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SHORT NOTE

Arbuscular mycorrhizal fungi in non-grazed, restored and over-grazed grassland in the Inner Mongolia steppe

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Abstract Arbuscular mycorrhizal (AM) fungal diversity was investigated in non-grazed, restored and over-grazed (fenced) plots of a grassland in the Inner Mongolia steppe. Plant cover and variety differ between the plots, being highest in the non-grazed to lowest in the over-grazed plots. A total of 19 AM fungal taxa belonging to six genera were found based on spores isolated from field samples and trap cultures. One belonged to *Acaulospora*, one to *Archaeospora*, one to *Entrophospora*, one to *Gigaspora*, 12 to *Glomus* and three to *Scutellospora*. *Glomus* was the dominant genus in all plots, and *Glomus geosporum* was the dominant species, whilst *G. albidum* and *G. etunicatum* were dominant in the restored plot. *Scutellospora* was the second dominant genus in the non-grazed plot with *Scutellospora calospora* being the dominant species. The mean spore density and mean species richness of AM fungi were significantly decreased by long-term over-grazing. The Sorenson's similarity coefficients of AM fungal community composition ranged from 0.5 to 0.64 among the three types of plot management. The results suggest that the AM fungal diversity is greatly affected by long-term over-grazing and that fencing of degraded areas partly restores plant cover and AM fungal diversity in grassland ecosystems.

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Introduction

Arbuscular mycorrhizal (AM) fungi are the largest component (mycelia and spores) of the microbial biomass in soil (Miller et al. 1995). It has been suggested that they play an important ecological role in determining plant biodiversity and species composition in terrestrial ecosystems (van der Heijden et al. 1998). Likewise, the composition of AM fungal communities may be affected by plant diversity (Eom et al. 2001; Börstler et al. 2006), as well as environmental factors such as soil nutrient content and land use (Landis et al. 2004; Hijri et al. 2006).

The Inner Mongolia steppe, situated at the eastern end of the Eurasian steppe zone, is the largest grassland in China and significantly contributes to Chinese economy and ecology. In recent years, severe degradation and desertification were found because of intense human activities such as grazing, mowing and crop cultivation in the grassland. About one third of available grassland areas are degraded in China. In the Xilin River Basin of the Inner Mongolia steppe, the total degraded area increased from 7,191.3 km^2 in 1985 to 7,689.3 km^2 (72% of the total Basin) in 1999 (Tong et al. 2004). In this area, sheep over-grazing is one of the most important causes of widespread degradation and desertification (Cui et al. 2005). Grazing by herbivores can substantially influence the dynamics of plant communities altering primary production, decomposition of organic matter, cycling and distribution of nutrients and competitive relationships among plant species (McNaughton 1985; Fahnestock and Detling 1999). Concurrently, AM fungal colonization and diversity can be affected by different

managements of grassland such as mowing, fertilization, restoration or grazing (Bethlenfalvay et al. 1985; Eom et al. 2001; Börstler et al. 2006).

To understand the mechanisms underlying degradation, restoration and maintenance of natural grassland ecosystems, permanent plots with different treatments were constructed in the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) of the Chinese Academy of Sciences in the Xilin River Basin of Inner Mongolia. Many ecological studies concerning animals and plants have been carried out in the IMGERS (Kang and Chen 1995; Bai et al. 2004). However, AM fungi, which can have important ecological functions in grassland ecosystems (Smith and Read 1997), have not been studied.

A study was conducted in three permanent plots in the IMGERS, non-grazed, restored and over-grazed, to understand how AM fungal species composition varies in response to the different management systems of a natural grassland ecosystem in the Inner Mongolia steppe.

Materials and methods

Study site

The IMGERS (43°26′–44°08′N, 116°04′–117°05′E) is located in a typical steppe zone of the Inner Mongolia Plateau. It has a semi-arid continental, temperate steppe climate with dry springs and moist summers. Annual mean temperature is 2°C, annual precipitation is 350 mm and more than 70% of annual precipitation occurs between May and August. The altitude is 1,180–1,250 m above sea level, and the soil type is Chestnut.

Leymus chinense (Trin.) Tzvel. is a typical zonal vegetation type in the Inner Mongolia steppe. Three permanent plots, i.e. non-grazed, restored and over-grazed, originating from types of different land use, were selected for this study. The non-grazed plot (approx. 600×400 m), a typical *L. chinense* steppe, was fenced in 1979. At present, there are approximately 25 grass species dominated by *Achnatherum sibiricum* (L.) Keng, *Carex korshinskyi* Kom., *L. chinense* and *Stipa grandis* P. Smirn.; plant community cover is about 80%. The restored plot (approx. 900×300 m), a severely degraded *L. chinense* steppe because of long-term free grazing, was fenced in 1983. This plot was designed for natural restoration, and there are now approximately 15 plant species dominated by *Artemisia frigida* Willd., *Agropyron cristatum* (L.) Gaertner, *Caragana microphylla* Lam. and *C. korshinskyi*; plant community cover is about 60%. The over-grazed plot (approx. 800×300 m), a severely degraded *L. chinense* steppe, was fenced in 1989 then designed for over-grazing (6.7 sheep hm^2) from May to October every year. There are approximately ten plant species dominated by *Allium bidentatum* Fisch. ex Prokh., *A. frigida*, *Kochia prostrata* (L.) Schrad and *Potentilla acaulis* L.; plant community cover is about 20%.

Sampling procedure

Thirty-two soil samples associated with plant roots were randomly collected in September 2005, at sampling points 5 m apart on the middle line transect of each plot. Each soil sample was about 1 kg, taken to a depth of 20 cm. Root systems were isolated from the soil samples, which were then air dried in the shade for 1 week, sieved through a 2-mm sieve, stored at 4°C and processed within 3 months. Because the same plant species were collected in more than one soil sample, a total of 96 (32 for each plot) soil samples from roots of 26 plant species were collected. Of these, 20, eight and 17 plant species were common to the non-grazed, over-grazed and restored plots, respectively.

Trap culture establishment

Trap cultures were set up in a greenhouse to provide fresh spores for identification of AM fungi and encourage sporulation of species present only as hyphae in field samples. Roots isolated from the soil samples were chopped into fragments (<2-cm long) and mixed thoroughly with associated soil. The planting substratum consisted of a 1:1 mixture of the soil sample with root fragments and steam-sterilized sand (1 h, 15 psi). The mixture was poured into plastic pots (10×10 cm). Seeds of clover (*Trifolium repens* L.) were surface sterilized (1% sodium hypochlorite, 5 min), and an average of 25 seeds were sown into each pot after 24-h incubation with water. The mix in each pot was covered with autoclaved sand to prevent unintentional dispersal of AM fungi. All seedlings were grown in greenhouse under natural ambient light and temperature conditions (about 31°C day/17°C night) for 4 months. soil samples were taken and air dried for spore isolation.

Spore isolation and identification of AM fungi

One hundred grams of air-dried soil from each field sample or trap culture was used for spore isolation, using the wet-sieving and decanting method of Gerdemann and Nicolson (1963), modified by Daniels and Skipper (1982). AM fungi were identified following the descriptions of Schenck and Pérez (1988), the information on the International Culture Collection of Arbuscular and Vesicular–Arbuscular Mycorrhizal Fungi (www.invam.caf.wdu.edu) and the original species descriptions with their emendations. At least 20 spores of each species were used for identification. Spores

were first mounted in water for morphological measurements. Melzer's reagent and cotton blue were used in the identification. The permanent slides were mounted in polyvinyl-lacto-glycerol, sealed with nail varnish and stored in the Herbarium Mycologicum Academiae Sinicae in Beijing, China.

Data analysis

AM fungal composition in field samples was evaluated based spore isolation frequency (IF), density, relative abundance (RA), species richness and importance value (IV). IF was calculated as the percentage of samples from which spores of a particular genus or species were isolated. Spore density (spores per 100 g air-dried soil) was calculated from direct counts of spores. RA was calculated as the number of spores of a particular genus or species divided by the total number of spores. Species richness was defined as the number of AM fungal species per 100 g air-dried soil sample (Koske 1987). Spore biovolume (Dickman et al. 1984) was calculated by multiplying the average spore density of a species in a zone by the average volume of an individual spore, and this value is expressed as volume of spores/100 g air-dried soil. The spore volume was calculated from the equation, $\mathrm{vol} = \frac{4}{3}\pi r^3$. Relative spore biovolume (RB) was calculated as the biovolume of spores of a particular genus or species divided by the total biovolume of spores (Dickman et al. 1984). IV was calculated as $\mathrm{IV} = \mathrm{IF} + \mathrm{RA} + \mathrm{RB}$ (Koske 1987). The degree of dominance was described as: dominant species or genus (IV≥50%), common species or genus (10%<IV<50%) and rare species or genus (IV≤ 10%). To evaluate the degree of community similarity of AM fungi between two sites, Sorenson's coefficient (C_S) was employed and calculated as $CS = 2\mathrm{j}/(\mathrm{a} + \mathrm{b})$, where j is the number of AM fungus species co-existing in two plots, a is the total number of AM fungus species in one plot and b is the total number of AM fungus species in the other plot.

Data on AM fungal spore density and species richness were analyzed using one-way analysis of variance to test the difference among the three plots (SPSS, Chicago, IL). The statistically significant difference was determined at $p<0.05$ level.

Results

AM fungal composition

A total of 19 taxa belonging to six genera of AM fungi were found based on spores isolated from the field samples and trap cultures. Most fungi identified from field samples sporulated in trap culture. However, spores of *Archaeospora trappei* were not isolated from field samples, but they were obtained in trap culture. Of the AM fungi obtained in trap cultures, one belonged to *Acaulospora*, one to *Archaeospora*, one to *Entrophospora*, one to *Gigaspora*, 12 to *Glomus* and three to *Scutellospora* (Table 1).

Glomus was the dominant genus in the three plots, and *Scutellospora* was the second dominant genus in the non-grazed plot (Table 2). *Glomus geosporum* was dominant in the three plots. *G. albidum* and *G. etunicatum* were the dominant species in the restored plot, and *Scutellospora calospora* was dominant in the non-grazed plot.

Different numbers of AM fungal species were isolated from the three plots, i.e., 16 from non-grazed, 11 from restored and ten from over-grazed plots. The Sorenson's similarity coefficient (C_S) of AM fungal composition was highest between non-grazed and restored plots (0.64) and between restored and over-grazed plots (0.63) and lowest (0.5) between non-grazed and over-grazed plots.

AM fungal spore density and species richness

Mean spore density of AM fungi was significantly ($p<0.05$) higher in non-grazed (21.1±2.6/100 g air-dried soil) than in over-grazed (8.3±1.2/100 g air-dried soil) plots, but there were no significant differences between non-grazed and restored (14.6±1.9/100 g air-dried soil) plots or between restored and over-grazed plots. Mean species richness of AM fungi was significantly ($p<0.05$) higher in non-grazed (2.6±0.2) than in the other two plots, whilst no significant difference was observed between the restored (1.7±0.2) and over-grazed (1.3±0.2) plots.

Non-*Glomus* species, such as *Acaulospora tuberculata*, *Entrophospora infrequens*, *Gigaspora* sp., *S. calospora*, and *S. pellucida*, were drastically affected by grazing, and spores were not detected in field samples from the over-grazed plot (Table 1). Within *Glomus* species, spore densities of *G. fasciculatum*, *G. geosporum*, *G. intraradices* and *G. mosseae* were significantly ($p<0.05$) higher in non-grazed plots than in the other two plots, but there was no significant difference between restored and over-grazed plots. The spore density of *G. etunicatum* was significantly ($p<0.05$) higher in restored as compared to non-grazed plots. There were no significant differences in spore densities of *G. aggregatum*, *G. albidum*, *G. ambisporum* and *Glomus* sp. 1 among the three plots.

Discussion

The number of plant species in the grassland plots of the Inner Mongolia steppe decreased from 25 to 10 because of long-term over-grazing, and plant community cover was

Table 1 The importance value (IV=isolation frequency relative abundance relative biovolume) and spore density (Density, per 100 g air-dried soil, mean±SE, n=32) of AM fungi isolated from the three plots

Species	Non-grazed plot		Restored plot		Over-grazed plot	
	IV	Density	IV	Density	IV	Density
Acaulospora tuberculata Janos & Trappe	9.4	0.2				
Archaeospora trappei (R.N. Ames & Linderman) J.B. Morton & D. Redecker[+]						
Entrophospora infrequens (I.R. Hall) R.N. Ames & R.W. Schneid.	4.6	0.2				
Gigaspora sp.	9.2	0.03				
Glomus aggregatum N.C. Schenck & G.S. Sm.		0[a]	4.4	0.2±0.2[a]	15.5	0.8±0.8[a]
G. albidum C. Walker & L.H. Rhodes	15.9	0.4±0.2[a]	65	3.3±1.8[a]	32.1	0.5±0.3[a]
G. ambisporum G.S. Sm. & N.C. Schenck	22.1	1.4±0.9[a]	4.7	0.2±0.2[a]		0[a]
G. etunicatum W.N. Becker & Gerd.	24.3	0.8±0.3[a]	70.2	4.5±1.6[b]	46.4	1.6±0.7[ab]
G. fasciculatum (Thaxt.) Gerd. & Trappe	39.6	2.2±0.7[a]	4.3	0.2±0.2[b]		0[b]
G. geosporum (T.H. Nicolson & Gerd.) C. Walker	192.3	11.6±1.7[a]	141.2	6.2±1.3[b]	160.9	4.2±0.8[b]
G. intraradices N.C. Schenck & G.S. Sm.	35.7	1.6±0.8[a]	5.1	0.2±0.2[b]	5.6	0.1±0.1[b]
G. mosseae (T.H. Nicolson & Gerd.) Gerd. & Trappe	15.5	0.4±0.2[a]		0[b]	4.4	0.1±0.1[b]
Glomus sp. 1		0[a]	16.9	0.6±0.4[a]	7.2	0.2±0.2[a]
Glomus sp. 2	4.6	0.2±0.2[ab]		0[a]	36.9	0.9±0.4[b]
Glomus sp. 3	5	0.8				
Glomus sp. 4	3.5	0.2				
Scutellospora calospora (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders	64.7	1.6±0.4[a]	24.9	0.6±0.3[b]		0[b]
S. pellucida (T.H. Nicolson & N.C. Schenck) C. Walker & F.E. Sanders	6.7	0.2±0.2[a]	16.5	0.3±0.2[a]		0[a]
Scutellospora sp.					8.6	0.1

Plus sign, only isolated from trap culture
Values in the same line with different alphabetical letter are significantly different at $p<0.05$ level

greatly reduced from 80 to 20%. Such decreases in aboveground plant biomass would reduce the carbon-source capacity of plants to such a degree that there was insufficient carbon to meet the demands of AM fungi, leading to reductions in mycorrhizal colonization.

A high number (19 taxa) of AM fungal species were found in the grassland plots of the Inner Mongolia steppe, in comparison to similar studies of arid and semi-arid grassland ecosystems in North America and Tibet (Hetrick and Bloom 1983; Stutz et al. 2000; Gai et al. 2006). The abundance of sporulating AM fungi was affected by grazing in the grassland ecosystem with lower species number and spore density in over-grazed as compared to non-grazed plots. Decreased AM fungal diversity and spore density was likewise reported in a grazed Nevada rangeland (Bethlenfalvay et al. 1985). Reductions in AM fungal diversity in over-grazed plots have been ascribed to reductions in plant diversity by long-term over-grazing (Landis et al. 2004; Uhlmann et al. 2004). Within this context, the number of plant species in the grassland plots of the Inner Mongolia steppe decreased from 25 to 10 because of long-term over-grazing, and plant community cover was greatly reduced from 80 to 20%. Such decreases in aboveground plant biomass by over-grazing would reduce the carbon-source capacity of plants to meet demands of the AM fungi, and the subsequent effects on AM fungal diversity and abundance may influence ecosystem stability in Inner Mongolia steppe.

Table 2 The importance value (IV=isolation frequency relative abundance relative biovolume) of AM fungal genera isolated from the three plots

Genus	Non-grazed plot	Restored plot	Over-grazed plot
Acaulospora	9.5		
Entrophospora	4.7		
Gigaspora	9.5		
Glomus	253	267.7	277.2
Scutellospora	70.1	41.7	13.4

AM fungi can have different tolerance and competitive ability to the pressure of grazing. The observation that non-*Glomus* species are particularly affected by over-grazing in the Inner Mongolia steppe grassland studied here concords with findings that intensified management intensities in arable lands affect non-*Glomus* species (Jansa et al. 2002; Oehl et al. 2004; Hijri et al. 2006). Although *Glomus* species such as *G. fasciculatum*, *G. geosporum*, *G. intraradices* and *G. mosseae* were sensitive to over-grazing, other *Glomus* species were not significantly affected by long-term over-grazing in the present study. Similarly, *G. geosporum* and *G. mosseae* were only found in a lightly grazed site in an arid region of Namibia and

not in moderately or heavily grazed sites (Uhlmann et al. 2006). It has been suggested that (1) reduced photosynthesis or plant species diversity affects AMF communities and species numbers and (2) AM fungi in association with grazing-tolerant plant species produce more spores than those in the rhizosphere of grazing-sensitive species (Allen et al. 1989; Eom et al. 2001).

In conclusion, there was a high number of AM fungi in the semi-arid grassland of Inner Mongolia at IMGERS. However, AM fungal community composition was conspicuously affected by long-term over-grazing, and AM fungal species showed different responses to this grassland management practice. Decreases in aboveground plant biomass by over-grazing with subsequent effects on AM fungal diversity and abundance may influence ecosystem stability in the Inner Mongolia steppe grassland.

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