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Diversity of arbuscular mycorrhizal fungal spore communities and its relations to plants under increased temperature and precipitation in a natural grassland

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Arbuscular mycorrhizal fungi (AMF) form mutualisms with most plant species, and therefore, understanding how AMF communities will respond to climate change is essential for predictions of changes in plant communities. To evaluate the impact of global climate change on AMFs and plant-AMF interactions in a natural grassland in Inner Mongolia, both artificial warming and watering treatments were assigned to experimental plots. Our results indicate that (1) warming and precipitation significantly affected the relative spore abundance of abundant sporulating AMF species; (2) the relative abundance of weak sporulating AMF species and AMF diversity decreased under experimental warming; (3) evidence was found that the composition of the AMF community in a given year might be correlated with plant community composition in the following year; and (4) grasses and forbs showing different preferences to *Claroideoglomus etunicatum* or *Ambispora gerdemannii* dominated plots. Our results imply that climate change appears to induce changes in AMF assemblages with knock-on effects on grassland plant communities. AMF communities may play a much more important role than we have thought in the responses of ecosystem to global climate changes.

global warming, precipitation increase, *Arbuscular mycorrhiza*, plant-AMF relations, grassland ecosystem

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Climate change refers to any change in climate over time, whether due to natural variability or as a result of human activity [1]. In the next century, global mean temperature is predicted to increase by 1.8–4.0°C, and mean annual precipitation is also predicted to change in many area of the world [1]. To understand the consequences of global climate change, research into how different ecosystems and their components will respond to global warming and altered precipitation patterns is essential. Grasslands are one of the most important ecosystems in the world, occurring in almost all climatic zones and covering nearly 20% of the global land surface [2]. In China, grasslands cover approximately 4×10^8 hm^2, 41.7% of the Chinese territory. The majority of these grasslands are found in northern China [3] under a semi-arid to arid cold climate. Both temperature and precipitation limit the productivity of agriculture and animal husbandry in these areas [4]. As many components of grassland ecosystems, including composition of aboveground plant community [5,6], root performances and lifespan [7], structure of soil microbial community [8,9], and soil and microbial respiration [10–12] are very sensitive to climatic change, understanding how grasslands will change with changing climatic conditions is essential for the sustainable development of agriculture and stockbreeding in northern China. Mycorrhizal fungal communities are an important component of grassland ecosystems and act as a

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carbon sink for up to 15% of primary production [13]. Grasses often form symbioses with arbuscular mycorrhizal fungi (AMF) [14], and AMF abundance tends to be much higher in grasslands than in other ecosystems [15]. Characterization of the mycorrhizal community could therefore be crucial for understanding the response of grassland ecosystems to climate changes [16,17].

While field studies have examined the effects of elevated CO_2 on mycorrhizal communities, little research has addressed the effects of other environmental factors, such as temperature and precipitation [16,17]. Similarly, most field experiments testing for warming effects in natural ecosystems have focused on changes in the aboveground vegetation, rather than the mycorrhizal fungal communities [17]. While enhanced extraradical mycelium (ERM) and root colonization of AMF have been observed in some warming experiments [18,19], increased temperature also showed negative or no significant effects on AMF in other studies [20,21], and the variety of heating approaches, the different ecosystems studied and the distinct consequences of warming such different systems may be reasons for such different results [22]. To better predict how grasslands will respond to a changing climate, studies of how key environmental factors, such as temperature and precipitation, influence the growth and reproduction of AMF are critical [21]. It is also necessary to quantify how much variation exists in the response of different AMF species to factors of environmental change such as soil temperature and moisture, as both plant and fungal species show differential migratory capabilities [16].

Since the diversity of AMF plays a key role in plant biodiversity and ecosystem functioning [23,24], it will be important to understand its responses to climate change. Biogeographic patterns of AMF species occurrence along environmental gradients suggest that temperature and moisture control both the distribution of AMF communities [25–27], However, the immediate responses of AMF communities in natural ecosystems to global warming and altered precipitation rates remain poorly understood.

Spore morphology methods were mainly used before the molecular methods introduced in studying AMF community. Although molecular methods such as DNA cloning and sequencing, DGGE, T-RFLP, and pyrosequencing revolutionized AMF research, due to the PCR bias, it is a little difficult for quantitative analysis of AMF. Therefore spore morphology methods still has its advantage in quantitative analysis of AMF among different experimental treatments [28]. Spore records are subject to bias because sporulation of AMF depends on biotic and abiotic conditions, and AMF species in the "spore community" may thus not always reflect their abundance in soils or plant roots [29]. However, sporulation is an important aspect of AMF life cycles and essential to propagule availability and even survival of AMF species, it is necessary to know the response of "spore community" to environmental factors when evaluate the impact of climate change on AMF community.

In this study, we investigated how higher temperatures and increased precipitation influence the spore production and colonization structures of mycorrhizal fungi. Infrared heaters were used to increase temperatures, and 120 mm water (about 30% of mean annual precipitation) was added to experimental plots to mimic the future warmer and wetter conditions.

The specific questions addressed were: (1) Are there any effects of increased temperature and precipitation on spore production and colonization of AMF communities in grasslands? (2) Does the spore production of different AMF species, including both spore-abundant and non-abundant species, respond similarly to environmental change? If not, how and to what extent do warmer temperatures and increased precipitation affect mycorrhizal communities in grassland ecosystems? (3) Since global climate change may affect both plant and AMF communities, will the relationship between plant and AMF communities be altered under the climatic conditions of the future?

1 Materials and methods

1.1 Study site

The study site was situated in a natural grassland in Duolun County of Inner Mongolia (42°02′N, 116°17′E, 1324 m a.s.l.). The mean annual precipitation in this area is 387.2 mm, with peaks in July and August. The mean monthly air temperature ranges from −15.9°C in January to 19.9°C in July, with an annual average of 2.1°C. Mean monthly soil surface temperatures parallel the air temperatures, and range from −16.3°C in January to 24.2°C in July. The soils in the study sites are chestnut (Chinese classification) or Calcic-orthic Aridisol (US soil taxonomy classification system). The total organic carbon and total nitrogen determined in 2006 are 15.4±1.9 and 1.51±1.77 mg g^{-1} in soil. The pH of the soil is 6.84±0.07, and the bulk density is 1.31±0.03 g cm^{-3}.

The experiment plots were established in a fenced grassland dominated by native grasses, *Chloris virgata* Swartz, *Cleistogenes squarrosa* Keng, *Eragrostis pilosa* Beauv., *Gagea pauciflora* Turcz., *Gueldenstaedtia stenophylla* Bunge, *Leymus chinensis* Tzvel., *Melilotoides ruthenica* Sojak, *Potentilla* spp., *Setaria viridis* Beauv, and *Stipa krylovii* Roshev [30].

1.2 Field treatments

The experimental design is described in detail in Niu et al. [30]. The experiment used a paired, nested design with precipitation as the primary factor and warming as the secondary factor. There were three pairs of 10 m×15 m plots: one plot in each pair was assigned as the increased precipitation treatment and the other as the control. At each precipitation

plot, 15 mm of water was added weekly in July and August using a watering system to mimic a 120 mm increase in precipitation (about 30% of the mean annual precipitation at the study site). Within each 10 m×15 m plot, four 3 m×4 m subplots were treated as the warmed and control subplots with two replicates. The subplots were randomly assigned to warming or control treatments. Artificial heating of plots in the field was accomplished with 1600 W overhead (2.5 m in height) infrared heaters (MSR-2420 infrared radiators, Kalglo Electronics Inc, USA) and a power input was about 100 W m^{-2}. Mean temperatures at 10 cm soil depth from May of 2005 to December of 2006 in control, W (warming), P (increased precipitation) and WP (warming plus increased precipitation) treatments are 7.15, 8.47, 7.04, and 8.09°C, respectively.

1.3 Sampling procedure

One soil cylinder (15 cm in depth, 8 cm in diameter) were taken from each plot at the beginning (May 25), middle (August 1), and end (September 22) of the growing season in 2005, and in the middle of the growing season (August 4) in 2006. All live roots were collected in each soil cylinder for determining AM colonization. Soil samples were stored at 4°C prior to spore isolation.

1.4 AM structure examination

Root samples were rinsed with tap water, cleared in 10% (w/v) KOH (20 min, 92°C), acidified in lactic acid (3 min), and stained (20 min, 92°C) with 0.5% acid fuchsin. Fifty root fragments (*ca* 1 cm long) were mounted on slides in a polyvinyl alcohol solution [31] and examined with a compound microscope (Olympus BH-2) at × 100–400 magnification for the presence of AM fungal structures (i.e. arbuscules, vesicles, hyphal coils, and intercellular nonseptate hyphae). The percentage of root length colonized by AMF structures was estimated according to the magnified line-intersect method [32].

1.5 Spore separation and identification

Arbuscular mycorrhizal fungal spores were isolated from the soil using the wet-sieving and decanting method of Gerdemann and Nicolson, modified by Daniels and Skipper [33]. AMF were identified according to current taxonomic criteria [34–37], and information given on the INVAM web page (http://www.invam.caf.wvu.edu). At least 20 spores of each species were used for identification. Morphological characteristics were first determined in water, and then in Melzer's reagent. Cotton blue was also used to aid identification. Permanent slides were prepared with Polyvinyl-lacto-glycerol (PVLG), sealed with nail varnish, and stored in the Herbarium Mycologicum Academiae Sinicae (HMAS) in Beijing.

1.6 Spore composition of the AMF community

The relative abundance (RA) of mycorrhizal fungal species was calculated as $RA_i=N_i/N$, where N_i is the spore number of species i, and N is the total spore number of all AMF species in one 100-g air dried soil sample. The difference in relative abundance (DRA) between two mycorrhizal fungal species was calculated as DRA=RA_1–RA_2, where RA_1 and RA_2 are the relative spore abundances of species 1 and species 2, respectively. Relative abundance of infrequent sporulating species was defined as sum of 1 minus RA of three spore dominant species (*Claroideoglomus etunicatum*, *Ambispora gerdemannii*, and *Glomus albidum*).

The species diversity of mycorrhizal communities was estimated using Simpson's diversity index (H): $H= 1-\sum p_i^2$, where p_i is the relative proportion of spores of species i in the community [38]. Seasonal changes in DRA, the relative abundance of non-dominant species, and H were calculated as the difference between middle and early samples (first half of the growing season), and between the final and middle samples (second half of the growing season). Inter-annual changes of these measures were calculated as the difference between those of the mid-season samples of 2006 and 2005.

1.7 Statistical analysis

Mantel tests were conduct with the PC-ORD version 5 [39] to test for a correlation between plant and AMF community composition in 2005 and 2006. The significance of the standardized Mantel statistic (r) was evaluated by randomization (1000 Monte-Carlo permutations). Effects of year and season on relative abundances of different AMF species and impacts of precipitation and warming on intra- and inter-annual changes of abundant sporulating species, total relative abundance of infrequent sporulating species and Simpson's diversity index of AMF spore community were evaluated using general linear model in SPSS (SPSS for windows, version 16.0, SPSS Inc, Chicago, USA). Impacts of Block, precipitation, warming, and sampling time on colonization of AM structures were evaluated using the repeated-measures ANOVA (aov function) in R software (version 2.12.2). Indicator species analysis was carried out using PC-ORD version 5 [39] to assess the preferences of AMF spores to warming, precipitation increase and seasons [40].

2 Results

2.1 AMF colonization in response to heating and watering

Arbuscular mycorrhizal structures were observed in all collected roots (Table 1). However, there were no significant differences in the quantity of AM structures or in the percentage of root length colonized (PRLC) by AM fungal

Table 1 Density of vesicles, arbuscules, hyphal coils, and percentage root length colonization (PRLC) of AMF[a)]

Treatment[b)]	Vesicles (No./cm)	Arbuscules (No./cm)	Hyphal coils (No./cm)	PRLC (%)
May 25, 2005				
Control	11.4±1.8	38.1±8.8	4.5±0.6	73.2±4.4
Warming	11.5±1.8	30.6±9.5	6.6±2.4	67.7±5.5
Aug. 1, 2005				
Control	6.6±2.4	11.8±5.5	4.7±1.7	53.0±6.1
Warming	4.3±1.5	3.9±0.9	3.3±1.1	50.3±2.8
Precipitation increase	9.2±1.9	5.1±1.3	3.1±0.7	40.0±2.9
Warming + Precipitation increase	7.8±1.8	7.9±1.8	4.3±1.9	41.1±5.4
Sep. 22, 2005				
Control	6.3±3.0	15.5±6.2	2.7±0.7	58.2±8.7
Warming	5.4±1.3	13.6±4.3	2.1±0.8	62.3±5.2
Precipitation increase	6.2±2.5	8.4±3.6	5.2±3.3	47.7±8.6
Warming + Precipitation increase	8.3±2.5	3.8±1.3	1.9±0.8	45.7±7.3
Aug. 4, 2006				
Control	5.2±1.7	1.0±0.9	1.4±0.7	39.3±8.8
Warming	5.9±2.4	2.6±2.0	0.6±0.2	29.9±5.7
Precipitation increase	6.1±2.4	0.4±0.2	0.8±0.4	37.3±8.4
Warming + Precipitation increase	5.4±1.6	2.1±1.8	1.5±0.8	36.1±7.8
Significance (*F*-values)				
Block (*B*)	0.645	29.771*	0.370	12.253
Precipitation(*P*)	0.067	3.348	0.304	8.440
Warming (*W*)	1.428	0.064	0.027	1.035
Sampling time (*T*)	5.347**	20.215***	4.207**	21.378***
P×*W*	0.270	2.946	1.248	0.098
P×*T*	1.409	0.349	0.171	0.704
W×*T*	0.383	0.121	0.401	1.191
$P \times W \times T$	0.338	1.725	0.978	0.734

a) Data are presented as Mean ± SE, n=6, F-values of the repeated-measures ANOVA were shown. b) Watering was not carried out on May 25, 2005, and the plots in treatments of Precipitation (*P*) and Warming plus Precipitation (*WP*) were added to control and Warming (*W*), respectively. *, P<0.005; **, P<0.01; ***, P<0.01.

structures between treatments. Significant intra-annual changes were found in the number of arbuscules and vesicles (P=0.002 and <0.001, respectively), and in total PRLC (P=0.001). Significant fewer hyphal coils (P=0.027) and less total PRLC (P=0.032) were found in August 2006 as compared with August 2005 (Table 1).

2.2 AMF spore community composition

A total of 22 AMF morpho-species, belonging to 11 genera (*Glomus*, *Claroideoglomus*, *Rhizophagus*, *Septoglomus*, *Funneliformis*, *Diversispora*, *Ambispora*, *Acaulospora*, *Entrophospora*, *Scutellospora* and *Gigaspora*), were found at the study site (Table 2). Spores of two genera *Claroideoglomus* and *Ambispora* had higher spore production, accounting for 38.4% and 36.4% of the total number of spores. Spores of *Glomus* were the third in spore production, and spores of *Entrophospora*, *Scutellospora*, and *Gigaspora* averaged less than 0.3% each. Three "spore abundant" species (*Claroideoglomus etunicatum*, *Ambispora gerdemannii*, and *Glomus albidum*) accounted for over three quarters of the AMF spores in the natural conditions.

The results of Indicator Species Analysis using PC-ORD are shown in Table 3. *Am. gerdemannii* was identified as an indicator species for increased precipitation, as it was found to sporulate more in plots with increased precipitation. *G. albidum* and *R. fasciculatum* were most abundant in 2005, while *Am. gerdemannii*, *G. aggregatum*, and *S. calospora* were most abundant in 2006. With respect to seasonal fluctuation in abundance, spores of *Am. gerdemannii* and *F. caledonium* were significantly higher in Spring, while spores of *C. etunicatum*, *R. fasciculatum*, and *G. warcupii* did not become abundant until the fall.

2.3 Different responses of *C. etunicatum* and *Am. gerdemannii* to climate changes

To evaluate the influence of elevated temperatures and increased precipitation on spore production of *C. etunicatum* vs. *Am. gerdemannii*, intra- and inter-annual changes of

Table 2 The relative abundance (RA) of AMF species at the grassland study site in Inner-Mongolia at four sampling times[a)]

AM Fungal species	May 25, 2005	Aug. 1, 2005	Sept. 22, 2005	Aug. 4, 2006	Year	Season
Claroideoglomus etunicatum	29.6±2.4	35.4±2.1	48.4±3.2	41.4±2.3	2.81	13.59***
Ambispora gerdemannii	39.3±2.4	32.9±3.4	23.6±3.4	40.0±3.1	2.60	6.39**
Glomus albidum	17.2±2.6	20.1±3.2	19.0±2.3	10.8±1.4	7.14**	0.36
Funneliformis badium	5.9±1.6	5.6±1.2	–	4.9±1.2	0.19	7.40**
Rhizophagus fasciculatum	1.8±0.7	2.6±1.1	4.4±1.7	0.1±0.1	2.60	1.58
Funneliformis caledonium	3.4±2.3	0.6±0.3	–	0.0±0.0	0.76	2.36
Glomus warcupii	–	–	1.6±1.1	–	0.00	2.76
Septoglomus constrictum	0.2±0.2	1.2±0.5	–	0.3±0.3	4.43*	4.87**
Glomus aggregatum	–	–	–	0.8±0.4	9.05**	0.00
Diversispora versiforme	0.8±0.8	–	–	–	0.00	1.31
Rhizophagus intraradices	–	–	0.7±0.7	–	0.00	1.40
Rhizophagus clarum	–	–	0.7±0.7	0.5±0.3	0.09	1.13
Glomus sp.1	–	0.3±0.3	0.1±0.1	–	1.88	1.02
Acaulospora sp.1	0.9±0.3	0.4±0.2	0.9±0.4	0.3±0.2	0.00	1.40
Acaulospora mellea	0.0±0.0	0.0±0.0	0.2±0.2	–	0.03	2.30
Acaulospora sp.2	0.5±0.4	0.5±0.3	–	–	0.05	1.27
Acaulospora sp.3	0.2±0.1	0.2±0.1	–	–	2.52	1.68
Acaulospora sp.4	0.1±0.1	–	–	–	2.21	1.29
Acaulospora sp.5	–	–	0.0±0.0	–	0.00	1.31
Entrophospora infrequens	–	–	0.3±0.2	0.2±0.1	1.10	1.50
Scutellospora calospora	0.1±0.1	0.1±0.1	–	0.7±0.3	5.70*	0.21
Gigaspora gigantea	–	0.0±0.0	–	–	1.98	1.31

a) Mean values and SE (%) of 24 samples are shown. Effects of year and season were evaluated using general linear model in SPSS (version 16.0) and *F*-values were shown. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. "–" indicates that the species is absent and "0.0" represents the rounded value of a very low RA.

Table 3 Indicator species for different treatments, years and seasons, according to indicator species analysis with a maximum indicator value (*IV*)[a)]

Grouped by	Preferred group	Indicator species	*IV*	*P*
Precipitation				
	Increased precipitation	*Ambispora gerdemannii*	54.8	0.023
Year				
	2005	*Glomus albidum*	60.8	0.006
	2005	*Rhizophagus fasciculatum*	41.8	0.003
	2006	*Ambispora gerdemannii*	55.6	0.030
	2006	*Glomus aggregatum*	16.7	0.005
	2006	*Scutellospora calospora*	22.2	0.003
Season				
	Spring	*Ambispora gerdemannii*	39.6	0.012
	Spring	*Funneliformis caledonium*	30.6	0.001
	Autumn	*Claroideoglomus etunicatum*	41.6	0.000
	Autumn	*Rhizophagus fasciculatum*	31.6	0.019
	Autumn	*Glomus warcupii*	12.5	0.031

a) Significant *P*-values indicate significant preferences to increased precipitation, specific year or seasons.

these two abundant sporulating species were calculated. Significant impacts of precipitation were found in the first half of the growth season (from May 25 to August 1) (see the left four columns in Figure 1(a) and (b)). Under ambient precipitation, the relative abundance of *C. etunicatum* increased and the relative abundance of *Am. gerdemannii* decreased; increased precipitation significantly reduced the increase of relative abundance of *C. etunicatum* (see the left four columns in Figure 1(a)) and alleviated the decrease of the relative abundance of *Am. gerdemannii* (see the left four columns in Figure 1(b)) during the first half of the growth season. Warming showed no significant effects on relative abundance of *C. etunicatum* and *Am. gerdemannii*. Both warming and increased precipitation had no significant

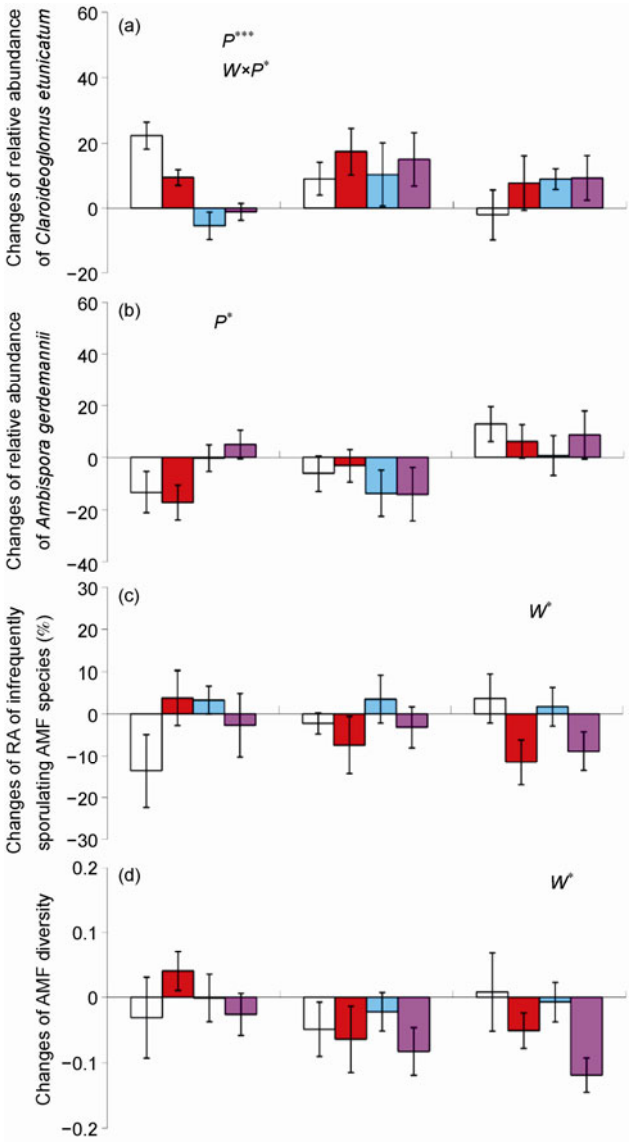

Figure 1 Intra- and inter-annual changes in relative abundance of two abundant sporulating AMF species, (a) *Claroideoglomus etunicatum* and (b) *Ambispora gerdemannii*; (c) the relative abundance (RA) of infrequently sporulating AMF species; and (d) species diversity (Simpson's Diversity Index) of the AMF community. Treatments include control (□), artificial warming (*W*, ■), enhanced precipitation (*P*, ■) and both warming and enhanced precipitation (*WP*, ■). Bars represent 1 SE. The significant impacts of *W*, *P* and *W*×*P* are shown as * $P<0.05$, *** $P<0.001$.

effect on inter-annual variation in the relative abundance of *C. etunicatum* and *Am. gerdemannii*.

To evaluate the influence of warming and precipitation on spore proportion of *C. etunicatum* and *Am. gerdemannii*, DRA (Difference of Relative Abundance) between the two dominant species were calculated (Table 4). *Am. gerdemannii* was the most abundant species and *C. etunicatum* took the second place on May 25. At the end of the growth season, however, *C. etunicatum* took the first place and *Am. gerdemannii* was at the second place. Owing to the proportion shifts of *C. etunicatum* and *Am. gerdemannii*, a significantly lower DRA between *C. etunicatum* and *Am. gerdemannii* was found in the warming treatment (*W*) on May 25 and in precipitation treatments (*P* and *WP*) on August 1 in 2005 as compared with control. Warming and increased precipitation had significant effects on DRA between the two species at early and middle stage of the growth season in 2005, respectively.

2.4 Effects of warming and watering on infrequent sporulating AMF and diversity

Both the relative abundance of infrequent sporulating species and species diversity differed between the warming and increased precipitation treatments (Figure 1(c) and (d)). Neither the warming nor increased precipitation treatments significantly affected the amount of intra-annual variations in abundance of rare species (see the left and middle four columns in Figure 1(c)). Warming had a significant effect on the inter-annual variation in the relative abundance of spore-non-abundant species (see the right four columns in Figure 1(c)).

The impact of both warming and increased precipitation on species diversity was similar to the effects of these treatments on the relative abundance of infrequent sporulating species (Figure 1(d)). While neither treatment altered the pattern of intra-annual variation (see the left and middle four columns in Figure 1(d)), the warming treatment significantly affected the pattern of inter-annual changes in the species diversity of AMF fungi (see the right four columns in Figure 1(d)). After one year, the proportion of spore-non-abundant AMF species in the WP treatment was significantly lower than in the control (one-way ANOVA, $P<0.05$; also see the right four columns in Figure 1(d)). As species diversity and the proportion of rare species tend to be correlated, the similar patterns observed for these two variables were not unexpected.

2.5 The relationship between plant and AMF community compositions

The results of the Mantel test on the associations between compositions of the plant and AMF community are presented in Table 5. The plant communities in 2005 and 2006 were significantly correlated as it is expected in experimental plots in which the vegetation is left from one year to the other. However, compositions of AMF communities in the experimental plots were not significantly correlated, indicating that sporulation varies greatly between years. No significant correlation was found between the plant and the AMF communities in 2006, and a marginal correlation ($P=0.11$) between plant and the AMF communities were found in 2005. Interestingly, the plant community in 2006 was significantly positively correlated with the AMF community in 2005, indicating that AMF community may exert influences on plant communities.

Table 4 Difference of relative abundance (DRA) between two abundant sporulating AMF species, *Claroideoglomus etunicatum* and *Ambispora gerdemannii*, under warming and precipitation treatment[a)]

Treatments	2005		2006	
	May 25[b)]	Aug. 1	Sep. 22	Aug. 4
Control	–2.1±5.2	23.3±8.0	38.1±6.8	0.8±12.6
Warming	–17.3±5.1	6.2±4.0	26.7±10.5	15.3±3.1
Precipitation increase	–	–5.9±11.3	18.2±11.3	2.4±9.1
Warming + Precipitation increase	–	–13.4±9.0	15.7±17.6	–12.7±11.9
Significance (*P*-values)				
Warming (*W*)	0.047*	0.162	0.770	0.971
Precipitation (*P*)	–	0.009**	0.996	0.198
$W \times P$	–	0.580	0.758	0.151

a) Data are presented as Mean ± SE. b) Watering was not carried out on May 25, 2005, and the plots in treatments of Precipitation (*P*) and Warming plus Precipitation (*WP*) were added to control and Warming (*W*), respectively.

Table 5 Mantel test on intra- and inter-annual association between plant and AMF community composition at a Mongolian Grassland site[a)]

	Plant-2005	Plant-2006	AMF-2005	AMF-2006
Plant-2005	1.000			
Plant-2006	0.703***	1.000		
AMF-2005	0.121^{+}	0.154*	1.000	
AMF-2006	0.053	0.006	0.057	1.000

a) Standardized Mantel statistics (*r*) are given: +, * and *** indicate significance at the 0.15, 0.05 and 0.001 level, respectively.

The relationships between two abundant sporulating AMF species and the ground cover by two dominant plant functional groups (i.e. grasses and forbs) are shown in Figure 2. As the abundance of *C. etunicatum* increases, relative to that of *Am. gerdemannii*, the percent cover of grass significantly decreases, whereas forbs significantly increased.

For a more detailed analysis we divided the plots into *C. etunicatum* dominated (DRA $\geqslant$ 10%), *Am. gerdemannii* dominated (DRA $\leqslant$ –10%), and remaining plots (–10%< DRA<10%). The mean relative ground cover by plant species in these *C. etunicatum*-dominated and *Am. gerdemannii*-dominated plots were then plotted in Figure 3. Linear regressions were calculated for grasses and forbs, whose slopes differ significantly ($t = 9.84$, $P < 0.01$). The steep and

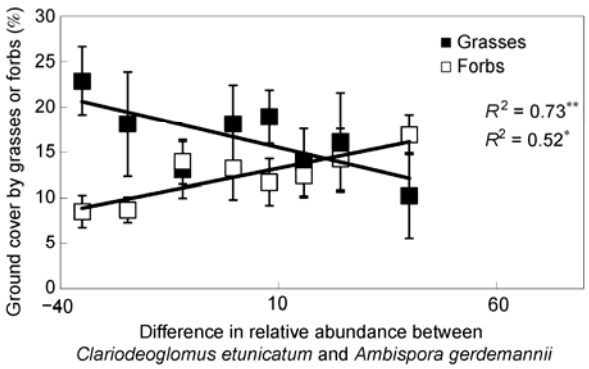

Figure 2 Relationship between ground cover of grasses and forbs and the difference in relative abundance (DRA) between *Claroideoglomus etunicatum* and *Ambispora gerdemannii*. Pearson correlation coefficients (*R*) are given. * and ** indicate significance at the 0.05 and 0.01 level, respectively.

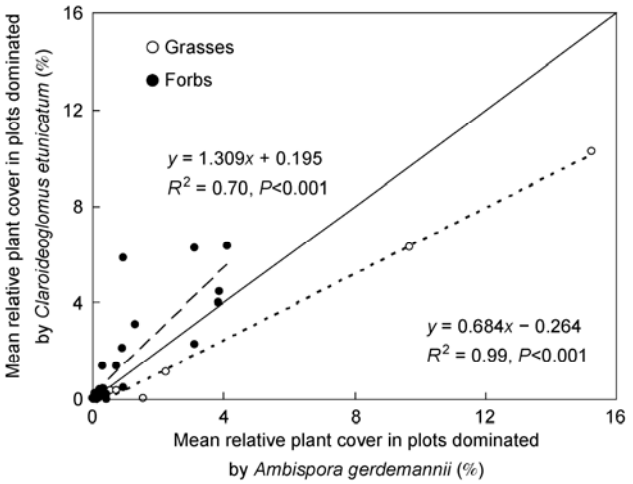

Figure 3 Mean relative coverage of grasses and forbs in *Ambispora gerdemannii* and *Claroideoglomus etunicatum* dominated plots. Dotted and dash lines show the linear regressions for grasses and forbs.

shallow regression slopes denote different preferences of grasses and forbs to *C. etunicatum* and *Am. gerdemannii* dominated microsites.

Preference index of plant species to *C. etunicatum* dominated plots, defined as the ratio of the mean relative coverage of a plant species in *C. etunicatum* dominated plots to the mean relative cover of it in both *C. etunicatum*- and *Am. gerdemannii*-dominated plots, were shown in Figure 4. Members of Gramineae (grasses) exhibited significantly lower preference index than species in Compositae, Fabaceae, Liliaceae, and Rosaceae. An *F*-test on this preference

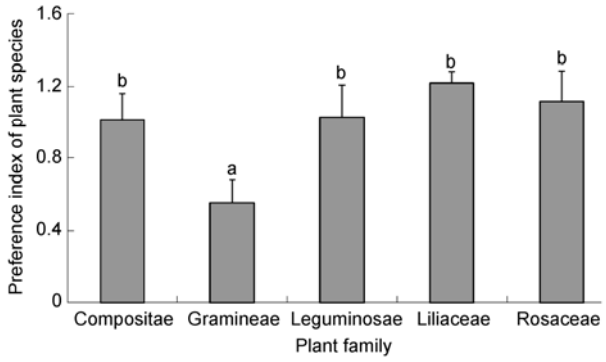

Figure 4 Preference index of members of different plant families to *Claroideoglomus etunicatum* dominated plots. The bars represent means and 1SE of preference index. Different letters denote significant differences (P<0.05) according to one-way ANOVA.

data suggests that plants within a family show more similar preferences in choosing plots dominated by different AMF species than plants from different families, since the variation between families was significantly higher than that within families (F=3.816, P=0.018).

3 Discussion

How mycorrhizal plant-fungal associations will be affected by changing climate has become a key challenge for mycorrhizal research in the new millennium [41]. Global climate change may have direct or plant-soil-mediated indirect effects on the physiology of mycorrhizal fungi (e.g. hyphal growth, sporulation, nutrient uptake) and the species composition of these below-ground communities [42,43]. While many factors, including plant nutrient, light intensity, and plant community composition, have been suggested to influence the development of arbuscular mycorrhizal fungi and the formation of mycorrhizae [44–51], soil temperature and moisture are undoubtedly two major factors [52,53]. It has been documented that increase of soil temperature may enhance growth of extraradical mycorrhizal hyphae, root length colonized by mycorrhizal fungi, fungal TRFs in soil and plant roots [19,54,55]. Changes in precipitation and soil moisture could also affect fungal abundance and litter decomposition [56]. Since the optimal growth temperatures and moistures may vary among fungal species [57], global warming and concurrent changes in precipitation progress could also affect the competitive balance between different AMF species and composition of AMF communities.

3.1 Fluctuations in the occurrence of rare AMF species and community diversity

In our study, artificial warming significantly reduced the relative abundances of species sporulating little and/or rarely and the species diversity of the AMF community (Figure 1), perhaps as a result of spore-abundant species performing better on average at higher temperatures. If abundant sporulating species and rarely sporulating respond differently to climate change, the competitive balance between dominant and rare species may be altered, and the species diversity may change. Negative impacts on AMF community diversities may affect the functioning of grassland ecosystems, given there are positive correlations between plant and AMF communities [23].

Rare species are important components of ecosystems, and their abundance, along with species richness can be important determinants of ecosystem functioning. Compared to dominant species, rare species face heightened extinction risks under unfavorable or stressful conditions.

Although spore abundance often does not completely reflect the fungal biomass in roots and soils [58–60], spore abundance of AMF species is still a good indicator of reproductive success. A decrease in spore density, especially for a species with low spore production, likely indicates reduced population viability which could result in local extinction. The species richness and species diversity of an AMF community depend, therefore, much on the occurrence of species with low sporulation rates.

3.2 Environmental factors and abundant sporulating AMF species

Our results showed that both the warming and water-addition treatments influenced the relative sporulation between *C. etunicatum* and *Am. gerdemannii*. The effects of these treatments were strongest during the first half of the growing season (Figure 2). This result implies that, in comparison with *C. etunicatum*, *Am. gerdemannii* prefers warmer and wetter early-season soil conditions. Compared to rare species, dominant species usually face a smaller threat of extinction through changes in their environment. Similarly, higher temperatures and increased precipitation may thus only alter the proportion of abundant sporulating AMF species, but not decide whether sporulation occurs or not. Higher temperatures and increased precipitation could therefore change the composition of AMF communities by altering the relative spore production of different AMF species.

It has been suggested that the AMF species composition, but not necessarily the species richness, is related to above ground plant biodiversity in ecosystems [61]. The changes in relative abundance of common species should therefore be involved in the plant-AMF interactions. Positive or negative feedbacks are expected to occur between plant and mycorrhizal fungal communities [49,62], with implications for ecosystem functioning. In these feedback models, the magnitude of the benefit for both plant and fungal partners is the key to determine the structure and stability of both plant and fungal communities [62].

We found that the DRA between *C. etunicatum* and *Am. gerdemannii* was negatively correlated with the percent cover of grasses, and positively correlated with the percent

cover of forbs, which implies that shifts in the dominance of spore-abundant AMF species may be related to the structural changes of the plant community. The different responses of grasses and forbs to the DRA between AMF species might result from asymmetrical benefits (to the grasses vs. forbs) from these two spore-abundant AMF species or different host preferences of these two AMF species to grasses and forbs. In grasslands, compensatory effects between functional groups are thought to be an important mechanism maintaining ecosystem stability [63]. Our results indicate that AMFs may be involved in creating these compensatory effects. In a status report for the BIOLOG project in Europe, which seeks to examine mycological responses to climate change, Hempel et al. [64] found that suppressing AMFs with fungicide significantly increased plant ^{15}N-nitrate uptake in grasses, while reducing it in herbs. Possible explanation was that fungicide-sensitive and fungicide-resistant AMF species benefit herbs to a different degree than grasses different amounts, which agree with our observations.

This study has shown the existence of different preferences of grasses and forbs to habitats dominated by different AMF species. Compared with the numerous plant species, the number of AMF species is quite limited. Many AMF species are considered to have broad host range, and some distribute in many areas of the world. Although lack of specificity, whether any preference exist in the plant-AMF associations is still not known. Our results suggest that members of different plant families may have preferences to assemblages with different dominant AMF species. Changes in the occurrence of dominant AMF species could thus induce changes in plant community composition.

3.3 The role of AMF community, driver or passenger?

Hart et al. [65] gave the "driver/passenger hypothesis", in which mycorrhizal fungal community could act as either driver or passenger in the interaction with plant community. In the driver hypothesis, interactions within mycorrhizal fungal communities are responsible for changes in the plant community, while in the passenger hypothesis, mycorrhizal community dynamics are only a by-product of changes in the plant community [65].

In studies which correlate plant and mycorrhizal fungal diversity, it is usually impossible to determine whether plant diversity causes mycorrhizal diversity or *vice versa* [66]. Although it has been suggested that mycorrhizal diversity may determine the diversity and productivity of an ecosystem [23,24], many researchers believe that plants can regulate mycorrhizal diversity and community structure [66–68]. In feedback models, a "circular" interaction is implied between plant and AMF communities [62,69]. That is, plant and AMF diversity are interdependent, serving as both cause and effects in these models. These feedback models should be extended to consider the effects of environmental change, as environmental variables may influence both plant and AMF communities.

Under future climate change, the relative sensitivities of plant and AMF communities to environmental signals will be the key to determining cause and effect. In detail, the more sensitive mycorrhizal partner (i.e. either the plant or the AMF) will respond immediately to any environmental change, and will therefore act as the cause of change in diversity. We have found that the plant communities were more stable than the AMF communities, perhaps because most plant species are perennial in this grassland ecosystem. However, we also found that the plant community composition in 2006 was correlated rather with the mycorrhizal community in 2005 than that in 2006, implying that changes in AMF community might cause changes in the plant community the following year. Our result seems to support the diver hypothesis and the relative sensitivities of the plant and AMF communities may be the key in determining who the driver is in the plant-AMF interactions. In ecosystems subjected to frequent disturbance (e.g. flood, drought, or extreme temperatures), the more sensitive AMF communities will probably act as the driver and play a much important role in the plant-AMF feedbacks.

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