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Different responses of arbuscular mycorrhizal fungal community to day-time and night-time warming in a semiarid steppe

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Abstract Arbuscular mycorrhizal (AM) fungi form mutualistic symbioses with most plant species and play important roles in ecosystems. Knowledge of the response of AM fungi to temperature change will improve our understanding of the function of AM fungal community under global climate change scenarios in ecosystems. The effects of constant warming on AM fungal communities have been investigated previously, but responses to asymmetrical warming over 24-h periods have never been documented in natural ecosystems. In this study, we examined AM fungal communities in a full factorial design including day-time and night-time warming in a semiarid steppe in northern China. Day-time and 24-h warming, but not night-time warming, significantly increased AM fungal spore density. In contrast, none of the three warming regimes had a significant effect on AM fungal extra radical hyphal density. A total of 161 operational taxonomic units (OTUs) of AM fungi were recovered by 454 pyrosequencing of 18S rDNA. Day-time, night-time, and 24-h warming all significantly increased AM fungal OTU richness. Some AM fungal OTUs showed a significant bias toward day-time, night-time or 24-h warming. The AM fungal community composition was significantly affected by night-time warming, but not by day-time and 24-h warming. Our finding highlighted different responses of AM fungal spore density and community composition to asymmetrical warming. This study might improve our understanding of ecosystem functioning of AM fungal community under global climate change scenarios in a semiarid steppe ecosystem.

Keywords Arbuscular mycorrhiza · Abundance · Community composition · Day-time warming · Night-time warming · Pyrosequencing · Semiarid steppe

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1 Introduction

Global warming is one of the greatest challenges that we face in this century [1]. Temperature manipulation studies have shown that warming affects plant communities [2, 3] and soil microorganisms [4–6]. As an important component of the soil microbial community, arbuscular mycorrhizal (AM) fungi form symbiotic associations with most terrestrial plant species [7]. In these AM associations, fungi use plants' photosynthetic carbon for their growth and function [8]. In return, AM fungi improve plant nutrient uptake and resistance to abiotic stresses and hence could affect plant community diversity, productivity, composition, and ecosystem functioning [9, 10]. It is accepted that AM associations, which function as vital links between aboveground

and belowground biotic communities, are influenced by rising temperature in ecosystems [4, 11–14].

Some studies have reported that warming has positive effects on AM fungal extraradical hyphal (ERH) density [6, 15]. In contrast, other studies have shown that warming has negative or neutral effects on AM fungal ERH density [4, 12] and AM fungal spore density [4]. In addition, some studies found that warming did not significantly affect soil AM fungal community composition in a UK grassland [11], but significantly altered soil AM fungal community composition in an alpine meadow on the Qinghai-Tibetan plateau in China [4]. However, to date, all manipulative experiments on AM fungal communities have involved increasing temperatures over the entire 24-h period.

Climate warming is expected to have different effects on daily minimum and maximum temperatures, with a tendency for greater night-time than day-time warming [16]. A growing body of evidence from long-term observations and manipulative experiments has demonstrated different impacts of increasing daily minimum and maximum temperatures on plant biomass and crop yield [17, 18]. In 2006, a full factorial design experiment involving day-time and night-time warming was established in a semiarid steppe in northern China [19], a grassland that is particularly sensitive to climate change [2]. Given that day-time and night-time warming have different effects on ecosystem productivity [19], carbon allocation to roots and overall root lifespan [20], plant phenology [21], and soil respiration [22], we hypothesize that they will also have different effects on the AM fungal community in this ecosystem in northern China.

To understand better the effect of day-time and night-time warming on the AM fungal community, we measured AM fungal spore density and ERH density from soil samples in the 5-year day-time and night-time warming experiment described above. The AM fungal community composition in soil was examined using 454 pyrosequencing of 18S rDNA. We tested the different effects of day-time and night-time warming on AM fungal abundance, diversity and community composition in this semiarid steppe ecosystem. This study provides insights into the role of AM fungi under asymmetrical warming scenarios in natural ecosystems.

2 Materials and methods

2.1 Study site, experimental design and sampling

This study was conducted in a semiarid steppe ecosystem in Duolun County, Inner Mongolia, northern China (42°02′N, 116°17′E, 1,324 m above sea level). This site is located in a temperate zone with a monsoon climate and an annual mean temperature of 2.1 °C and precipitation of 385.5 mm (~86 % between May and September). The dominant plants are *Stipa krylovii*, *Artemisia frigida*, *Potentilla acaulis*, *Cleistogenes squarrosa*, *Allium bidentatum*, and *Agropyron cristatum*. The soil is classified as Haplic Calcisols according to the Food and Agricultural Organization of the United Nations (FAO) classification [19].

The detailed experimental design has been described by Wan et al. [19]. Briefly, in a complete random block design including six treatments each with six replicates, 36 plots (3 m × 4 m each) were arranged in a 6 × 6 matrix. The distance between any two adjacent plots was 3 m. In this study, we selected four treatments: (1) control (no warming), (2) day-time (06:00–18:00, local time) warming, (3) night-time (18:00–06:00) warming, and (4) constant (24-h) warming. Warming plots were heated using MSR-2420 infrared radiators (Kalglo Electronics, Bethlehem, PA, USA) suspended 2.25 m above the ground. One "dummy" heater of the same size as the infrared radiator was installed in each control plot to simulate the shading effect of the heater. All the heaters used for the warming treatments were set at a radiation output of 1,600 watts. The differences in mean soil temperature between the heated and control plots were 0.82 ± 0.16 °C during day-time warming and 0.95 ± 0.14 °C during night-time warming. The warming treatment commenced on April 23, 2006.

On August 10, 2010, three soil cores (20 cm deep, 3.5 cm diameter) were randomly collected from each plot and mixed as one composite sample. A total of 24 samples (4 treatments × 6 replicates) were used in this study. Fresh soil was sieved (1 mm sieve) to remove roots and debris. Soils for DNA extraction were stored at −80 °C and for determining AM fungal spore density and physicochemical properties were air-dried and stored at 4 °C until they were analyzed.

2.2 Soil and plant variables

Soil samples were dried for 24 h at 105 °C to determine gravimetric moisture. Soil pH (soil:water = 1:2.5, W/V) was determined with a glass electrode (Thermo Orion T20, USA). Soil organic carbon (SOC) was measured by the dichromate oxidation and titration method [23]. Total soil nitrogen (N) was determined by the Kjeldahl digestion method using an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden), while NH_4^+-N and NO_3^--N (2 mol/L KCl extraction) were determined using a Continuous Flow Analyzer (SAN++, Skalar, Netherlands) according to manufacturer's instructions. Soil available phosphorus (P) was determined by Olsen method [24]. Aboveground net primary productivity (ANPP, g m^{-2}), belowground net primary productivity (BNPP, g m^{-2}), and plant community composition were determined based on the methods of Xia et al. [25]. Information of soil and plant in each treatment is summarized in Table S1.

2.3 AM fungal spore density and ERH density

AM fungal spores were extracted from 20.0 g air-dried soil from each sample with distilled water using the wet-sieving and decanting method [26] and counted under 40× magnification (Nikon 80i, Japan). Extraradical hyphae were extracted from 4.0 g fresh soil from each sample using the membrane filter method [27] and were separated into AM and non-AM fungal hyphae based on their morphology and staining color [28]. AM hyphal length was measured using a grid-line intersect method by observing 135 fields of view for each filter under 200× magnification (Nikon 80i, Japan).

2.4 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, Inc. USA) according to the manufacturer's instructions. Genomic DNA for 454 pyrosequencing was amplified using a two-step PCR procedure. The first amplification with primers GeoA-2 [29] and NS4 [30] was carried out in a final 25-Ml reaction solution including 2.5 μL 10× PCR buffer, 1.5 mmol/L $MgCl_2$, 200 μmmol/L of each dNTP, 0.75 μmmol/L of each primer, 1.5 U *Taq* polymerase (TaKaRa, Japan), and 1 μL template DNA. The thermal cycling was followed by 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 and extension at 72 °C for 1.5 min, and then a final extension at 72 °C for 10 min. The product of the first amplification was diluted with sterilized deionized water by a factor of 20, and 1.0 μL of the resulting solution was used as the 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 [31] and AML2 [32] linked to sequencing adaptors A and B, respectively. A bar-code sequence, 10 base pairs (bp) in length, was inserted between the A adaptor and NS31 primer sequence (Table S2). The nested PCR products were then loaded onto a 1 % agarose gel (BIOWEST, Spain) with 1.0× TAE buffer (40 mmol/L Tris base, 20 mmol/L glacial acetic acid, and 1 mmol/L EDTA, pH 8.0), visualized after Goldview staining (Applied Biosystems, USA) under ultraviolet light, and then purified using an Axygen PCR Product Gel Purification Kit (Axygen, California, USA). The purified PCR products were measured using a fluorescence spectrophotometer (TBS 380, Promega, USA), 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, CT, USA). The raw sequence data were submitted to the Sequence Read Archive of the National Center for Biotechnology Information, USA (NCBI, accession No. SRA071517).

2.5 Bioinformatics analysis

The noise generated during the sequencing process was removed using the shhh.flow command in Mothur 1.31.2 [33]. Subsequently, the denoised sequences, with no valid primer sequence or DNA tag, containing ambiguous bases, homopolymers with >8 bases, or with an average quality score <25 were removed using the trim.seqs command in Mothur. As the average read quality score dropped below 25 after the 420th bp, the remaining longer sequences were chopped to 400 bp to assure read quality [33]. Potential chimeras were detected using "chimera uchime" command in Mothur, referencing the chopped sequence itself [33], the SILVA database [34], and the MaarjAM database [35], respectively. The remaining non-chimeric sequences were clustered into different OTUs at a 97 % similarity level [36] using the CD-HIT-EST [37]. The representative sequences (the most abundant one for each OTU) from the OTUs were blasted against the NCBI nt database, and all non-AM fungal OTUs were removed from the dataset (identified on the basis of the closest BLAST hit not annotated as "glomeromycota") as well as OTUs with <5 reads to reduce the risk of artificially inflating richness due to sequencing error [38]. To assure the remaining OTUs are of AM fungal origin, a neighbor joining tree was constructed in MEGA v5 [39], based on the representative sequences of OTUs obtained in this study and reference sequences of Glomeromycota downloaded from GenBank (Fig. S1). To eliminate the influence of the different read numbers (from 1,793 to 3,410 reads among the samples) on AM fungal community, the number of sequences per sample was normalized to the smallest sample size using without-replacement method by the "normalized.shared" command in Mothur [33]. The cumulative number of AM fungal OTUs was calculated using the "rarefy" function in the package Vegan in R 2.15.1 [40].

2.6 Statistical analysis

The abundance of a given AM fungal OTU is defined as the number of reads of that OTU in a sample. AM fungal OTU richness is defined as the number of OTUs in a sample. A two-way ANOVA was used to examine the effects of day-time warming, night-time warming, and their interaction on AM fungal spore density, ERH density, and OTU richness. Significant differences (mean ± SE, $n = 6$) between the treatments were compared using Tukey's HSD test at $P < 0.05$.

Prior to the following analysis of AM fungal community composition, the abundance of every OTU was Hellinger transformed to down weight the influence of rare OTUs.

Permutational multivariate analysis of variance using distance matrices (PERMANOVA) was carried out to evaluate the effects of day-time warming, night-time warming, and their interaction on AM fungal community composition. To elucidate dissimilarity in AM fungal community composition among plots, non-metric multidimensional scaling (NMDS) with the Bray–Curtis dissimilarity measurement was employed. Using the "envfit" function with 999 permutations, the four treatments were fitted as centroids, and day-time warming, night-time warming, plant species richness, ANPP, BNPP, soil pH, moisture, SOC, total N, C:N, NH_4^+-N, NO_3^--N, and available P were fitted as vectors onto the NMDS graph in order to examine how the AM fungal community composition was related to these variables.

Bray–Curtis dissimilarities were calculated to construct the distance matrices of plant community composition, plant diversity, and biomass (including species richness, ANPP, and BNPP), soil (pH, moisture, SOC, total N, C:N, N:P, NH_4^+-N, NO_3^--N, and available P), day-time warming, and night-time warming. Mantel tests with 9,999 random permutations were carried out to explore the responses of AM fungal community composition dissimilarity to these distance matrices. Partial Mantel tests with 9,999 random permutations were carried out to explore the relationship of AM fungal community composition with plant, soil, day-time warming, and night-time warming, after the influences of other distance matrices were partialled out.

The number of variables for plant community composition was reduced through principal component analysis (PCA), and the first six PCs representing 83.9 % of total variation in plant community composition were retained for subsequent analyses (Table S3). The varpart function was used to partition the variation of AM fungal community dissimilarity by day-time warming, night-time warming, soil (pH, moisture, SOC, total N, C:N, N:P, NH_4^+-N, NO_3^--N, and available P), and plant (PC1-PC6, species richness, ANPP, and BNPP).

NMDS, *envfit*, PCA, and variation partition were carried out in the package *vegan* [40]. Mantel test and partial Mantel test were carried out in the package *ecodist* [41]. All statistical analyses were carried out in R 2.15.1 [42].

3 Results

3.1 AM fungal spore density and ERH density

Day-time and night-time warming had significant effects on AM fungal spore density, but only their significant interactive effect was observed on AM fungal ERH density (Table 1). Compared with the control treatment, day-time and 24-h warming significantly increased AM fungal spore density by 34.8 % and 51.3 %, respectively (Fig. 1a). The AM fungal ERH density, ranged from 0.87 ± 0.05 to 1.08 ± 0.03 (mkg^{-1} DW), was not significantly different among the four treatments (Fig. 1b).

Table 1 Two-way ANOVAs examining the effects of day-time warming (D), night-time warming (N) and their interaction (D × N) on arbuscular mycorrhizal fungal extraradical hyphal (ERH) density, spore density and OTU richness

Source of variation	*df*	ERH density		Spore density		OTU richness	
		F	*P*	*F*	*P*	*F*	*P*
D	1.00	0.31	0.58	10.60	<0.01	5.67	0.03
N	1.00	0.22	0.65	7.19	0.01	17.37	<0.01
D × N	1.00	6.53	0.02	0.60	0.45	6.91	0.02

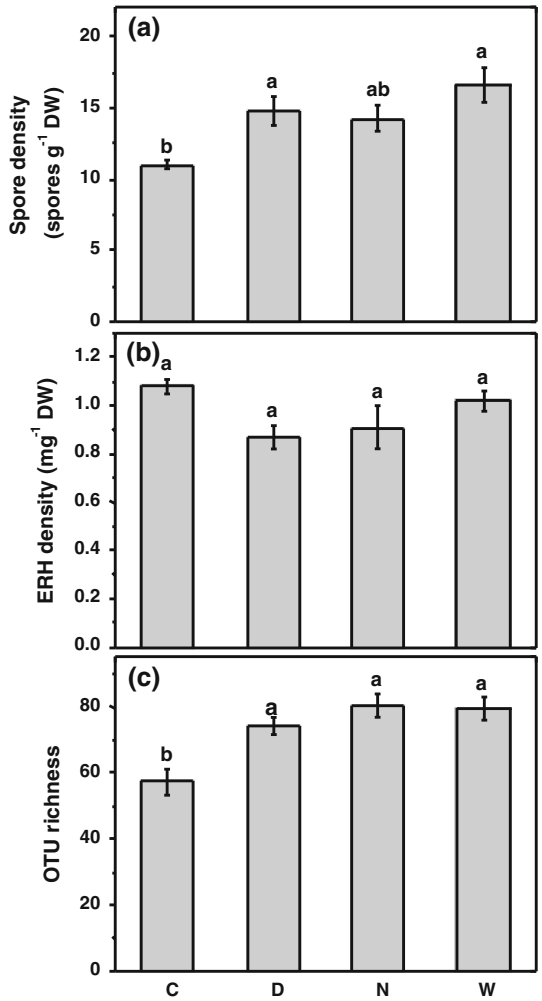

Fig. 1 **a** Arbuscular mycorrhizal (AM) fungal spore density; **b** AM fungal extraradical hyphal (ERH) density; and **c** AM fungal OTU richness under different treatments. The values are mean ± standard error ($n = 6$). Bars with different letters indicate significant differences at $P < 0.05$. C: control, D: day-time warming, N: night-time warming, W: 24-h warming

3.2 Pyrosequencing analysis and identification of AM fungi

A total of 178,482 reads were retained from 226,549 raw reads after a denoising step; subsequently 85,442 reads were removed after a trimming step. Of the remaining 93,040 reads, 9,011 potential chimeras were removed from the dataset. Of the 84,029 non-chimeric reads, 20,434 were of non-fungal origin, and the remaining 63,595 fungal reads were assigned to 1,348 operational taxonomic units (OTUs), based on a 97 % sequence similarity. Of these 1,348 OTUs, 1,063 (62,401 reads) belonged to the AM fungi, and 285 (1,194 reads) were of non-AM fungal origin. As the number of AM fungal reads ranged from 1,793 to 3,410 among the samples, the read numbers were normalized to 1,793, which resulted in a normalized dataset containing 1,063 AM fungal OTUs (43,502 reads). Of the 1,063 AM fungal OTUs, 902 (<5 reads each) were removed from the dataset, and the remaining 161 OTUs with ≥5 reads (42,238 reads) were used for the following analyses.

Among the 161 AM fungal OTUs, 134 (10,549 reads) were recovered from the control treatment, 137 (10,567 reads) from the day-time warming treatment, 146 (10,569 reads) from the night-time warming treatment, and 145 (10,553 reads) from the 24-h warming treatment (Fig. 2a). Of the 161 OTUs, 140 (99.6 % of total reads)

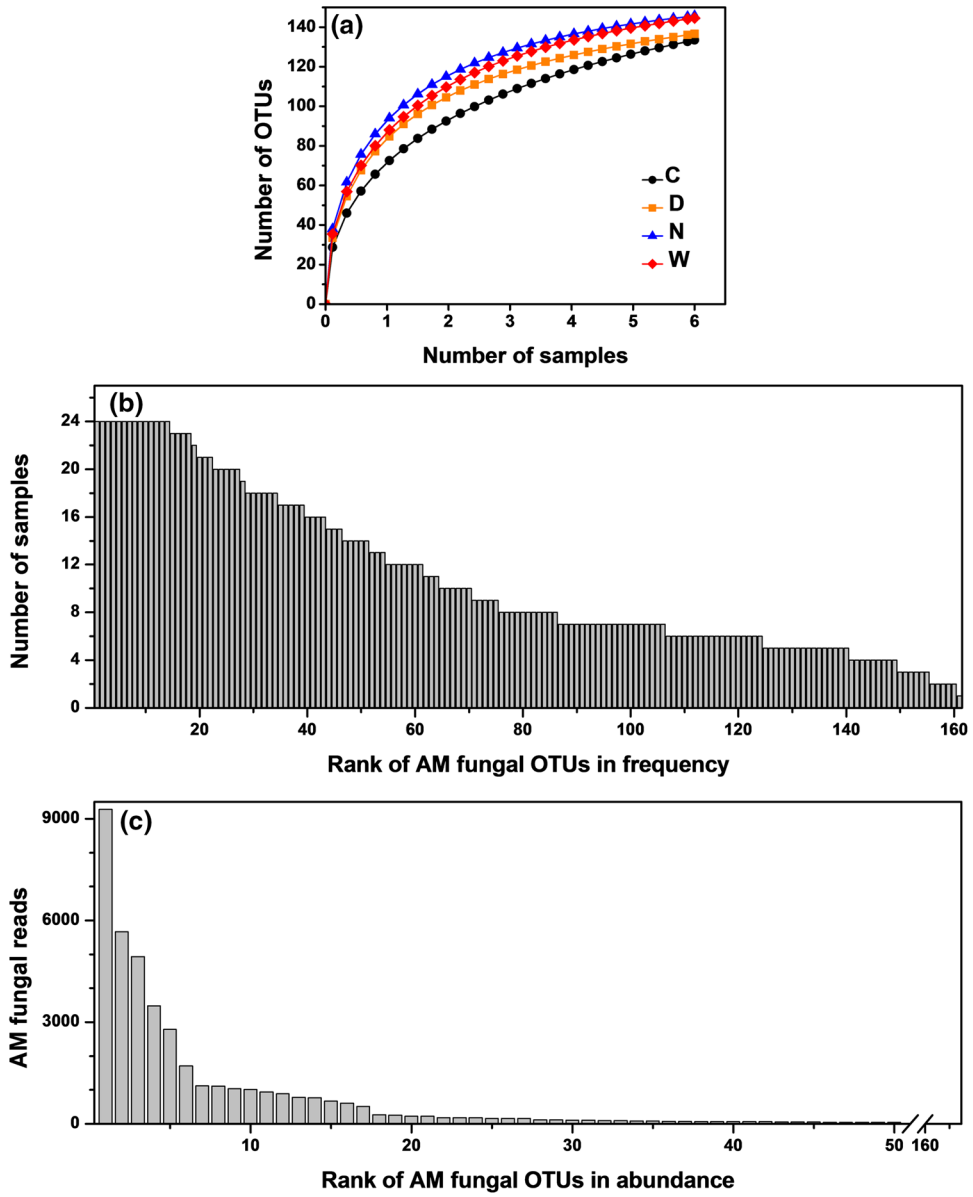

Fig. 2 (Color online) **a** Rarefaction curves for observed arbuscular mycorrhizal (AM) fungal OTUs among the different treatments; **b** rank of the AM fungal OTU in frequency; and **c** rank of the AM fungal OTU in abundance. C: control, D: day-time warming, N: night-time warming, W: 24-h warming

occurred in ≥5 samples, and 21 (0.4 %) in ≤ 4 samples (Fig. 2b). The 17 most abundant OTUs accounted for 88.3 % of the total AM fungal reads, and the remaining 144 OTUs only accounted for 11.7 % (Fig. 2c). Among the 161 AM fungal OTUs, 156 belonged to Glomeraceae (including 51 *Glomus* OTUs, 26 *Funneliformis* OTUs, four *Rhizophagus* OTUs, five *Sclerocystis* OTUs, 70 Glomeraceae OTUs), two to Claroideoglomeraceae (two *Claroideoglomus* OTUs), one to Paraglomeraceae (one *Paraglomus* OTU), one to Diversisporaceae (one *Redeckera* OTU), and one to Archaeosporaceae (one *Archaeospora* OTU, Fig. S1).

3.3 AM fungal community

Day-time warming, night-time warming, and their interaction had significant effects on AM fungal OTU richness (Table 1). Compared with the control treatment, day-time, night-time, and 24-h warming significantly increased AM fungal OTU richness by 29.4 %, 39.8 %, and 38.4 % (Fig. 1c).

Day-time warming, night-time warming, or their interaction had significant effect on ten of 161 AM fungal OTUs (Table 2). For example, day-time and night-time warming significantly decreased the abundance of OTU1 by 30.1 % and 28.4 % compared with the control treatment (Fig. 3a). In contrast, the abundances of the other nine OTUs were significantly increased by day-time, night-time, and/or 24-h warming compared with the control treatment, i.e., 142.9 %–210.8 % as a result of day-time warming, 170.2 %–764.4 % as a result of night-time warming, and 151.6 %–1234.9 % as a result of 24-h warming (Fig. 3b–j).

Table 2 Two-way ANOVAs examining the effects of day-time warming (D), night-time warming (N), and their interaction (D × N) on the abundance (read numbers, sqrt-transformed) of arbuscular mycorrhizal (AM) fungal OTUs

AM fungus	D		N		D×N	
	F	*P*	*F*	*P*	*F*	*P*
OTU1	1.97	0.18	1.45	0.24	4.79	0.04
OTU8	0.30	0.59	9.74	0.01	0.02	0.89
OTU18	7.43	0.01	0.79	0.39	0.47	0.50
OTU19	1.03	0.32	0.09	0.76	4.76	0.04
OTU21	1.01	0.33	13.41	<0.01	<0.01	0.97
OTU26	1.75	0.20	1.17	0.29	4.65	0.04
OTU30	0.41	0.53	4.96	0.04	1.25	0.28
OTU40	2.30	0.15	0.96	0.34	7.51	0.01
OTU44	2.06	0.17	8.72	0.01	1.43	0.25
OTU48	2.30	0.15	6.58	0.02	1.82	0.19

PERMANOVA analysis showed that the AM fungal community composition was affected by night-time warming ($F = 20210$, $R^2 = 0.090$, $P < 0.001$), but not by day-time warming ($F = 1.138$, $R^2 = 0.046$, $P = 0.252$), and the interaction between day-time and night-time warming ($F = 1.236$, $R^2 = 0.050$, $P = 0.142$). In addition, NMDS analysis indicated that the AM fungal community composition was affected by treatments ($R^2 = 0.306$, $P = 0.014$, Fig. 4). Furthermore, the AM fungal community composition was related to SOC ($R^2 = 0.416$, $P = 0.004$), total N ($R^2 = 0.352$, $P = 0.015$), NH_4^+-N ($R^2 = 0.288$, $P = 0.020$), and night-time warming ($R^2 = 0.431$, $P = 0.002$; Fig. 4).

Mantel tests showed that the AM fungal community composition was related to soil ($R = 0.452$, $P = 0.005$) and night-time warming ($R = 0.062$, $P = 0.049$; Table 3). Furthermore, the AM fungal community composition was related to soil ($R = 0.396$, $P = 0.009$), after the effects of plant, day-time warming, and night-time warming were partialled out (Table 3). In contrast, the AM fungal community composition was related to night-time warming ($R = 0.087$, $P = 0.035$), after the effects of plant, soil, and day-time warming were partialled out (Table 3).

Variation partition showed that total 22 % of the variation in AM fungal community composition was explained (Fig. 5). Of these, plant (11.45 % variation explained) and soil (12.24 %) accounted for most of the explained AM fungal community composition variation, but the influences of night-time warming (2.6 %) and day-time warming (0.04 %) were weak (Fig. 5).

4 Discussion

Day-time and 24-h warming, but not night-time warming, had significant positive effects on AM fungal spore density (Fig. 1a). The different effects of three warming regimes on AM fungal spore density may be explained as the reason in which day-time and 24-h warming, but not night-time warming, significantly increase root non-structural carbohydrate content and prolong overall root lifespan during the growing season at the study site [20]. Similarly, Volder et al. [43] found that constant warming had a greater positive effect on root length production than increased night-time warming in a flat fallow field in Australia. Because AM fungi use plants' photosynthetic carbon for their growth [8], any factors that affect root growth and carbon allocation to roots could influence AM fungal spore density [7].

Compant et al. [13] reviewed 135 studies and concluded that AM fungal ERH density responded differently to warming under various experimental conditions. For

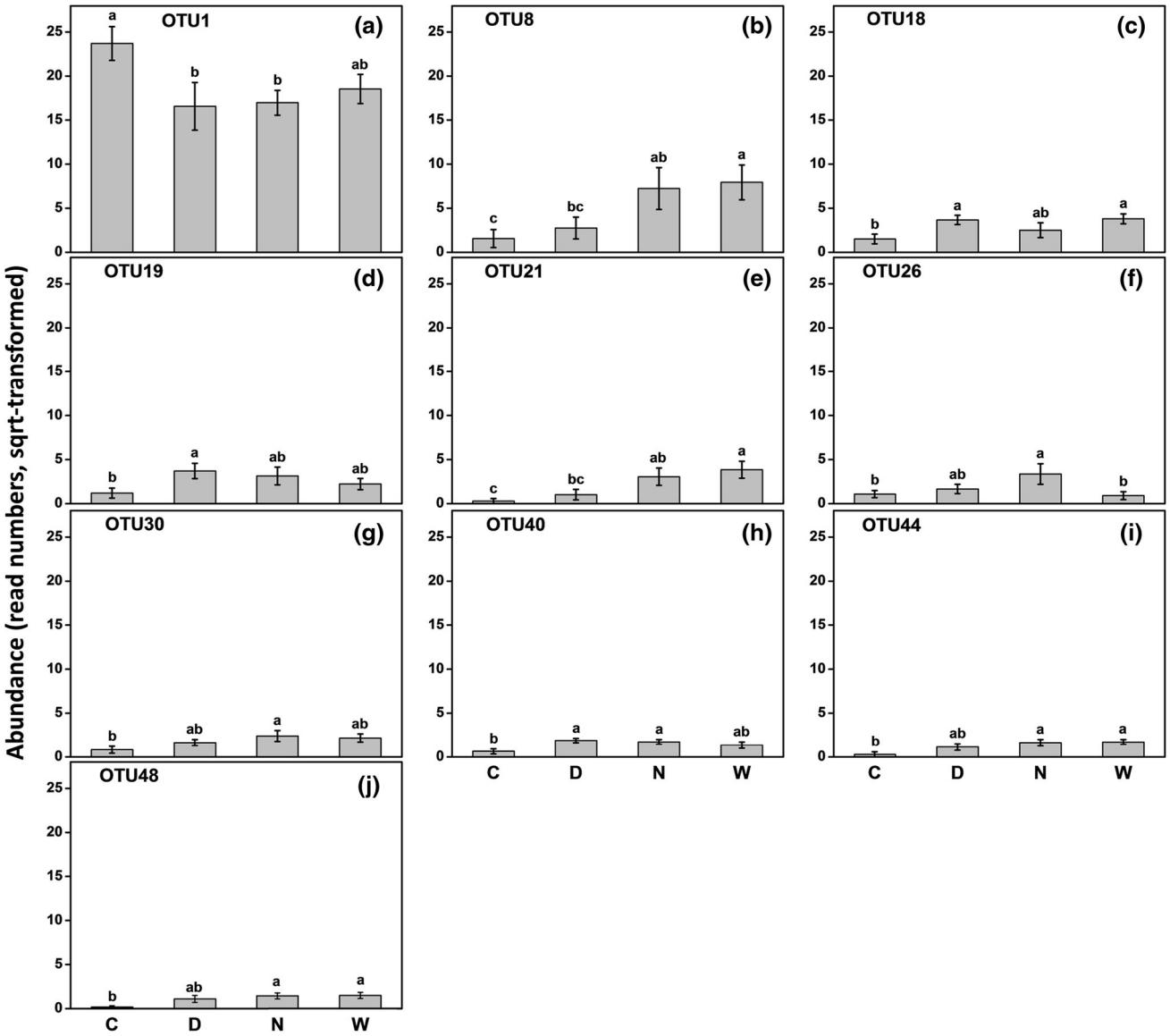

Fig. 3 Abundance (read numbers, sqrt-transformed) of arbuscular mycorrhizal fungal OTUs under different treatments. The values are mean ± standard error ($n = 6$). Bars with different letters indicate significant differences at $P < 0.05$. C: control, D: day-time warming, N: night-time warming, W: 24-h warming

example, warming conspicuously increased AM fungal ERH density in a grassland [27] and a glasshouse [15], where water availability was relatively abundant, whereas AM fungal ERH density was significantly decreased under summer drought condition in a limestone pasture [12] and by an increase in soil temperature in the meadow of a semiarid mixed conifer forest [44]. However, we found that day-time, night-time and 24-h warming did not significantly affect AM fungal ERH density in the semiarid steppe (Fig. 1b). Similarly, Yang et al. [4] found that AM fungal ERH density was not significantly influenced by constant warming in an alpine meadow on the Qinghai-Tibetan plateau. These inconsistent results suggest that different environmental conditions might play a crucial role in determining warming effect on AM fungal ERH density.

High AM fungal diversity (161 OTUs) was found in the semiarid steppe using 454-pyrosequencing compared with previous studies in grassland ecosystems [4, 45, 46]. Day-time, night-time, and 24-h warming significantly increased AM fungal OTU richness (Fig. 1e). Similarly, AM fungal species richness is positively correlated with

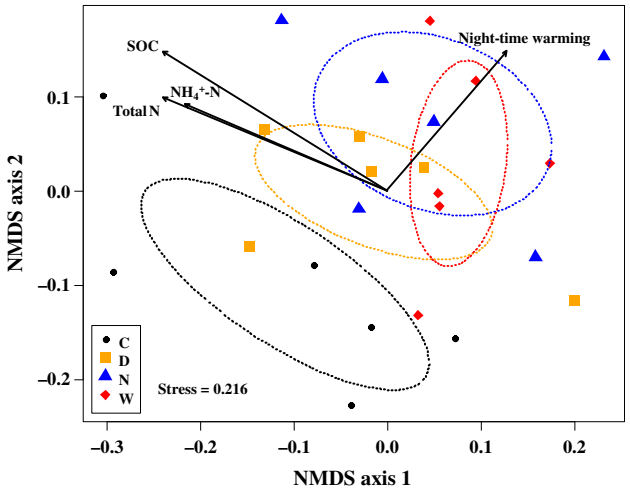

Fig. 4 (Color online) Non-metric multidimensional scaling (NMDS) of arbuscular mycorrhizal fungal community composition under different treatments ($R^2 = 0.306$, $P = 0.014$). Ellipses in the plot represent 95 % CIs around the average values for samples from each treatment. Significant soil organic carbon (SOC), total nitrogen (N), NH_4^+-N and night-time warming are presented as vectors on the ordination graph ($P < 0.05$ in *envfit*). C: control, D: day-time warming, N: night-time warming, W: 24-h warming

Table 3 Mantel and partial Mantel tests of the matrix of arbuscular mycorrhizal (AM) fungal community with the matrices of plant, soil, day-time warming, and night-time warming

Matrix	Component variable	Mantel test		Partial Mantel test	
		R	*P*	*R*	*P*
Plant community composition	Species composition	0.115	0.202	0.113	0.202
Plant diversity & biomass	ANPP[a], BNPP[b], species richness	0.203	0.062	0.062	0.268
Soil	pH, moisture, SOC[c], total N[d], NH_4^--N, NO_3^+-N, C:N, available P[e]	0.452	0.005	0.396	0.009
Day-time warming	Day-time warming	0.000	0.435	0.004	0.406
Night-time warming	Night-time warming	0.062	0.049	0.087	0.035

[a] ANPP: aboveground net primary productivity

[b] BNPP: belowground net primary productivity

[c] SOC: soil organic carbon

[d] Total N: total nitrogen

[e] Available P: available phosphorus

temperature in the root zones of three major plant species growing in the barrier dunes extending from northern New Jersey to Virginia, USA [47]. However, the AM fungal OTU richness was not significantly affected by 24-h warming in an alpine meadow on the Qinghai-Tibetan Plateau, China [4]. These results indicate that the effect of warming on AM fungal diversity varied in different ecosystems.

The AM fungal community composition was significantly affected by night-time warming, but not by day-time and 24-h warming in the semiarid steppe (Fig. 5). Similarly, Heinemeyer et al. [11] found that constant soil warming had no significant effect on AM fungal community composition in a UK grassland. The different effects of the three warming regimes on the AM fungal community may be the result of different changes in ecosystem processes. At our study site, day-time and night-time warming had different effects on ecosystem productivity [19], non-structural carbohydrate allocation to roots and overall root lifespan [20], plant phenology of early- and late-blooming species [21], and soil respiration [22]. These different responses of the semiarid steppe ecosystem to day-time and night-time warming could result in different changes in the AM fungal community, as findings in previous studies [5, 6, 15, 45]. In addition, the AM fungal community was significantly affected by soil SOC, total N, and NO_4^+-N (Fig. 5). Indeed, previous studies demonstrated that AM fungal communities were structured by soil factors [45, 48, 49]. However, Yang et al. [4] reported that constant warming significantly altered soil AM fungal community composition in an alpine meadow on the Qinghai-Tibetan plateau, China. These inconsistent results suggest that different environmental conditions might also have played a crucial role in determining warming effect on the AM fungal community composition.

In summary, although the effect of constant warming on the AM fungal community has been investigated in previous studies [4–6, 11, 12, 14], this is the first study to reveal the different effects of day-time versus night-time warming in natural ecosystem. We found that day-time and 24-h warming, but not night-time warming, had significant positive effects on AM fungal spore density. The three warming regimes significantly increased AM fungal richness, but did not significantly change AM fungal ERH density. Some AM fungal OTUs showed a significant bias toward day-time, night-time, or 24-h warming. The AM fungal community composition was significantly affected by night-time warming, but not by day-time and 24-h warming. Our finding highlighted different responses of AM fungal abundance and community composition to asymmetrical warming. This study might improve our understanding of ecosystem functioning of AM fungal community under global climate change scenarios in a semiarid steppe ecosystem.

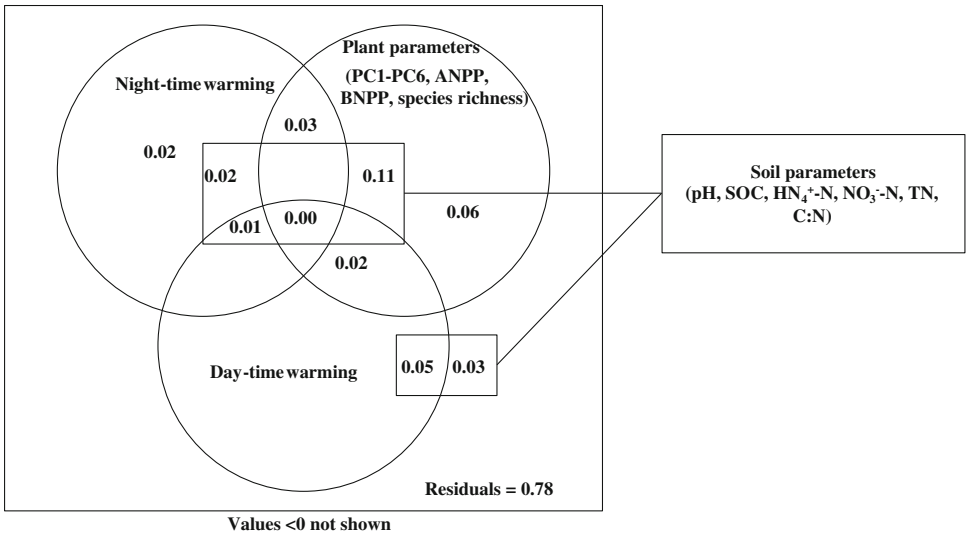

Fig. 5 Pure and shared effects of day-time warming, night-time warming, soil and plant parameters on arbuscular mycorrhizal fungal community as derived from variation partitioning analysis. Numbers indicate the proportion of explained variation. ANPP: aboveground net primary productivity, BNPP: belowground net primary productivity, SOC: soil organic carbon, TN: total nitrogen

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Conflict of interest The authors declare that they have no conflict of interest.

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