0.0005$). Colonization rates of AM fungi in *S. krylovii* ranged from 29.1 to 80.7% with average of 53.1%. The AM fungal colonization rate was significantly correlated to the total soil N (Fig. 2b; $R = 0.521$, $P < 0.01$) and the total plant biomass (Fig. 2c; $R = 0.45$, $P < 0.05$). Meanwhile, no correlation was found between the AM fungal colonization ratio and the poaceous biomass or the poaceous biomass ratio.

3.3. *Arbuscular mycorrhizal fungal phylotypes*

In total, 627 AM fungal partial SSU rDNA sequences of approximately 800 bp. were obtained. Considering the phylogenetic division by the first NJ analysis of the obtained

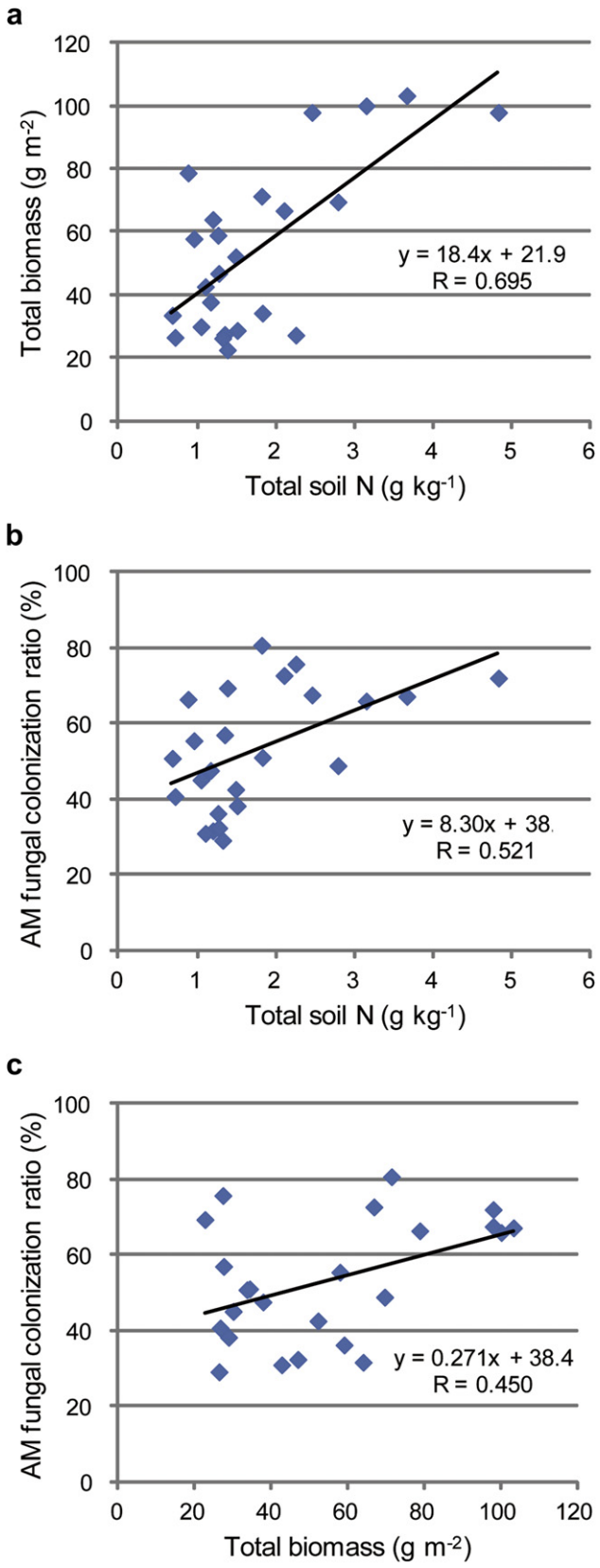

Fig. 2 – Correlations between total soil N and total plant biomass (a), between total soil N and colonization rate of AM fungi (b), and between total plant biomass and colonization rate of AM fungi (c).

sequences, 623 sequences were divided into 10 phylotypes so that each phylotype had a sequence similarity of more than 97%. In each phylotype, some representative sequences were selected to maintain the tree topology, and ML analysis with similar sequences in the GenBank database was performed. The resulting tree (lnL = −3517.12237; Fig. 3) showed that the almost all sequences obtained belonged to *Glomus*-group A. The 10 phylotypes were named Glo1–Glo10. The four independent sequences (U03-03, H03-19, H07-14, H08-01) without inclusion into any phylotypes were also shown in Fig. 3. Sequences of *G. intraradices* Schenck & Smith and *G. irregulare* Błaszk., Wubet, Renker & Buscot were included in phylotype Glo3. The distribution of AM fungal phylotypes in the sampling plots is shown in Table S2. Significant correlations were not obtained between total biomass or grass biomass ratio and the numbers of AM fungal phylotypes. Significant correlations were neither obtained between soil chemical properties (pH, available P, total C and total N) and the numbers of AM fungal phylotypes. It was also found that most of the phylotypes include AM fungi previously detected in high altitude regions in the Eurasian Continent (Fig. 3). The AM fungal sequences (GU238322, GU238331, GU238394, GU238395, GU238405) obtained from Tibet Plateau with above 4500 m of mean altitude (Liu et al. 2011) were included into Glo3, Glo8 and Glo9. The sequences (JN009130, JN009221, JN009273, JN009404, JN009415) obtained from Tibet Plateau with 3500 m of altitude (Liu et al. 2012) were also included into Glo2, Glo3, Glo9 and Glo10. Other sequences from Tibet Plateau (JN009494, JN009529, JN009568, JN009614) were also found to be included into Glo2, Glo3, Glo6 and Glo7. Two sequences (EU350060, EU350066) from Loess Plateau region of northwestern China with 2400 m of altitude (Liu et al. 2009) were included into Glo3 and Glo8. Furthermore, sequences from alpine plants in Salzburg, Austria (AM946787, AM946811, AM946841, AM946851, AM946867, AM946879, AM946928) were included into Glo2, Glo3, Glo4, Glo6, Glo7, and Glo10.

3.4. *Multivariate analysis*

Using DCA for data of AM fungal distribution, the length of the community composition gradients of the first axis was computed to be 2.59. From this result, we used PCA as the linear ordination method. In PCA, the eigenvalues of the first and second axes were 0.260 and 0.155, respectively. The cumulative percentage variance of species data showed that the first two PCA axes explain 41.5% of the variability in species data. The ordination diagram obtained (Fig. 4) shows relationships between the AM fungal distribution and the environmental variables. The ordination indicate that the AM fungi in Glo5, Glo8 and Glo10 relatively prefer the nutrient rich soil conditions with higher soil pH, while those in Glo3, Glo7 and Glo9 are the opposites. However, the Monte Carlo Permutation Tests on RDA showed that only soil pH had a significant effect on the distribution of the AM fungal phylotypes (Table 3).

4. Discussion

Almost all AM fungi detected in roots of *S. krylovii* in Mongolian steppe were in *Glomus*-group A, which was divided into

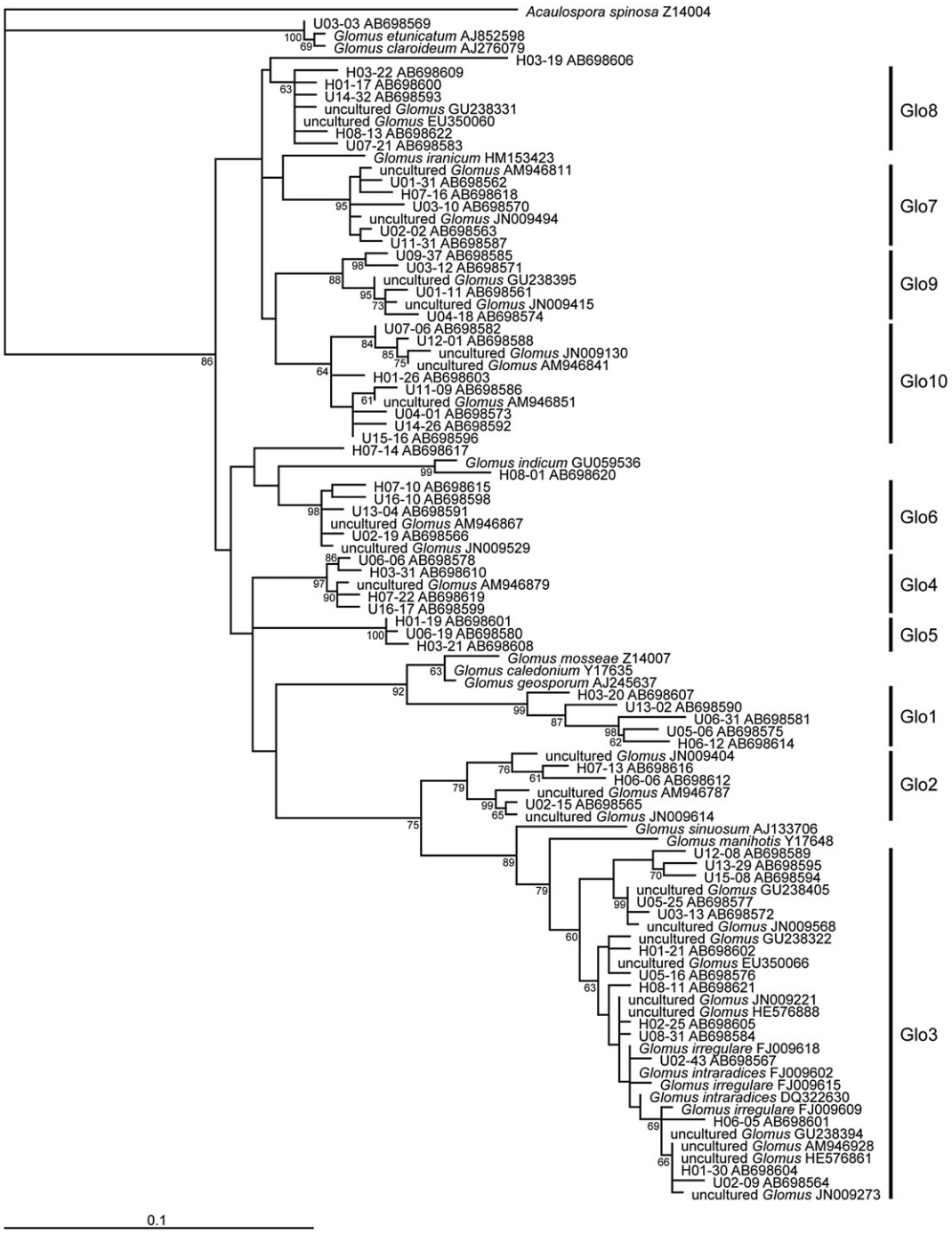

Fig. 3 – Maximum likelihood phylogenetic tree based on partial sequences of small subunit of nuclear ribosomal RNA gene (SSU rDNA) of arbuscular mycorrhizal fungi in *Glomus* obtained from *Stipa krylovii* in steppe vegetations in Uvurkhangai and Hustai in Mongolia and the GenBank database. For the sequences from *Stipa krylovii*, representative ones of the phylotypes (Glo1–Glo10) and four independent ones were analyzed. The tree is rooted to *Acaulospora spinosa* (Z14004) in Glomeromycota. The sequence numbers relate to sample number (U1–U16, H1–H8) and the clone numbers. Bootstrap values are shown where they exceed 60% (1,000 replications). The scale is shown so that evolutionary distances can be inferred. Accession numbers are given for all sequences.

10 phylotypes based on the sequences of SSU rDNA. Most of the phylotypes did not form clades with morphologically identified AM fungal species in the Genbank database. This result indicates that some of them may be novel AM fungal species. Meanwhile, diverse AM fungal spores have previously been isolated from steppe vegetation. For example, Tian et al. (2009b) found 27 species belonging to seven genera: *Acaulospora*, *Ambispora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Paraglomus*,

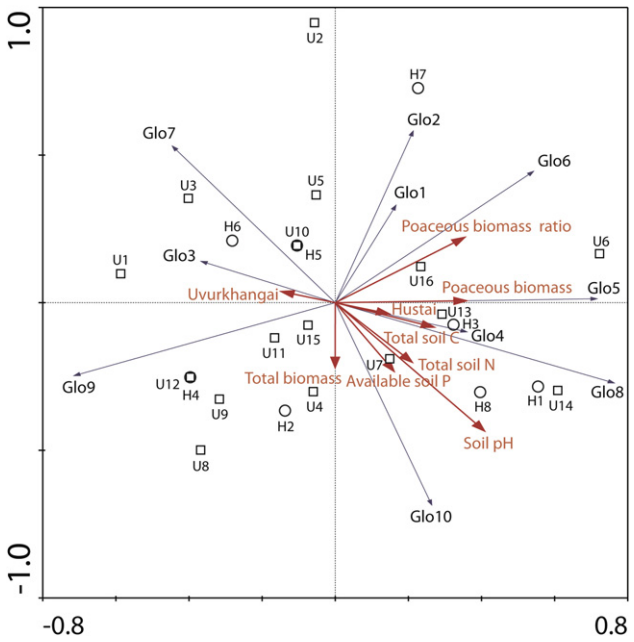

Fig. 4 – Diagram of a principal component analysis (PCA) on arbuscular mycorrhizal fungal communities with environmental variables, sampling regions, soil chemical properties, and plant biomass in steppe vegetations in Mongolia. The samples from Uvurkhangai are shown as squares, and those from Hustai are shown as circles. The eigenvalues of the first and second axes were 0.260 and 0.155, respectively.

and *Scutellospora*, of AM fungi from 54 plant species in the steppe of eastern Inner Mongolia. Peng et al. (2010) also isolated 15 species of AM fungal spores in *Glomus*, *Acaulospora*, and *Scutellospora* from *Stipa* steppe in north Tibet. Because the primer set used in this study is applicable to diverse AM fungi (Lee et al. 2008), the examination of only one plant species, *Stipa krylovii*, may explain the apparent predominance of *Glomus*-group A fungi. However, further study is required to explain the inconsistency in AM fungal communities between spore analyses and molecular studies. Among the AM fungal phylotypes, the one forming a clade with *G. intraradices* and *G. irregulare*, named Glo3, was the most dominant. *Glomus intraradices* is an AM fungal species known as a generalist distributed in various environments (Öpik et al. 2006), and is often dominant in arid environments (Alguacil et al. 2009; Yamato et al. 2009). *Glomus irregulare* is an AM fungal species recently distinguished from *G. intraradices* (Błaszkowski and Czerniawska 2008; Stockinger et al. 2009). However, the two AM fungal species were not distinguished in the phylogeny based on the partial sequences of SSU rDNA region in this study. Most of the AM fungal phylotypes determined in this study include AM fungi previously detected in high altitude regions in the Eurasian Continent. These AM fungi may be tolerant to severe cold temperature.

Table 3 – Result of Monte Carlo permutation tests on redundancy analysis (RDA) for the effects of environmental variables on arbuscular mycorrhizal fungal distribution.

Environmental variables	*F* value	*P* value
Soil pH	2.44	0.010
Poaceous biomass ratio	1.52	0.148
Poaceous biomass	1.45	0.161
Total soil C	0.91	0.528
Available soil P	0.63	0.809
Total soil N	0.49	0.907
Total biomass	0.48	0.909
Uvurkhangai	0.39	0.945
Hustai	0.39	0.946

The steppe vegetation examined consisted of various herbaceous plant species of Poaceae, Asteraceae, Liliaceae, Fabaceae, Chenopodiaceae, etc. The sampling was conducted in two separate regions, Uvurkhangai and Hustai, in which annual precipitation was higher in Hustai where the soils are significantly more fertile than those in Uvurkhangai (Table S2). The plant biomass was higher in Hustai than Uvurkhangai, which may be because of the higher precipitation and more fertile soil conditions in Hustai.

Significant correlations were not found for the relationship between the biomass values and number of AM fungal phylotypes. RDA analysis suggested that the effects of environmental variables on AM fungal distribution were not significant except soil pH (Table 3). From these results, it was suggested that the grazing had little effect on AM fungal proliferation, diversity and community structure in the steppe vegetation examined in Mongolia. Meanwhile, significant correlations were found among soil N, total biomass and AM ratio (Fig. 2). The direct effect of soil N on AM fungi is not clear, but higher plant biomass may be required for the proliferation of AM fungi in the environment.

Acknowledgments

We thank the staff and students at the Center for Ecosystem Study, Mongolian State University of Agriculture for their help in sample collection. The Ministry of Agriculture, Forestry and Fisheries, Japan, granted permission to import the soil samples from Mongolia. This study was supported by the Global COE Program “Advanced utilization of fungus/mushroom resources for sustainable society in harmony with nature” from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.myc.2012.09.006.

REFERENCES

Alguacil MM, Roldán A, Torres MP, 2009. Assessing the diversity of AM fungi in arid gypsophilous plant communities. *Environmental Microbiology* 11: 2649–2659.

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ, 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25: 3389–3402.

Augé RM, 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza* 11: 3–42.

Ba L, Ning J, Wang D, Facelli E, Facelli JM, Yang Y, Zhang L, 2012. The relationship between the diversity of arbuscular mycorrhizal fungi and grazing in a meadow steppe. *Plant and Soil* 352: 143–156.

Błaszkowski J, Czerniawska B, 2008. *Glomus irregulare*, a new arbuscular mycorrhizal fungus in the Glomeromycota. *Mycotaxon* 106: 247–267.

Börstler B, Renker C, Kahmen A, Buscot F, 2006. Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. *Biology and Fertility of Soils* 42: 286–298.

Brundrett M, Bougher N, Dell B, Grove T, Malajczuk N, 1996. *Working with mycorrhizas in forestry and agriculture*. Australian Centre for International Agriculture Research, Canberra.

Felsenstein J, 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O, 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* 59: 307–321.

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW, 1998. Ploughing up the wood wide web? *Nature* 394: 431.

Hooland SM, 2003. *Analytic rarefaction 1.3.User's guide and application*. Published at. https://www.uga.edu/_strata/software/AnRare/Readme.html.

Jigjidsuren S, Johnson DA, 2003. *Forage plants of Mongolia*. Admon, Ulan Bator.

Jirout J, Tříska J, Růžičková K, Elhottová D, 2009. Disturbing impact of outdoor cattle husbandry on community of arbuscular mycorrhizal fungi. *Communications in Soil Science and Plant Analysis* 40: 736–745.

Koide RT, 1991. Tansley review no. 29 nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytologist* 117: 365–386.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG, 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23: 2947–2948.

Lee J, Lee S, Young JPW, 2008. Improved PCR primers for the detection and identification of arbuscular mycorrhizal fungi. *FEMS Microbial Ecology* 65: 339–349.

Liu Y, He L, An L, Helgason T, Feng H, 2009. Arbuscular mycorrhizal dynamics in a chronosequence of *Caragana korshinskii* plantations. *FEMS Microobial Ecology* 67: 81–92.

Liu Y, He J, Shi G, An L, Öpik M, Feng H, 2011. Diverse communities of arbuscular mycorrhizal fungi inhabit sites with very high altitude in Tibet Plateau. *FEMS Microbial Ecology* 78: 355–365.

Liu Y, Shi G, Mao L, Cheng G, Jiang S, Ma X, An L, Du G, Johnson NC, Feng H, 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytologist* 194: 523–535.

Morton JB, Bentivenga SP, Bever JD, 1995. Discovery, measurement and interpretation of diversity in arbuscular endomycorrhizal fungi (Glomales, Zygomycetes). *Canadian Journal of Botany* 73: S25–S32.

Öpik M, Moora M, Liira J, Zobel M, 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology* 94: 778–790.

Page RDM, 1996. An application to display phylogenetic trees on personal computers. *Computer Application in the Biosciences* 12: 357–358.

Peng Y, Yang M, Cai X, 2010. Influence of soil factors on species diversity of arbuscular mycorrhizal (AM) fungi in *Stipa* steppe of Tibet Plateau. *Chinese Journal of Applied Ecology* 21: 1258–1263.

Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 404–425.

Schüßler A, Schwarzott D, Walker C, 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research* 105: 1413–1421.

Simon LM, Lalonde TD, Bruns TD, 1992. Specific amplification of 18S fungal ribosomal genes from vesicular-arbuscular endomycorrhizal fungi colonizing roots. *Applied Environmental Microbiology* 58: 291–295.

Stockinger H, Walker C, Schler A, 2009. '*Glomus intraradices* DAOM197198' a model fungus in arbuscular mycorrhiza research, is not *Glomus intraradices*. *New Phytologist* 183: 1176–1187.

ter Braak CFJ, Smilauer P, 2002. *CANOCO Reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5)*. Biometris, Wageningen.

Tian H, Gai JP, Zhang JL, Christie P, Li XL, 2009a. Arbuscular mycorrhizal fungi in degraded typical steppe of Inner Mongolia. *Land Degradation & Development* 20: 41–54.

Tian H, Gai JP, Zhang JL, Christie P, Li XL, 2009b. Arbuscular mycorrhizal fungi associated with wild forage plants in typical steppe of eastern Inner Mongolia. *European Journal of Soil Biology* 45: 321–327.

Ulziikhutag N, 1989. *Review of flora of Mongolia*. Ulaanbaatar, Ulaanbaatar.

van der Heijden MGA, Boller T, Wiemken A, Sanders IR, 1998a. Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. *Ecology* 79: 2082–2091.

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR, 1998b. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69–72.

Wang B, Qiu YL, 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299–363.

Yamato M, Ikeda S, Iwase K, 2009. Community of arbuscular mycorrhizal fungi in drought-resistant plants, *Moringa* spp., in semiarid regions on Madagascar and Uganda. *Mycoscience* 50: 100–105.