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A preliminary survey of the arbuscular mycorrhizal status of grassland plants in southern Tibet

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Abstract We report for the first time the arbuscular mycorrhizal (AM) status of native plant species and AM fungal diversity in the grasslands of southern Tibet. A total of 51 soil samples were collected from the rhizospheres of the dominant plant species, and AM fungal structures were observed in 18 (82%) of 22 plant species examined. Vesicles and aseptate hyphae were the structures most frequently observed in the plant roots. After trap culture for 5 months, 25 AM fungal taxa were identified in the soil samples collected, of which nine belonged to *Glomus*, ten to *Acaulospora*, one to *Entrophospora* and five to *Scutellospora*. The frequency of occurrence of different genera and species varied greatly. *Glomus* was the dominant genus, and the most frequent and abundant species was *Glomus mosseae*. Over the whole sampling area, spore density in the rhizosphere soil of different host plant species ranged from 2 to 66 per 20 g air-dried soil. Overall AM fungal species richness was 2.10 and species diversity was 2.35. AM fungal diversity was also compared among the four different land use types (farmland and normal, disturbed and highly disturbed montane scrub grassland). Spore densities in the farmland and normal grassland were much higher than in the grasslands that had been degraded to varying degrees. The species richness in normal grassland was the highest of the four land use types examined. Species

diversity varied from 1.99 to 0.94 and was highest in normal grassland, intermediate in degraded grassland and farmland, and lowest in the highly disturbed grassland.

Keywords AM fungal structure · Species diversity · Grassland · Tibet

Introduction

Tibet is located to the southwest of China (26°44'–36°32', N 78°25'–99°06'E). It is the largest and highest plateau in the world. The average altitude is 4,500 m above sea level and it is sometimes called the 'roof of the world'. High mountains including the Kunlun Mountains, the Kela Kunlun ranges and the steep Hengduan ranges surround it.

Due to the extreme environment, grassland is quite important for the Tibetan way of life. As one of China's five important pastoral areas, Tibet has 82.07 million hectares of grassland, of which 70.77 million hectares are usable. Tibetan grasslands belong to the arid or semi-arid type of high-altitude frigid zone. The harsh geographical and climatic conditions have led to the natural desertification of the pastures. Large-scale grazing has also exacerbated the degeneration of the pastures and reduced the growth of grass, giving rise to further ecological degradation. The restoration of prairie communities in Tibet has received considerable attention in recent years. These restoration efforts often involve planting prairie species in highly disturbed habitats.

It is well-established that arbuscular mycorrhizal (AM) fungi are ubiquitous and abundant in grasslands worldwide (Richter et al. 2002; Hartnett and Wilson 2002; Stutz et al. 2000). They can benefit plants by increasing the uptake of nutrients, especially phosphorus (Jayachandran and Shetty 2003), increasing drought tolerance (Klironomos et al. 2001; Bever et al. 2001) and potentially protecting roots from plant pathogens (Graham 2001). In recent years, the importance of AM fungal diversity for plant diversity, productivity and ecosystem processes has been recognised (van der Heijden et al. 1998; Bever 2002; Burrows and

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Pfleger 2002). Numerous studies have focused on the diversity of AM fungi in grasslands and their role in the restoration of highly disturbed areas (Smith et al. 1998; Anken et al. 2004). These studies have shown that AM fungi exert a significant influence on plant community structure and dynamics in grasslands and other terrestrial ecosystems (Koide and Dickie 2002; Ferrol et al. 2004). The work of van der Heijden et al. (1998) in the grasslands of Europe and North America has indicated that increasing the diversity of AM fungi might directly increase the diversity of plants.

However, the effects of AM fungi on their host plant communities are not absolute but context-dependent, varying with host species, plant life history stage, resource availability, and abiotic conditions (O'Connor et al. 2002). The taxonomic or genotypic composition of the fungal community may also be important (Hartnett and Wilson 2002). For these reasons, a rehabilitation approach for revegetation of degraded ecosystems must begin with the evaluation of the mycorrhizal status and with the isolation, identification and characterization of the native AM fungi in the target area.

There have been few studies reported on the AM fungal diversity in areas of high altitude. Read and Haselwandter (1981) investigated the nival zone 3,000 m above in the Austrian Alps and found that *Ranunculus glacialis* appeared to be free of all fungal colonization. However, at lower altitudes where there is a consistent snow-free season and a continuous vegetation cover, AM colonization may occur. Mullen and Schmidt (1993) found AM colonization of *Ranunculus adoneus* at 3,500 m in the alpine zone of the Colorado Front Range. There have been no published reports on the AM status of grassland plants in Tibet, and the aim of the present preliminary study was to investigate the mycorrhizal status of native plants and AM fungal diversity on the Tibetan plateau.

Materials and methods

Study sites and sampling procedure

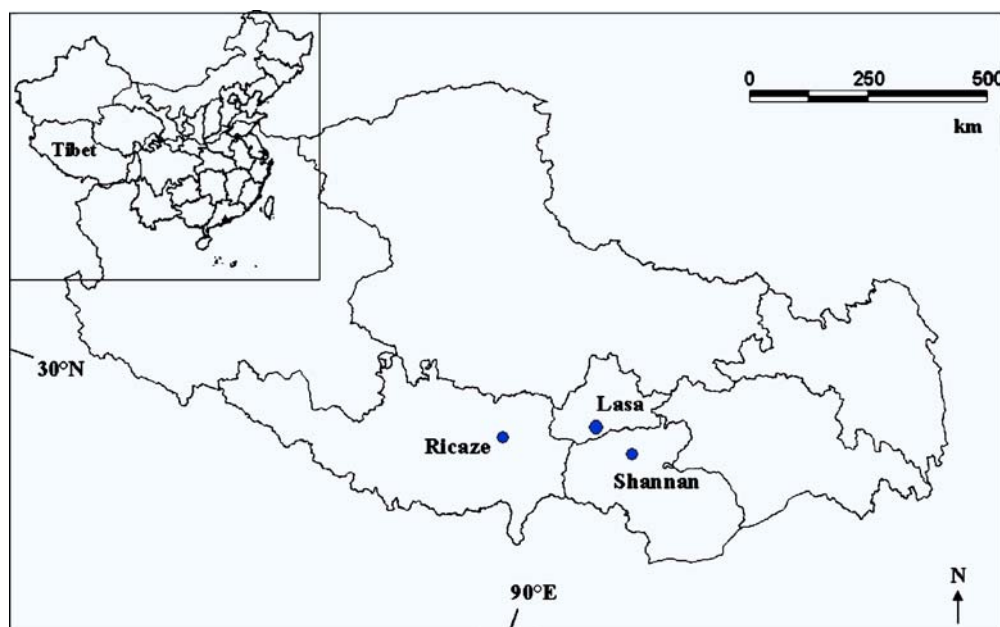
The investigation was conducted in the Brahmaputra middle reaches of South Tibet (28°–31°N, 87°–93°E), which represents the important area for agriculture and animal husbandry. The sampling sites included regions of Lhasa, Rikaze and Shannan (Fig. 1), which belong to semi-arid type of temperate plateau climate. The altitude is from 3,500 to 4,800 m and 80% of the land area is meadow. The mean annual temperature is 0–8°C and the annual precipitation 304–542 mm. The natural vegetation types from valley to alp, in turn, are montane scrub grassland dominated by Gramineae, alpine steppe dominated by Leguminosae and Gramineae, and the alpine meadow dominated by *Kobresia*. There is still some cultivated farmland in the valley. The grasslands here are seriously disturbed especially the montane scrub grassland and alpine steppe.

A total of 51 soil samples with associated roots were collected in the target areas from 17 to 22 September 2003, of which six were in farmland, 30 in montane scrub grassland, eight in alpine steppe and seven in alpine meadow. Approximately 2 kg soil was collected from the rooting zone of 22 dominant plant species to a depth of 20 cm, and at least three individual plants of each species were up-rooted. The soil samples were air-dried, passed through a 2-mm sieve and stored at 4°C before analysis.

Root staining

Root samples were rinsed with tap water, cleared in 10% (w/v) KOH (30 min, 90°C), acidified in lactic acid (10 min), and stained with 0.5% Trypan blue (Phillips and Hayman 1970). Fifty pieces of 0.5- to 1-cm root

Fig. 1 Map showing the location of the sampling sites in the southern grasslands of Tibet. Three regions, Lhasa, Rikaze and Shannan, contained target areas



fragments were examined per sample for their arbuscular mycorrhizal status and presence of fungal structures under a compound microscope at magnifications of $\times 100$ –400.

Spore extraction and counting

Spores were isolated from air-dried soil using the method described by Daniels and Skipper (1982). Twenty grams of soil were taken from each sample and wet-sieved. AM fungal spores were counted on a grid pattern dish under a binocular stereomicroscope.

Establishment of trap cultures

Trap cultures were established from fresh soil samples mixed with autoclaved sand in a ratio of 2:1. Two kilograms of

mixture per pot were used for the culture of one sample. Clover (*Trifolium repens* L.) and sorghum (*Sorghum vulgare* Pers.) were used as host plants. Cultures in 51 pots were grown in a greenhouse for 5 months and then harvested.

Identification of AM fungi

Spores of AM fungi isolated from the field soil and trap cultures were mounted on glass slides in polyvinyl lactoglycerol (PVLG) or PVLG + Melzer's reagent (1:1, v/v). Spores were examined microscopically and identified according to current taxonomic criteria (Schenck and Perez 1990) and using information from INVAM on the internet (<http://www.invam.caf.wvu.edu>).

Table 1 Arbuscular mycorrhizal fungal status in the roots of native plants in south Tibet

Host plant species	Fungal structures	Colonization rate (%)	Spore density (number per 20 g soil)
Asteraceae			
<i>Ajania pallsiana</i>	v, h	13.1	6
Chenopodiaceae			
<i>Salsola nepalensis</i>	v, h	10.1	8
Cruciferae			
<i>Brassica campestris</i> (associated with <i>Avena sativa</i>)	N	0	56
Cyperaceae			
<i>Carex capillacea</i> var. <i>linzensis</i> (associated with <i>Poa annua</i> and <i>Potentilla chinensis</i>)	v, h	25.0	10
<i>Carex tibetica</i>	v, h	30.9	18
<i>Cyperus compressus</i>	ar, v, h	21.5	66
<i>Kobresia humilis</i>	v, h	35.2	24
Gramineae			
<i>Agrostis hugoniana</i>	ap, ar, v, h	31.6	28
<i>Avena sativa</i> (associated with <i>Brassicacampestris</i>)	N	0	56
<i>Festuca rubra</i>	ar, v, h	31.7	50
<i>Hordeum vulgare</i> var. <i>trifurcatum</i>	v, h	41.2	60
<i>Imperata cylindrica</i>	ap, ar, v, h	43.3	38
<i>Poa annua</i>	v, h	27.0	26
<i>Poa palustris</i>	ar, v, h	31.8	16
<i>Poa pratensis</i>	v, h	25.5	30
<i>Stipa glareosa</i>	ar, v, h	32.1	2
Leguminosae			
<i>Caragana versicolor</i>	v, h	23.5	12
<i>Medicago sativa</i>	ap, ar, v, h, c	50.1	18
<i>Oxytropis tibetica</i>	ap, v, h	40.5	44
Polygonaceae			
<i>Fagopyrum esulentum</i>	N	0	12
Rosaceae			
<i>Potentilla chinensis</i> (associated with <i>Poa annua</i> and <i>Carex capillacea</i> var. <i>linzensis</i>)	v, h	12.0	10
Tamaricaceae			
<i>Tamarix chinensis</i>	N	0	10

ap Appressoria, ar arbuscules, h aseptate hyphae, v vesicles, c coils, N not detected

Table 2 Genera and species of arbuscular mycorrhizal fungi (AMF) isolated from grassland soils in southern Tibet

Genus	Species
<i>Glomus</i>	<i>G. aggregatum</i> Schenck & Smith, <i>G. etunicatum</i> Becker & Gerdemann, <i>G. geosporum</i> (Nicol. & Gerd.) Walker, <i>G. intraradices</i> Schenck & Smith, <i>G. luteum</i> Kennedy, Stutz, et Morton, <i>G. mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe, <i>G. rubiformis</i> (Gerd. & Trappe) Almeida & Schenck, <i>G. versiforme</i> (Karsten) Berch, <i>G. sp.1</i>
<i>Acaulospora</i>	<i>A. appendicula</i> Spain, Sieverding & Schenck, <i>A. delicata</i> Morton, <i>A. elegans</i> Trappe & Gerdemann, <i>A. lacunosa</i> Morton, <i>A. spinosa</i> Walker & Trappe, <i>A. mellea</i> Spain & Schenck, <i>A. scrobiculata</i> Trappe, <i>A. sp.1</i> , <i>A. sp.2</i> , <i>A. sp.3</i>
<i>Entrophospora</i>	<i>E. infrequens</i> (Hall) Ames & Schneider
<i>Scutellospora</i>	<i>S. aurigloba</i> (Hall) Walker & Sanders, <i>S. erythropha</i> (Koske & Walker) Walker & Sanders, <i>S. calospora</i> (Nicol. & Gerd.) Walker, <i>S. spherica</i> Koske & Walker, <i>S. pellucida</i> (Nicol. & Schenck) Walker & Sanders

Data analysis

AM fungal composition was evaluated by spore density, relative abundance, isolation frequency and species richness in field soil. Spore density was expressed as numbers of AM fungal spores per 20 g dried soil. Relative abundance was defined as the percentage of numbers of spores of the particular species or genera. Isolation frequency was calculated as the percentage of samples from which a particular genus or species was isolated. Species richness was defined as the numbers of AM fungal species per soil sample. Genus richness was defined as the numbers of AM fungal species in the particular genus per soil sample. Species diversity was calculated by the Shannon–Weiner index as follows:

$$\text{Shannon - Weiner index} = -\sum (P_i \ln[P_i])$$

where $P_i = n_i/N$, n_i = number of individuals in species i , and N = total number of individuals in all species.

Results

Root colonization by AM fungi

AM fungal structures, i.e. arbuscules, vesicles, intraradical aseptate hyphae, appressoria and intracellular coils were observed in 18 (82%) of 22 plant species (Table 1). However, the colonization pattern and rate varied among the plant species. Vesicles and aseptate hyphae were the most frequent structures present in the plants studied. Vesicles were observed in the roots of 18 plant species (82%), whereas arbuscules were observed only in seven species

Table 3 Isolation frequency (F , %), genus richness (richness, mean±SE) and relative abundance (RA, %) of the four AM fungal genera isolated from grassland soils in southern Tibet

Genus	F	Richness	RA
<i>Glomus</i>	86.7	1.43±0.18	68.2
<i>Acaulospora</i>	26.7	0.40±0.14	28.6
<i>Scutellospora</i>	20.0	0.23±0.09	2.0
<i>Entrophospora</i>	3.3	0.03±0.03	1.2

(32%). Appressoria were present in *Agrostis hugoniana*, *Imperata cylindrica*, *Medicago* sp. and *Oxytropis tibetica*. Intracellular coils were found in *Medicago* sp. only. No AM fungal structures were observed in *Tamarix chinensis*, *Fagopyrum esculentum* or *Avena sativa* associated with *Brassica campestris*. Colonization rate varied from 0 to 50.1% among the plant species.

AM fungal diversity

Twenty-five AM fungal taxa were identified in the soil samples collected from southern Tibet, of which 21 were identified down to species level and four to genus level. Of the 25 taxa, nine belonged to *Glomus*, ten to *Acaulospora*, one to *Entrophospora* and five to *Scutellospora* (Table 2).

AM fungi belonging to the genus *Glomus* were dominant and *Entrophospora* was rare, both in frequency and relative abundance (Table 3). Species richness of *Glomus* was highest, followed by the second dominant genus, *Acaulospora*. The most frequent and most abundant species was *Glomus mosseae* (Table 4).

The spore density in the rhizosphere soils of different hosts ranged from 2 to 66 per 20 g of soil (Table 1). Species richness in the sampling area was 2.10, and the fungal diversity was 2.35.

AM fungal diversity in normal and disturbed soils

AM fungal diversity was detected and compared in cultivated farmland, normal montane scrub grassland, degraded montane scrub grassland and seriously degraded

Table 4 Isolation frequency (F , ≥10%) and relative abundance (RA, %) of AM fungal species isolated from grassland soils in southern Tibet

Species	F	RA
<i>Acaulospora lacunosa</i> Morton	10.0	4.2
<i>Acaulosporamellea</i> Spain & Schenck	10.0	4.1
<i>Glomus etunicatum</i> Becker & Gerdemann	20.0	5.6
<i>Glomus geosporum</i> Walker	23.3	8.2
<i>Glomus intraradices</i> Schenck & Smith	20.0	6.3
<i>Glomus mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	63.3	39.8

Table 5 Spore density (density, number per 20 g air-dried soil, mean±SE), species richness (richness, mean±SE) and fungal diversity (diversity, Shannon–Weiner index) of AM in natural and disturbed soils

Land use type	Number of samples	Density	Richness	Diversity
Farmland	6	39±13	1.75±0.65	1.50
Montane scrub grassland	10	34±11	2.71±0.49	1.99
Degraded montane scrub grassland	12	20±7	1.72±0.38	1.25
Highly degraded montane scrub grassland	8	11±3	1.68±0.30	0.94

montane scrub grassland. The spore densities in the farmland and normal montane scrub grassland were much higher than in the grasslands that were degraded to different degrees. The AM species richness of normal montane scrub grassland was highest. AM fungal diversity varied from 1.99 to 0.94, with normal grassland having the highest species richness, degraded grassland and farmland intermediate, and the highly degraded grassland the lowest (Table 5).

Discussion

In natural ecosystems, almost all the plants in modern taxa form mycorrhizal associations. However, there are still some exceptions both between and within plant families that fail to associate with mycorrhizal fungi. In our preliminary study, AM fungal structures were observed in 82% of the surveyed plants in the southern grassland of Tibet. Most plant species in the target habitats were in the Gramineae, Leguminosae, Rosaceae, Asteraceae, Cyperaceae, Polygonaceae, Cruciferae, Chenopodiaceae and Tamaricaceae. All plants sampled belonging to the Gramineae, Leguminosae, Rosaceae and Asteraceae were found to be mycorrhizal. However, for members of the Chenopodiaceae, Cruciferae, Polygonaceae and Tamaricaceae, which are generally considered to lack mycorrhizal associations or in which they are found only very rarely (Hirsch and Kapulnik 1998), the results were quite different. No mycorrhizal structures were observed in *Fagopyrum esulentum* (Polygonaceae). *Brassica campestris* (Cruciferae) growing together with *A. sativa* showed no mycorrhizal structures in our survey. *T. chinensis* (Tamaricaceae) was also non-mycorrhizal. However, *Carex tibetica*, *Carex capillacea* var. *linzensis*, *Kobresia humilis* and *Cyperus compressus* (Cyperaceae) were all observed to form mycorrhizal structures. This agrees with Muthukumar et al. (2004), who reviewed the mycorrhizal status of the sedges and concluded that the Cyperaceae could not be regarded as a non-mycorrhizal family, and that the mycorrhizal status of its members was greatly influenced by environmental conditions. Vesicles and hyphae were also formed in the roots of *Salsola nepalensis* (Chenopodiaceae).

Previous surveys of the species richness of AM fungi in degraded arid and semi-arid environments worldwide may have underestimated species richness in these ecosystems if a prolonged period of trap culturing was not performed (Johnson 1993; Azcón-Aguilar et al. 2002). In fact, it is now accepted that the low species richness often reported for arid and semi-arid ecosystems by extraction of spores from field soil reflects limitations in the sporulation patterns under field conditions (Stutz and Morton 1996; Stutz et al. 2000). We conducted trap cultures for 5 months in our study to propagate the AM fungi in the soil samples. Twenty-five species of AM fungi associated with 22 plant species were isolated and identified in the arid or semi-arid grassland soil samples from high-altitude sites, and the results are similar to former grassland studies. For example, 23 AM fungal species associated with 25 plant species were found in a mown grassland ecosystem by Bever et al. (1996), and Smith et al. 1998 identified at least 20 species of AM fungi from a undisturbed prairie in Isanti County, MN, USA.

Drought can exert some influence on AM fungal composition. Stutz et al. (2000) studied the AM species composition and distribution in two arid regions (Chihuahua and Sonoran deserts) and semi-arid grassland in North America. They found only *Glomus* and *Acaulospora* species in the grasslands, with most species in *Glomus*. In the present study, we found four genera of AM fungi in the southern Tibetan grasslands, but *Glomus* and *Acaulospora* were the dominant genera, comprising 97% of the species found. At species level, *G. mosseae*, *Glomus intraradices* and *Glomus etunicatum*, which were the dominant or commonest species in our study, were also found by Stutz et al. (2000) in their semi-arid grassland.

The presence and infectivity of mycorrhizal fungi in the soil may be greatly reduced by soil disturbance (Jasper et al. 1991; Boddington and Dodd 2000). We also found that species richness and diversity were somewhat lower in disturbed soils than in undisturbed grassland soils and decreased with increasing severity of disturbance. Spore density in the montane scrub grassland showed a similar trend to species richness. Species diversity and richness in farmland was much lower than in natural montane scrub grassland in our survey, presumably due to soil tillage in the farmland (Jansa et al. 2002).

There have been few reports on the effects of high altitude on AM fungal diversity. Zhang et al. (1994) found that *Scutellospora* did not occur above an altitude of 3,000 m when they investigated AM fungal communities in North China (Xinjiang, Jilin and Beijing). However, we found five species of *Scutellospora* above 3,500 m. Mullen and Schmidt (1993) studied AM colonization of *R. adoneus* at an altitude of 3,500 m in the alpine zone of the Front Range in Colorado. They found light colonization by AM fungi throughout the year, but arbuscules were present only during the short growing season and this may have been related to the period of active P uptake by the host plant. There is a need for further studies on the dynamics of AM fungal colonization and nutrient uptake in extreme natural environments to obtain more information on the ecological

significance of the associations. Further work is also required to understand the potential role of arbuscular mycorrhizae in the restoration of disturbed habitats.

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