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ARTICLE

Increased precipitation, rather than warming, exerts a strong influence on arbuscular mycorrhizal fungal community in a semiarid steppe ecosystem[1]

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Abstract: Knowing the responses of arbuscular mycorrhizal (AM) fungi to warming and increased precipitation are critical for understanding how biodiversity is maintained and how the ecosystem functions under global climate-change scenarios in natural ecosystems. In this study, AM fungal communities were examined in a 6 year experiment with warming and increased precipitation, in a semiarid steppe in northern China. Only the increased precipitation, regardless of warming, significantly increased AM fungal extra-radical hyphal density, compared with the control treatment. AM fungal spore density was significantly increased by the combination of warming and increased precipitation, and increased precipitation-only treatments, but not by warming alone. A total of 36 operational taxonomic units (OTUs) of AM fungi were recovered by 454 pyrosequencing of 18S rDNA. Only increased precipitation, regardless of warming, significantly decreased AM fungal OTU richness and Shannon diversity index, and yet significantly increased AM fungal Bray–Curtis dissimilarity index, compared with the control treatment. AM fungal community composition was significantly affected by increased precipitation via water availability, but not by warming. Our findings demonstrated that the AM fungal community responded more strongly to water availability than to warming in the semiarid steppe ecosystem.

Key words: arbuscular mycorrhizal fungi, increased precipitation, warming, 454 pyrosequencing, 18S rDNA.

Résumé : La caractérisation de la réponse des champignons mycorhizes arbusculaires (MA) au réchauffement et à l'accroissement des précipitations est importante afin comprendre somment se maintient la diversité et comment fonctionne un écosystème, selon des scénarios de changements à l'échelle planétaire dans les écosystèmes naturels. Dans cette étude, des communautés de champignons MA ont été examinées au cours d'une expérience échelonnée sur 6 ans au cours de laquelle elles ont été soumises à un réchauffement et à des précipitation accrues dans une steppe semi-aride du nord de la Chine. Des précipitations accrues, indépendamment du réchauffement, contrairement au réchauffement seul, augmentaient significativement la densité extraradicale des hyphes des champignons MA. La densité des spores des champignons MA était significativement accrue par la combinaison du réchauffement et des précipitations importantes, mais pas par le réchauffement seul ou les précipitations seules. Un total de 36 unités taxonomiques opérationnelles (OTU) de champignons MA ont été trouvées par le pyroséquençage 454 de l'ADNr 18S. Une augmentation des précipitations, indépendamment du réchauffement, contrairement au réchauffement seul, diminuait significativement la richesse en OTU des champignons MA et l'indice de diversité de Shannon, mais augmentait significativement l'indice de distance de Bray–Curtis des champignons MA. La composition de la communauté de champignons MA était significativement affectée par l'augmentation des précipitations à travers la disponibilité en eau, mais pas par le réchauffement. Les résultats des auteurs ont démontré que la communauté de champignons MA répondait davantage à la disponibilité en eau qu'au réchauffement dans un écosystème de steppe semi-aride. [Traduit par la Rédaction]

Mots-clés : champignons mycorhizes arbusculaires, augmentation des précipitations, réchauffement, pyroséquençage 454, ADNr 18S.

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Introduction

Arbuscular mycorrhizal (AM) fungi are symbiotic partners of more than 80% of terrestrial plant species (Smith and Read 2008). In the AM association, the plant supplies photosynthetic carbon (C) for the growth and functions of AM fungi, and this could affect AM fungal communities. In return, AM fungi enhance host-plant nutrient and water uptake and resistance to abiotic stresses, and therefore can exert influence on host-plant communities (van der Heijden et al. 1998; Birhane et al. 2012) and consequently influence ecosystem processes and functioning (Rillig 2004). It is accepted that AM associations, which function as vital links between aboveground and belowground biotic communities, are influenced by environmental variation such as warming and increased precipitation in ecosystems (Liu et al. 2009; Yang et al. 2011*a*; Kim et al. 2014, 2015; Li et al. 2015).

Mean global temperature has increased by ~0.76 °C since 1850 and is predicted to rise by an additional 1.8–4.0 °C by the end of this century (IPCC 2007). Temperature-manipulation studies have shown that warming affects plant and soil microbial communities in ecosystems (Rillig et al. 2002; Heinemeyer et al. 2004; Hoeppner and Dukes 2012; Yang et al. 2013). As an important component of soil microorganisms, the AM fungal community has shown a variety of responses to warming in ecosystems (Rillig et al. 2002; Gavito et al. 2003; Staddon et al. 2003; Heinemeyer et al. 2004; Yang et al. 2013; Kim et al. 2014, 2015). For example, warming has been shown to have positive or neutral effects on AM fungal richness and spore density (Yang et al. 2013; Kim et al. 2014, 2015), and positive, negative, or neutral effects on AM fungal extra-radical hyphal (ERH) density (Rillig et al. 2002; Gavito et al. 2003; Staddon et al. 2003; Yang et al. 2013; Kim et al. 2014, 2015). Meanwhile, warming significantly influenced AM fungal community composition on the Qinghai–Tibet Plateau in China (Yang et al. 2013), but not in grasslands of the UK or Inner Mongolia in China (Heinemeyer et al. 2004; Kim et al. 2015).

Water availability is the primary limiting factor for biodiversity and ecosystem functioning in arid and semiarid ecosystems (Fuzy et al. 2008). Water-manipulation studies have shown that increased precipitation affects plant and microbial communities in ecosystems (Liu et al. 2009; Yang et al. 2011*a*; Sun et al. 2013*a*; Li et al. 2015; Zhang et al. 2015). For example, increased precipitation has been shown to have positive or neutral effects on the density of AM fungal ERH (Trent et al. 1994; Li et al. 2015; Propster and Johnson 2015), and neutral effects on AM fungal spore density and diversity (Li et al. 2015) in grassland ecosystems. In addition, increased precipitation significantly changed AM fungal community composition in grassland ecosystems (Sun et al. 2013*a*; Li et al. 2015). While the separate effects of warming alone or increased precipitation alone on AM fungal communities have received considerable attention in previous studies, to our knowledge, the combined and interactive effects of these two factors on AM fungal communities have been poorly documented from natural ecosystems.

The responses of AM fungal ERH density, spore density, diversity, and community composition to warming and increased precipitation may predict changes in community structure and function in ecosystems (van der Heijden et al. 2008). For example, AM fungal hyphae are responsible for host-plant nutrient and water uptake and re-distribution of nutrients among plant individuals, which mediate plant co-existence (Smith and Read 2008; van der Heijden et al. 2008). AM fungal spores and hyphae secret a glomalin-related soil protein with a residence time in the soil of 6–42 years, thereby contributing to soil C sequestration (Rillig et al. 2001). In addition, because different AM fungal species have various functional traits, their diversity and community composition affect plant diversity, productivity, and community composition (van der Heijden et al. 1998). Therefore, knowing the response of AM fungi to warming and increased precipitation is critical for understanding how biodiversity is maintained and ecosystems function under global climate change scenarios in natural ecosystems.

The Inner Mongolian steppe covers 313 million hm^2 in northern China, where the semiarid grassland is particularly sensitive to climate change and anthropogenic activities (Wan et al. 2009). Significant changes in both temperature and precipitation have been reported in this area (Gong et al. 2004). To simulate the changes of temperature and precipitation, a controlled warming and increased precipitation experiment was established in a semiarid steppe ecosystem in northern China to study the responses of the biotic community and ecosystem functioning in 2005. Previous studies have shown that warming and increased precipitation had different effects on plant community structure and productivity (Niu et al. 2008; Yang et al. 2011*b*), soil microbial biomass and respiration (Liu et al. 2009), and soil C storage, decomposition, and cycling (Niu et al. 2008; Song et al. 2012; Zhou et al. 2013). However, limited information is available on how an AM fungal community responds to warming and increased precipitation in the semiarid steppe ecosystem.

To better understand the response of AM fungi to warming and increased precipitation, AM fungal ERH density and spore density were measured in a 6 year experiment with warming and increased precipitation in the semiarid steppe ecosystem in northern China. The AM fungal community composition in soil was examined using 454 pyrosequencing of 18S rDNA. The aims of this study were to test for the different effects of warming and increased precipitation on AM fungal spore density, ERH density, diversity, and community composition. This study provides insight into the role of AM fungi under global climate change scenarios in semiarid steppe ecosystems.

Materials and methods

This study was conducted in a semiarid steppe ecosystem in Duolun County, Inner Mongolia, northern China (42°02′N, 116°17′E, 1324 m a.s.l.). This site is located in a temperate zone with a mean annual temperature of 2.1 °C and mean annual precipitation of 385.5 mm (~86% occurring between May and September). The dominant plants are *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*. The soil is composed of Haplic Calcisols (Wan et al. 2009).

The experimental design has previously been described in detail by Niu et al. (2008). Briefly, 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 × 15 m^2 blocks; one block in each pair was assigned as the increased precipitation treatment and the other as the control. Within each 10 × 15 m^2 block, four 3 × 4 m^2 plots were treated as the warmed and control plots with two replicates. For increased precipitation, a total amount of 120 mm precipitation (approximately 30% of mean annual precipitation), at a rate of approximately 15 mm per week, was applied by sprinklers in July and August from 2005 onwards. For warming, plots were heated by MSR-2420 infrared radiators (Kalglo Electronics, Bethlehem, Pennsylvania, USA) with a radiation output of 1600 W suspended 2.25 m above the ground since 28 April 2005. The annual mean temperatures were 3.89 ± 0.15 °C in warming treatments and 2.1 ± 0.15 °C in no-warming treatments. Therefore, there were six replicates for each treatment (control, warming, increased precipitation, warming plus increased precipitation).

On 12 August 2011, three soil cores (20 cm deep, 3.5 cm diameter) were randomly collected from each plot and mixed as one composite sample. Fresh soil was sieved (1 mm sieve) to remove roots and debris. Soils for AM fungal ERH density and DNA extraction were stored at −80 °C until analysis. Soil for AM fungal spore density was air-dried and stored at 4 °C until analysis. For each plot, soil pH, moisture, available phosphorus (P), NH_4^+-N and NO_3^--N were measured since 2005, and information on the soil variables for August 2011 is presented in the Supplementary data, Table S1[2].

AM fungal spore density and ERH density

AM fungal spores were extracted from 20 g air-dried soil from each sample with distilled water using the wet-sieving and decanting method (Daniels and Skipper 1982) and counted under 40× magnification (80i microscope, Nikon). Extra-radical hyphae were extracted from 4 g of each fresh soil sample using the membrane filter method (Rillig et al. 1999) and were separated into AM and non-AM fungal hyphae based on their morphology and staining color (Miller et al. 1995). AM fungal hyphal length was measured using a grid-line intersect method by observing 135 fields of view for each filter under 200× magnification (80i microscope).

DNA extraction, PCR, and 454 pyrosequencing

Genomic DNA was extracted from 0.5 g frozen soil by a direct bead-beating extraction method using an UltraClean Soil DNA isolation kit (MoBio Labs) according to the manufacturer's instructions. Amplicons of the 18S rDNA region for 454 pyrosequencing was generated by a two-step PCR procedure. The first amplification with primers GeoA-2 (Schwarzott and Schussler 2001) and NS4 (White et al. 1990) was carried out in a final 25 μL reaction solution containing 2.5 μL 10× PCR buffer, 1.5 mmol·L^{-1} $MgCl_2$, 200 μmol·L^{-1} of each dNTP, 0.75 μmol·L^{-1} of each primer, 1.5 U *Taq* polymerase (TaKaRa), and 1 μL of template DNA. The thermal cycling followed an initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 45 s, annealing at 54 °C for 1 min, extension at 72 °C for 1.5 min, and a final extension at 72 °C for 10 min. The product of the first amplification was diluted 20× with sterilized deionized water, and 1.0 μL of the resulting solution was used as template for the nested PCR. Conditions for the nested PCR were similar to the first PCR, except for an annealing temperature of 58 °C, 1 min extension, 30 cycles, and primers NS31 (Simon et al. 1992) and AML2 (Lee et al. 2008) linked to sequencing adaptors A and B, respectively. A bar-code sequence, 10 bases in length, was inserted between the A adaptor and NS31 primer sequence (Supplementary data, Table S2). The nested PCR products were then loaded onto a 1% agarose gel (BIOWEST) with 1.0× TAE buffer (40 mmol·L^{-1} Tris, 20 mmol·L^{-1} glacial acetic acid, and 1 mmol·L^{-1} EDTA; pH 8.0), visualized after Goldview staining (Applied Biosystems) under ultraviolet light, and then purified using an Axygen PCR Product Gel Purification Kit (Axygen, Union City, California, USA). The purified PCR products were measured using a fluorescence spectrophotometer (TBS 380; Promega), and 50 ng of DNA from each of the 24 samples were pooled and adjusted to 10 ng·$μL^{-1}$. The pooled products were subjected to 454 pyrosequencing on a Roche Genome Sequencer FLX Titanium (454 Life Sciences, Branford, Connecticut, USA). The raw sequence data were submitted to the Sequence Read Archive of the National Center for Biotechnology Information, USA (NCBI, accession No. SRP029357).

Bioinformatics analysis

The noise generated during the sequencing process was removed using the shhh.flow command in Mothur 1.31.2 (Schloss et al. 2009). Subsequently, the denoised sequences with no valid primer sequence or DNA tag,

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjb-2015-0210.

Table 1. Two-way ANOVA examining the effects of warming (*W*), increased precipitation (*P*), and their interaction (*W*×*P*) on arbuscular mycorrhizal fungal extra-radical hyphal (ERH) density, spore density, OTU richness, Shannon diversity index (*H′*), Pielou's evenness index (*J*), and Bray–Curtis dissimilarity index (BC).

		ERH density		Spore density		OTU richness		*H′*		*J*		BC	
Source of variation	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
W	1	0.025	0.877	0.123	0.730	0.270	0.609	0.001	0.971	1.187	0.289	0.417	0.521
P	1	67.715	<0.001	14.670	0.001	24.067	<0.001	22.172	<0.001	11.123	0.003	130.485	<0.001
W×*P*	1	1.316	0.265	0.007	0.934	0.003	0.955	0.138	0.714	1.293	0.269	0.396	0.531

containing ambiguous bases, homopolymers with >8 bases, or with an average quality score <25 were removed using the trim.seqs command in Mothur (Schloss et al. 2009). As the average read quality score dropped below 25 after 420 bp, the remaining longer sequences were chopped to 400 bp to assure read quality (Schloss et al. 2009). Potential chimeras were detected using the "chimera uchime" command in the Mothur, referencing the chopped sequence itself (Schloss et al. 2009), the SILVA database (Pruesse et al. 2007), and the Maarj*AM* database (Öpik et al. 2010), respectively. The remaining nonchimeric sequences were dereplicated, sorted, clustered, and mapped into OTUs at a 97% similarity level using UPARSE (Edgar 2013). To assure the OTUs were of AM fungal origin, a neighbor joining tree was constructed in MEGA version 6 using the Kimura 2-parameter model with 9999 replicates (Tamura et al. 2013), based on the representative sequences of OTUs obtained in this study and reference sequences of Glomeromycota downloaded from GenBank. To eliminate the effects of different read numbers among the plots on the deduced AM fungal community composition, the number of sequences per plot was normalized to the smallest sample size using the sub.sample command in Mothur (Schloss et al. 2009).

Statistical analysis

Prior to the following analysis on AM fungal community, the abundance (read number) of every OTU was Hellinger transformed. AM fungal Pielou's evenness index, Shannon diversity index for each sample, and Bray–Curtis dissimilarity index between samples were calculated from Hellinger-transformed dataset. A 2-way analysis of variance (ANOVA) was used to examine the effects of increased precipitation, warming, and their interaction on AM fungal spore density, ERH density, OTU richness, Pielou's evenness index, Shannon diversity index, and Bray–Curtis dissimilarity index. Significant differences between treatments were compared using Tukey's HSD test at $P < 0.05$.

To evaluate the effects of treatments and soil variables on AM fungal community composition, permutational multivariate ANOVA using distance matrices (PERMANOVA) was carried out using the adonis function in the package vegan with 9999 permutations (Oksanen et al. 2013). Furthermore, the AM fungal community composition was subjected to nonmetric multidimensional scaling (NMDS) in the vegan package (Oksanen et al. 2013) to elucidate Bray–Curtis dissimilarities in AM fungal community composition among plots. Using the envfit function in the package vegan with 9999 permutations (Oksanen et al. 2013), the four treatments were fitted as centroids and increased precipitation, warming, and soil variables (pH, moisture, NH_4^+-N, NO_3^--N, and available P) were fitted as vectors onto the NMDS graph to elucidate how AM fungal community composition was related to these variables. Euclidean dissimilarities were calculated to construct the distance matrices of increased precipitation, warming, soil moisture, pH, NH_4^+-N, NO_3^--N, and available P. Mantel tests with 9999 random permutations were carried out in the vegan package (Oksanen et al. 2013) to explore the responses of AM fungal community composition dissimilarity to these distance matrices. Partial Mantel tests with 9999 random permutations were carried out in the vegan package (Oksanen et al. 2013) to explore the relationship of AM fungal community composition with warming, increased precipitation, soil moisture, pH, NH_4^+-N, NO_3^--N, and available P, after the influences of other distance matrices were partialled out. The varpart function in the vegan package (Oksanen et al. 2013) was used to partition the variation of AM fungal community dissimilarity by warming, increased precipitation, and soil (including pH, moisture, NH_4^+-N, NO_3^--N, and available P).

Results

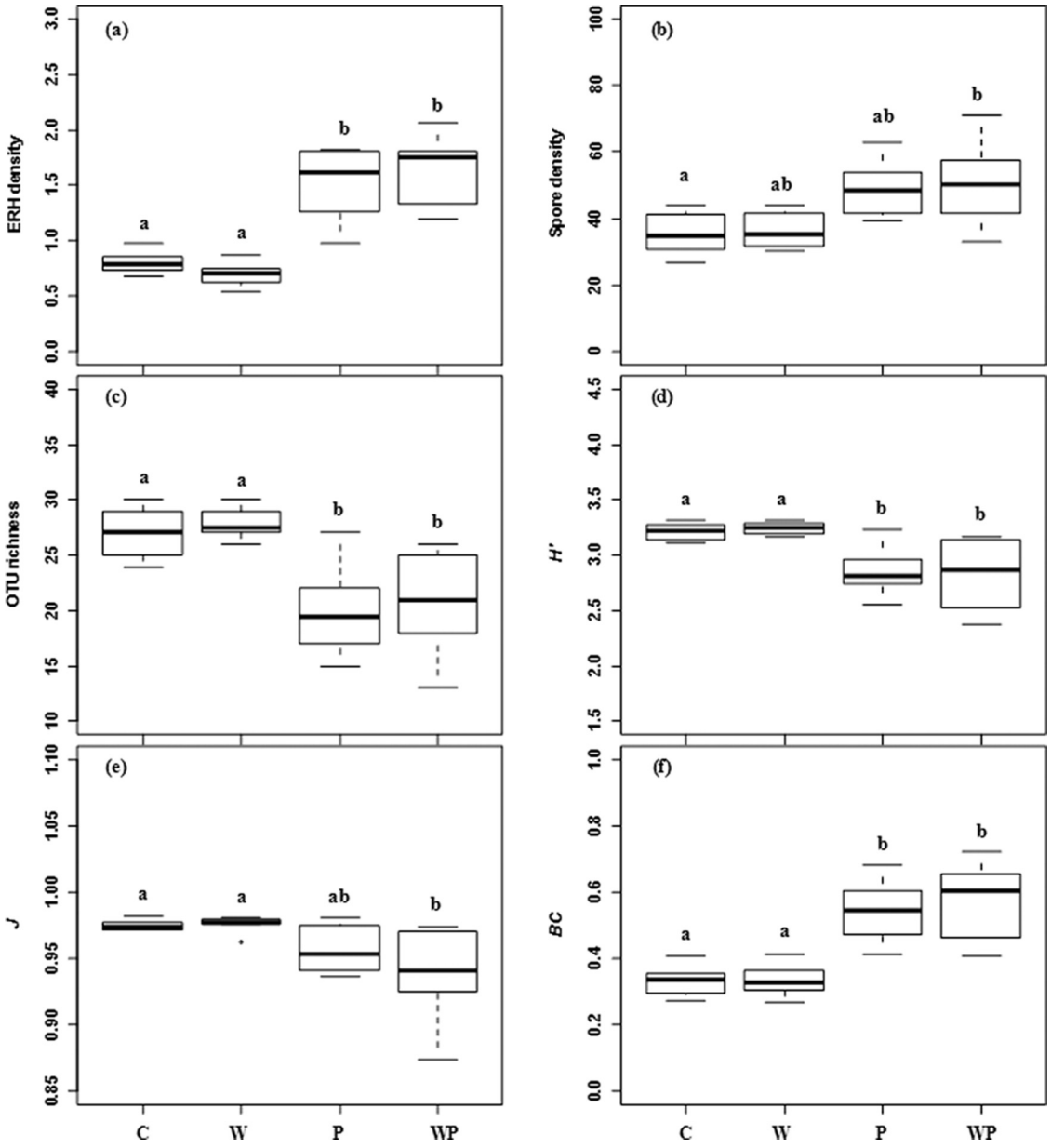
AM fungal spore density and ERH density

Two-way ANOVA showed that increased precipitation, but not warming, had a significant effect on AM fungal ERH density and spore density (Table 1). For example, increased precipitation and warming plus increased precipitation significantly increased AM fungal ERH density by 89%–105% compared with the control treatment, and by 116%–135% compared with the warming treatment (Fig. 1*a*). Compared with the control treatment, AM fungal spore density was significantly increased by 43.7% by warming plus increased precipitation treatment (Fig. 1*b*).

Pyrosequencing analysis and identification of AM fungi

After controlling for sequence quality, 36 911 nonchimeric AM fungal reads were retained from 94 996 raw reads and clustered into 36 OTUs at a 97% similarity level. As the AM fungal read numbers ranged from 564 to 2493

Fig. 1. Arbuscular mycorrhizal fungal (*a*) extra-radical hyphal (ERH) density, (*b*) spore density, (*c*) OTU richness, (*d*) Shannon diversity index (*H'*), (*e*) Pielou's evenness index (*J*), and (*f*) Bray–Curtis dissimilarity index (BC) under different treatments. Data are the mean ± SE. Bars without shared letters indicate significant differences at $P < 0.05$. *C*, control; *W*, warming; *P*, increased precipitation; *WP*, *W* plus *P*.



among the 24 plots, the read numbers were normalized to 564, resulting in a normalized dataset containing 36 AM fungal OTUs (13 536 reads). Among the 36 OTUs, 31 belonged to Glomeraceae, two to Claroideoglomeraceae, two to Diversisporaceae, and one to Paraglomeraceae (Fig. 2). Among the 36 AM fungal OTUs, 34 (3384 reads) were recovered from the control treatment, 33 (3384 reads) from the warming treatment, 36 (3384 reads) from increased precipitation treatment, and 35 (3384 reads) from warming plus increased precipitation treatment (Supplementary data, Fig. S1*a*). The 10 most abundant OTUs accounted for 85.5% of the total AM fungal reads (Supplementary data, Fig. S1*b*). Of the 36 AM fungal OTUs, 26 occurred in ≥12 (50% of total) samples and two in <5 (8.3% of total) samples (Fig. S1*c*).

AM fungal community

Two-way ANOVA showed that increased precipitation, but not warming, had a significant effect on AM fungal OTU richness, Shannon diversity index, Pielou's evenness index, and the Bray–Curtis dissimilarity index (Table 1). Compared with the control treatment, increased precipitation and warming plus increased precipitation treatments significantly decreased AM fungal OTU richness by 15.4%–16.7% (Fig. 1*c*) and Shannon diversity index by 7.7%–9.8% (Fig. 1*d*). Compared with the warming treatment,

Fig. 2. Neighbor-joining analysis of 18S rDNA of arbuscular mycorrhizal fungal OTUs obtained in the study with reference sequences of Glomeromycota in MEGA. The phylogram is rooted with an outgroup *Paraglomus brasilianum*. The GenBank accession number of reference sequences and the sequence name of OTUs obtained in this study are given in parentheses. Bar indicates 0.01 expected changes per site.

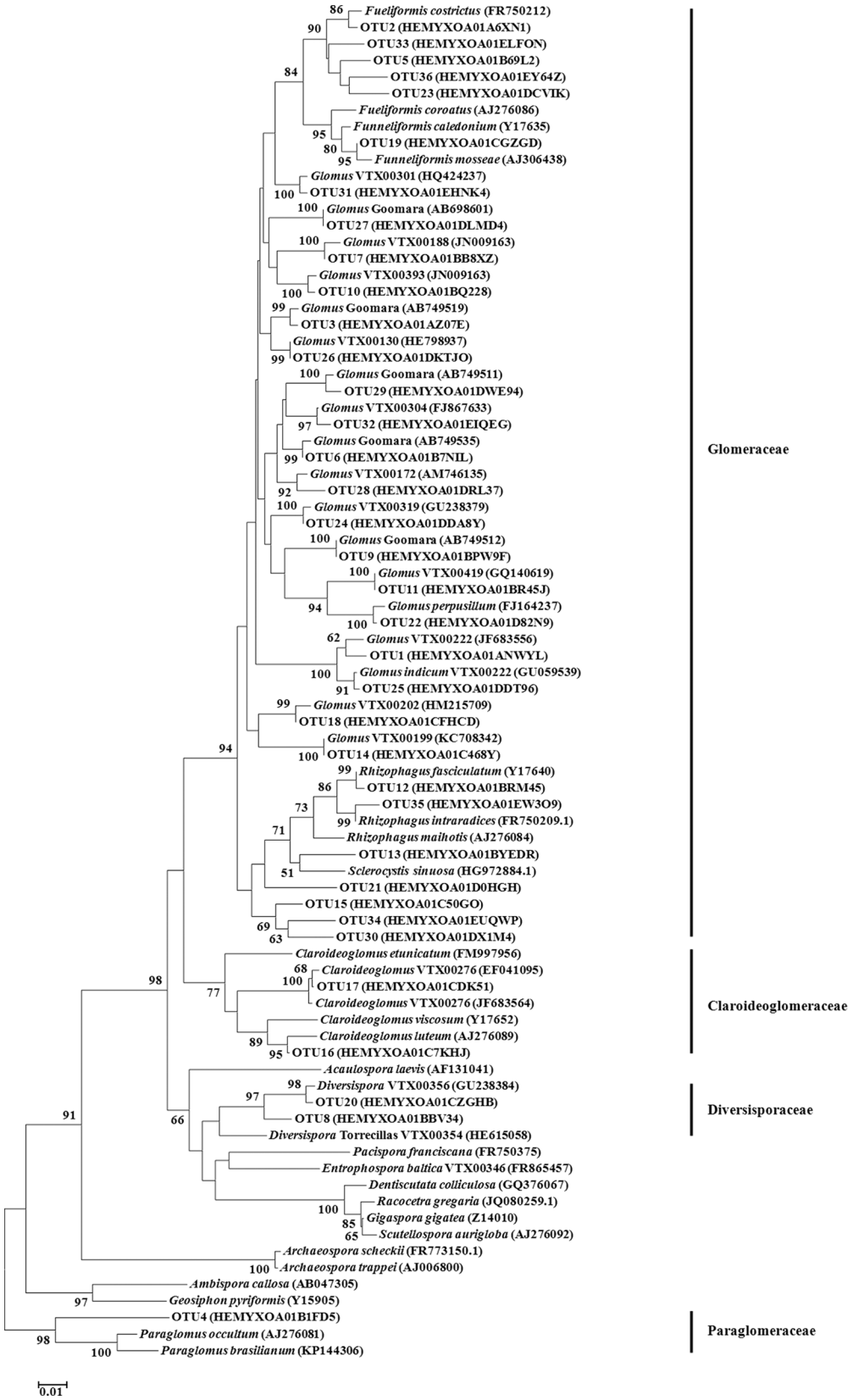
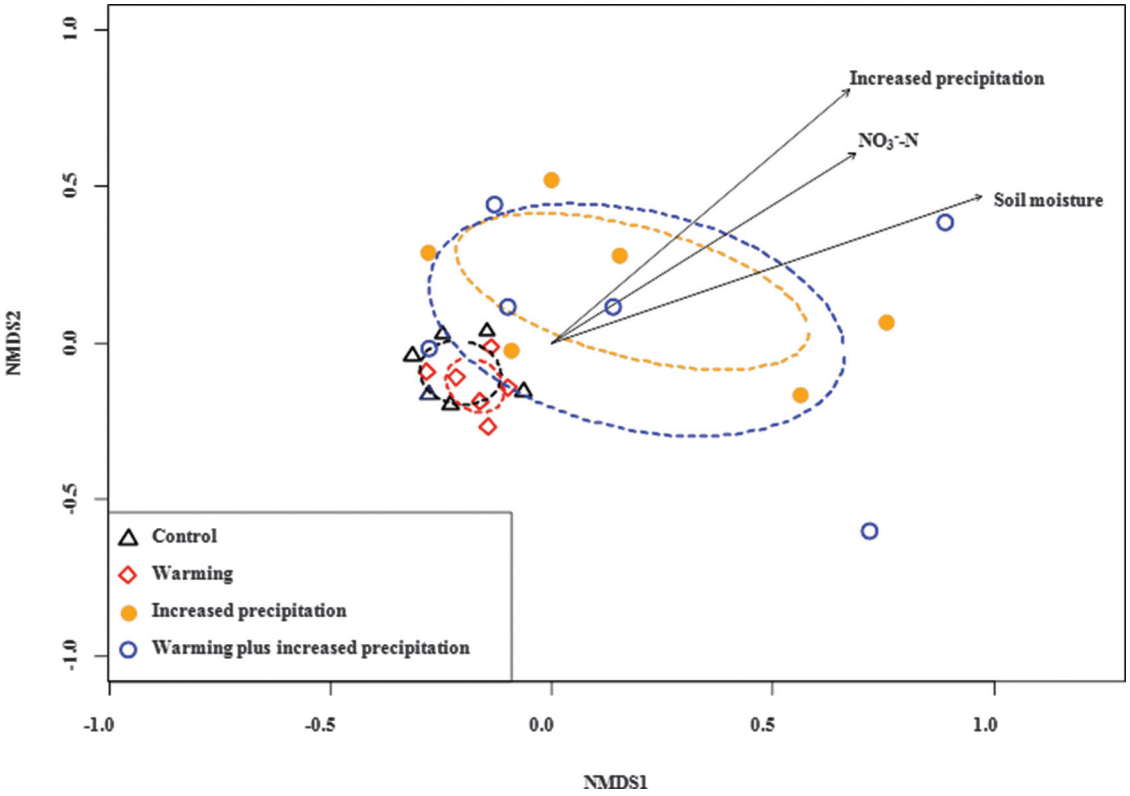


Fig. 3. Nonmetric multidimensional scaling (NMDS) of arbuscular mycorrhizal fungal community composition under different treatments (R^2 = 0.283, P = 0.017). Ellipses in the plot represent 95% confidence intervals around the average values for samples from each treatment. Significant soil moisture, NO_3^--N and increased precipitation are presented as vectors on the ordination graph (P < 0.05 in envfit). [Colour online.]



increased precipitation and warming plus increased precipitation treatments significantly decreased AM fungal OTU richness by 18.0%–19.3% (Fig. 1*c*) and Shannon diversity index by 8.7%–10.9% (Fig. 1*d*). Warming plus increased precipitation treatment significantly decreased Pielou's evenness index for AM fungi by 4.7% compared with the control treatment, and by 4.9% compared with the warming treatment (Fig. 1*e*). Increased precipitation and warming plus increased precipitation treatments significantly increased the Bray–Curtis dissimilarity index for AM fungi by 64.2%–71.8% compared with the control treatment, and by 64.0%–71.7% compared with the warming treatment (Fig. 1*f*).

PERMANOVA showed that the AM fungal community composition was affected by increased precipitation (F = 5.788, R^2 = 0.183, P < 0.001) and soil moisture (F = 5.856, R^2 = 0.185, P = 0.002). In addition, NMDS analysis indicated that the AM fungal community composition was affected by treatments (R^2 = 0.514, P < 0.001; Fig. 3). Furthermore, the AM fungal community composition was related to increased precipitation (R^2 = 0.527, P < 0.001), soil moisture (R^2 = 0.819, P < 0.001), and NO_3^--N (R^2 = 0.589, P < 0.001; Fig. 3).

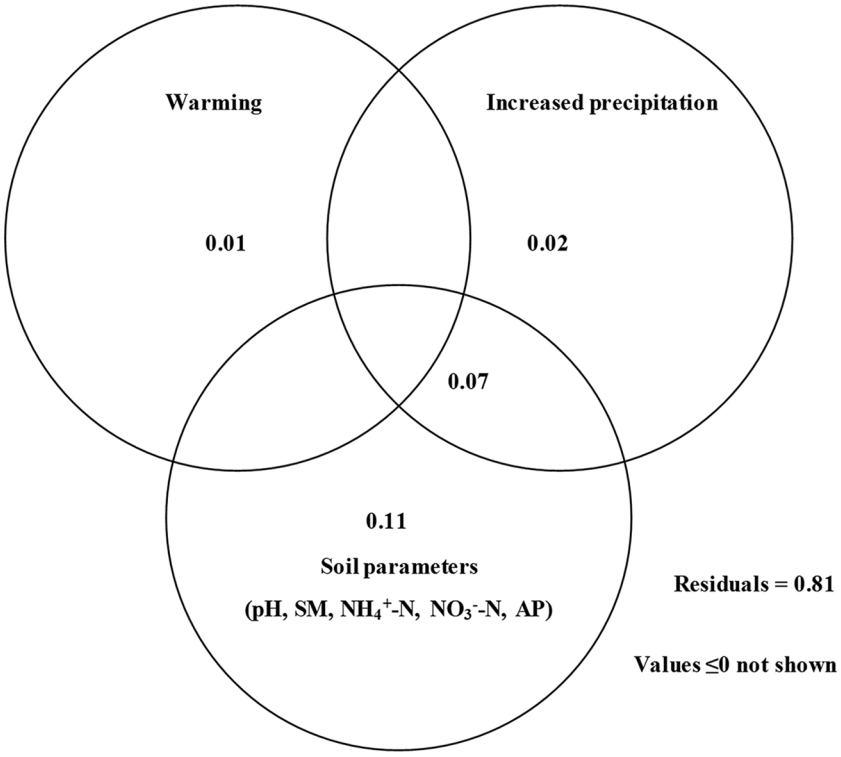
Mantel tests showed that the AM fungal community composition was related to increased precipitation (R = 0.267, P < 0.001), NO_3^--N (R = 0.454, P < 0.001), and soil moisture (R = 0.730, P < 0.001; Table 2). Furthermore, the AM fungal community composition was related to soil moisture (R = 0.682, P < 0.001), after the effects of warming, increased precipitation, soil pH, available P, NH_4^+-N and NO_3^--N were partialled out (Table 2).

Table 2. Mantel and partial Mantel tests of the matrix of arbuscular mycorrhizal fungal community composition with the matrices of warming, increased precipitation, soil moisture, pH, available phosphorus, NH_4^+-N, and NO_3^--N.

	Mantel test		Partial Mantel test	
Matrix	*R*	*P*	*R*	*P*
Warming	−0.013	0.621	0.038	0.152
Increased precipitation	0.267	<0.001	−0.201	0.961
pH	−0.219	0.975	−0.174	0.929
Soil moisture	0.730	<0.001	0.682	<0.001
Available phosphorus	−0.115	0.730	−0.063	0.583
NH_4^+-N	0.018	0.363	−0.057	0.679
NO_3^--N	0.454	<0.001	−0.123	0.767

Variation partition showed that 19% of total variation in AM fungal community composition was explained (Fig. 4). Of these, increased precipitation (9% variation explained) had a stronger influence than warming (1%). Furthermore, most of the increased precipitation effect (7%) was shared with soil parameters (Fig. 4). Addition-

Fig. 4. Pure and shared effects of warming, increased precipitation, and soil parameters on arbuscular mycorrhizal fungal community as derived from variation partitioning analysis. Numbers indicate the proportion of explained variation. SM, soil moisture; AP, available phosphorus.



ally, soil factors independently explained 11% of variation in the AM fungal community (Fig. 4).

Discussion

The response of AM fungal ERH density to warming and increased precipitation is of particular significance for understanding soil nutrient cycling and biodiversity maintenance under global climate change scenarios in natural ecosystems. We found that increased precipitation, but not warming, significantly increased AM fungal ERH density, regardless of warming in the semiarid steppe. This result is consistent with the previous field studies showing that increased precipitation has a positive effect on AM fungal ERH density in an arid ecosystem (Trent et al. 1994), and warming exerts a neutral effect on AM fungal ERH density in grassland ecosystems (Yang et al. 2013; Kim et al. 2014, 2015). The positive effect of increased precipitation on AM fungal ERH density may result from the alleviated water stress of AM fungi in this semiarid grassland ecosystem (Trent et al. 1994). In addition, increased precipitation may lead to more products of photosynthesis being allocated to root AM fungi (Smith and Read 2008) in exchange for the soil nutrients and minerals absorbed by the AM fungus, which would be more needed for plant growth stimulated by increased precipitation (Niu et al. 2008; Yang et al. 2011*a*). In support of this explanation, root turnover was found to be faster under the increased precipitation treatment than that in control and warming treatments in this study site (Bai et al. 2010).

The response of AM fungal spore density to warming and increased precipitation is of particular significance for understanding soil C cycling under global climate change scenarios in natural ecosystems. We found that AM fungal spore density was significantly increased by the combination of warming and increased precipitation, but not by the warming-only and increased precipitation-only treatments in this study. Similarly, AM fungal spore density was increased by plastic film mulching treatment that simultaneously increased soil temperature and water availability in the Loess Plateau, China (Liu et al. 2012*a*), but was not affected by warming (Yang et al. 2013; Kim et al. 2014, 2015) or increased precipitation (Li et al. 2015) in grassland ecosystems. As the stress of warming was enhanced but the stress of drought was alleviated in the warming plus increased precipitation treatment, this study suggests that AM fungal sporulation was influenced by the trade-off between different environmental stresses (Augé 2001).

The response of AM fungal diversity and community composition to warming and increased precipitation would predict the shifts in plant diversity, productivity and community composition under global climate change scenarios in natural ecosystems (van der Heijden et al. 2008). We found that increased precipitation regardless of warming, but not warming alone, had negative effects

on AM fungal OTU richness and Shannon diversity index, but had a positive effect on the AM fungal Bray–Curtis dissimilarity index in this study. Similarly, Yang et al. (2013) found that warming did not have a significant effect on AM fungal richness and Shannon diversity index in the Qinghai–Tibet Plateau. The negative effect of increased precipitation on AM fungal diversity may be attributed to the loss of niche spaces caused by the alleviation of water stress in the increased precipitation treatment, as AM fungal diversity decreases with decreasing niche spaces (Dickie 2007; Gao and Guo 2013). Meanwhile, the loss of niche selection would lead to the increase of stochasticity of AM fungi (Lindström and Langenheder 2012), resulting in the increase of AM fungal Bray–Curtis dissimilarity index along with increased precipitation in this study.

AM fungal community composition was significantly affected by increased precipitation, but not by warming in this semiarid steppe. Previous studies have similarly demonstrated that AM fungal community composition was significantly affected by increased precipitation (Sun et al. 2013*a*; Li et al. 2015), but not by warming (Heinemeyer et al. 2004; Kim et al. 2015) in grassland ecosystems. Furthermore, increased precipitation, but not warming, significantly altered soil moisture and NO_3^--N (Supplementary data, Table S1), and it has been shown in our and previous studies that AM fungal communities are structured by soil moisture and NO_3^--N in diverse ecosystems (Liu et al. 2012*b*; Sun et al. 2013*b*; Zheng et al. 2014).

In conclusion, increased precipitation without and with warming, but not warming alone, had positive effects on AM fungal ERH density and Bray–Curtis dissimilarity index, but had negative effects on AM fungal OTU richness and Shannon diversity index. The combination of warming and increased precipitation, but not increased precipitation alone or warming alone, had a positive effect on AM fungal spore density. AM fungal community composition was significantly affected by increased precipitation via soil moisture, but not by warming. Our findings demonstrate that the AM fungal community responded more strongly to water availability than to warming in the semiarid steppe ecosystem. This study highlights the risk of losing AM fungal diversity and community stability under increased precipitation scenario in the semiarid steppe ecosystem.

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