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ARBUSCULAR MYCORRHIZAL FUNGI IN DEGRADED TYPICAL STEPPE OF INNER MONGOLIA

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi may have some potential use in the restoration of degraded grassland through beneficial effects on plant growth and soil quality. A field investigation was conducted in three grassland sites of typical steppe in Inner Mongolia. The three plant communities, one of which was undegraded, one moderately degraded and the third severely degraded, were studied by collecting soil samples and samples of four plant species that occurred in all three sites. The percentage of root length colonized by AM fungi was estimated and the species composition and diversity of AM fungus spores recovered from the soil were determined using spore morphological characteristics. Although differences between the sites may have been due partly to other factors, it is likely that the degree of degradation was an important factor. No decline was found in the AM colonization of the roots of the indicator plant species in the moderately or severely degraded plant communities, and two plant species showed higher colonization status in the two degraded areas. *Glomus geosporum* and *Scutellospora calospora* were the dominant AM fungi in the undegraded steppe, while *G. geosporum* and *Glomus aggregatum* dominated the two degraded sites which also had low spore densities, species richness and diversity indices. However, different AM species showed different distributions among the three plant communities and the results indicate that both biotic and abiotic factors were important in determining the AMF communities, with biotic factors possibly the more important. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS: arbuscular mycorrhizal fungi; grassland degradation; diversity; spore communities; Mongolia

INTRODUCTION

Land degradation is a major environmental problem worldwide and has recently become particularly severe in areas of rapid economic development in China (Jiang et al., 2006). Grasslands cover 3.93 million km² in China, comprise 41 per cent of the total land area, and are considered to be one of the most important natural renewable resources because of their ecological and economic importance. However, large-scale land degradation has occurred across the vast grasslands of Inner Mongolia, resulting from inappropriate anthropogenic activities such as excessive cutting and over-grazing (Li, 1997). Recent surveys have shown that nearly 90 per cent of the grasslands now are degraded to various extents in this region (Wu and Loucks, 1992). Grassland degradation may alter the regional environment and directly affect the livelihood of millions of people who have lived in the region for generations (Yoshino, 2001).

A large number of factors have been identified that may contribute to grassland degradation. However, numerous studies have indicated that overgrazing is one of the most important causal factors (Green, 1989; Zhou et al., 2002; Christensen et al., 2004; Zhou et al., 2005). Grazing by herbivores can substantially influence the dynamics of plant communities by altering primary production, decomposition of organic matter (OM), the cycling and distribution of nutrients and competitive relationships among plant species (McNaughton, 1985; Fahnestock and Detling, 1999).

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Degraded grasslands therefore often exhibit severe defoliation of the herbage, changes in plant species composition, depletion of soil fertility and declining primary productivity.

Arbuscular mycorrhizal (AM) fungi can form a substantial part of microbial communities in soil and can form mutualistic associations with the roots of the majority of terrestrial plant species, thereby acting as extensions of plant root systems and increasing nutrient uptake, especially of phosphorus in soils of low fertility (Smith and Read, 1997). Mycorrhizal fungi may help govern plant community structure and successional trajectories. In fact, both plant productivity and diversity have been shown to increase with increasing diversity of mycorrhizal fungi (Grime et al., 1987; van der Heijden et al., 1998). This role of AM fungi may be most pronounced in the restoration of disturbed areas where secondary succession is taking place. Rosales et al. (1997) held the view that restoration programs in degraded areas should take mycorrhizae into account, reintroducing them or manipulating the soils to increase the mycorrhizal inoculum. It is known that communities of AMF occur which vary in species composition, species number and, therefore, in AMF biodiversity during the process of vegetation succession (Oehl et al., 2003; Aldrich-Wolfe, 2007; Artz et al., 2007). Accordingly, the application of AM fungi in degraded grassland restoration projects should begin with a thorough understanding of the distribution and diversity of the indigenous AM fungi in the field.

In the present study, a field survey was conducted of colonization of roots by AM fungi and AMF species diversity, inferred from the morphological characterization of AM fungus spores, in three grassland sites of varying degree of degradation in the typical steppe of Inner Mongolia. The aims were to determine (1) whether the different sites had different amounts of AM plant root colonization, (2) variation in the AM fungus spore communities in the three sites and (3) the main environmental factors that influence the AM fungus spore communities in the steppe grassland communities.

MATERIALS AND METHODS

Study Area

The survey was conducted in a typical steppe area of Xilin River Basin (43°26′–44°34′N, 115°30′–117°12′E), Xilin Gol League of Inner Mongolia municipality, northwest China. This region covers an area of about 10 000 km² and declines gradually topographically from the east (with the highest elevation of 1505 m) to the west (with the lowest elevation of 902 m) (Chen, 1988). The region has a semiarid, continental, temperate steppe climate with dry springs and moist summers. The annual mean temperature increases from southeast to northwest, ranging from 0.5 to 2.1°C (Chen, 1988). The lowest average temperature (January) is −17°C, and the highest (July) is 18°C. Total annual precipitation decreases gradually from 400 mm in the southeast to 250 mm in the northwest (Chen, 1988). More than 70 per cent of the annual precipitation occurs from June to August. The major soil type is a Kastanozem soil (Wang and Cai, 1988). Grazing is the main land use and fertilizers are very seldom used.

The degree of grassland degradation in different parts of the study area is related to their location in relation to a local inhabited site. The local inhabitants usually leave their livestock to graze freely on the grassland around their houses from June to October every year. With increasing grazing radius, the grazing pressures on the steppe become progressively lighter, eventually forming a gradient of degradation in the long term. The undegraded steppe community is often dominated by Leymus chinensis (Trin.) Kitag, changing to Agropyron michnoi Roshev when the grassland becomes degraded, and then Artemisia frigida Willd and Potentilla acaulis L. with further degradation. L. chinensis, A. michnoi, A. frigida and P. acaulis were also used as indicator plants of different degradation intensity. Besides, the degraded steppe community is often associated with low vegetation cover and height (Liu et al., 2002).

Sampling Methods

Sampling took place from 20 to 22 September 2006. Before sampling, three steppe communities representing undegraded, moderately degraded and severely degraded grassland along a southward transect from the human settlement were marked off. The undegraded grassland, the southermost on the transect, is a permanent
experimental plot established by the Inner Mongolia Grassland Research Station in 1979 and administered by the Chinese Academy of Sciences. This site is a *L. chinensis* steppe community and now appears to be representative of natural climax steppe communities after about 27 years of enclosure (Bai *et al*., 2004). The severely degraded community, dominated by *A. frigida* and *P. acaulis*, is the nearest to the inhabited site (<100 m). The moderately degraded community, dominated by *A. michnoi*, occupies an intermediate position along the transect.

A 3 km long transect stretched across the three sites from the human settlement southwards. In each site, there were 30 1×1 m² quadrats along the transect with a minimum distance between adjacent quadrats of 25 m. Soil samples were collected by dividing each quadrant into four 0.25 m² squares and taking four cores to 30 cm depth from the centre of each square. The four cores were mixed to give one composite soil sample from each quadrant. A total of 90 composite soil samples were collected from the three steppe communities. The root materials belonging to the four indicator plants were also sampled in each community along the transect. Four replicate samples from each plant species were collected to give four samples per plant species per community. During the collection of individual plants, care was taken that the roots could be positively identified as belonging to a particular plant. Soil samples were air-dried before extraction, counting and identification of AM fungal spores. Root samples were taken to the laboratory and stored frozen (0°C) until colonization of roots by AM fungi was determined.

**Measurement of Vegetation Parameters**

Some basic vegetation parameters such as cover, height, plant species richness (PSR) and species diversity index (*PH*') in each quadrant of the three steppe communities were measured and are shown in Table I. Total plant cover was estimated visually and the height of one individual of each plant species in the quadrats was measured in centimetres with a ruler, and the average value as the vegetation height of that quadrat. Species number and the total number of individuals of each plant species in each quadrat were counted. Plant Shannon–Weiner index (*PH*') was calculated for each quadrat using the Equation (1), where \( P_i = n_i/N \), \( n_i = \) number of individuals of species \( i \), and \( N = \) total number of individuals in all species.

\[
H' = - \sum (P_i) \ln(P_i)
\]  

(1)

**Soil Analysis**

Selected chemical properties of a sub-sample of the composite soil sample from each quadrant, namely OM, available (Olsen) P (*P*) and pH (water, 1:2.5 soil/water) were determined and the values are shown in Table I.

### Table I. Characters of soil and vegetation community of each quadrant in the three different degraded steppe communities

<table>
<thead>
<tr>
<th>Site (degree of degradation)</th>
<th>Soil</th>
<th>Dominant plant species</th>
<th>Vegetation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Olsen P (<em>P</em>)</td>
<td>Organic matter (OM) (per cent)</td>
<td>pH (water, 1:2.5 soil/water)</td>
</tr>
<tr>
<td>Undegraded</td>
<td>4.48 b</td>
<td>3.58 a</td>
<td>7.48 a</td>
</tr>
<tr>
<td>Moderate</td>
<td>7.94 a</td>
<td>3.00 b</td>
<td>7.31 b</td>
</tr>
<tr>
<td>Severe</td>
<td>5.38 b</td>
<td>1.53 c</td>
<td>7.27 b</td>
</tr>
</tbody>
</table>

Numbers in the same column followed by a different letter are significantly different according to LSD test of multiple comparisons \( p < 0.05 \) \((n = 30)\).
Assessment of AM Colonization

Roots were washed carefully with tap water and cut into segments about 1 cm long. About 0.5 g root segments were cleared in 10 per cent (w/v) KOH at 90°C in a water bath for 60 min. After cooling, the root samples were washed and stained with 0.05 per cent (w/v) Trypan blue (McGonigle et al., 1990). Thirty 1-cm-long root segments were mounted on slides in a polyvinyl alcohol-lactic acid-glycerol solution (Koske and Tessier, 1983) and examined at 100–400× magnification under a Nikon YS100 microscope. The percentage of root length colonized was calculated according to the method of Trouvelot et al. (1986). The colonization data for specific AM structures are expressed as percentage of root length.

Analysis of AM Fungus Spores

Spores or sporocarps were extracted from 50 g sub-samples of air-dried soil by wet sieving followed by flotation-centrifugation in 60 per cent sucrose (Dalpé, 1993). The finest sieve used was 40 μm and the spores were collected on a grid patterned (4 × 4 mm²) filter paper. After washing three times with distilled water to spread them evenly over the entire grid, the spores were counted using a dissecting microscope at 30× magnification. A sporocarp was counted as one unit. For observation and identification of spore characters, spores were mounted on glass slides in polyvinyl alcohol-lactoglycerol (PVLG) + Melzer’s reagent. Spores were examined microscopically and identified according to taxonomic criteria (Schenck and Perez, 1990) and taxonomic information from two websites on the internet (http://invam.caf.wvu.edu and http://www.lrz-muenchen.de/~schuessler/amphylo/).

One composite soil sample was analysed for AM fungus spores for each of the 90 quadrats. With the data obtained, we calculated: (1) spore density (SD), measured as total number of spores occurring in 50 g (air-dry weight) soil, (2) AM species richness (ASR), measured as the total number of different AM fungus spore species occurring in 50 g (air-dry weight) soil, (3) frequency of occurrence (F), calculated as the percentage of samples from which a determined species was isolated, (4) relative abundance (RA), calculated as the ratio between the number of spores of a particular species to the total number of spores and (5) the Shannon–Weiner diversity index (\( AH^F \)) was calculated following the same method as for plant species diversity with Equation (1).

Statistical Analysis

The AM colonization and spore community data in the 30 quadrats of each of the three grassland sites were combined and analysed by one-way ANOVA and means were compared by least significant difference (LSD) at the 0.05 level using the software SPSS 11-5. The Pearson correlation coefficient was employed to determine the relationships between AM fungus spore community indices and some biotic and abiotic parameters.

Canonical correspondence analysis (CCA) was used to analyse the relationship between AM fungal distribution pattern and environmental variables using the CANOCO 4.5 software application (ter Braak and Ř milauer, 2002). CCA analysis was selected because the gradient lengths in previous detrended correspondence analysis (DCA) reached 4.78, and therefore a unimodal rather than linear model was used. The species data matrix was composed of the spore number of a particular AM species in each 50 g air-dried field soil sample. Any AM species that occurred fewer than three times overall were abandoned because of their small contribution to the total variance. The environmental data matrix was composed of the values of three soil variables (pH, Olson P and OM) and two vegetation variables (average cover and height of the vegetation) in each sample. During the CCA procedure, the data were log-transformed and the Monte Carlo permutation test for significance at the 0.01 level was used. Biplots were created using CanoDraw 4-1 to display the ordination results (ter Braak and Ř milauer, 2002).

RESULTS

AM Colonization

All the four indicator plant species collected from all three steppe communities were colonized by AM fungi. AM colonization of A. frigida was much higher (19-8 per cent) in the severely degraded steppe than in the undegraded and moderately degraded sites (8-1 and 8-0 per cent, respectively). The abundance of vesicles within the roots also
increased significantly from 0.45 and 1.8 per cent in the undegraded and moderately degraded sites to 7.9 per cent in the severely degraded steppe, but the abundance of arbuscules did not change among the three plant communities (Figure 1C). The abundance of vesicles within the roots of *P. acaulis* was also much higher in the severely degraded site than the other two (Figure 1D). Neither the root colonization nor the abundance of vesicles or hyphal coils/arbuscules in *L. chinensis* or *A. michnoi* showed any significant differences among the three plant communities due to large variability (Figure 1A and B).

**AM Fungal Species Composition**

A total of 47 AM fungal species belonging to seven genera were identified morphologically from the three communities, and 38, 28 and 20 from the undegraded, moderately and severely degraded grasslands, respectively.

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**Figure 1. AM root colonization of the four indicator plant species (A): Leymus chinensis (Trin.) Kitag; (B): Agropyron cristatum Roshev; (C): Artemisia frigida Willd; (D): Potentilla acaulis L. in different grassland communities. Values are means and standard errors. n = 4. Bars followed by the same letter are not different among degradation intensities (p < 0.05). UD, undegraded; M, moderately degraded; S, severely degraded.**

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Of the seven genera, *Glomus*, which had the highest species number, RA and frequency, was the dominant genus in the investigation (Table II). *Glomus geosporum* was the dominant \((F > 50 \text{ per cent})\) AM species in all the three sites; and *Scutellospora calospora* was another dominant species in the undegraded steppe, while *Glomus aggregatum* was dominant in the moderately and severely degraded plant communities (Table III).

Of the total of 47 AMF species, 14 were found only in the undegraded, 5 only in the moderately degraded and 1 only in the severely degraded steppe (Table III). Among the total of 12 AM species occurring in all the three sites, 6 species had lower frequencies in the moderately degraded or undegraded steppes. The frequency of *G. aggregatum* and *Glomus etunicatum* increased in the moderately degraded and severely degraded sites (Table III). *Glomus heterosporum*, *Glomus hoi* and *Glomus tenerum* had the highest frequencies in the moderately degraded steppe.

### Spore Abundance, Species Richness and the Diversity of AM Fungi

The total spore densities in the soil are shown in Figure 2A. The spore densities were significantly different among the three grassland communities. The values in the moderately and severely degraded sites decreased by 60 and 84 per cent compared to the undegraded site.

Spore abundance of particular AM genus or species was varied among the three steppe communities. At the generic level, the relative spore abundance of *Appendicispora* and *Scutellospora* showed a distinct decrease in the moderately or severely degraded sites. In contrast, the spore abundance of *Glomus* showed the opposite trend (Table II). At species level, of all the 12 AM fungal species which occurred in all the three steppe communities, the RA of 9 species showed an increasing trend in both the degraded communities. Two species showed the opposite trend, and the remaining one showed no change (Table III).

The ASR and Shannon–Weiner diversity index decreased significantly in both degraded sites (Figure 2B and C). The number of AM species per 50 g air-dried soil decreased by 28-1 and 54-7 per cent in the moderately and severely degraded areas, respectively. The Shannon–Weiner diversity index of the AM species also declined correspondingly by 19-0 and 39-3 per cent.

### Influence of Environmental Variables on AM Fungus Spore Community

Correlation analyses between AM fungus spore community and environmental variables are shown in Table IV. All three AM fungus spore community indices (ASR, \(AH^f\) and SD) had significant positive correlation with soil OM content and height and cover of the vegetation, but had no significant positive correlation with PSR. ASR and SD had the highest correlation coefficient with height, and \(AH^f\) had the highest correlation coefficient with OM. SD and \(AH^f\) also had significant positive correlations with soil pH. ASR and \(PH^f\) were negatively correlated.

---

**Table II. Relative abundance (RA), frequency (F) and species number of the total AMF genera identified from the three grassland sites**

<table>
<thead>
<tr>
<th>AMF genus</th>
<th>Species number</th>
<th>Frequency (F) (per cent)</th>
<th>Relative abundance (RA) (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UD  M  S</td>
<td>UD  M  S</td>
<td>UD  M  S</td>
</tr>
<tr>
<td><em>Acaulospora</em></td>
<td>4   4   3</td>
<td>30  30  10</td>
<td>3-46 3-06 4-51</td>
</tr>
<tr>
<td><em>Appendicispora</em></td>
<td>1  1   1</td>
<td>10  3-3  3-3</td>
<td>1-86 0-44 0-75</td>
</tr>
<tr>
<td><em>Entrophospora</em></td>
<td>1  1   0</td>
<td>10  10  0</td>
<td>1-06 1-31 0</td>
</tr>
<tr>
<td><em>Glomus</em></td>
<td>26  18  14</td>
<td>100 100 100</td>
<td>80-59 92-58 90-23</td>
</tr>
<tr>
<td><em>Kuklospora</em></td>
<td>1   1   0</td>
<td>10  10  0</td>
<td>0-8 1-31 0</td>
</tr>
<tr>
<td><em>Paraglomus</em></td>
<td>1   1   0</td>
<td>6-7  3-3  0</td>
<td>0-53 0-44 0</td>
</tr>
<tr>
<td><em>Scutellospora</em></td>
<td>4  2   2</td>
<td>70  6-67 13-3</td>
<td>11-70 0-87 4-51</td>
</tr>
</tbody>
</table>

UD, undegraded; M, moderately degraded; S, severely degraded.
### Table III. Frequency (F) and relative abundance (RA) of the AM fungus species identified from the three grassland communities

<table>
<thead>
<tr>
<th>AM species</th>
<th>Frequency (F) (per cent)</th>
<th>Relative abundance (RA) (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acaulospora delicata Walker, Pfeiffer &amp; Bloss</td>
<td>UD: 0, M: 6-7, S: 3-3</td>
<td>UD: 0, M: 0.87, S: 1.50</td>
</tr>
<tr>
<td>Acaulospora longula Spain &amp; Schenck</td>
<td>UD: 6-7, M: 6-7, S: 3-3</td>
<td>UD: 1.59, M: 0.87, S: 1.50</td>
</tr>
<tr>
<td>Acaulospora nicolsonii Walker, Reed &amp; Sanders</td>
<td>UD: 6-7, M: 0, S: 0.79</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Acaulospora rugosa Morton</td>
<td>UD: 6-7, M: 0, S: 0.79</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Acaulospora sp.</td>
<td>UD: 3-3, M: 0, S: 0.44</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Acaulospora spinosa Walker &amp; Trappe</td>
<td>UD: 3-3, M: 6-7, S: 0</td>
<td>UD: 0.26, M: 0.87, S: 0</td>
</tr>
<tr>
<td>Appendicispora appendiculata Spain, Oehl &amp; Sieverd</td>
<td>UD: 0, M: 3-3, S: 0</td>
<td>UD: 0.44, M: 0.75, S: 0</td>
</tr>
<tr>
<td>Appendicispora leptoticha C. Walker, Vestberg &amp; Schuessler</td>
<td>UD: 10-0, M: 0, S: 1.85</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Entrophospora infrequens (Hall) Ames &amp; Schneider</td>
<td>UD: 10-0, M: 10-0, S: 1.06</td>
<td>UD: 1.31, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus albicum Walker &amp; Rhodes</td>
<td>UD: 16-7, M: 3-3, S: 0</td>
<td>UD: 1.32, M: 0.87, S: 0</td>
</tr>
<tr>
<td>Glomus ambisporum Smith &amp; Schenck</td>
<td>UD: 6-7, M: 0, S: 0.53</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus caledonia (Nicol. &amp; Gerd) rappe &amp; Gerde</td>
<td>UD: 26-7, M: 0, S: 3.97</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus claroideum Schenk &amp; Smith</td>
<td>UD: 43-3, M: 26-7, S: 5.82</td>
<td>UD: 10.48, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus clarum Nicolson &amp; Schenck</td>
<td>UD: 26-7, M: 0, S: 11.90</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus constrictum Trappe</td>
<td>UD: 10-0, M: 6-7, S: 0.79</td>
<td>UD: 0, M: 3-0, S: 0</td>
</tr>
<tr>
<td>Glomus convolutum Gerdemann &amp; Trappe</td>
<td>UD: 10-0, M: 0, S: 1.59</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus delhiense Mukerji, Blattacharjee, &amp; Tewari</td>
<td>UD: 23-3, M: 0, S: 2.12</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus deserticola Trappe, Bloss &amp; Menge</td>
<td>UD: 6-7, M: 3-3, S: 0.53</td>
<td>UD: 0, M: 0, S: 0.75</td>
</tr>
<tr>
<td>Glomus diaphanum Morton &amp; Walker</td>
<td>UD: 6-7, M: 0, S: 0.87</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus eburneum Kennedy, Stuta et Morton</td>
<td>UD: 6-7, M: 0, S: 0.53</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus etunicatum Becher &amp; Gerdemann</td>
<td>UD: 23-3, M: 30-0, S: 3.97</td>
<td>UD: 6-11, M: 15.04, S: 0</td>
</tr>
<tr>
<td>Glomus fasciculatum (Thaxter) Gerd. &amp; Trappe emend. Walker &amp; Koske</td>
<td>UD: 13-3, M: 0, S: 3.3</td>
<td>UD: 0, M: 1.59, S: 0</td>
</tr>
<tr>
<td>Glomus heterosporum Smith &amp; Schenck</td>
<td>UD: 10-0, M: 16-7, S: 10-0</td>
<td>UD: 1.06, M: 2.18, S: 2-26</td>
</tr>
<tr>
<td>Glomus hoi Berch &amp; Trappe</td>
<td>UD: 10-0, M: 26-7, S: 10-0</td>
<td>UD: 1.32, M: 3.49, S: 2-26</td>
</tr>
<tr>
<td>Glomus intraradices Schenk &amp; Smith</td>
<td>UD: 23-3, M: 30-0, S: 6-7</td>
<td>UD: 2.65, M: 4-80, S: 2-26</td>
</tr>
<tr>
<td>Glomus lacteum Rose &amp; Trappe</td>
<td>UD: 6-7, M: 3-3, S: 0.53</td>
<td>UD: 0, M: 0, S: 2-26</td>
</tr>
<tr>
<td>Glomus magnificaule Hall</td>
<td>UD: 26-7, M: 13-3, S: 4-50</td>
<td>UD: 4-37, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus manihotis Howeler, Sieverding &amp; Schenck</td>
<td>UD: 10-0, M: 6-7, S: 1.59</td>
<td>UD: 0, M: 0, S: 0.87</td>
</tr>
<tr>
<td>Glomus maculosum Miller &amp; Walker</td>
<td>UD: 0, M: 3-3, S: 0</td>
<td>UD: 0, M: 0, S: 0.44</td>
</tr>
<tr>
<td>Glomus monosporum Gerdemann &amp; Trappe</td>
<td>UD: 0, M: 6-7, S: 3-3</td>
<td>UD: 0, M: 0, S: 0.87</td>
</tr>
<tr>
<td>Glomus mosseae (Nicol. &amp; Gerd.) Gerdemann &amp; Trappe</td>
<td>UD: 33-3, M: 36-7, S: 20-0</td>
<td>UD: 5-82, M: 12-23, S: 6-78</td>
</tr>
<tr>
<td>Glomus multicauze Gerdemann &amp; Bakshi</td>
<td>UD: 0, M: 3-3, S: 0</td>
<td>UD: 0, M: 0, S: 0.44</td>
</tr>
<tr>
<td>Glomus radiatum (Thaxter) Trappe &amp; Gerd</td>
<td>UD: 6-7, M: 0, S: 1.06</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus reticulatum Bhattacharjee &amp; Mukerji</td>
<td>UD: 3-3, M: 0, S: 0.53</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus tortuosum Schenck &amp; Smith</td>
<td>UD: 3-3, M: 0, S: 2-65</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Glomus versiforme (Karsten) Berch</td>
<td>UD: 10-0, M: 3-3, S: 2-38</td>
<td>UD: 0, M: 0, S: 0.44</td>
</tr>
<tr>
<td>Kuklospora kentinensis Oehl &amp; Sieverd</td>
<td>UD: 10-0, M: 10-0, S: 0.79</td>
<td>UD: 1.31, M: 0, S: 0</td>
</tr>
<tr>
<td>Paraglomus occultum (Walker) Morton</td>
<td>UD: 6-7, M: 3-3, S: 0.53</td>
<td>UD: 0, M: 0, S: 0.44</td>
</tr>
<tr>
<td>Scutellopsora calospora (Nicol. &amp; Gerd) Walker &amp; Sanders</td>
<td>UD: 56-7, M: 3-3, S: 13-3</td>
<td>UD: 9-79, M: 0.44, S: 3.76</td>
</tr>
<tr>
<td>Scutellopsora cerradensis Spiian &amp; Miranda</td>
<td>UD: 3-3, M: 0, S: 0.26</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
<tr>
<td>Scutellopsora erythropa (Koske &amp; Walker) Walker &amp; Sanders</td>
<td>UD: 0, M: 3-3, S: 0</td>
<td>UD: 0, M: 0, S: 0.44</td>
</tr>
<tr>
<td>Scutellopsora pellucida Nicolson &amp; Schenck</td>
<td>UD: 16-7, M: 0, S: 3-3</td>
<td>UD: 1.32, M: 0, S: 0.75</td>
</tr>
<tr>
<td>Scutellopsora sp.</td>
<td>UD: 3-3, M: 0, S: 0.26</td>
<td>UD: 0, M: 0, S: 0</td>
</tr>
</tbody>
</table>

UD, undegraded; M, moderately degraded; S, severely degraded.
Figure 2. Total spore density (SD) (A), AM spore species richness (ASR) (B) and Diversity of AM species (A Shannon-Wiener index) (C) in 50 g air-dried soil from each quadrat in the three grassland communities. Values are means and standard errors, n = 30. Bars followed by the same letter are not significantly different among degradation intensities (p < 0.05). UD, undegraded; M, moderately degraded; S, severely degraded.

CCA analysis showed the relationship between environmental variables and the species distribution pattern of AM fungi. The coordinate from the first two ordination axes explained 76.4 per cent (the first axis, 50.3 per cent and the second axis, 26.1 per cent) of the variance that could be explained by the variables in the CCA study, as shown in Figure 3. The significance (according to the Monte Carlo permutation tests) of all canonical axes was $P = 0.004$, indicating that the environmental factors had a significant influence on the distribution of the AM fungus spore species.

In the biplots of Figure 3, the length of the vector describes the relative significance of the correlation of that variable with the axes, and the angle between a vector and any axis is a measure of the degree of correlation of the

![Figure 3. Ordination diagram from the CCA analysis of the relationship between the distribution of AMF spore community and environmental variables in the grassland degraded gradient. The environmental variables were represented by vectors, where $P$: Olson P of soil; pH: the pH of the soil; OM: the organic matter of the soil; cover: the average cover of the vegetation; height: the average height of the vegetation. The AM species are represented by empty triangles, where A.I: Acaulospora longula; A.n: Acaulospora nicolsonii; A.re: Acaulospora rehmi; A.ru: Acaulospora rugosa; A.s: Acaulospora spinosa; Ap.l: Appendicispora leptoticha; E.i: Entrophospora infrequens; G.ag: Glomus aggregatum; G.al: Glomus albidum; G.ca: Glomus caledonium; G.cl: Glomus claroideum; G.co: Glomus constrictum; G.conv: Glomus convolutum; G.d: Glomus delhiense; G.de: Glomus deserticola; G.et: Glomus etunicatum; G.f: Glomus fasciculatum; G.ge: Glomus geosporum; G.h: Glomus heterosporum; G.ho: Glomus ho; G.i: Glomus intraradices; G.l: Glomus lacteum; G.mag: Glomus magnicaule; G.man: Glomus manihotis; G.mn: Glomus monosporum; G.mus: Glomus musae; G.r: Glomus radiatum; G.te: Glomus tenerum; G.to: Glomus tortuosum; G.v: Glomus versiforme; K.k: Kuklospora kentinensis; P.o: Paraglomus occultum; S.ca: Scutellospora calospora; S.p: Scutellospora pellulida.](image)

**NB**: ASR, species richness of AM fungi; $AH'$, Shannon–Weiner index of AM fungi; SD, AM fungus spores density; pH, pH of soil; OM, organic matter content of soil; $P$, Olson P of soil; height, average height of vegetation; cover, average cover of vegetation; PSR, plant species richness in a quadrat; $PH'$, plant Shannon–Weiner index.

*Correlation significant at $p < 0.01$.*
variable with that axis. Thus, the differences most strongly correspond with the biotic factor, the average height of the vegetation along the first axis, and the Monte Carlo test for this axis was \( P = 0.006 \). Cover and OM were also positively correlated with the first axis, but height determined the distribution of the AM fungal species along this axis. The second axis was positively correlated with both pH and \( P \), but pH had a higher correlation with the distribution of AM community and also a higher degree of correlation with this axis. Both the Olson P and the height of the vegetation had a rather low degree of negative correlation with the first and second axes, respectively.

**DISCUSSION**

**AM Colonization**

Conventional wisdom might lead us to expect less AM colonization of host plants in the grazed grassland because the loss of leaf area might result in a decreased source capacity that would be insufficient to satisfy both root and AM fungal sink demands (Harley and Smith, 1983; Bethlenfalvay and Dakessian, 1984; Gehring and Whitham, 1994; Dhillion and Gardsjord, 2004). However, in the present investigation, colonization of roots by AM fungi of the four indicator plant species was not significantly lower in the degraded grassland in which the degradation which was mainly due to over-grazing. One possible explanation might be the high grazing tolerance of the plants (all four plant species are recognized as grazing-tolerant forages) (Gehring and Whitham, 1994; Saito et al., 2004). Furthermore, the percentage length of *A. frigida* roots with AM colonization increased in the severely degraded grassland (Figure 1C). Some recent studies have reported similar results (Busso et al., 2001; Grigera and Oesterheld, 2004). Effects of grazing on mycorrhizal colonization are regarded as species dependent (Wallace, 1981; Bethlenfalvay et al., 1985). Trends of increasing abundance of AMF vesicles within the roots of the indicator plants in the severely degraded steppes were also found (Figure 1). Titus and Lepš (2000) found that mowing can significantly increase the number of AMF vesicles in *Potentilla* spp. Some other studies have shown that plant stress may cause an increase in the abundance of vesicles (Reece and Bonham, 1978; Cooke et al., 1993; Duckmanton and Widden, 1994). Vesicles have storage or propagule functions and can support the regrowth of intercellular hyphae when appropriate conditions occur (Smith and Read, 1997). In stressful environments, AM fungi may have a tendency to invest more energy in storage structures needed for their survival (Turnau et al., 1996). In the present study, over-grazing may have been an important factor influencing the increase in AMF vesicles in the severely degraded grassland.

**AM Fungus Spore Communities**

We identified a total of 47 AM species according to the morphological characters of the spores. This number is much larger than those reported in semiarid Mediterranean ecosystems (Ferrol et al., 2004) and semiarid areas in Brazil (Silva et al., 2005) where only 23 and 21 AM species were identified. However, similar high AM fungal diversity can also be found in other semiarid or arid regions. Forty-four AM species were isolated from semiarid grasslands of Namibia (Uhlmann et al., 2004), 45 from a temperate region of Europe (Oehl et al., 2003) and 43 from an arid area in southwest China (Zhao and Zhao, 2007). Our results also revealed that *Glomus* was the dominant AM genus in all three steppe communities. *G. aggregatum* and *G. geosporum* were the dominant species in both moderately and severely degraded sites. Numerous other studies have shown *Glomus* to be dominant in arid or semiarid areas (Frank et al., 2003; Ferrol et al., 2004; Shi et al., 2007). *G. aggregatum* and *G. geosporum* are also frequently found in arid or semiarid ecosystems (Panwar and Tarafdar, 2006; Shi et al., 2006; Uhlmann et al., 2006).

The degraded grassland areas had significantly lower AMF species diversity. Thirty-eight AM fungal species were found in the undegraded grassland, and only 28 and 20 species in the moderately and severely degraded areas (Table III). Either the ASR or the Shannon–Weiner diversity index appeared to decrease sharply with increasing intensity of grassland degradation (Figure 2B and C). These results are consistent with an earlier study by Eom et al. (2001), who also found that grazing markedly reduced AM fungal species diversity. However, the responses of different AMF species to degradation are varied (Table III). Some AMF species such as *Acaulospora delicate,*
Glomus clarum and G. etunicatum had higher spore RA in moderately or severely degraded grassland while some AM species such as Appendicispora leptoticha, Glomus caledonium and S. calospora showed a decreasing trend of spore RA. Furthermore, there were also some species that occurred only in particular steppe communities (Table III). Similar results were reported by Eom et al. (2001) and Uhlmann et al. (2006). It was supposed that different AMF species may have different sensitivities to photosynthetic stress (Bethlenfalvay and Dakessian, 1984). However, the morphological method used in the present study, which is based on the AM fungus spores, has limitations in that spore production is highly dependent on physiological parameters of the AMF and on environmental conditions. In recent years, molecular identification techniques have been used increasingly in studies on the ecology of arbuscular mycorrhizae because they offer the opportunity to identify AMF in any given root sample without the need for spores (Redecker et al., 2003). In combination with the classic morphological analyses of spores, molecular identification of AMF by ribosomal sequences is highly promising and should provide a workable strategy to better characterize AMF communities.

Influence of Environmental Variables on AM Fungus Spore Communities

The distribution of AM fungal species is affected by various factors including both biotic and abiotic factors (Hayman, 1982). Some studies indicated that abiotic factors may be more important than biotic factors for establishing population patterns (Mohammad et al., 2003; Panwar and Tarafdar, 2006). However, other studies suggested that abiotic factors alone are not sufficient in explaining AM species distribution, and some biotic factors have to be taken into account (Kurle and Pfleger, 1996; Uhlmann et al., 2004). In the degraded grassland ecosystems, both soil and vegetation characteristics may influence the AM fungal community.

The present study suggests that abiotic factors had important influences on AM fungus spore communities and both soil pH and OM had significant correlations with AM fungus spore communities (Table IV) and this supports a number of earlier studies (Cuenca and Meneses, 1996; Hinojosa et al., 2005; Panwar and Tarafdar, 2006). According to the CCA analysis, pH also played an important role in influencing the distribution of the AM fungus spore community (Figure 3). The soil pH was lower in the moderately and severely degraded sites (Table I) and the disappearance of some common or rare AM species may due in part to the change in soil pH because it is known that some AM fungal species seem to be sensitive to changes in soil pH (Porter et al., 1987; Kurle and Pfleger, 1996; Coughlan et al., 2000).

Moreover, higher correlations were found between biotic factors and AM fungus spore communities. Both the ASR and SD had the highest correlations with the average height of the vegetation (Table IV). The CCA analysis of the distribution of AM fungus spore community and environmental variables also indicated that among the five environmental factors examined, vegetation height associated with vegetation cover was most related to the distribution of the AM fungus spore community (Figure 3). This suggests that the defoliation caused by grazing may be a major factor influencing the distribution of the AM fungus spore community. The loss of photosynthetic tissue through defoliation may reduce the synthetic capacity of plants (Davidson and Milthorpe, 1966) and may also negatively affect arbuscular mycorrhizal function since the fungal partner in the association is entirely dependent on carbon fixed by the host plant (Harley and Smith, 1983). Saito et al. (2004) found that defoliation can significantly affect the community structure of AM fungi within the roots of grazing-intolerant plant species.

The changes in vegetation structure and plant diversity resulting from grazing in the degraded grasslands may also influence the AM fungal community (Barni and Siniscalco, 2000; Johnson et al., 2003). However, in the present study neither PSR nor plant diversity (PH) was negatively affected by grassland degradation (Table I). In addition, correlation analysis indicates that the AM fungus spore community was not related to PSR or plant diversity index (PH'), except for a negative correlation between ASR and plant diversity index (PH') (Table IV). However, much more complex mechanisms were involved in this process and a field investigation based on morphological characters of AM fungus spores extracted from the soil is not adequate to elucidate this. Perhaps molecular methods used to detect the dynamics of AM communities within the roots during the changes in vegetation communities will be a useful approach in future studies.

The results of this study clearly suggest that the amounts of colonization of roots by AM fungi did not decrease in the degraded steppe communities. Some plant species even exhibited higher percentage of colonization of roots by
AM fungi or greater abundance of vesicles. However, AM fungus spore numbers and species diversity distinctly decreased in the degraded communities, whereas there were still quite a few AM species that adapted to the stressful environment of the degraded grassland. Both biotic and abiotic factors had significant influence on AMF communities, and biotic factors were possibly more important. However, the present study was based on the spore morphological method and could not assess the AM community within the roots. It is important to learn more about the molecular ecology and intricacies of the association in the further future research.

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REFERENCES


