

# Arbuscular mycorrhizal fungi in degraded typical steppe of Inner Mongolia

Tian, H., Gai, J. P., Zhang, J. L., Christie, P., & Li, X. L. (2009). Arbuscular mycorrhizal fungi in degraded typical steppe of Inner Mongolia. Land Degradation and Development, 20(1), 41-54. DOI: 10.1002/ldr.876

# Published in:

Land Degradation and Development

**Queen's University Belfast - Research Portal:** Link to publication record in Queen's University Belfast Research Portal

#### **General rights**

Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

#### LAND DEGRADATION & DEVELOPMENT

Land Degrad. Develop. 20: 41-54 (2009)

Published online 31 October 2008 in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/ldr.876

# ARBUSCULAR MYCORRHIZAL FUNGI IN DEGRADED TYPICAL STEPPE OF INNER MONGOLIA

# H. TIAN<sup>1</sup>, J. P. GAI<sup>1\*</sup>, J. L. ZHANG<sup>1</sup>, P. CHRISTIE<sup>1,2</sup> AND X. L. LI<sup>1</sup>

<sup>1</sup>Department of Plant Nutrition, China Agricultural University, 2 Yuanmingyuan West Road, Beijing 100094, P.R. China <sup>2</sup>Agricultural and Environmental Science Department, Queen's University Belfast, Newforge Lane, Belfast BT9 5PX, UK

Received 10 April 2008; Revised 29 June 2008; Accepted 30 June 2008

#### 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 km<sup>2</sup> 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).

<sup>\*</sup> Correspondence to: J. P. Gai, Department of Plant Nutrition, China Agricultural University, 2 Yuanmingyuan West Road, Beijing 100094, P.R. China.

E-mail: gaijp@cau.edu.cn

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 ( $43^{\circ}26'-44^{\circ}34'N$ ,  $115^{\circ}30'-117^{\circ}12'E$ ), Xilin Gol League of Inner Mongolia municipality, northwest China. This region covers an area of about 10 000 km<sup>2</sup> 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  $2 \cdot 1^{\circ}C$  (Chen, 1988). The lowest average temperature (January) is  $-17^{\circ}C$ , and the highest (July) is  $18^{\circ}C$ . 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 southernmost 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  $30.1 \times 1 \text{ m}^2$  quadrats along the transect with a minimum distance between adjacent quadrats of 25 m. Soil samples were collected by dividing each quadrat into four  $0.25 \text{ m}^2$  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 quadrat. 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 quadrat 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.

$$H' = -\sum \left(P_i\right) \ln(P_i) \tag{1}$$

#### Soil Analysis

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

Site (degree of degradation)		Soil		Vegetation								
	Olsen P (P) $(mg kg^{-1})$	Organic matter (OM) (per cent)	pH (water, 1:2·5 soil/water)	Dominant plant species	Cover (per cent)	Height (cm)	Plant species richness (PSR)	Plant Shannon–Weiner index (PH')				
Undegraded	4.48 b	3.58 a	7·48 a	Leymus chinensis (Trin.) Kitag	70·7 a	28·1 a	11.6 b	1.25 b				
Moderate	7.94 a	3.00 b	7·31 b	Agropyron michnoi Roshev	50.5 b	5.6 b	8.3 c	1.13 b				
Severe	5.38 b	1.53 c	7.27 b	Artemisia frigida Willd; Potentilla acaulis L.	34.6 c	5·1 c	13·6 a	1.61 a				

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

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  $100-400 \times$  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 flotationcentrifugation in 60 per cent sucrose (Dalpé, 1993). The finest sieve used was 40  $\mu$ m and the spores were collected on a grid patterned (4 × 4 mm<sup>2</sup>) 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') 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 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 Šmilauer, 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 Šmilauer, 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



Figure 1. AM root colonization of the four indicator plant species (A): *Leynus 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.

Copyright © 2008 John Wiley & Sons, Ltd.

LAND DEGRADATION & DEVELOPMENT, 20: 41–54 (2009) DOI: 10.1002/ldr

AMF genus		Species number		]	Frequency (F) (per cent)	Relative abundance (RA) (per cent)			
	UD	М	S	UD	М	S	UD	М	S
Acaulospora	4	4	3	30	30	10	3.46	3.06	4.51
Appendicispora	1	1	1	10	3.3	3.3	1.86	0.44	0.75
Entrophospora	1	1	0	10	10	0	1.06	1.31	0
Glomus	26	18	14	100	100	100	80.59	92.58	90.23
Kuklospora	1	1	0	10	10	0	0.8	1.31	0
Paraglomus	1	1	0	6.7	3.3	0	0.53	0.44	0
Scutellospora	4	2	2	70	6.67	13.3	11.70	0.87	4.51

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

UD, undegraded; M, moderately degraded; S, severely degraded.

(Tables II and III). 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 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 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  $28 \cdot 1$  and  $54 \cdot 7$  per cent in the moderately and severely degraded areas, respectively. The Shannon–Weiner diversity index of the AM species also declined correspondingly by  $19 \cdot 0$  and  $39 \cdot 3$  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' 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' had the highest correlation coefficient with OM. SD and AH' also had significant positive correlations with soil pH. ASR and PH' were negatively correlated.

Table III.	Frequency	(F)	and	relative	abundance	(RA)	of	the	AM	fungus	species	identified	from	the	three	grassland
communit	ies															

AM species	Fre (	quency (per cent	(F) )	Relative abundance (RA) (per cent)		
	UD	М	S	UD	М	S
Acaulospora delicata Walker, Pfeiffer & Bloss	0	0	3.3	0	0	1.50
Acaulospora longula Spain & Schenck	0	6.7	6.7	0	0.87	1.50
Acaulospora nicolsonii Walker, Reed & Sanders	16.7	6.7	3.3	1.59	0.87	1.50
Acaulospora rehmii Sieverding & Toro	6.7	0	0	0.79	0	0
Acaulospora rugosa Morton	6.7	0	0	0.79	0	0
Acaulospora sp.	0	3.3	0	0	0.44	0
Acaulospora spinosa Walker & Trappe	3.3	6.7	0	0.26	0.87	0
Appendicispora appendicula Spain, Oehl & Sieverd	0	3.3	3.3	0	0.44	0.75
Appendicispora leptoticha C. Walker, Vestberg & Schuessler	10.0	0	0	1.85	0	0
Entrophospora infrequens (Hall) Ames & Schneider	10.0	10.0	0	1.06	1.31	0
Glomus aggregatum Schenck & Smith emend. Koske	26.7	76.7	50.0	5.29	16.16	15.79
Glomus albidum Walker & Rhodes	16.7	3.3	0	1.32	0.87	0
Glomus ambisporum Smith & Schenck	6.7	0	0	0.53	0	0
Glomus caledonium (Nicol. & Gerd) rappe & Gerde	26.7	0	0	3.97	0	0
Glomus claroideum Schenk & Smith	43.3	26.7	0	5.82	10.48	0
Glomus clarum Nicolson & Schenck	26.7	0	0	11.90	0	0
Glomus constrictum Trappe	10.0	0	6.7	0.79	0	3.01
Glomus convolutum Gerdemann & Trappe	10.0	0	0	1.59	0	0
Glomus delhiense Mukerji, Blattacharjee, & Tewari	23.3	0	0	2.12	0	0
Glomus deserticola Trappe, Bloss & Menge	6.7	0	3.3	0.53	0	0.75
Glomus diaphanum Morton & Walker	0	6.7	0	0	0.87	0
Glomus eburneum Kennedy, Stuta et Morton	6.7	0	0	0.53	0	0
Glomus etunicatum Becher & Gerdemann	23.3	30.0	33.3	3.97	6.11	15.04
Glomus fasciculatum (Thaxter) Gerd. & Trappe emend. Walker & Koske	13.3	0	3.3	1.59	0	0.75
Glomus geosporum (Nicol. & Gerd.) Walker	66.7	60.0	53.3	13.23	16.59	29.32
Glomus heterosporum Smith & Schenck	10.0	16.7	10.0	1.06	2.18	2.26
Glomus hoi Berch & Trappe	10.0	26.7	10.0	1.32	3.49	2.26
Glomus intraradices Schenck & Smith	23.3	30.0	6.7	2.65	4.80	2.26
Glomus lacteum Rose & Trappe	6.7	3.3	6.7	0.53	0.44	2.26
Glomus magnicaule Hall	26.7	13.3	0	4.50	4.37	0
Glomus manihotis Howeler, Sieverding & Schenck	10.0	6.7	0	1.59	0.87	0
Glomus maculosum Miller & Walker	0	3.3	0	0	0.44	0
Glomus monosporum Gerdemann & Trappe	0	6.7	3.3	0	0.87	0.75
Glomus mosseae (Nicol. & Gerd) Gerdemann & Trappe	33.3	36.7	20.0	5.82	12.23	6.77
Glomus multicaule Gerdemann & Bakshi	0	3.3	0	0	0.44	0
Glomus radiatum (Thaxter) Trappe & Gerd	6.7	0	0	1.06	0	0
Glomus reticulatum Bhattacharjee & Mukerji	3.3	0	0	0.53	0	0
Glomus tenerum Tandy emend. McGee	26.7	40.0	23.3	3.44	10.92	8.27
Glomus tortuosum Schenck & Smith	3.3	0	0	2.65	0	0
Glomus versiforme (Karsten) Berch	10.0	3.3	3.3	2.38	0.44	0.75
Kuklospora kentinensis Oehl & Sieverd	10.0	10.0	0	0.79	1.31	0
Paraglomus occultum (Walker) Morton	6.7	3.3	0	0.53	0.44	0
Scutellospora calospora (Nicol. & Gerd) Walker & Sanders	56.7	3.3	13.3	9.79	0.44	3.76
Scutellospora cerradensis Spian & Miranda	3.3	0	0	0.26	0	0
Scutellospora erythropa (Koske & Walker) Walker & Sanders	0	3.3	0	0	0.44	0
Scutellospora pellucida Nicolson & Schenck	16.7	0	3.3	1.32	0	0.75
Scutellospora sp.	3.3	0	0	0.26	0	0

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 (AH') (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.

Copyright © 2008 John Wiley & Sons, Ltd.

AM community parameters		Soil propertie	S	Vegetation parameters					
	pH	ОМ	Р	Height	Cover	PSR	Ρ <i>Η</i> ′		
ASR	0.182	0.470**	-0.081	0.524**	0.506**	-0.125	-0.362**		
AH' SD	0·338** 0·504**	0·501** 0·698**	$-0.027 \\ -0.162$	0·438** 0·777**	0·424** 0·643**	$0.104 \\ -0.095$	$-0.088 \\ -0.147$		

Table IV. Correlation analysis between AM community and some abiotic and biotic variables; n = 90; correlation coefficients are Pearson coefficients

*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.

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; 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 rehmii; A.ru: Acaulospora rugosa; A.s: Acaulospora spinosa; Ap.I: Appendicispora leptoticha; E.i: Entrophospora infrequens; G.ag: Glomus aggregatum; G.al: Glomus albidum; G.c: Glomus caledonium; G.c: Glomus claroideum; G.cl: Glomus clarum; G.co: Glomus constrictur; G.g: Glomus geosporum; G.h: Glomus heterosporum; G.h: Glomus delhiense; G.de: Glomus manihotis; G.l: Glomus lacteum; G.magi Clomus magnicaule; G.man: Glomus manihotis; G.mon: Glomus monosporum; G.m: Glomus mosseae; G.r. Glomus radiatum; G.t: 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 pelluclda.* 

Copyright © 2008 John Wiley & Sons, Ltd.

LAND DEGRADATION & DEVELOPMENT, 20: 41–54 (2009) DOI: 10.1002/ldr 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 photosynthate 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 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 Siniscalo, 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.

#### ACKNOWLEDGEMENTS

This work was funded by the National Natural Science Foundation of China (Project 40571078) and the British Council with the UK Department for International Development through their Development Partnerships in Higher Education program (Project DelPHE 1.64). We also thank the Inner Mongolia Grassland Ecosystem Research Station, Chinese Academy of Sciences, for permission to use the sampling sites.

#### REFERENCES

- Aldrich-Wolfe L. 2007. Distinct mycorrhizal communities on new and established hosts in a transitional tropical plant community. *Ecology* 88: 559–566.
- Artz RRE, Anderson IC, Chapman SJ, Hagn A, Schloter M, Potts JM, Campbell CD. 2007. Changes in fungal community composition in response to vegetational succession during the natural regeneration of cutover peatlands. *Microbial Ecology* 54: 508–522.
- Bai YF, Han XG, Wu JG, Chen ZZ, Li LH. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. *Nature* **431**: 181–184.
- Barni E, Siniscalo C. 2000. Vegetation dynamics and arbuscular mycorrhiza in old-field successions in the western Italian Alps. *Mycorrhiza* **10**: 63–72.
- Bethlenfalvay GJ, Dakessian S. 1984. Grazing effects on mycorrhizal colonization and floristic composition of the vegetation on a semiarid range in northern Nevada. *Journal of Range Management* **37**: 312–316.
- Bethlenfalvay GJ, Evans RA, Lesperance AL. 1985. Mycorrhizal colonization of crested wheatgrass as influenced by grazing. *Agronomy Journal* **77**: 233–236.
- Busso CA, Briske DD, Olalde-Portugal V. 2001. Root traits associated with nutrient exploitation following defoliation in three coexisting perennial grasses in a semi-arid savanna. *Oikos* **93**: 332–342.
- Chen ZZ. 1988. Topography and Climate of Xilin River Basin (in Chinese). Science Press: Beijing.
- Christensen L, Coughenour MB, Ellis JE, Chen ZZ. 2004. Vulnerability of the Asian typical steppe to grazing and climate change. *Climate Change* **63**: 351–368.
- Cooke MA, Widden P, O'Halloran I. 1993. Development of vesicular-arbuscular mycorrhizae in sugar maple (*Acer saccharum*) and effects of base-cation amendment on vesicle and arbuscle formation. *Canadian Journal of Botany* **71**: 1421–1426.
- Coughlan AP, Dalpe Y, Lapointe L, Piche Y. 2000. Soil pH-induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research* **30**: 1543–1554.
- Cuenca G, Meneses E. 1996. Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil* 183: 315–322.
- Dalpé Y. 1993. Vesicular-arbuscular mycorrhiza. In *Soil Sampling and Methods of Analysis*, Carter MR (ed.). Lewis Publishers: Boca Raton; 287–301.
- Davidson JL, Milthorpe FL. 1966. The effect of defoliation on the carbon balance in Dactylis glomerata. Annals of Botany 30: 185-198.
- Dhillion SS, Gardsjord TL. 2004. Arbuscular mycorrhizas influence plant diversity, productivity, and nutrients in boreal grasslands. *Canadian Journal of Botany* 82: 104–114.
- Duckmanton L, Widden P. 1994. Effect of ozone on the development of vesicular-arbuscular mycorrhizae in sugar maple saplings. *Mycologia* 86: 181–186.
- Eom AH, Wilson GWT, Hartnett DC. 2001. Effects of ungulate grazers on arbuscular mycorrhizal symbiosis and fungal community structure in tallgrass prairie. *Mycologia* **93**: 233–242.
- Fahnestock JT, Detling JK. 1999. The influence of herbivory on plant cover and species composition in the Pryor Mountain Wild Horse Range, USA. *Plant Ecology* **144**: 145–157.
- Ferrol N, Calvente R, Cano C, Barea JM, Azcón-Aguilar C. 2004. Analysing arbuscular mycorrhizal fungal diversity in shrub-associated resource islands from a desertification-threatened semiarid Mediterranean ecosystem. *Applied Soil Ecology* **25**: 123–133.

Frank DA, Gehring CA, Machut L, Phillips M. 2003. Soil community composition and the regulation of grazed temperate grassland. *Oecologia* **137**: 603–609.

- Gehring CA, Whitham TG. 1994. Interactions between above-ground herbivores and the mycorrhizal mutualists of plants. *Trends in Ecology and Evolution* **9**: 251–255.
- Green DR. 1989. Rangeland restoration projects in western New South Wales. Australian Rangeland Journal 11: 110-116.
- Grigera G, Oesterheld M. 2004. Continuous grazing is associated with an increase of mycorrhizal colonization at the community level. *Journal of Range Management* 57: 601–605.

Copyright © 2008 John Wiley & Sons, Ltd.

- Grime JP, Mackey JML, Hillier SH, Read DJ. 1987. Floristic diversity in a model system using experimental microcosms. *Nature* **328**: 420–422. Harley JL, Smith SE. 1983. *Mycorrhizal Symbiosis*. Academic Press: London.
- Hayman DS. 1982. Influence of soils and fertility on activity and survival of vesicular arbuscular mycorrhizal fungi. *Phytopathology* **72**: 1119–1125.
- Hinojosa MB, Carreira JA, Garcia-Ruiz R, Dick RP. 2005. Microbial response to heavy metal-polluted soils: Community analysis from phospholipid-linked fatty acids and ester-linked fatty acids extracts. *Journal of Environmental Quality* **34**: 1789–1800.
- Jiang GM, Han XG, Wu JG. 2006. Restoration and management of the Inner Mongolia grassland require a sustainable strategy. Ambio 35: 269– 270.
- Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Booth RE, Grime JP, Young JPW, Read DJ. 2003. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist* **161**: 503–515.
- Koske RE, Tessier B. 1983. A convenient, permanent slide mounting medium. Mycological Society of America Newsletter 34: 59.
- Kurle JE, Pfleger FL. 1996. Management influences on arbuscular mycorrhizal fungal species composition in a corn-soybean rotation. *Agronomy Journal* **88**: 155–161.
- Li B. 1997. The degradation of grassland in North China and its countermeasure (in Chinese, with English abstract). *Scientia Agricultura Sinica* **30**: 1–10.
- Liu ZL, Wang W, Hao DY, Liang CZ. 2002. Probes on the degeneration and recovery succession mechanisms of Inner Mongolia steppe (in Chinese, with English abstract). *Journal of Arid Land Resources and Environment* 1: 84–91.
- McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA. 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist* **115**: 495–501.

McNaughton SJ. 1985. Ecology of a grazing ecosystem: The Serengeti. Ecological Monographs 55: 259-294.

- Mohammad MJ, Hamad SR, Malkawi HI. 2003. Population of arbuscular mycorrhizal fungi in semi-arid environment of Jordan as influenced by biotic and abiotic factors. *Journal of Arid Environments* 53: 409–417.
- Oehl F, Sieverding E, Ineichen K, Mäder P, Boller T, Wiemken A. 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. *Applied and Environmental Microbiology* **69**: 2816–2824.
- Panwar J, Tarafdar JC. 2006. Distribution of three endangered medicinal plant species and their colonization with arbuscular mycorrhizal fungi. *Journal of Arid Environments* **65**: 337–350.
- Porter WM, Robson AD, Abbott LK. 1987. Field survey of the distribution of vesicular-arbuscular mycorrhizal fungi in relation to soil pH. *Journal of Applied Ecology* 24: 659–662.
- Redecker D, Hijri I, Wiemken A. 2003. Molecular identification of arbuscular mycorrhizal fungi in roots: Perspectives and problems. Folia Geobotanica 38: 113–124.
- Reece PE, Bonham CD. 1978. Frequency of endomycorrhizal infection in grazed and ungrazed blue grama plants. *Journal of Range Management* **31**: 149–151.
- Rosales J, Cuenca G, Ramírez N, De Andrade Z. 1997. Native colonizing species and degraded land restoration in La Gran Sabana, Venezuela. *Restoration Ecology* **5**: 147–155.
- Saito K, Suyama Y, Sato S, Sugawara K. 2004. Defoliation effects on the community structure of arbuscular mycorrhizal fungi based on 18s rDNA sequences. *Mycorrhiza* 14: 363–373.
- Schenck NC, Perez Y. 1990. Manual for the Identification of Vesicular-Arbuscular Mycorrhizal Fungi. INVAM: University of Florida, Gainesville, FL.
- Shi ZY, Feng G, Christie P, Li XL. 2006. Arbuscular mycorrhizal status of spring ephemerals in the desert ecosystem of Junggar Basin, China. *Mycorrhiza* 16: 269–275.
- Shi ZY, Zhang LY, Li XL, Feng G, Tian CY, Christie P. 2007. Diversity of arbuscular mycorrhizal fungi associated with desert ephemerals in plant communities of Junggar Basin, northwest China. Applied Soil Ecology 35: 10–20.
- Silva GA, Trufem SFB, Saggin Junior OJ, Maia LC. 2005. Arbuscular mycorrhizal fungi in a semiarid copper mining area in Brazil. *Mycorrhiza* **15**: 47–53.
- Smith SE, Read DJ. 1997. Mycorrhizal Symbiosis, 2nd edn. Academic Press: London, UK.
- ter Braak CJF, Šmilauer P. 2002. CANOCO Reference Manual and Canodraw for Windows User's Guide: Software for Canonical Community Ordination, 4.5 edn. Microcomputer Power: Ithaca, NY.
- Titus JH, Lepš J. 2000. The response of arbuscular mycorrhizae to fertilization, mowing, and removal of dominant species in a diverse oligotrophic wet meadow. *American Journal of Botany* 87: 392–401.
- Trouvelot A, Kough JL, Gianinazzi-Pearson V. 1986. Mesure dutaux de mycorrhization VA d'un systeme radiculaire. Recherche de methodes d'estimation ayant une signification functionnelle. In *Physiological and Genetic Aspects of Mycorrhizae*, Gianinazzi-Pearson V, Gianinazzi S (eds). INRA: Paris; 217–221.
- Turnau K, Miszalski Z, Trouvelot A, Bonfante P, Gianinazzi S. 1996. Oxalis acetosella as a monitoring plant on highly polluted soils. In Mycorrhizas in Integrated Systems Form Genes to Plant Development, Azcon-Aguilar C, Barea JM (eds). In Proceedings of the Fourth European Symposium on Mycorrhizas. COST Edition, European Commission: Brussels, Luxembrug; 483–486.
- Uhlmann E, Görke C, Petersen A, Oberwinkler F. 2004. Arbuscular mycorrhizae from semiarid regions of Namibia. *Canadian Journal of Botany* **82**: 645–653.
- Uhlmann E, Gorke C, Petersen A, Oberwinkler F. 2006. Arbuscular mycorrhizae from arid parts of Namibia. *Journal of Arid Environments* 64: 221–237.
- van der Heijden M, Klironomos J, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders I. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* **396**: 69–72.
- Wallace LL. 1981. Growth, morphology and gas exchange of mycorrhizal and nonmycorrhizal Panicum coloratum L., a C<sub>4</sub> grass species, under different clipping and fertilization regimes. Oecologia 49: 272–278.
- Wang JW, Cai WQ. 1988. Studies on Genesis, Types and Characteristics of the Soils of the Xilin River Basin (in Chinese). Science Press: Beijing.

Copyright © 2008 John Wiley & Sons, Ltd.

#### H. TIAN ET AL.

- Wu JG, Loucks O. 1992. Grasslands and Grassland Sciences in Northern China (U.S. National Research Council, ed.). National Academy Press: Washington, DC.
- Yoshino M. 2001. Relationship between land degradation and sand dust storm occurrence, aeolian sand transport and its damages in east Asia during the recent years. In *Integrated Land Management in Dry Areas*, Adeel Z (ed.). (UNU Desertification Series No. 4), Kinkosha Printers: Tokyo; 119–136.
- Zhao DD, Zhao ZW. 2007. Biodiversity of arbuscular mycorrhizal fungi in the hot-dry valley of the Jinsha River, southwest China. *Applied Soil Ecology* **37**: 118–128.
- Zhou G, Wang Y, Wang S. 2002. Response of grassland ecosystems to precipitation and land use along the Northeast China Transect. *Journal of Vegetation Science* 13: 361–368.
- Zhou HR, Zhao XQ, Tang YH, Song G, Li Z. 2005. Alpine grassland degradation and its control in the source region of the Yangtze and Yellow River, China. *Japanese Society of Grassland Science* **51**: 191–203.