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Diversity and zonal distribution of arbuscular mycorrhizal fungi on the northern slopes of the Tianshan Mountains

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The arbuscular mycorrhizal (AM) fungal status of the 20 most common plant species distributed in 4 vegetation types (meadow steppe, desert steppe, steppe desert and typical desert) on the northern slopes of the Tianshan Mountains was investigated. Samples of the plant species and their rhizosphere soils were collected from the 4 vegetation zones and examined to compare their mycorrhizal status, AM fungal spore densities, biovolumes, and community structures. 28 AM fungal species were isolated from the rhizosphere soils: of these, 5 belonged to Acaulospora, 1 to Archaeospora and 22 to Glomus. 5 AM fungi, *Glomus aggregatum*, *G. claroideum*, *G. deserticola*, *G. etunicatum* and *G. sinuosum*, were observed in all 4 zonal types. No significant differences were observed in mean proportion of root length colonized by AM fungi among the plant species within each zonal type. Comparing the 4 zonal types, *Plantago minuta* (84.5%) in steppe desert and *Eremopyrum orientale* (83.1%) in typical desert showed the highest root colonizations rates. AM fungal spore densities and biovolumes were significantly different in the different zonal types. AM fungal spore densities and biovolumes, species richness and diversity were highest in meadow steppe and lowest in typical desert.

northern slopes, Tianshan Mountains, arbuscular mycorrhizal fungi, spore densities, diversity, zonal distribution

The concept of the ecosystem is predominantly rooted in biogeochemical processes with emphasis on the transfers of materials and energy between abiotic and biotic compartments^[1]. The pivotal involvement of arbuscular mycorrhizal (AM) fungi in plant mineral nutrition^[2,3] places these fungi at the abiotic/biotic interface in ecosystems. Rillig^[4] reviewed the pathways of influence of AM fungi to ecosystem processes and recognized 4 aspects in which they act: (1) by influencing plant community composition, (2) by influencing soil microbial communities, (3) by mediating changes in (individual) host physiology and resource capture, and (4) by direct effects of the AM fungal mycelium and its products in soil. As a result of this unique situation AM fungi have been described as “keystone mutualists” in terres-

trial ecosystems^[5].

AM fungi establish broadly distributed and possibly symbiotic associations with the majority (80%) of higher plant species^[3] and may potentially have played an important role in terrestrial ecosystems for over 460 Ma^[6]. They are responsive to many perturbations that act at the ecosystem level such as factors of global change^[7–10], agricultural management practices^[11–13], or pollution (e.g., heavy metals^[14]) and nitrogen deposition^[15].

The relationship between terrestrial ecosystems and

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climate change is one of the most important fields in the study of global change and the relationship between vegetation and climate change is one of the main research focuses^[16–18]. The degree of biological response in different climatic regions to global change on a range of temporal and spatial scales has, therefore, become a topical research area in recent years. Of particular interest, is the sensitivity of ecosystems in arid areas^[19]. AM symbionts must be considered in investigations of plant biodiversity and ecosystem function^[20]. Consequently, the relationship between AM fungal diversity and global change has become an important scientific focus.

Xinjiang Autonomous Region, located in the hinterland of Eurasia, has unique landforms that play an important role in the climatic conditions in this arid part of northwest China. Global change and violent disturbances resulting from human activities have resulted in significant climatic and environmental changes in Xinjiang^[21] and these responses to global climate change have been studied in some detail^[22]. Both temperature and precipitation appear to have increased on the northern slopes of the Tianshan Mountains over the last two decades and the climate has changed from a warm-dry type to a warm-humid type^[23]. This has led to much

interest in the question of how ecosystems and plant communities respond to climate change^[19] and, because they play such an important role in the ecosystem, the responses of AM fungi may be crucial. The objectives of the present survey were to investigate AM fungal colonization and developmental status of desert ephemerals and AM fungal communities in 4 different vegetation types on the northern slopes of the Tianshan Mountains to elucidate the relationship between AM fungal diversity and global change.

1 Materials and Methods

1.1 Study sites

AM fungal diversity was investigated in 4 vertical zonal types on the northern slopes of the Tianshan Mountains in May 2005 (Table 1). Samples of roots and soil (ca. 100 g) were collected from the rhizosphere of each plant listed in Table 1 in each zonal type. 20 individuals of each plant species were collected and these were separated into 4 portions. Care was taken during collection of individual plants to ensure that roots could be clearly identified as belonging to a particular plant. Samples were taken to the laboratory for determination

Table 1 Descriptions of sampling sites in the 4 vegetation types

Zonal type	Site location and elevation	Soil type	Soil physico-chemical properties				Host plant species
			Organic matter (g/kg)	Available P (mg/kg)	pH	Electrical conductivity (mS/cm)	
Meadow steppe	44°31.906'N 81°18.375'E 2100 m	Chestnut soil	58.92	9.73	8.03	0.55	<i>Potentilla</i> sp. <i>Stipa capillata</i> <i>Androsace</i> sp. <i>Taraxacum</i> sp.
Desert steppe	44°36.898'N 81°45.089'E 1100 m	Light chestnut soil	15.42	10.43	8.15	0.33	<i>Allium polyrrhizum</i> <i>Orostachys</i> sp. <i>Cleistogenes songgorica</i> <i>Stipa glareosa</i>
Steppe desert	44°37.324'N 81°51.004'E 830 m	Grey brown desert soil	5.15	6.23	8.73	0.33	<i>Ceratoides latens</i> <i>Salsola arbuscula</i> <i>Allium polyrrhizum</i> <i>Cleistogenes songgorica</i> <i>Plantago minuta</i> <i>Trigonella arcuata</i> <i>Nonea caspica</i>
Typical desert	44°27.010'N 83°55.098'E 525 m	Gobi desert	5.00	4.26	7.94	0.82	<i>Plantago minuta</i> <i>Erodium oxyrrhynchum</i> <i>Cancrinia discoidea</i> <i>Heliotropium ellipticum</i> <i>Lactuca</i> sp. <i>Eremopyrum orientale</i> <i>Arnebia guttata</i> <i>Atriplex dimorphostegia</i>

of the proportion of total root length colonized by AM fungi. Soil samples were air-dried prior to extraction, counting and identification of spores using standard methods.

1.2 Assessment of AM colonization

Fresh roots were processed by washing them with water to remove soil, followed by clearing with 10% (w/v) KOH in a 90°C water bath for between 15–30 min, depending on the degree of lignification of the roots and their pigmentation. The cooled root samples were washed, cut into 0.5- to 1.0-cm-long segments and stained with 0.5% (w/v) acid fuchsin^[24]. The percentage of root length colonized by AM fungal structures was determined using the magnified line-intersect method of McGonigle et al.^[25].

1.3 Recovery and counts of AM fungal spores

Spores or sporocarps were extracted from 30 g air-dried subsamples of each soil sample by wet sieving followed by flotation-centrifugation in 50% sucrose^[26]. The finest sieve used was 53 µm. Spores were collected on grid-patterned (4 mm × 4 mm) filter paper, washed three times with distilled water to spread them evenly over the entire grid and counted using a dissecting microscope at ×30 magnification. A sporocarp was counted as one spore.

1.4 Numbers and distribution of AM fungal spores

Species richness, spore density and frequency of AM fungi were expressed as follows: spore density (SD)= number of AM fungal spores in 30 g soil; species richness (SR) = number of AM fungal taxa found in 30 g soil sample; frequency (F) = (number of samples in which the species or genus was observed/total samples)×100%. Species diversity was assessed by the Shannon-Weiner index and Simpson index as follows:

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

$$\text{Simpson index } (D) = 1 - \sum (P_i)^2;$$

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

Species evenness was assessed as follows: Shannon-Weiner index $J = \text{Shannon } H / \ln SR$

Spore biovolume was calculated as $V = 1/6\pi D^3$ for species with spherical spores, or as $V = 1/6\pi D_1 D_2^2$ (where D_1 is the larger dimension and D_2 is the smaller dimension) for species with elongated spores^[27].

1.5 Identification of AM fungi

Spore identification was based mainly on spore size, colour, wall structure and hyphal attachment^[28]. Spore identification was corroborated by comparisons with descriptions from the reference cultures in INVAM (<http://invam.cag.wvu.edu>) and the IBG (International Bank for the Glomales, formerly named La Banque Europeenne de Glomales). AM fungal spores were classified according to the taxonomic system of Morton and Redecker^[29].

1.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to test for differences in AM fungal index in the 4 different vegetation zones using the SPSS software package version 11.0. Pairs of mean values were compared using least significant difference (LSD) at the 5% level.

2 Results

2.1 Comparison of AM fungal colonization status, spore densities and biovolume in the 4 zonal types

AM fungal colonization status, spore densities and biovolumes in the 4 zonal types are shown in Figure 1. The mean mycorrhizal colonization rates in meadow steppe, desert steppe, steppe desert and typical desert were 28.1%, 19.6%, 33.7% and 23.3%, respectively and no significant differences were observed among the 4 zonal types ($p>0.05$; Figure 1(a)). Spore densities and biovolumes were significantly higher in meadow steppe than in the other three zonal types, with mean values of 75.0 spores /30 g soil and 9.03 mm³/30 g soil (Figure 1(b), (c)). The lowest spore densities and biovolumes were found in typical desert, with an average of 7.1 spores and 0.55 mm³/30 g soil (Figure 1(b), (c)).

The mycorrhizal colonization rates of all plant species and spore densities of their rhizosphere soils in all zonal type are shown in Table 2. The mycorrhizal colonization rates of *Plantago minuta* (84.5%) in steppe desert and *Eremopyrum orientale* (83.1%) in typical desert were significantly higher than those of the other plant species. The highest spore densities found were associated with *Taraxacum* sp. in meadow steppe, with an average of 150 spores/30 g soil.

2.2 Comparison of AM fungal species richness, diversity and species evenness in the 4 vegetation zones

Species richness, diversity and species evenness of AM

Figure 1 Mean mycorrhizal colonization rate (a), spore density (b) and biovolume (c) of AM fungi in 4 different vegetation zones. Error bars denote $\pm$ SE. Mean values with the same letter are not significantly different by LSD at the 5 % level.

fungi were calculated to give the values in Figure 2. Species richness and diversity of AM fungi showed similar trends, with the highest values in meadow steppe and lowest in typical desert (Figure 2(a), (b)). AM fungal species evenness did not show any significant differences among the 4 vegetation types (Figure 2(c)).

2.3 Comparison among AM fungal species

All 28 AM fungal species belonging to three genera, including 1 species of *Archaeospora*, 5 in *Acaulospora* and 22 in *Glomus*, were isolated and identified in rhizosphere soil samples from the 4 vegetation types on the northern slopes of the Tianshan Mountains (Table 3).

Meadow steppe, steppe desert and typical desert had 14, 12 and 14 species, respectively, representing 3 AM fungal genera. Only *Glomus* species (13 total) were isolated and identified from desert steppe, with no species of *Archaeospora* or *Acaulospora* detected. Among the 28 AM fungal species, only 5 (*G. aggregatum*, *G.*

Figure 2 Species richness (a), diversity (b) and equality of uniformity (c) of AM fungi in the 4 vegetation types. Error bars denote $\pm$ SE. Mean values with the same letter are not significantly different by LSD at the 5% level. H, Shannon-Weiner index; D, Simpson index.

claroidium, *G. deserticola*, *G. etunicatum* and *G. sinuosum*) were observed in all 4 vegetation zones.

3 Discussion

AM fungi are ecologically important root symbionts of most terrestrial plants. They are a critical component of terrestrial ecosystems and can have important effects on nutrient acquisition by most land plants. Additionally, colonization by AM fungi can increase plant survivorship under abiotic and biotic stresses such as soil disturbance, biological invasion and environmental pollution. So far, more than 200 AM fungal species have been identified and reported. The distributions of some species are very wide, including mountains, grasslands and arid areas^[30,31]. The International Bank for the Glomeromycota (IBG (<http://www.bio.ukc.ac.uk/beg/>)) states, “The majority of plants, strictly speaking, do not have roots; they have mycorrhizas. The study of plants without their

Table 2 Mycorrhizal colonization rates of each host plant species and spore densities of their rhizosphere soils in 4 different vegetation zones¹⁾

Zonal type/Host plant species	Mycorrhizal colonization rate (percentage of root length colonized)	Spore density (Number/30 g soil)
Meadow steppe		
<i>Potentilla</i> sp.	36.7 c	30 c
<i>Stipa capillata</i>	2.9 h	60 b
<i>Androsace</i> sp.	52.2 b	60 b
<i>Taraxacum</i> sp.	20.5 def	150 a
Desert steppe		
<i>Allium polyrrhizum</i>	26.4 cde	30 c
<i>Orostachys</i> sp.	27.3 cd	25 c
<i>Cleistogenes songgorica</i>	12.6 fgh	50 b
<i>Stipa glareosa</i>	12.2 fgh	20 cde
Steppe desert		
<i>Ceratoides latens</i>	3.7 h	30 c
<i>Salsola arbuscula</i>	12.0 fgh	50 b
<i>Allium polyrrhizum</i>	20.2 defg	20 cde
<i>Cleistogenes songgorica</i>	58.7 b	4 f
<i>Plantago minuta</i>	84.5 a	4 f
<i>Trigonella arcuata</i>	35.2 c	11def
<i>Nonea caspica</i>	22.0 def	20 cde
Typical desert		
<i>Plantago minuta</i>	15.3 efgh	1 f
<i>Erodium oxyrrhynchum</i>	36.0 c	23 cd
<i>Cancrinia discoidea</i>	15.3 efgh	4 f
<i>Heliotropium ellipticum</i>	7.6 h	8 ef
<i>Lactuca</i> sp.	8.0 gh	3 f
<i>Eremopyrum orientale</i>	83.1 a	6 f
<i>Arnebia guttata</i>	10.3 fgh	4 f
<i>Atriplex dimorphostegia</i>	11.0 fgh	8 ef

1) Within each column, mean values followed by the same letter are not significantly different by LSD at the 5% level.

Table 3 AM fungal species in the different zonal types¹⁾

AM fungal species	Meadow steppe	Desert steppe	Steppe desert	Typical desert
<i>Acaulospora. bireticulata</i> Rothwell & Trappe			+	
<i>A. denticulata</i> Sieverding & Toro	+			
<i>A. elegans</i> Trappe & Gerdemann			+	
<i>A. foveata</i> Trappe & Janos	+			
<i>A. scrobiculata</i> Trappe	+			
<i>Archaeospora leptoticha</i> (Schenck & Smith) Morton & Redecker	+		+	+
<i>Glomus aggregatum</i> Schenck & Smith	+	+	+	+
<i>G. caledonium</i> (Nicol. & Gerd.) Trappe & Gerd.	+	+		
<i>G. canadense</i> (Thaxter) Trappe & Gerd.				+
<i>G. chimonobambusae</i> Wu & Liu				+
<i>G. claroideum</i> Schenck & Smith	+	+	+	+
<i>G. clarum</i> Nicol. & Schenck		+		+
<i>G. deserticola</i> Trappe, Bloss & Menge	+	+	+	+
<i>G. etunicatum</i> Becker & Gerdemann	+	+	+	+
<i>G. fulvum</i> (Berk. & Broome) Trappe & Gerd.				+
<i>G. geosporum</i> (Nicol. & Gerd.) Walker			+	+
<i>G. halonatum</i> Rose & Trappe	+			
<i>G. hoi</i> Berch & Trappe		+		
<i>G. lacteum</i> Rose & Trappe		+		
<i>G. macrocarpum</i> Tulasne & Tulasne	+			+
<i>G. microaggregatum</i> Koske, Gemma & Olexia	+		+	
<i>G. microcarpum</i> Tulasne & Tulasne		+	+	+
<i>G. mosseae</i> (Nicol. & Gerd.) Gerd. & Trappe	+		+	
<i>G. nanolumen</i> Koske & Gemma				+
<i>G. reticulatum</i> Bhattacharjee & Mukerji		+		
<i>G. sinuosum</i> (Gerd. & Bakshi) Almeida & Schenck	+	+	+	+
<i>G. trimurales</i> Koske & Halvorson		+		
<i>G. verruculosum</i> Blaszkowski		+		

1) +, AM fungi were present in soil samples.

mycorrhizas is the study of artefacts”.

AM fungi form associations with plant roots and, therefore, they are inevitably affected by their host plants^[32,33] and by environmental factors^[34–36]. All common plant species in the 4 vegetation types on the northern slopes of the Tianshan Mountains were found to form arbuscular mycorrhizal associations, indicating that AM fungi are widely distributed in the region. The high mycorrhizal colonization rates found in all 4 zonal types indicate that AM fungi may play an important role in the ecology of the host plants. Extensive mycorrhizal hyphal networks in the soil may provide an important pathway for the flow of nutrients and energy between plants^[37–40]. However, the mycorrhizal colonization rate differed among host plant species and vegetation types; the mycorrhizal colonization rate of the same plant species varied among the vegetations zones. For example, mycorrhizal colonization rates of *Plantago minuta* averaged 84.5% in steppe desert and 15.3% in typical desert. AM fungal biological yield may be expressed by spore densities and biovolumes^[27]. Similar trends were observed in both spore density and biovolume in the 4 vegetation types as evidenced by their significant positive relationship ($r=0.81$, $p<0.01$). The present findings indicate that mycorrhizal colonization rates and spore densities are influenced not only by different host plants, but also by different environmental conditions in accordance with the conclusions of Wang et al.^[41].

Species richness and diversity of AM fungi differed among the 4 vegetation zones. This difference may be due to both host plant species and environmental factors because many studies have shown that soil organic matter content and available P and altitude significantly affect the diversity of AM fungi^[35,42–44]. The differences in AM fungal diversity and species richness between meadow steppe and typical desert may be attributable to soil nutrients, rather than altitude of host plant species, because the soil organic matter and available P contents were higher in meadow steppe than in typical desert. No significant differences in AM fungal species evenness were observed among the 4 different vegetation zones.

The 28 AM fungal species representing three genera were isolated from the rhizosphere soils of the most common plant species and identified. 22 species of *Glomus* appeared to be dominant on the northern slopes of the Tianshan Mountains. In contrast, *Archaeospora* and *Acaulospora* represented only 3.6% and 17.9% of the species present. This provides strong support for the conclusions of other researchers, who have suggested that *Glomus* species tend to be the dominant AM fungi in arid ecosystems. In addition, the genus *Glomus* is the largest of all the AM fungal genera in the Glomales^[45]. No relationship was found between root colonization rate and spore density among the 4 vegetation types ($r=-0.15$, $p>0.05$) and this finding agrees with some previous reports from studies in other ecosystems^[46,47].

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