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Arbuscular mycorrhizal status of spring ephemerals in the desert ecosystem of Junggar Basin, China

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Abstract A survey was made of the arbuscular mycorrhizal (AM) status of 73 spring ephemeral plant species that grow in the desert ecosystem of Junggar Basin, northwest China. The proportion of AM colonization ranged from 7 to 73% with a mean value of 30%. A total of 65 plant species studied were AM with coils/arbuscules or vesicles and the remaining eight species were possibly AM with no coils/arbuscules or vesicles but with fungal mycelia in the root cortex. AM fungal spores were isolated from rhizosphere samples of all 73 plant species and identified. The mean spore density was 22 per 20 ml of air-dried soil, ranging from 0 to 120. Colonization and spore density of perennials were slightly higher than of annuals and varied among different plant families. A total of 603 AM fungal spore (or sporocarp) specimens were isolated belonging to six genera, *Acaulospora*, *Archaeopora*, *Entrophospora*, *Glomus*, *Paraglomus*, and *Scutellospora*; *Glomus* was the dominant genus. We conclude that spring ephemerals may be highly dependent on AM associations for survival in the very infertile and arid soils of this desert ecosystem.

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Keywords Arbuscular mycorrhiza · Spring ephemeral plants · Root colonization · Spore density · Desert ecosystem

Introduction

We use the term "ephemerals" here to include both annual and perennial species with a very short period of growth aboveground. The aboveground parts of the perennial ephemerals usually die at the end of the growing period but the belowground structures (bulbs, corms, or tubers) enter a period of dormancy until the subsequent time of sprouting. The dominant plant species in Gurbantunggut Desert in the Junggar Basin, northwest China, the ephemerals, grow for a short period each year from early spring (late March) to early summer (late May). These plants can take advantage of the favorable soil moisture conditions and warm temperatures in spring to grow rapidly to the flowering stage within about 2 months before arid conditions return (Zhang 2002a). In April, May, and early June, the mean cover of spring ephemerals reaches 13.9, 40.2, and 14.1%, respectively, with a mean plant height of 10–20 cm (Wang et al. 2003). The plants that play a very important role in the stabilization of sand dunes are pioneer species during the rehabilitation of degraded desert areas (Wang et al. 2003) and can reduce the frequency of occurrence and intensity of sandstorms.

In natural ecosystems, the majority of higher plant roots are associated with arbuscular mycorrhizal (AM) fungi belonging to the phylum Glomeromycota (Schüβler et al. 2001). Disturbance of the vegetation cover is the first visible indication of a desertification process, but damage to physical, chemical, and biological soil properties are known to occur concomitantly (Requena et al. 2001). With regard to biological components, land degradation is usually associated with reduction in belowground microbial diversity and/or activities governing the biogeochemical cycles of the major plant nutrients (Kennedy and Smith 1995). In particular, desertification reduces the diversity and inoculum potential of AM fungi (Jasper et al. 1991). As a result, arbuscular mycorrhizas of desert plants have been the subject of a number of studies in recent years (O'Connor et al. 2001; Titus et al. 2002; Collier et al. 2003; Ferrol et al. 2004), but to our knowledge there are no detailed published studies of the incidence of AM in spring ephemerals in desert ecosystems. We therefore conducted a systematic survey of the biodiversity of AM fungi and AM colonization of spring ephemeral plant communities in the Junggar Basin from March to July 2004 to understand the AM status of these important plants.

Materials and methods

Geography of the study area

The Junggar Basin (34°09′–49°08′N, 73°25′–96°24′E) forms a triangle between the Altai and Tianshan mountain ranges. It extends 1,100 km in length from east to west, and up to 800 km at its widest part, covering an area over 300,000 km^2 in northern Xinjiang Autonomous Region. Gurbantunggut Desert, located in the hinterland of the Junggar Basin and with an area of 48,800 km^2, is the second largest desert and the largest fixed and semifixed desert in China. Remote from the oceans and surrounded by high mountains, the area has a typical continental climate characterized by low precipitation, low specific humidity, long winters and short springs and autumns, ample sunshine and a wide temperature range. The region has an average annual precipitation of 200 mm, and a high free surface evaporation of 1,400–1,700 mm. The annual average temperature ranges from −4 to 9°C with mean temperatures of −20 to −15°C and 22 to 26°C in January and July, respectively. The frost-free period in the study area is about 150 days per annum.

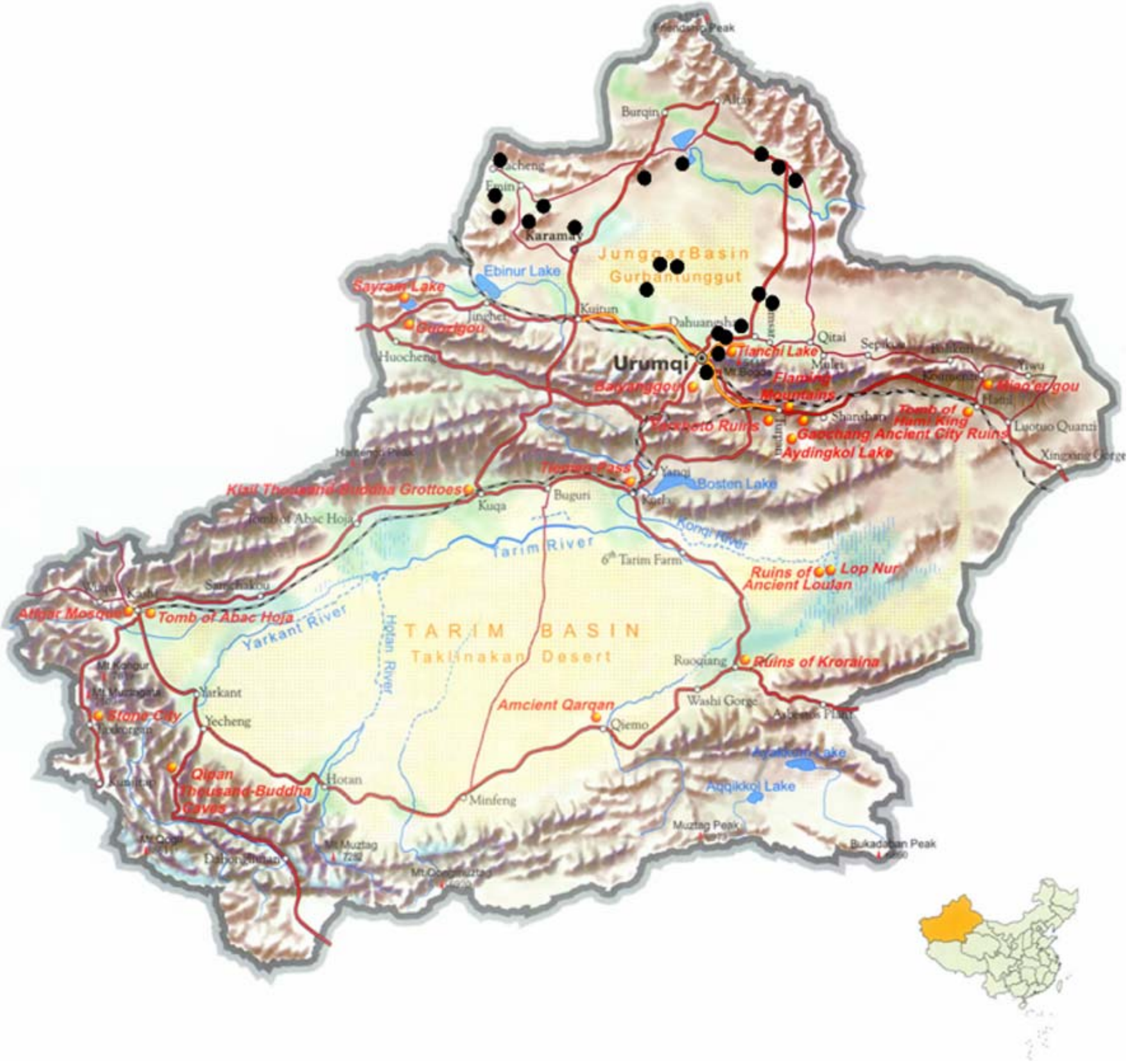

Fig. 1 Map showing the sampling sites in Junggar Basin, Xinjiang Autonomous Region, and northwest China. This map was downloaded from http://www.muztagh.com/map-of-china/large-map-xinjiang.htm

Collection of soil and root samples

The locations of the sampling sites were determined using an eTrex C portable GPS [Garmin (Asia) Corporation, Taiwan] and are shown on the relief map of Xinjiang in Fig. 1. The top ca. 1–2 cm of the soil surface was removed and soil and root samples were collected. Care was taken during collection of individual plants that the roots could be positively identified as belonging to a particular plant. The entire plants were dug out by trowel to ensure that the roots remained connected to the shoots after sample collection. Samples were taken to the laboratory for determination of AM root colonization. Soil samples (ca. 1,000 g) associated with the plants removed were collected. The soil samples were air-dried before extraction, counting, and identification of AM fungal spores.

Assessment of AM colonization

Fresh roots (ca. 0.2 g) were washed free of soil and cleared in 10% (w/v) KOH at 90°C in a water bath for 20–30 min, the exact time depending on the degree of lignification of the roots and their pigmentation. The root subsamples were cooled, washed, and cut into 0.5 to 1.0-cm-long segments and stained with 0.5% (w/v) acid fuchsin (Biermann and Linderman 1981). Fifty root fragments (ca. 1 cm long) were mounted on slides in polyvinyl alcohol–lactic acid–glycerol (Koske and Tessier 1983) and examined at ×100–400 magnification using an Olympus BX50 microscope equipped with an automatic photomicrographic system for the presence of AM fungal structures. The proportion of root length colonized was calculated according to the method of Trouvelot et al. (1986). If at least one root

Table 1 Fungal colonization of roots of spring ephemerals showing mean percentage colonization of root length with standard errors

Family	Life form	Species, percentage of colonization, species with hyphae only and therefore of uncertain AM status (?)
Liliaceae	P	*Allium setifolium*: 12±2(?); *Eremurus inderiensis*: 35±8; *Gagea alberti*: 23±5; *G. bulbifera*: 28±3; *G. divaricata*: 22±3; *G. fedtschenkoana*: 14±2; *G. granulosa*: 22±8; *G. sacculifera*: 33±8; *G. stepposa*; 24±8; *G. tenera*: 68±9; *Tulipa iliensis*: 33±9; *T. schrenkii*: 35±7
Primulaceae	P	*Androsace septentrionalis*: 42±6
Plantaginaceae	A	*Plantago minuta*: 55±8
Labiatae	A	*Eremostachys molucelloides*: 59±10; *Nepeta micrantha*: 40±35; *N. ucranica*: 12±2
Euphorbiaceae	A	*Chrozophora sabulosa*: 21±5; *Euphorbia pilosa*: 72±2; *E. turkestanica*: 22±5
Fabaceae	A	*Astragalus ammophilus*: 16±4; *A. arpilobus*: 18±3; *A. oxyglottis*: 31±7; *A. stalinskyi*: 26±3; *Trigonella arcuata*: 26±6
	P	*A. lithophilus*: 16±1
Gramineae	A	*Bromus tectorum*: 39±4; *Eremopyrum orientale*: 33±6; *E. triticeum*: 24±9; *Schismus arabicus*: 29±6
	P	*Poa bulbosa*: 16±3
Zygophyllaceae	A	*Zygophyllum lehmannianum*: 17±3
Compositae	A	*Amberboa turanica*: 16±2; *Crepis tectorum*: 18±2; *Heteracia szovitsii*: 9±4; *Koelpinia linearis*: 52±8; *Lactuca altaica*: 54±4; *Senecio subdentatus*: 31±5
	P	*Cancrinia chrysocephala*: 17±1; *C. discoidea*: 25±4; *Echinops sphaerocephalus*: 35±8; *Scorzonera circumflexa*: 28±8; *S. pusilla*: 45±17; *Tragopogon kasahstanicus*: 43±11; *T. elongates*: 10±1
Chenopodiaceae	A	*Atriplex dimorphostegia*: 14±2(?); *Corispermum lehmannianum*: 33±10(?); *Salsola praecox*: 9±2(?)
Ranunculaceae	A	*Adonis sp.*: 20(?); *Ceratocephalus testiculatus*: 18±4
Geraniaceae	A	*Erodium oxyrrhynchum*: 30±6
	P	*Geranium transversale*: 21±5
Solanaceae	A	*Hyoscyamus pusillus*: 13±4(?)
Umbelliferae	P	*Ferula dissecta*: 41±11; *F. ferulaeoides*: 60±19; *F. soongarica*: 35±8; *F. sp.1*: 53±16; *F. sp.2:* 41±10; *F. syreitschikowi*: 53±17; *Soranthus meyeri*: 53±0
Cyperaceae	P	*Carex physodes*: 35±9
Amaryllidaceae	P	*Ixiolirion tataricum*: 30±7
Scrophulariaceae	A	*Veronica campylopoda*: 24±6
Papaveraceae	A	*Hypecoum parviflorum*: 21±6(?)
	P	*Corydalis glaucescens*: 23±7
Iridaceae	P	*Iris songarica*: 19±4
Boraginaceae	A	*Arnebia decumbens*: 13±1; *Asperugo procumbens*: 27±14; *Heliotropium acutiflorum*: 12±5(?); *Lappula spinocarpa*: 20±6; *Lithospermum arvense*: 43±11; *Nonea caspica*: 37±9;
	P	*Arnebia sp.*: 7

A Annual, *P* Perennial

Plants with roots that contained hyphae but no coils/arbuscules or vesicles are shown as "?" and are only possibly mycorrhizal. Further information, with full binomials and authorities, percentage of root length with coils/arbuscules or vesicles, and spore densities in rhizosphere soil samples around roots of the individual plant species, are given as Electronic Supplementary Material on the Springer Website (http://www.springerlink.com) segment was observed to contain coils/arbuscules or vesicles, then the plant was recorded as an AM plant and denoted as “+”. If the root cortex was found to be colonized by fungal mycelia without coils/arbuscules or vesicles, the plant was recorded as possibly AM and denoted as “?”. The colonization data for specific structures are expressed as a percentage of root length.

Extraction and counting of AM fungal spores

The AM fungal spore numbers in the rhizosphere soil of 73 spring ephemerals were isolated and counted in a total 336 soil samples. Spores or sporocarps were extracted from 20-ml air-dried subsamples of each soil sample in triplicate by wet sieving followed by flotation–centrifugation in 50% sucrose (Dalpé 1993). The finest sieve used was 53 µm. The spores were collected on a grid patterned (4×4 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. The number of spores is expressed as the mean±standard error of three replicates. For observation and identification of spore characters, spores were mounted on glass slides in polyvinyl alcohol-lactoglycerol (PVLG) and PVLG + Melzer’s reagent and then identified to species using current taxonomic criteria (Schenck and Perez 1990) and information published by INVAM on the internet (http://invam.caf.wvu.edu).

Statistical analysis

The data were analyzed using SPSS software version 11.0.

Results

The fungal colonization status of 73 species of spring ephemerals belonging to 20 families is summarized in Table 1. Further details are provided as “Electronic Supplementary Material” (http://www.springerlink.com). Fungi were found to colonize the roots of the majority of plant species examined. The overall mean colonization rate was 30% and ranged widely from 7 to 72%. Coils/arbuscules and vesicles were observed in 58 and 64 species with an average of 4 and 7%, respectively (shown in the “Electronic Supplementary Material”). Coils/arbuscules were most abundant in the roots of *Plantago minuta* (Plantaginaceae) and vesicles in *Androsace septentrionalis* (Primulaceae). In summary, of the 73 spring ephemerals investigated, 89% (65 species) formed AM. Another eight taxa (11% of the total) contained fungal hyphae but not coils/arbuscules or vesicules. These were recorded as possibly AM. No plants were recorded as nonmycorrhizal. Among the plant species, one belonged to the Liliaceae, three to the Chenopodiaceae (a family often considered to be nonmycorrhizal or rarely AM), and the remaining four species belonged to the Ranunculaceae, Solanaceae, Papaveraceae, and Boraginaceae. Some of the ephemerals in families, traditionally considered to be nonmycorrhizal or rarely forming mycorrhizas, were colonized by AM fungi. For example, *Carex physodes* (Cyperaceae) was typically colonized (Table 1).

The AM fungal spore density varied from 0 to 120, with a mean of 22 (shown in the “Electronic Supplementary Material”). Overall, 603 AM fungal spore (or sporocarp) samples belonging to six genera were obtained. Some spore types were identified to species according to their morphological characters but most of the specimens were identified to genus level because it was difficult to identify them to species owing to a lack of distinguishable fine taxonomic characters. As a result, the frequency of AM fungal occurrence was analyzed statistically only at the genus level. *Glomus* was the dominant genus, with a frequency of occurrence of 83.1% over all the spore samples examined (Table 2). The second genus in dominance was *Acaulospora* with a frequency of occurrence of 12%. The frequencies of occurrence of members of the other four genera were very low (Table 2).

The proportion of root length colonized, the occurrence of coils/arbuscules and vesicles, and the spore densities of perennials were all slightly higher than those of annuals (Fig. 2). Coils/arbuscules or vesicles were observed in 18

Table 2 AM fungal genera identified and their times of spore presence and frequency of occurrence in soil associated with ephemeral plants

AM fungal genus	Times of spore presence	Frequency of occurrence (%)
Acaulospora	70	12
Archaeopora	11	2
Entrophospora	6	1
Glomus	501	83
Paraglomus	13	2
Scutellospora	2	<1
Total	603	100.0

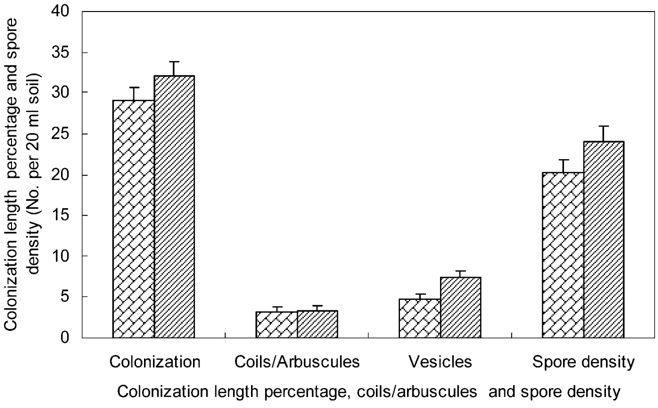

Fig. 2 Comparison of colonization status and spore density between annual and perennial spring ephemerals. Data are means and standard errors. “▩: Annual species”, “▨: Perennial species”

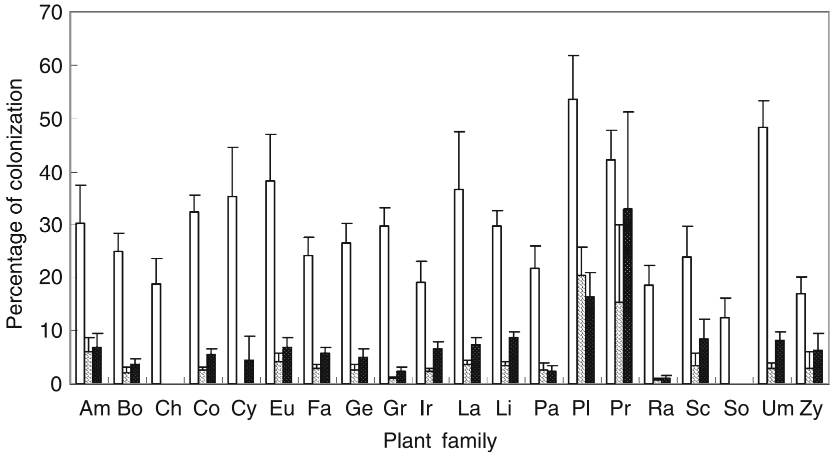

Fig. 3 Colonization status of different families of spring ephemeral plants “☐: Colonization proportion”, “▨: Coils/Arbuscules”, “■: Vesicles”. *Am* Amaryllidaceae, *Bo* Boraginaceae, *Ch* Chenopodiaceae, *Co* Compositae, *Cy* Cyperaceae, *Eu* Euphorbiaceae, *Fa* Fabaceae, *Ge* Geraniaceae, *Gr* Gramineae, *Ir* Iridaceae, *La* Labiatae, *Li* Liliaceae, *Pa* Papaveraceae, *Pl* Plantaginaceae, *Pr* Primulaceae, *Ra* Ranunculaceae, *Sc* Scrophulariaceae, *So* Solanaceae, *Um* Umbelliferae, *Zy* Zygophyllaceae. Data are means and standard errors

out of 20 families (Fig. 3). Plantaginaceae had the highest colonization (54%) and occurrence of arbuscules (20%). The lowest root colonization rate occurred in the Solanaceae (13%) (Fig. 3). The highest spore densities were observed in the Plantaginaceae with 43 spores per 20 ml air-dried soil and the lowest in the Euphorbiaceae, with only five spores per 20 ml soil (Fig. 4).

Discussion

Trappe (1987) estimated that about 70% of angiosperms form arbuscular mycorrhizas, and most of these are herbaceous plants. AM fungi can play an important role in plant survival and in the community stability of vegetation in natural ecosystems (Gange et al. 1993; Francis and Read 1994; Azcón-Aguilar and Barea 1997; Hartnett and Wilson 2002; Moraes et al. 2004). Plant community structure and AM status and colonization might be used to monitor desertification and soil degradation. Several studies have been conducted in recent years on AM fungi in desert ecosystems (e.g., O’Connor et al. 2001; Titus et al. 2002; Collier et al. 2003; Ferrol et al. 2004).

Herbaceous ephemerals are widely distributed in many deserts and can grow well in very arid, infertile, and windy environments. They form important plant communities in the Jungggar Basin in early spring. About 196 individuals of *Eremurus inderinsis*, 135 of *Erodium oxyrrhynchum*, and 81 of *Carex physodes* were found per square meter in the Gurbantunggut Desert (unpublished data). Numerous studies have been reported on the functional significance, biological characteristics, and distribution of spring ephemerals (Mao and Zhang 1994; Li 2000; Zhang 2002a–c; Wang et al. 2003, 2004) but so far there is little information on the relationship between AM fungi and spring ephemerals. In the present study, hyphae but neither coils/arbuscules nor vesicles were observed in the roots of *Atriplex dimorphostegia*, *Corispermum lemannianum*, or *Salsola praecox,* which belong to the Chenopodiaceae. The occurrence of mycorrhizal colonization of species in the Chenopodiaceae has occasionally been reported (Allen 1983; Allen et al. 1989; Aguilera et al. 1998; O’Connor et al. 2001) although this family is usually thought to be nonmycorrhizal. *Carex physodes* (Cyperaceae), a species typically found at the top of sand dunes, was observed to form AM. Muthukumar et al. (2004) have pointed out that the number of plants in the Cyperaceae reported to form arbuscular mycorrhizas has been steadily increasing over a wide range of biomes including the savannas of Venezuela and China (Lovera and Cuenca 1996; Li et al. 2004) and Indian semiarid tropical grasslands (Muthukumar and Udaiyan 2002).

The higher colonization and spore densities of perennials than of annuals that we observed support the findings of Collier et al. (2003) in the Chihuahuan Desert and O’Connor et al. (2001) in the Simpson Desert, indicating that plants in stressed environments might rely on AM for survival. Colonization rates and spore densities also differed among the plant families. This may be attributed to a range of host plant characteristics including root morphology, genetics, and phenology (Baylis 1975; Lorgio et al. 1999; Eom et al. 2000).

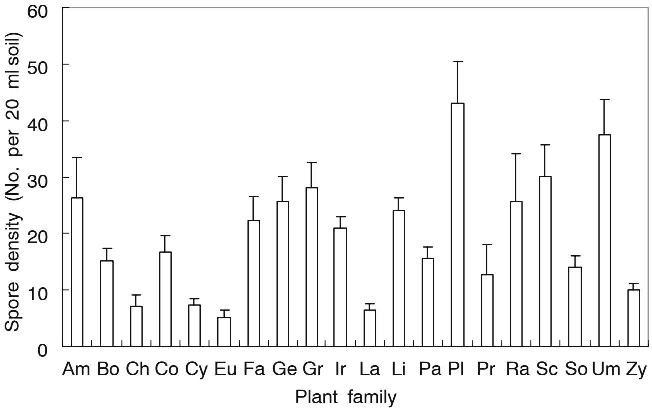

Fig. 4 Spore densities in different families of spring ephemerals. *Am* Amaryllidaceae, *Bo* Boraginaceae, *Ch* Chenopodiaceae, *Co* Compositae, *Cy* Cyperaceae, *Eu* Euphorbiaceae, *Fa* Fabaceae, *Ge* Geraniaceae, *Gr* Gramineae, *Ir* Iridaceae, *La* Labiatae, *Li* Liliaceae, *Pa* Papaveraceae, *Pl* Plantaginaceae, *Pr* Primulaceae, *Ra* Ranunculaceae, *Sc* Scrophulariaceae, *So* Solanaceae, *Um* Umbelliferae, *Zy* Zygophyllaceae. Data are means and standard errors

It has been reported that both spore production and species richness of AM fungi are lower in arid climates than in other ecosystems (Rose 1981; Pond et al. 1984), and decrease as aridity increases (Stahl and Christensen 1982; Stutz and Morton 1996). The mean spore density of 22 spores per 20 ml of soil in our study was lower than reports from other ecosystems (Zhao et al. 2001; Li et al. 2004). However, our results are similar to those from other desert ecosystems such as the Mojave (Titus et al. 2002) and Negev (He et al. 2002) deserts.

AM fungi belonging to the genus *Glomus* seem to be dominant in the rhizosphere of spring ephemerals in Junggar Basin. This provides strong support for the conclusions of other workers that AM fungi belonging to *Glomus* tend to be dominant in arid ecosystems (Lamont 1982; Pande and Tarafdar 2004).

It is well known that AM fungi can increase plant nutrient uptake (Tarafdar and Praveen-Kumar 1996), water use efficiency (Augé and Stodola 1990) and resistance to abiotic stress under certain conditions. The association may therefore be expected to be especially important in desert ecosystems. Most plant families in arid regions have been found to form AM associations (Trappe 1981). Our results would seem to indicate that desert spring ephemerals may benefit from these associations. Why so many desert ephemeral plant species that finish their life span (even if only aboveground) in such short periods of time sacrifice their valuable carbohydrates to the associated fungi and how the AM fungi complete their life span during such a short time span are interesting questions. Changing the host plants via underground hyphal links may be an important mechanism as suggested by Lapointe and Molard (1997). However, unlike other ecosystems, few herbaceous plants survive in the sand dunes in summer in the Gurbantunggut Desert. The annuals die and the perennials enter a dormant period followed by senescence of aboveground tissues. Nevertheless, the roots of perennials could harbor AM fungi. The AM fungi must also germinate (presumably from spores) all at the same time and rapidly colonize the roots when spring arrives. The symbionts may depend on each other for survival in these extreme environments but further studies will be required to elucidate the mechanisms operating in desert ecosystems.

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