Elevation determined the change of Arbuscular Mycorrhizal Fungal community caused by warming

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Background. Global warming affects the growth and communities of plants, which has consequences for soil-plant associated microorganisms. Arbuscular mycorrhizal fungi (AMF) are distributed widely and form symbiotic relationships with more than 80% of vascular plants. **Methods.** A warming experiment was conducted in-situ by open-top chamber (OTC) for exploring the effect of global warming on AMF community structure in the Qinghai-Tibet Plateau (QTP) of the most sensitive region to climate changes. Four elevations were selected including 3000 m, 3500 m, 3800 m, and 4170 m for ensuring the accurate findings. **Results.** AMF richness at the level of OTUs was increased from 36 to 45.67 by warming when all elevations were calculated. AMF richness was increased markedly by warming only in the elevation of 3500 m, while it was not significant in other three elevations. Warming did not alter notably the diversity of AMF when it was assessed by Shannon, Ace, and Simpson evenness index. Further, we discover that *Glomus* and *Acaulospora* were the dominated genera through their relative abundance and occurrence frequency of AMF by observing different genera in CK and OTC at different elevations. **Conclusion.** Warming changed significantly AMF richness. The effects of warming on AMF community varied depend on elevations. The occurrences of AMF in different genera were also presented the different responses to warming in four elevations.

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ABSTRACT

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Methods. A warming experiment was conducted in-situ by open-top chamber (OTC) for exploring the effect of global warming on AMF community structure in the Qinghai-Tibet Plateau (QTP) of the most sensitive region to climate changes. Four elevations were selected including 3000 m, 3500 m, 3800 m, and 4170 m for ensuring the accurate findings.

Results. AMF richness at the level of OTUs was increased from 36 to 45.67 by warming when all elevations were calculated. AMF richness was increased markedly by warming only in the elevation of 3500 m, while it was not significant in other three elevations. Warming did not alter notably the diversity of AMF when it was assessed by Shannon, Ace, and Simpson evenness index. Further, we discover that *Glomus* and *Acaulospora* were the dominated genera through their relative abundance and occurrence frequency of AMF by observing different genera in CK and OTC at different elevations.

Conclusion. Warming changed significantly AMF community. The effects of warming on AMF community varied depend on elevations. Moreover, the occurrences of AMF in different genera were also presented the different responses to warming in four elevations.

Subjects Biodiversity, Ecology, Ecosystem Science, Mycology; Soil Science

Keywords Arbuscular mycorrhizal fungi, warming, Qinghai-Tibet Plateau, AMF community, AMF richness

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are distributed widely and forms symbiotic relationships readily with more than 80% of vascular plants (*Wang et al., 2012; Yang et al., 2012; Li et al., 2020*). There are many physiological and ecological influences which are beneficial on the growth of arbuscular mycorrhizal plants (*Phillips & Hayman, 1970; Colla, 2008; Zhang, 2009; Ren et al., 2017; Bi, Xiao & Sun, 2019*). Hashem *et al. (2018)* found that AMF can improve the phenomenon of the uptake of N and P decreased significantly under drought stress. *Shi et al. (2014)* reported that mycorrhizal fungi absorbed carbon from host plants to exchange nutrient for transfer to the roots. Mycorrhizal plants were able to transfer more photosynthate from shoot to roots than non-mycorrhizal plants (*Fitter, 1991; Marschner, Crowley & Higashi, 1997; Kucey and Paul, 1982*).

Further, *Yang et al. (2010)* reported that AMF are important components of soil and functional links between plants and soil. *Tisdall* (*1991*) discovered that AMF can stabilize the soil structure through soil aggregation processes. And it indicated that AM symbiosis can improves nutrient and water supply to host plants (*Wang, Pokharel & Chen, 2019; Sarmiento-López et al., 2020; Parniske, 2008; Wu et al., 2010; Song et al., 2015*). *Hardie* (*1985*) reported that AMF can promote plants to absorb soil moisture and increase the resistance to water stress. Many studies also suggested that AMF may promote plant growth through enhance tolerance to stress, such as drought and salinity (*Yang et al., 2016; Xiang et al., 2016; McKibben & Henning, 2018; Higo et*

al., 2019; Zhang et al., 2019; Wu et al., 2021; Zhang et al., 2019). *Diagne et al. (2020)* insisted that AMF enhance some of essential ecosystem processes and play a crucial role in plant growth in stressed conditions. *Chen et al. (2020)* indicated that AMF might improve plant drought resistance. *Mickan et al., (2016)* suggested that AMF may alleviate plant water stress.

Mycorrhizas also play an important role in biodiversity of plants and ecosystem functions (*He et al., 2010; Zhao et al., 2017*). The differences in AMF composition can change plant community and diversity (*van der Heijden et al., 1998, 2004; Pagano, Cabello & Scotti, 2010*). *Lin et al. (2015)* indicated that the influence of AM on plant diversity and competitiveness would vary with the change of the number of fungi and plant types. Therefore, the biodiversity and community of AMF has been widely attended due to its vital role in the origin, evolution, distribution, survival, growth, and development of plants and ecosystem (*Liu & Wang, 2003; Wang & Qiu, 2006; Mcguire et al., 2008; Hiiesalu, 2014*). *Schußler et al. (2001)* found that AMF established an independent Glomeromycota based on taxonomic status from phylum to order. *Borstler et al. (2006)* estimated that there were at least 1250 species of AMF worldwide. *Opik et al. (2013)* analyzed the AMF community of 96 plant roots and found 59 new virtual taxa (VT). *Grime et al. (1988)* suggested that mycorrhizal colonization significantly increased plant species diversity. Overall, the preservation of AM fungal diversity is important for plant diversity and productivity maintenance (*Moora & Zobel, 1996; Mahmoudi et al., 2019*).

With climate change, the influence of warming on AMF has also received more and more attentions. *Wu & Zou (2010)* showed that inoculated seedlings were significantly higher compared to un-inoculated ones at 25 °C but not at 15 °C and the symbiotic development of citrus seedling inoculated with AMF colonization were higher at 25 °C than at 15 °C. The study of *Gavito et al. (2005)* claimed that the transferred carbon from host plants to AMF was increased below 18 °C with warming and it was opposing above 18 °C.

However, they only studied the influence of warming on AMF in single elevation, which was probably difficult to evaluate accurately the responses of AMF to warming during climate changes. The most sensitive region to climate changes in the world is Qinghai-Tibet Plateau (QTP), where is a global biodiversity hotspot because it provides a natural “laboratory” for the development of natural science research with unique geographical environment (*Tian et al., 2009; Shi et al., 2015*). And it has been testified that there were abundant AMF diversity on QTP (*Gai et al., 2009*). Besides, the knowledge of how the AM fungal communities respond to warming is limited on the Qinghai-Tibetan Plateau. To better understand the effects of warming on AM fungal communities, we investigated the influence of warming on AMF community based on four elevations.

In this study, a warming experiment was conducted in-situ by open-top chamber (OTC) for exploring the effect of global warming on AMF community in the Qinghai-Tibet Plateau (QTP) of the most sensitive region to climate changes. Four elevations were selected including 3000 m, 3500 m, 3800 m, and 4170 m for ensuring the accurate findings to explore the influence of warming on AMF community by elevation and the associated temperature changes. Due to the temperature-sensitive nature of the Qinghai-Tibet Plateau, we have made the following

assumptions: (1) Warming significantly changes the AMF community. (2) Warming significantly changes AMF richness. (3) The changes of AMF community are consistent at four elevations after warming.

MATERIALS & METHODS

Site Description

The Qinghai-Tibet Plateau is a vast plateau in Central Asia covering most of the Tibet Autonomous Region and Qinghai Province in China. It occupies 2.5 million square kilometers. Called as "the roof of the world", it is the highest and biggest plateau of the world. The annual average temperature is -4 °C. Annual precipitation ranges from 100 mm to 300 mm. It was the main silk-road route from China to the west in the past (*http://www.chinatraveldepot.com/C274-Qinghai-Tibet-Plateau*). In our study, the main vegetation is *Kobresia pygmea* and the type of soil is meadow soil. The slope for each sampling site is less than 2º. In view of the uniqueness of climatic and topographical characteristics on QTP, this study selected samples between 29º50′36.49′′-29º54′26.70′′ north latitude and 102º0′42.50′′-102º02′9.50′′ east longitude on the eastern part of QTP (Table 1).

Table 1. The sampling sites and coordinates based on different elevations on the Qinghai-Tibet Plateau.

Experiment design and sample collection

Quadrats of 20 m × 20 m were positioned at four elevations of 3000 m, 3500 m, 3800 m and 4170 m on QTP. Each quadrat was divided into twenty-five of 4 m × 4 m little quadrats. We take three biological repetitions with non-adjacent randomly as control check treatment (CK) and OTC warming treatment by the way of artificial and simulated warming through Open-top chamber, respectively (*Gao & Li, 2019; Li et al., 2020*). Compared with other warming methods, it can ensure that the soil is basically undamaged and easy to repeated (*Klein, Harte & Zhao, 2004*). Its top and bottom are hexagonal and open with the side composed of six trapezoid-shaped plexiglass. We carried out a one-year warming test and all samples were taken in August and September of the next year without rain or snow. Small meteorological observation stations are set up at each altitude to monitor soil temperature and soil moisture. Instantaneous measurement of soil temperature and soil moisture is performed by fixed-point measurement using HOBO PRO temperature and soil moisture recorder. We selected meadow soils samples randomly with a punch to collect a soil column with diameter of 2 cm and depth of 0-20 cm. We mixed three soil columns as a sample and repeated three times in CK and OTC, respectively. Then, separating the root system from the soil and sealing with valve bags, respectively. Field experiments were approved by the Key Laboratory of Mountain Surface Processes and Ecological Regulation, Chinese Academy of Sciences (20160416).

DNA extraction and PCR amplification

Microbial DNA was extracted from soil samples, using the Fast DNA SPIN Kit for Soil (MP Biomedicals LLC, Santa Ana, CA, USA) according to manufacturer's protocols. The final DNA purification and concentration were determined by NanoDrop 2000 UV-vis spectrophotometer

(Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The extracted DNA was subjected to nested PCR by thermocycler PCR system (GeneAmp 9700, ABI, USA). The first PCR amplification was performed with primers AML1F (5'-ATCAACTTTCGATGGTAGGATAGA-3') and AML2R (5'-GAACCCAAACACTTTGGTTTCC-3') by an ABI GeneAmp® 9700 PCR thermocycler (ABI, CA, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 32 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. PCR reactions were performed in triplicate 20 μL mixture containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase and 10 ng of template DNA. The second PCR amplication used identical reaction conditions described above with the primers AMDGR (5'-CCCA ACTATCCCTATTAATCAT-3') and AMV4-5NF (5'-AAGCTCGTAGTTGAATTTCG-3'), and the following program: 3 min of denaturation at 95 °C, 30 cycles of 30 s at 95 °C, 30 s for annealing at 55 °C, and 45 s for elongation at 72 °C, and a final extension at 72 °C for 10 min. The resulted PCR products were extracted from a 2% agarose gel and further purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) and quantified using QuantiFluor™-ST (Promega, USA) according to the manufacturer's protocol.

Illumina MiSeq DNA sequencing

Purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq PE300 platform/NovaSeq PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database (Accession Number: PRJNA694003 http://www.ncbi.nlm.nih.gov/bioproject/694003).

Processing of sequencing data

Raw fastq files were demultiplexed by bcl2fastq, quality-filtered by fastp version 0.20.0 (*Chen et al., 2018*) and merged by FLASH version 1.2.7(*Magoč and Salzberg, 2011*) with the following criteria: (i) The 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, and the truncated reads shorter than 50 bp were discarded, reads containing ambiguous characters were also discarded;. (ii) Only overlapping sequences longer than 10 bp were assembled according to their overlapped sequence. The maximum mismatch ratio of overlap region is 0.2. Reads that could not be assembled were discarded; (iii) Samples were distinguished according to the barcode and primers, and the sequence direction was adjusted, exact barcode matching, 2 nucleotide mismatch in primer matching.

Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSEversion 7.1, and chimeric sequences were identified and removed (*Stackebrandt and Goebel, 1994; Edgar et al., 2013*). The taxonomy of each OTU representative sequence was analyzed by RDP Classifier version 2.2 against the maarjam081/AM database using confidence threshold of 70% (*Wang et al., 2007*).

The raw sequence data were deposited at NCBI, in the SRA database with the following accession: PRJNA694003 (http://www.ncbi.nlm.nih.gov/bioproject/694003).

Data Analysis

Total soil carbon, nitrogen, and sulphur were determined by an elemental analyser (GC IsolinkFlash 2000; Thermo Scientific, Waltham, MA, USA) analyzer. The concentration of total C, N, and S in soil are 6.96%, 0.55%, and 0.05%, respectively. Meanwhile, the C/N is 12.62. At the same time, it was found that the soil temperature was increased 1.4 °C. The dynamic range of the soil temperature was increased from 0.6 °C to 2.4 °C in different elevations. The soil moisture decreased 0.07 m^3. The soil moisture was increased 0.15 m^3 at 4170 m and decreased 0.11 m^3, 0.09 m^3, and 0.25 m^3 at 3000 m, 3500 m, and 3800 m.

The community was expressed by AMF richness, relative abundance and occurrence frequency in different elevations. AMF richness was calculated by the number of OTUs. The relative abundance of AM fungal genus was calculated as the percentage of the sequence number of OTUs in each genus divided by the total sequence number of OTUs in all genera at this altitude in CK or OTC. The occurrence frequency of AM fungal genus was defined as the percentage of the number of samples where this genus observed to the number of all samples in this genus. The rate of decrease = (the number of OTUs in CK - the number of OTUs in OTC)/ the number of OTUs in CK * 100%. The rate of increase = (the number of OTUs in OTC - the number of OTUs in CK)/ the number of OTUs in CK * 100%.

AMF diversity in different elevations were expressed and plotted by the index of Shannon, Ace, and Simpson evenness at the level of OTUs by Excel and Origin, respectively. The differences of AMF richness and AMF diversity in different elevations were analyzed by two-way ANOVA analysis and Duncan in SPSS 19.0 (*Shi et al., 2019*). We analyze the impact of environmental factors on AMF community after warming through RDA. We standardize the data by flattening according to the minimum number of sample sequences. The data of the percentage of relative abundance and occurrence frequency were subjected to square root transformation.

RESULTS

AMF richness at the level of OTUs

The Venn diagram intuitively showed AMF richness in CK (the treatment of control check) and OTC (the treatment of warming) (Figure 1). Warming increased AMF richness at the level of OTUs from 36 to 45.67 with the increase of 26.86% and. Among them, AMF richness of shared was 28 OTUs, which was 77.78% and 61.19% in CK and OTC, respectively. In CK, there were 8 unique OTUs, which was 22.22% of the total in CK. AMF richness of shared was 3.5 times to CK solely. In OTC, there were 17.67 unique OTUs, which was 38.69% of the total in OTC. And AMF richness of shared was 1.58 times to AMF richness in OTC solely. It showed that AMF richness was increased but has no significant effects after warming by two-way ANOVA analysis ($P = 0.052$).

Figure 1. The influence of warming on AMF richness. CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share. The similarity level was 97%. The data were statistically analyzed by ANOVA (warming: F = 7.509, *P* = 0.052).

AMF diversity index at the level of OTUs based on different elevations

There were different influences of warming on AMF richness with the elevations varied. It showed upward trend at 3000 m, 3500 m and 3800 m but showed downward trend at 4170 m after warming (Figure 2A). No matter in CK or in OTC, the highest AMF richness occurred at 3500 m. AMF richness in OTC is greater than that in CK at the elevations of 3000 m, 3500 m, and 3800 m, but it was opposite at 4170 m. That was, AMF richness was lower at the higher altitude after warming. Moreover, elevation had extremely significant effects on AMF richness (*P* = 0.000**). The interaction of elevations and warming also had a significant effect on AMF richness (*P* = 0.029*).The Shannon index has the same tendency to AMF richness (Figure 2B). At 3000 m, 3500 m, and 3800 m, the Shannon index in OTC were higher than that in CK, but showed opposite at 4170 m and none of them are significant. The Simpson evenness index has the same tendency to the Shannon index at 3000 m (Figure 2C). Meanwhile, it has the contrary tendency to Shannon index at 3500 m, 3800 m, and 4170 m.

At 3000 m and 3500 m, the Ace index in OTC were higher than that in CK, but it was opposite at 3800 m and 4170 m(Figure 2D). Elevation had significant effects on the Ace index (*P* = 0.045*).

Figure 2. AMF diversity index at the level of OTUs based on different elevations by warming. Error bars represent the standard error of the mean. Different lowercase letters above each column indicate significant difference, *P*<0.05. The data were statistically analyzed by ANOVA (elevations: F = 15.387, *P* = 0.000**; warming: F = 1.347, *P* = 0.263; elevations×warming: F = 3.874, *P* = 0.029*), Shannon index (elevations: F = 2.805, *P* = 0.073; warming: F = 0.682, *P* = 0.421; elevations×warming: F = 2.358, *P* = 0.110), Simpson evenness index (elevations: F = 0.768, *P* = 0.529; warming: F = 0.471, *P* = 0.502; elevations×warming: F = 1.594, *P* = 0.230), Ace index (elevations: F = 3.369, *P* = 0.045*; warming: F = 0.047, *P* = 0.832; elevations×warming: F = 1.578, *P* = 0.234).

The influence of warming on AMF community based on different elevations

Among the genera of *Ambispora*, *Unclassified* (Archaeosporales order), and *Paraglomus*, AMF richness of CK was identical with OTC (Table 2). The largest change in AMF richness was *Glomus*, which increased from 13.67 to 20.33 after warming. AMF richness of *Unclassified* (Unclassified order) was increased from 5.33 OTUs to 6.33 OTUs. For *Acaulospora*, AMF richness was increased from 10.67 OTUs to 12.33 OTUs. The smallest change in AMF richness was *Archaeospora*, which increased from 4 to 4.33.

In addition, there was a downward trend at 4170 m and the decline rate was 100%. However, there was an increasing trend at 3500 m and 3800 m. For *Archaeospora*, AMF richness was increased at 3800 m, but decreased at 3500 m and 4170 m. For *Acaulospora*, AMF richness trended to increase 7.28% and 26.38% at 3000 m and 3500 m, respectively. As for 4170 m, it decreased 42.86%. For *Glomus*, AMF richness was increased 116.5%, 58.53%, and 38.83% at

3000 m, 3500 m, and 3800 m, respectively. And it decreased at 4170 m., AMF richness of *Paraglomus* was increased at 3000 m and decreased at 4170 m. Moreover, the rate of increase on AMF richness at 3000 m was the same as the rate of decrease at 4170 m. For *Unclassified* (Unclassified order), AMF richness was increased at 3000 m and decreased at 3800 m and 4170 m.

Table 2. The influence of warming on AMF richness based on different elevations.
Notes:
CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

The beta-diversity of the AMF community was determined by the Bray-Curtis method (Figure. 3). In the 2-dimensional NMDS plots, soil samples collected from the four different elevations and two different treatments and separated from each other. ANOSIM based on the Bray-Curtis distance showed significant dissimilarities of the AMF communities among the four different elevations and two different treatments ($P = 0.001$).

Figure 3. Nonmetric multidimensional scaling (NMDS) of the influence of warming on AMF community at the level of OTU. The symbols represent the elevations of 3000 m, 3500 m, 3800 m, and 4170m. CK means the treatment of control check and OTC means the treatment of warming with Open-top chamber.

The relative abundance and occurrence frequency of AMF

For four different elevations, the relative abundance of *Acaulospora* and *Glomus* were the largest before and after warming (Table 3). Among the four different elevations, all genera had one thing in common, if the relative abundance was zero in CK, it would be still zero in OTC, except *Paraglomus* at 3000 m. Moreover, the trend of change on the relative abundance of *Acaulospora* and *Glomus* were opposite in four different elevations. At 3000 m, the relative abundance of other genera showed an increasing trend after warming, except *Glomus* was decreased from 79.4761% to 65.1349%. At 3500 m, the relative abundance of *Ambispora*, *Glomus*, and *Unclassified* (Unclassified order) showed an increasing trend after warming, but *Archaeospora* and *Acaulospora* were decreased. At 3800 m, the relative abundance of *Glomus* and *Unclassified* (Unclassified order) showed an increasing trend but the relative abundance of *Ambispora*, *Archaeospora*, *Unclassified* (Unclassified order), and *Acaulospora* showed a decreasing trend after warming. As for 4170 m, the relative abundance of *Archaeospora*, *Acaulospora* and *Unclassified* (Unclassified order) was increased but *Ambispora*, *Glomus* and *Paraglomus* was decreased. The relative abundance of *Glomus* decreased at 3000 m and 4170 m, but *Acaulospora* was opposite after warming.

Table 3. The influence of warming on the relative abundance of AMF based on different elevations.
Notes:
CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

For different elevations, the occurrence frequency of *Acaulospora* was always 100% (Table 4). The occurrence frequency of *Glomus* was always 100%, except at 4170 m in OTC. In CK, the occurrence frequency of *Acaulospora* and *Unclassified* (Archaeosporales order) was the same as that in OTC at different elevations, which seemed that warming had no affect on them. The occurrence frequency of *Unclassified* (Archaeosporales order) varied at four elevations, *Ambispora* and *Archaeospora* varied at three elevations, *Paraglomus* varied at two elevations and *Glomus* varied at only one elevations. For different elevations, the occurrence frequency of *Paraglomus* showed a tendency of increasing from 0 to33.33% at 3000 m but opposite at 4170 m. The occurrence frequency of *Unclassified* (Unclassified order) increased at 3000 m and 4170 m from 66.67% to 100%, but decreased at 3500 m and 3800 m. The occurrence frequency of *Ambispora* and *Archaeospora* decreased at 4170 m. But at 3500 m, the tendency of *Ambispora* and *Archaeospora* were opposite.

Table 4. The influence of warming on the occurrence frequency of AMF based on different elevations.
Notes:
CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

The influence of soil factors on AMF community by warming

For different elevations, RDA1 explained 59.16%, 69.55%, 95.26%, and 95.35% at 3000 m, 3500 m, 3800 m, and 4170 m, respectively. RDA2 explained 0.91%, 2.34%, 1.52%, and 0.56% at 3000 m, 3500 m, 3800 m, and 4170 m, respectively (Figure 4A - 4D). RDA1 increased from 59.16% to 95.35% with the elevation increased. As the elevations increased, the influence of C, N, S, and C/N were different. C, N, and C/N were positively correlated to RDA1 and RDA2. S was negatively correlated to RDA1 but positively correlated to RDA2 at 3000 m (Figure 4A). At 3500 m, C, N, and S were all positively correlated to RDA2 but negatively correlated to RDA1 (Figure 4B). C/N was negatively correlated to RDA2 but positively correlated to RDA1. C and N were negatively correlated to RDA1 and RDA2. S and C/N were negatively correlated to RDA2 but positively correlated to RDA1 at 3800 m (Figure 4C). At 4170 m, C, N, and S were negatively correlated to RDA1 and RDA2. C/N was negatively correlated to RDA2 but positively correlated to RDA1 (Figure 4D).

Figure 4. The influence of warming on RDA analysis at the level of genus based on different elevations. CK means the treatment of control check and OTC means the treatment of warming with Open-top chamber. A, B, C, and D represent the RDA analysis at 3000 m, 3500 m, 3800 m, and 4170 m, respectively.

DISCUSSION

The influence of warming on AMF, which have an inseparable relationship with plants, have got more and more attention. However, studies about the influence of warming on AMF were usually occurred at one elevation, which can't accurately reflect the change of AMF community in mountain area. We investigated the influence of warming on AMF community based on

different elevations by the way of in-situ through open-top chamber on the Qinghai-Tibet Plateau.

Gao et al. (2016) reported that warming had no effects on AMF community in the semiarid steppe ecosystem. It might related to *Jiang et al. (2018)*, who believed that AMF communities had no sensitivity to short-term climate change or *Heinemeyer et al. (2003)*, who reported that soil warming had no effect on AMF community might display seasonal dynamics in a native grassland. And *Millar &Bennett (2016)* believed that AMF community were influenced by plant community. In our study, we supported that warming increased AMF community because 17.67 new OTUs were found in OTC. However, with the elevations increased, AMF richness increased from 3000 m to 3800 m, but decreased at 4170 m. AMF richness was significantly effected at 3500 m but wasn't significantly effected in other three elevations. *Yang et al. (2013)* found that warming had no significant effect on AMF community, which was the same as our results at 3000 m, 3800 m and 4170 m. Meanwhile, our results was also similar to *Liu et al. (2016)*, who suggested that warming does not always lead to significant changes in fungal. We indicated that warming might not affect all elevations, but affect a particular elevation. It probably related to the soil moisture and temperature because the soil moisture was the largest but the change was the smallest at 3500 m whether in CK or in OTC and the soil temperature changed the smallest except 4170 m. *Sun et al. (2013)* suggested that soil moisture had influence on AMF. In addition, we don't know if there's an inflection point between 3800m and 4170 m, which needs further research.

The analysis of AMF diversity index including Shannon index, Ace index, and Simpson evenness index in different elevations found that warming had no significant effects on AMF diversity, which was consistent with *Yang et al. (2013)*, who also found that warming had no significant effects on Shannon diversity index. And it was the same as *Gai et al. (2012)*, who insisted that the Shannon-Weiner index was unaffected by elevation. *Geml et al. (2015)* and *Jiang et al. (2018)* also reported that diversity were not significantly affected by warming. *Kim et al. (2014)* indicated that the influences of warming on AMF diversity varied in different ecosystems. Therefore, we supported that warming has no effect on AMF diversity, which may be related to the ecosystem. Besides, *Li et al. (2014)* reported that plant species and elevation exert significant influences on AMF diversity. And *Shi et al. (2014)* reported that the diversity of the host plants determined AMF diversity. Therefore, we suggested that the reason of AMF diversity not increased might be that plant identity has played a huge role and warming had no effect on plant diversity.

Previous studies have shown that there are dominant genera in AMF communities, such as *Glomus* and *Acaulospora* (*Dobo et al., 2016; Belay, Vestberg & Assefa, 2013*). Our study found that the relative abundance and occurrence frequency of *Glomus* and *Acaulospora* were higher whether in CK or OTC than other genara except the occurrence requency of *Glomus* at 4170 m, which was the same as *Sturmer & Siqueira (2011)*, who reported that *Glomus* and *Acaulospora* were dominated species. And *Coutinho et al. (2015)* also reported that AMF species abundance was high, while *Glomus* and *Acaulospora* contributed significantly. *Mickan et al. (2017)* found

that *Glomus* was dominant in roots according to DNA sequencing. Further, the relative abundance of *Glomus* was decreased at 3000 m and 4170 m but increased at 3500 m and 3800 m after warming, which was opposite to *Acaulospora*, which was increased at 3000 m and 4170 m but decreased at 3500 m and 3800 m. *Yang et al. (2016)* also suggested that Acaulosporaceae increased with elevations increased, which were the same as our study of the relative abundance of *Acaulospora* (Acaulosporaceae Family) at 3000 m and 4170 m. It seemed that genus was differ in adaptability at different elevations after warming and there are also differences between different genera. Besides, studies had also reported that *Glomus* and *Acaulospora* were most in different plants, respectively (*Schenck & Kinloch, 1980; Blaszkowski, 1989*). Therefore, it indicated that *Glomus* and *Acaulospora* were also highly adaptable to different plants in mountainous areas.

The influence of warming on RDA analysis showed that RDA1 increased with elevation increased. At the same time, C and N are from positively correlated to RDA1 and RDA2 at 3000 m to be negatively correlated to RDA1 and RDA2 at 3800 m and 4170 m. C/N has a great effect at 3000 m, 3500 m, and 3800 m, but opposite at 4170m. And the influence of C, N, and S were greater at 4170 m than that of other three elevations. It indicated that soil factors might change the direction of action on AMF community. But none of them were significant.

Conclusions

In conclusion, warming changed significantly AMF community. The effects of warming on AMF community varied depend on elevations which consistent with our assumptions that warming significantly changes AMF community and the effect of warming on AMF community is different in different elevations. Moreover, the occurrences of AMF in different genera were also presented the different responses to warming in four elevations.

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Figure 1

Figure 1. The influence of warming on AMF richness .

CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share. The similarity level was 97%. The data were statistically analyzed by ANOVA (warming: F = 7.509, *P* = 0.052).

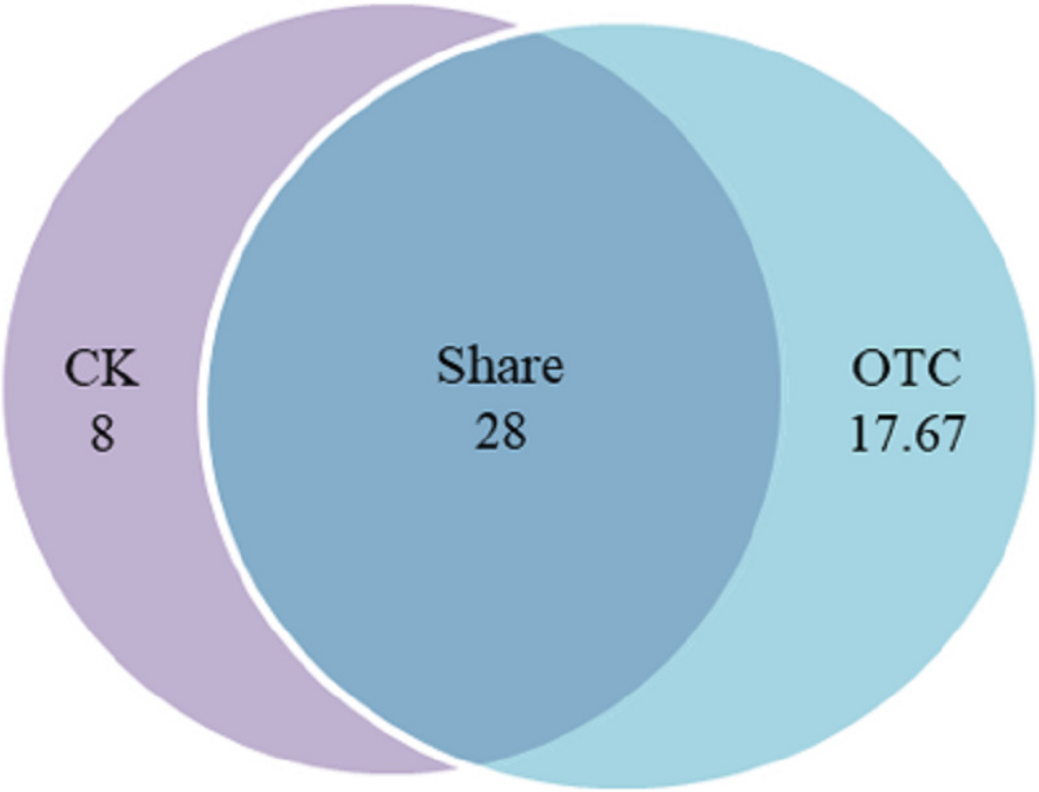
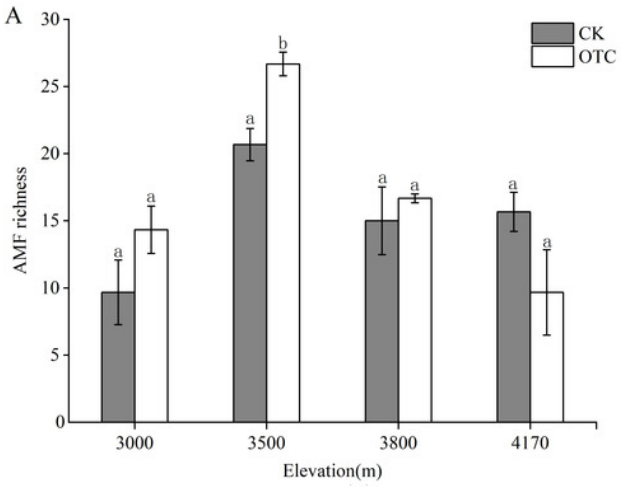
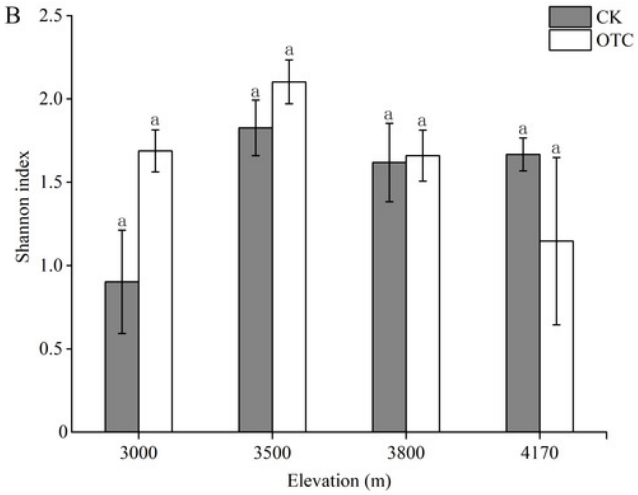


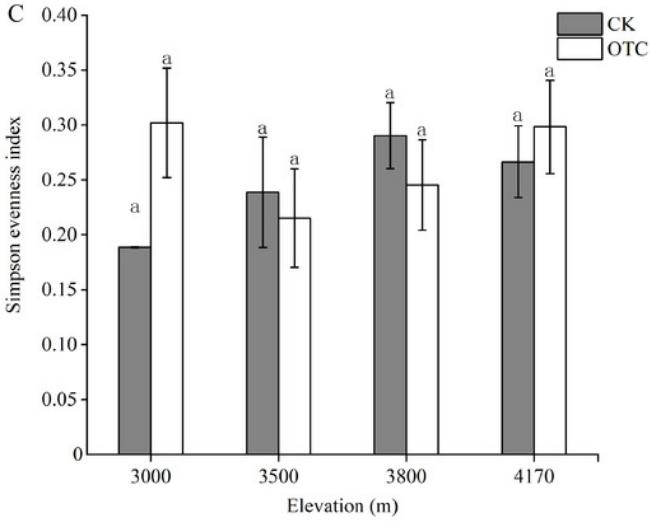
Figure 2

Figure 2. AMF diversity index at the level of OTUs based on different elevations by warming.

Error bars represent the standard error of the mean. Different lowercase letters above each column indicate significant difference, $P<0.05$. The data were statistically analyzed by ANOVA (elevations: F = 15.387, P = 0.000**; warming: F = 1.347, P = 0.263; elevations×warming: F = 3.874, P = 0.029*), Shannon index (elevations: F = 2.805, P = 0.073; warming: F = 0.682, P = 0.421; elevations×warming: F = 2.358, P = 0.110), Simpson evenness index (elevations: F = 0.768, P = 0.529; warming: F = 0.471, P = 0.502; elevations×warming: F = 1.594, P = 0.230), Ace index (elevations: F = 3.369, P = 0.045*; warming: F = 0.047, P = 0.832; elevations×warming: F = 1.578, P = 0.234).


A
AMF richness
CK
OTC
Elevation(m)
3000
3500
3800
4170


B
Shannon index
CK
OTC
Elevation (m)
3000
3500
3800
4170


C
Simpson evenness index
CK
OTC
Elevation (m)
3000
3500
3800
4170

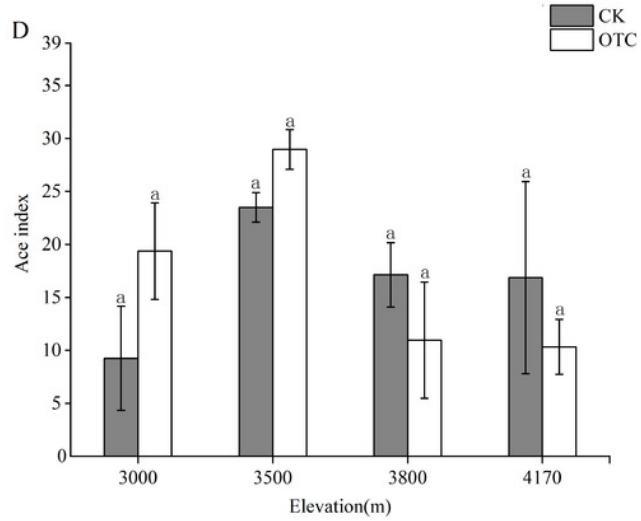

D
Ace index
CK
OTC
Elevation(m)
3000
3500
3800
4170

Figure 3

Figure 3. Nonmetric multidimensional scaling (NMDS) of the influence of warming on AMF community at the level of OTU.

The symbols represent the elevations of 3000 m, 3500 m, 3800 m, and 4170m. CK means the treatment of control check and OTC means the treatment of warming with Open-top chamber.

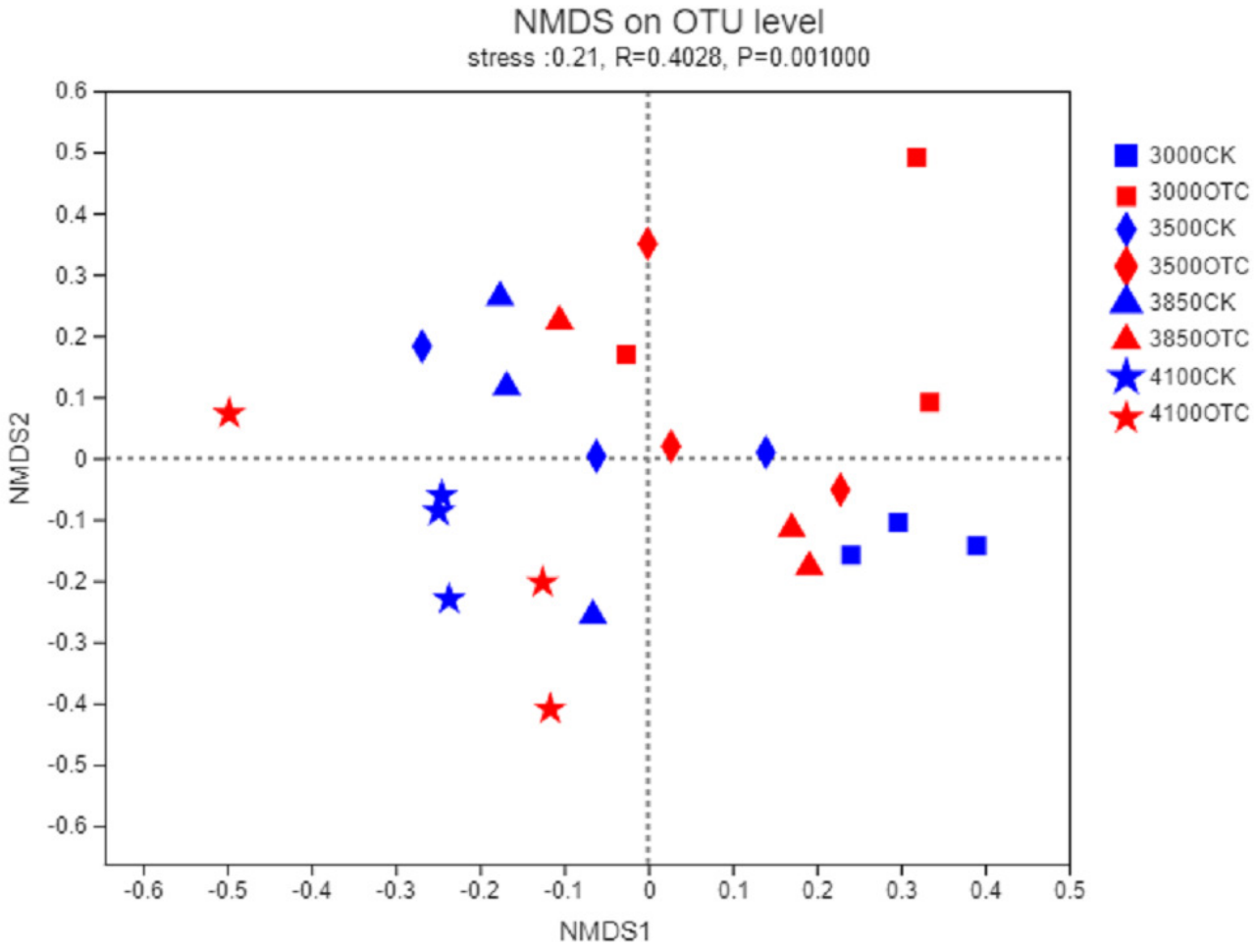


Figure 4

Figure 4. The influence of warming on RDA analysis at the level of genus based on different elevations.

CK means the treatment of control check and OTC means the treatment of warming with Open-top chamber. A, B, C, and D represent the RDA analysis at 3000 m, 3500 m, 3800 m, and 4170 m, respectively.

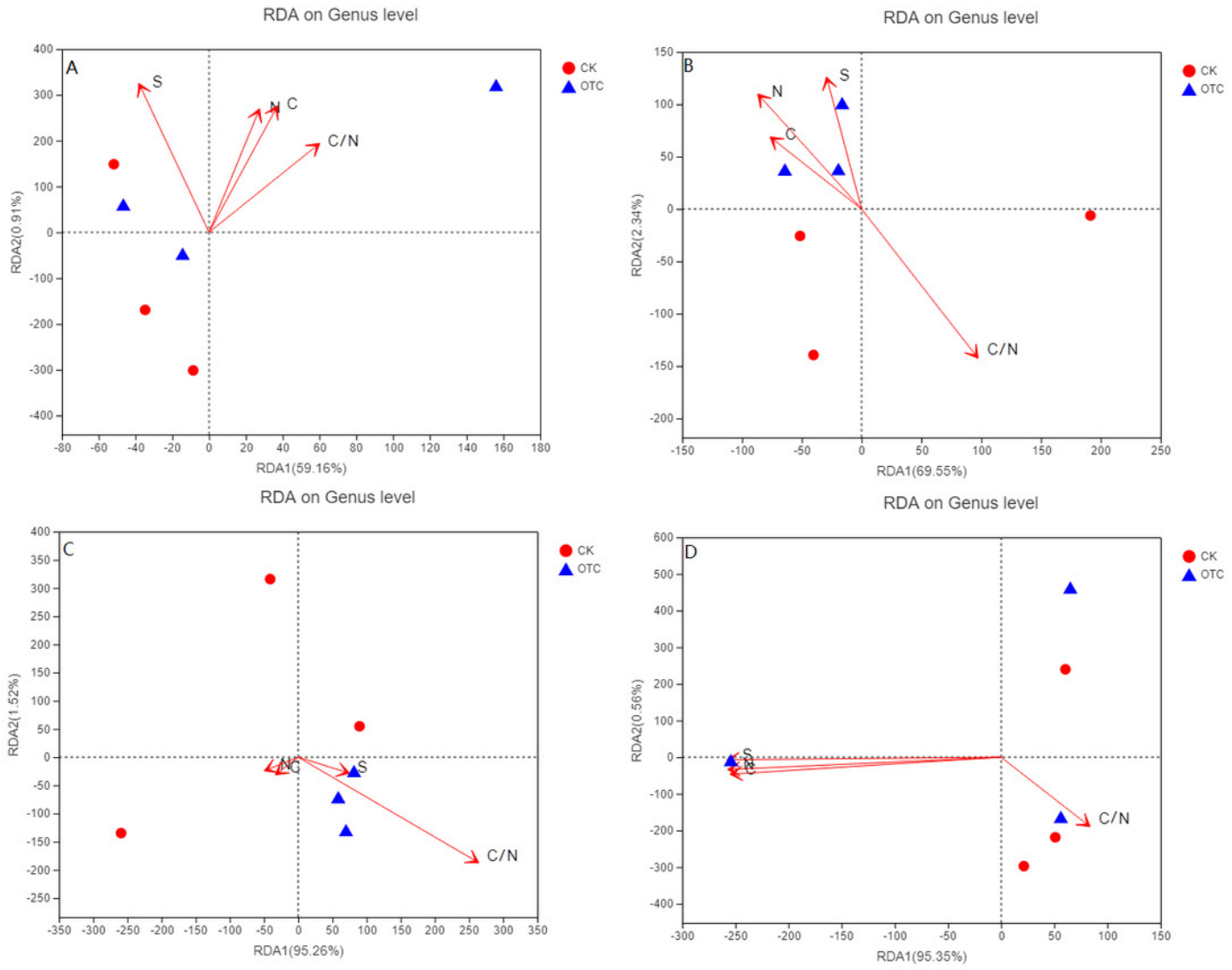


Table 1(on next page)

Table 1. The sampling sites and coordinates based on different elevations on the Qinghai-Tibet Plateau.

1

Elevation	Sample location	longitude	latitude
3000 m	Near the Redstone Observation Deck	E102º02´3.42´´	N29º50´36.49´´
3500 m	Near the Yajiageng Timber Checkpoint	E102º02´9.50´´	N29º51´42.90´´
3800 m	Near the rock	E102º01´2.30´´	N29º53´20.80´´
4170 m	Go up the Yajiageng Boundary Monument for 1 km	E102º0´42.50´´	N29º54´26.70´´

2

Table 2(on next page)

Table 2. The influence of warming on AMF richness based on different elevations.

Notes: CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

1

Order	Family	Genus	shared	treatments		Elevations							
				CK	OTC	3000 m		3500 m		3800 m		4170 m	
						CK	OTC	CK	OTC	CK	OTC	CK	OTC
Archaeosporales	Ambisporaceae	*Ambispora*	1.33	1.67	1.67	0	0	0.33	1	0.67	1	1	0
	Archaeosporaceae	*Archaeospora*	3	4	4.33	2.67	2.67	3	1	0.67	1.33	1	0.33
	Unclassified	*Unclassified*	0.33	0.33	0.33	0	0	0	0	0.33	0.33	0	0
Diversisporales	Acaulosporaceae	*Acaulospora*	9	10.67	12.33	4.33	4.67	6.33	8	5	5	7	4
Glomerales	Glomeraceae	*Glomus*	12	13.67	20.33	2	4.33	9.67	15.33	6	8.33	3.67	3
Paraglomerales	Paraglomeraceae	*Paraglomus*	0	0.33	0.33	0	0.33	0	0	0	0	0.33	0
Unclassified	Unclassified	*Unclassified*	1.33	5.33	6.33	0.67	2.33	1.33	1.33	2.33	0.67	2.67	2.33

2

Table 3(on next page)

Table 3. The influence of warming on the relative abundance of AMF based on different elevations.

Notes: CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

1

Order	Family	Genus	Relative abundance/%							
			3000 m		3500 m		3800 m		4170 m	
			CK	OTC	CK	OTC	CK	OTC	CK	OTC
Archaeosporales	Ambisporaceae	*Ambispora*	0	0	0.0490	0.0523	0.2286	0.0294	0.3756	0
	Archaeosporaceae	*Archaeospora*	0.8361	1.2770	0.4115	0.0523	0.1110	0.0327	0.0555	0.5814
	Unclassified	*Unclassified*	0	0	0	0	0.0621	0.0196	0	0
Diversisporales	Acaulosporaceae	*Acaulospora*	19.6551	32.8304	38.9477	28.3297	37.8633	22.0295	53.9715	55.3139
Glomerales	Glomeraceae	*Glomus*	79.4761	65.1349	60.5559	71.3894	60.8956	75.6614	44.0003	36.4655
Paraglomerales	Paraglomeraceae	*Paraglomus*	0	0.0033	0	0	0	0	0.0065	0
Unclassified	Unclassified	*Unclassified*	0.0327	0.7545	0.0359	0.1764	0.8394	2.2274	1.5906	7.6393

2

Table 4(on next page)

Table 4. The influence of warming on the occurrence frequency of AMF based on different elevations.

Notes: CK means the treatment of control check and OTC means the treatment of warming by Open-top chamber. Shared means the treatment of CK and OTC in share.

1

Order	Family	Genus	Occurrence frequency/%							
			3000 m		3500 m		3800 m		4170 m	
			CK	OTC	CK	OTC	CK	OTC	CK	OTC
Archaeosporales	Ambisporaceae	*Ambispora*	0	0	33.33	100	33.33	66.67	100	0
	Archaeosporaceae	*Archaeospora*	100	100	100	66.67	33.33	66.67	100	33.33
	Unclassified	*Unclassified*	0	0	0	0	33.33	33.33	0	0
Diversisporales	Acaulosporaceae	*Acaulospora*	100	100	100	100	100	100	100	100
Glomerales	Glomeraceae	*Glomus*	100	100	100	100	100	100	100	66.67
Paraglomerales	Paraglomeraceae	*Paraglomus*	0	33.33	0	0	0	0	33.33	0
Unclassified	Unclassified	*Unclassified*	66.67	100	66.67	33.33	100	33.33	66.67	100

2