

Different response of arbuscular mycorrhizal fungal communities in soil and *Elymus nutans* roots to long-term warming in alpine meadow

Bing Han

Wantong Zhang

Lu Yu

Xiaoting Wei

Huakun Zhou

Yiyi Geng

Xinmin Lu

Xinqing Shao (✉ shaoxinqing@163.com)

College of Grassland Science and Technology China Agricultural University <https://orcid.org/0000-0002-2604-0460>

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Abstract

Background and aims

Arbuscular mycorrhizal fungi (AMF) are symbiotic with most terrestrial plants and are important microorganisms that sustain the activity of alpine meadow ecosystems, while warming affects plant and microbial activity and soil nutrient cycling. However, the response of AMF to long-term warming is still poorly understood, especially in alpine meadows.

Methods

We conducted a 7-year field warming experiment to examine the effects of warming (+ 2°C) on the composition of AMF communities in soil and plant roots.

Results

The results showed that warming significantly affected AMF communities, increasing the α -diversity of soil AMF communities and the relative abundance of *Glomeraceae* and decreasing the relative abundance of *Paralomeraceae*, while the relative abundance and diversity of AMF communities within plant roots decreased. Warming increased soil organic carbon and effective phosphorus content and promoted mycorrhizal colonization. In addition, soil AMF communities were strongly influenced by SOC, and intraradical AMF communities were correlated with $\text{NH}_4^+\text{-N}$.

Conclusions

In conclusion, our results suggest that warming mainly changed the composition of soil AMF communities in alpine meadows and enhanced plant-AMF interactions.

Introduction

Since the Industrial Revolution in the 19th century, the massive human use of fossil fuels has increased the concentration of greenhouse gases in the atmosphere leading to a consistent increase in global temperatures, which are projected to rise further by about 1.8-5.0°C between 2000 and 2100 (IPCC 2017). The Qinghai-Tibet Plateau, known as the "third pole of the world", is extremely sensitive to climate change and has experienced striking warming in the past (Shi et al. 2021), making it an ideal place to study the response of terrestrial ecosystems to the global climate.

Arbuscular mycorrhizal fungi (AMF) belong to the subphylum Glomeromycotina of the phylum Mucoromycota (Spatafora et al. 2016). They can form mutually beneficial symbiotic relationships with 90% of plant species and are one of the most important soil microorganisms. Once plant roots were colonized by AMF, mycorrhizal could improve the ability of plants to absorb mineral nutrients, while providing phosphorus

essential for plant development and promoting the growth of host plants (Begum et al. 2019). In return, the host plant gave AMF carbohydrates and lipids to complete its life cycle. AMF can also enhance the plants' resistance to abiotic stresses such as high temperature, drought, and heavy metals (Bhantana et al. 2021; Zhang et al. 2022). Therefore, AMF can affect ecosystem processes by influencing plant communities (Averill et al. 2019; Bennett et al. 2017), rhizosphere microbial communities (Zhang et al. 2010; Liu et al. 2012) and regulating important soil nutrient cycles processes (Ji et al. 2019; Mei et al. 2019). However, the diversity of AMF and their response to climate change, as in the alpine meadow ecosystems of the Tibetan Plateau, are still poorly understood.

The abundance and composition of AMF communities depend to some extent on their propagation form (spores, infected root segments, extensive extraradical mycelium), which might be very sensitive to climate changes (Jiang et al. 2018; Ji et al. 2020). According to reports, AMF abundance and colonization have been reported to show different changes with increasing temperature (Cao et al. 2020), which can be positively correlated (Kim et al. 2015; Zavalloni et al. 2012), negatively correlated (Yang et al. 2021), or even unaffected (Shi et al. 2021). For example, warming increases the densities of external hyphae (Bunn et al. 2009) and spores (Rillig et al. 2002) and reduces mycorrhizal colonization (Hawkes et al. 2008). In addition, warming usually stimulates microbial activity and enhances the availability of soil mineral elements (Rustad et al. 2001; Zhang et al. 2020). High N efficiency will reduce the dependence of host plants on N uptake by AMF and change the abundance of AMF (Qiu et al. 2021; Treseder et al. 2018). However, in cold environments, warming may indirectly increase plant photosynthesis by stimulating hyphal growth (Cotton et al. 2015) thereby altering belowground carbon allocation (Heinemeyer et al. 2004) to promote colonization in roots. Further studies on the Qinghai–Tibet Plateau found that the effect of warming on soil fungal communities depends on the ecosystem type (Morgado et al. 2015; Semenova et al. 2015; Treseder et al. 2016), alpine meadow environments are more sensitive.

It is generally recognized that AMF community assembly is driven synchronously by the host plant and soil environmental filters (Caruso et al. 2012; Davison et al. 2015; Vályi et al. 2016; Xu et al. 2022). Studies had been found that the composition of AMF communities observed in the root (intraradical) and soil (extraradical) samples collected from the same site often differed because the biomass of AMF was distributed differently in and out of the roots (Gao et al. 2019; Ji et al. 2020; Stevens et al. 2020; Varela-Cervero et al. 2015). For one thing, Host plant species (Eom et al. 2000) and their characteristics (López-García et al. 2017) may regulate AMF communities, as some studies have found that host plants preferentially allocate carbohydrates to more efficient symbionts when providing photosynthetic products to AMF (Eom et al. 2000; Vandenkoornhuyse et al. 2002; Wyatt et al. 2014), this selective preference leads to different AMF community composition and diversity, resulting in unequal effects of AMF on growth rates of different plant species (Hausmann and Hawkes 2009). For another, the important functional role of AMF on their host plants can be affected by changes in environmental conditions (Staddon et al. 2004; Treseder et al. 2007), directly affecting AMF abundance and community diversity mainly by affecting AMF growth, and indirectly by altering host plant carbon (C) allocation and soil nutrient content to change community composition (Cotton 2018; Deveautour et al. 2018). Furthermore, soil properties, such as moisture, pH, and nutrient content, have been identified as important predictors of AMF relative abundance and community structure. (Davison et al. 2021; Johnson et al. 2010; Moora et al. 2014; Xu et al. 2022).

Due to the increase of livestock population in alpine pasture areas in recent years, the climate has warmed, rainfall has decreased, and the grassland ecological environment has gradually deteriorated (Jiang et al. 2018; Wei et al. 2021), resulting in a significant decrease in the proportion of good natural forage grass in alpine meadows, which has seriously affected the sustainable development of the local livestock industry and laid a huge hidden danger for the ecological security of the area (Xu et al. 2018; Zhang et al. 2019). Moreover, previous studies on AMF communities in alpine meadows had mainly focused on the N addition context (Liu et al. 2012; Jiang et al. 2018), and some related warming experiments have been short-term warming (Wei et al. 2021; Jiang et al. 2018) with few observational studies on long-term warming (Shi et al. 2021). Therefore, in this study, we explored the responses of AMF community structure and function to 7 years of warming treatments in an alpine meadow ecosystem on the Tibetan Plateau, analyzing changes in species composition and soil AMF communities throughout the plant community, as well as the abundance and composition of AMF communities within the roots of an individual dominant plant species (*Elymus nutans*). Plant and AMF diversity are relatively high (Jiang et al. 2018) in this region and ecosystem productivity is strongly limited by N effectiveness (Xu et al. 2015). We hypothesized that: H₁, Long-term warming alters soil AMF abundance and diversity; H₂, The effect of warming on AMF can be reflected at the single-plant-species scale; H₃, Plant and soil AMF communities do not respond to warming uniformly.

Materials And Methods

Site description

The experimental site was located in Haibei Autonomous Prefecture, Qinghai Province, China (36°92' N, 100°93' E, 3029 m a.s.l.). The area is a typical plateau continental climate with an average annual precipitation of about 410 mm. The year is divided into cold and warm seasons with an annual average temperature of 1.4°C. The highest temperature in the warm season (June to August) is about 15°C, and the lowest temperature in the cold season (December to February) is about -13°C. Annual mean temperature and annual mean precipitation from 1976 to 2016 are shown in Fig. S1. *Elymus nutans*, *Poa pratensis* L., *Kobresia humilis* and *Potentilla bifurca* are the main plant species. The soil is classified as Mat-Gryic Cambisol with a clay loam texture under the Chinese soil classification system (Ma et al. 2017) with 3.5 g kg⁻¹ soil total nitrogen, 40.9 g kg⁻¹ soil total carbon and 2.32 mg kg⁻¹ available phosphorus.

Experimental Design

In 2012, a flat alpine meadow was fenced to prevent livestock from disturbance and the dominant species in this meadow was *Elymus nutans*. In order to simulate the global warming environment in 2014, open-top chambers (OTC) (top diameter 1.5 m, height 0.7 m, bottom diameter 2.2 m) were permanently installed in the meadow. The meadow was divided into Control (CK) and Warming (W; +~2°C), with 4 replications of each treatment. Each experimental plot had a diameter of 2.2 m with a 3 m buffer zone in adjacent plots. Three extractor fans were installed at the top of each chamber to maintain a ~2 °C difference between the warmed and control plots. A multipoint soil (CS655-L30 decagon devices, USA) temperature and humidity meter was

used to automatically record soil temperature and humidity, and data were exported by an automatic data collector (CR800-XT).

Sample Collection In The Field

The plant community characteristics were investigated using the quadrat sampling method (1 × 1 m) in August 2021. Plant species richness in each experimental plot was recorded, with the frequency and coverage of each plant species being assessed. Then five soil cores (3.5 cm in diameter and 0–10 cm in depth) were randomly selected from each plot, and after removing rock from the cores, they were uniformly mixed and combined into one soil sample. The soil samples were divided into two parts: one part was frozen in liquid nitrogen and transported to the laboratory within 24 hours and stored at -80°C for AMF community analysis; the other part was sieved through a 2 mm sieve and air-dried for soil physical and chemical property analysis. To explore the effect of long-term warming on the AMF community at the individual-plant-species level, we sampled a dominant plant species, *E. nutans*. Five individuals of *E. nutans* were selected randomly from each plot, excavated and collected as one sample (Jiang et al. 2018). Plant samples for the analysis of AMF community and mycorrhizal colonization were stored at -80°C, while those for plant nutrient contents were dried at 65°C oven.

Determination Of Mycorrhizal Colonization Rate

Mycorrhizal colonization was examined by following the procedure of Koske and Gemma (1989). When the plants are excavated, select the fine roots and washed them to remove soil particles, weighed 0.5-1.0 g and cut them into 1 cm. Soaked them in KOH (10%, w/v) boiled until the roots were transparent. Then acidified in HCl (2%, v/v), and stained with trypan blue (0.05%, w/v). After 30 min, these roots were immersed in destaining solution (glycerol: lactic = 1:1). Finally, we mounted stained roots on slides and calculated AMF colonization using the magnified gridline intersect method (McGonigle et al. 1990) with a compound microscope under 100–400 magnification. A root intersection was considered colonized if hyphae, arbuscules or vesicles were present.

Analysis Of Soil Physicochemical And Plant Properties

The soil moisture content (SMC) was measured gravimetrically (dried at 105°C for 12 h) and soil pH was measured in 1 M KCl (1:5 w/v) by using a pH meter (Orion Star A215, Thermo Fisher Scientific, USA). NO_3^- -N and NH_4^+ -N concentrations were determined by a flow-through solution analyzer (Flowsys, Ecotech, Germany). Soil total C (TC) and total N (TN) were measured by a carbon-nitrogen element analyzer (Elementar, Hanau, Germany), as well as plant TC and TN. Soil active phosphorus (AP) was extracted from 2.5 g of dry soil with 0.5 M NaHCO_3 solution (pH 8.5) for 30 min and determined by colorimetric method (Bao, 2008). Soil organic carbon (SOC) was measured with an Organic Carbon Analyzer (Vario Macro, elementary, Germany) after 8 hours of fumigation with concentrated hydrochloric acid. HClO_4 - H_2SO_4 colorimetry was used to measure plant total phosphorus (TP) which was extracted by 0.5 mol/L NaHCO_3 .

Molecular Identification Of Amf

Microbial DNA was extracted from root and soil samples using the E.Z.N.A.® Soil DNA Kit (Omega Biotek, Norcross, GA, US) according to the manufacturer's statement. Final genomic DNA purification and concentration were determined by a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and DNA quality was checked by 1% agarose gel electrophoresis. Afterwards, the fungal 18S rRNA gene was amplified by PCR (BioRad S1000, Bio- Rad Laboratory, CA) with primer set AMV4.5NF/AMDGR (5'-AAGCTCGTAGTTGAATTTTCG-3'/5'-CCCAACTATCCCTATTAATCA T -3') according to the study of Lumini et al. 2009. PCRs were performed in a 20 µL mixtures containing 5 µl of buffer (5×), 0.25 µl of Fast pfu DNA Polymerase (NEB, USA), 2 µl (2.5 mM) of dNTPs, 1 µl (10 uM) of each Forward and Reverse primer, 2µl of DNA Template, and 8.75 µl of ddH₂O. The following PCR conditions were: initial denaturation at 98°C for 2min, denaturation with 30 cycles at 98°C for 15s, annealing at 55°C for 30s and extension at 72°C for 30s, with a final extension of 5 min at 72°C. PCR reaction mixtures were purified using the TruSeq Nano DNA Gel Extraction Kit (Illumina, USA) and quantified using the Agilent Bioanalyzer 2100 system (Agilent, USA). Sequencing libraries were generated according to the standard procedures of N (New England Biolabs, USA). Finally, the library was sequenced on the Illumina Hiseq 2500 platform.

Microbiome bioinformatics were performed with QIIME2 2019.4 (Bolyen et al.2018), with slight modification according to the official tutorials (<https://docs.qiime2.org/2019.4/tutorials/>). Multiplex the raw sequence data using the demux plugin and then cut the primers using the cutadapt plugin (Martin et al.2011). The sequences were then quality denoised, filtered, merged and chimeras removed using the DADA2 plugin (Callahan et al. 2016) The mafft (Katoh et al. 2002) was used for comparison with non-single amplicon sequence variants (ASV). Fasttree2 (Price et al. 2009) was used to construct the phylogeny. For the MAARJAM database (<https://www.maarjam.botany.ut.ee/>) (Bokulich et al. 2018), classifications were assigned to ASVs using the classify-sklearn naïve Bayes taxonomy classifier in the Feature Classifier plugin (Bokulich et al.2018).

Statistical analysis

To test the significance of the differences between warming and control treatment for various variables, two-tailed paired t-tests, Wilcoxon-test were employed by SPSS19.0. For Illumina MiSeq sequencing data, alpha diversity indices (Chao1, Faith's PD, species and Simpson diversity indices) were generated using QIIME2 (2019.4). Using the “qiime feature- table rarefy” function in QIIME2, all samples are thinned to the same sequence for downstream analysis based on 95% of the lowest sequence number to ensure that the samples are sequenced to the same depth. Sequencing reads are considered sufficient if the number of species no longer increases with the number of sequencing reads. Differences between treatments were compared using Kruskal-Wallis rank sum test and Dunn's test was used for pairwise comparison. For beta diversity analysis, Bray-Curtis distances were calculated and principal coordinate analysis (PCoA) was performed using the ‘vegan’ package in ‘R’ (Version 4.1.3) to visualize community similarity. The significant difference of AMF community among treatments was detected by permutational multivariate analysis of variance (PERMANOVA). Canonical correspondence analysis (CCA) was used to identify soil properties that predict changes in AMF communities. The Mantel test was used to assess the relationships between the

AMF community and the soil characteristics. CCA and Mantel tests were analyzed using the cca function in the 'vegan' package and the mantel.rtest function in the 'ade4' package in 'R' (version 4.1.3), respectively. Origin2018 (OriginLab, US) and Adobe Illustrator (2020) were used for plotting.

Result

AMF richness at the level of ASV

The Venn diagram analysis showed that 21 ASVs were shared by all samples and these ASVs accounted for 0.7% of the total ASVs observed (Fig. 1). Soil samples had more ASVs than root samples. There were 566 shared ASVs in soil samples, Soil_W treatment had the most ASVs. Root samples had 42 shared ASVs and Root_W only had 156 ASVs. Warming increased ASVs in soil samples, by 30.37% compared to Soil control, while decreased 22.39% in root warming samples.

AMF community composition

A total of 1,366,225 quality filtered AMF sequences were obtained in all samples after filtering, denoising, merging, chimerization and singleton removal, with reads ranging from 60,104 to 112,745 per sample for further analysis (Table S1).

The taxonomic distribution of the AMF was different among different treatments. At the order level, *Paraglomerales* and *Glomerales* are the two most dominant orders both in soil samples and root samples (Fig. 2a). The relative abundance of *Paraglomerales* in soil samples ranged from 28.28–70.99%, while *Glomerales* ranged from 18.34–54.61%. And in root samples, the relative abundance of *Glomerales* ranged from 44.68–18.77%. Only *Diversisporales* can be detected in soil samples, *Archaeosporales* can be detected in root samples. Warming significantly increased the relative abundance of *Glomeraceae* ($P < 0.05$) and decreased *Paralomeraceae* ($P < 0.05$) in Soil warming treatment (Fig. 2b). The relative abundance of *Glomeraceae* in root sample decreased ($P < 0.1$) as well as *Paralomeraceae* significantly reduced 44.29% compared to Root_CK treatment after warming affecting ($P < 0.05$). *Paraglomus* and *Glomus* were the dominant AMF genus (Fig. S2).

Effects of long-term warming on soil and plant characteristics

Seven years of warming changed soil characteristics (Table 1). Warming significantly increased SOC content ($P < 0.05$) but did not affect total carbon ($P > 0.05$). Soil pH decreased significantly under the warming treatment compared to Soil_CK treatment ($P < 0.1$). Long-term warming also influenced soil nitrogen concentrations, with TN, $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$ concentrations increasing, but the differences were not significant. In contrast, soil AP concentration increased significantly in the warming treatment ($P < 0.05$), by 41.81% compared to the control. The soil nitrogen to phosphorus ratio (N:P) decreased.

Table 1
Soil characteristics in different treatments

Treatments	pH	TN (mg g ⁻¹)	TC (mg g ⁻¹)	AP (mg kg ⁻¹)	SOC (mg g ⁻¹)	WC (%)	NH ₄ ⁺ - N (mg kg ⁻¹)	NO ₃ ⁻ - N (mg kg ⁻¹)	N:P	AN (mg g ⁻¹)
Soil_CK	8.29 ± 0.04	3.53 ± 0.10	41.00 ± 1.33	2.32 ± 0.30	28.08 ± 0.78	20.99 ± 0.60	3.84 ± 0.06	23.31 ± 1.26	12.45 ± 2.58	27.68 ± 2.65
Soil_W	8.05 ± 0.06*	3.73 ± 0.05	42.20 ± 0.20	3.29 ± 0.35**	31.78 ± 0.45**	20.38 ± 1.34	3.88 ± 0.29	25.91 ± 1.60	9.69 ± 2.92	30.33 ± 3.66
Data are means ± SE (n = 4). Soil_CK represent samples of control treatment and Soil_W represent samples of warming treatment. The differences between the control and warming were tested by two-tailed paired t-tests, indicated by *** when $P < 0.01$, ** when $P < 0.05$ or * when $P < 0.10$.										

At the plant community level, warming reduced the Shannon-Winener and Simpson index and increased the Pielou evenness index, but the differences were not significant (Table 2). At the individual plant level, warming significantly increased AMF colonization of *E. nutans* ($P < 0.01$), but all plant nutrient contents decreased, with a significant decrease of 15.54% in TN ($P < 0.05$) and no significant changes in TP and TC compared to the control.

Table 2

Changes in plant properties for whole plant communities and for single plant *Elymus nutans* after long-term warming treatment

	Plant community level			<i>Elymus nutans</i>			
	Shannon-Winener	Simpson	Pielou	Colonization (%)	TN (mg g ⁻¹)	TP (g kg ⁻¹)	TC (mg g ⁻¹)
Root_CK	2.064 ± 0.047	0.845 ± 0.013	0.881 ± 0.034	16.32 ± 2.13	10.23 ± 0.19	0.43 ± 0.02	443.6 ± 0.50
Root_W	1.964 ± 0.044	0.833 ± 0.011	0.894 ± 0.020	37.08 ± 3.99***	8.64 ± 0.45**	0.37 ± 0.05	442.4 ± 1.45
Data are means ± SE (n = 4). Root_CK and Root_W represent samples of control treatment and warming treatment, respectively. The differences between the control and warming were tested by two-tailed paired t-tests, indicated by *** when $P < 0.01$, ** when $P < 0.05$ or * when $P < 0.10$.							

Correlations between environmental factors and AMF communities

CCA and Mantel tests were conducted to determine the key environmental factors affecting AMF community structure. The environmental factors based on CCA analysis explained 62% and 56% of the variation in soil and plant root AMF community composition, respectively. Soil TN, SOC, and NO_3^- -N appeared to be the most important environmental factors affecting soil AMF communities, and warming affected soil AMF communities (Fig. 3a). Root CCA analysis showed SMC, AP and NH_4^+ -N arrows are longer than the others (Fig. 3b), which indicated that these variables had stronger effects on AMF communities than the others. Mantel test showed that soil AMF community was significantly correlated with soil TN ($R = 0.331$, $P < 0.05$) and SOC ($R = 0.274$, $P < 0.05$); Root AMF community was significantly correlated with NH_4^+ -N ($R = 0.791$, $P < 0.05$) and SMC ($R = 0.794$, $P < 0.05$, Table 3b).

Table 3
Correlations of AMF community composition (Bray-Curtis distance) with soil characteristics as revealed by Mantel test.

Environment factor	Soil AM		Root AM	
	R	P	R	P
pH	0.02466154	0.355	0.22914338	0.225
TN (g/kg)	0.33121460	0.037	-0.27159422	0.965
TC (g/kg)	0.13554589	0.168	-0.32420364	0.873
TP (g/kg)	-0.23519778	0.983	0.40014792	0.100
AP (mg/kg)	-0.19531326	0.917	0.44071687	0.183
SOC (g/kg)	0.27385163	0.037	-0.04251677	0.485
NO_3^- -N(mg/kg)	0.27662464	0.086	0.01010984	0.426
NH_4^+ -N (mg/kg)	-0.01614515	0.570	0.79060413	0.018
WC (%)	-0.11784507	0.759	0.79361792	0.048
AN (mg/kg)	0.27859569	0.089	0.11055393	0.321
N:P	-0.19981026	0.932	0.41436906	0.198

AMF community diversity and structure

The sparsity curve of observed-species stabilized with increasing sequence numbers for all samples (Fig. S3), indicating that the sequencing depth in this study was sufficient to obtain the majority of the AMF species. Warming increased the Chao1 index, Faith's PD, Simpson index and observed-species of soil samples ($P > 0.05$), while the Chao1 index, Faith's PD and observed-species of root samples were lower in the Root_W treatment ($P > 0.05$) (Fig. 4). The Simpson index of root samples was significantly higher in Root_CK treatment than in Root_W treatment ($P < 0.1$). In addition, the Chao1 index, Faith's PD and observed-species of AMF in the soil were significantly higher than those in the roots ($P < 0.01$).

Principal Co-ordinates Analysis (PCoA) showed that soil and plant samples were separated from each other (Fig. 5a). Warming significantly affected the soil AMF community ($P < 0.05$) and had no effect on plant. The two principal component axes explained more than 67% of the total variation (PCoA 1 = 43.5%, PCoA 2 = 24%). The permutational multivariate analysis of variance (PERMANOVA) demonstrated that the AMF community structure differed by sample type ($P < 0.01$) (Fig. 5b, Table S2).

Discussion

We found significant differences between the AMF communities in plant roots and soil AMF, such as AMF α -diversity and relative abundance, due to the fact that plants select AMF to different degrees during the growth and developmental stages depending on nutrient uptake, which inevitably leads to differences in the species and abundance of AMF in soil and roots (Ji et al. 2020). The higher abundance of soil AMF than in roots suggests that all AMF species in plants are derived from the soil fungal pool and selectively recruit AMF according to their needs (Bainard et al. 2011; Hassan et al. 2011; Torrecillas et al. 2012). In the present study *Glomeraceae* was more abundant in the roots than in the soil, which is in agreement with previous studies (Hart and Reader 2002). Several studies have reported that *Clarodeoglossum* and *Acaulospora* are the dominant genera in the central (Li et al. 2014) and southeastern (Liu et al. 2011) Tibetan Plateau, respectively. However, we found that *Glomeraceae* and *Paralomeraceae* are the dominant genera in the region, suggesting that the various dominant AMF genera on the Tibetan Plateau can be explained by site-specific differences in vegetation communities and environmental conditions, which can exert a strong selective force on AMF taxa (Liu et al. 2011). We also found that it is *Paraglossum* the core AM species of the region (Fig. S2), which is consistent with the finding of Senés-Guerrero and Schüßler (Senés-Guerrero and Schüßler 2016) that AMF communities have a conservative core species under different environmental conditions.

Long-term warming leads to a decrease in the relative abundance of soil AMF communities and the disappearance of some species, affirming our first hypothesis (H_1), but this, contrary to a study conducted in semi-arid grassland ecosystems, proved that it could be caused by differences in the type, magnitude and duration of warming and vegetation types. (Cao et al. 2020). Temperature can be considered as a natural selection that directly affects the relative abundance of soil AMF, leading to the extinction of some AMF families, thus affecting the recruitment of AMF by single classes of plants and changing the composition of AMF communities within roots (H_2), (Treseder et al. 2016; Antunes et al. 2012). The relative abundance of *Glomeraceae* in the soil was significantly increased under warming conditions (Fig. 2b), indicating that AMF communities in soil change with environment (Stevens et al. 2020), while warming greatly increased plant mycorrhizal colonization rates (Zavalloni et al. 2012), probably because *Glomeraceae* can reproduce faster by colonizing pieces of mycelium or mycorrhizal root fragments, whereas other AMF groups reproduce mainly by spores (Hart and Reader 2002).

In this study, warming significantly affected soil AMF communities, while there was no change in plant AMF communities (Fig. 5), verifying the third hypothesis (H_3) and consistent with previous studies. AMF communities in the root system are mainly affected by host plants, while AMF communities in the soil are more influenced by environmental factors (Stevens et al. 2020; Ji et al. 2020). Soil AMF diversity increased

due to warming (Fig. 4), which is more consistent with previously reported studies that warming positively affected soil AMF diversity (Fig. 4) (Gao et al. 2016; Kim et al. 2014). However, warming reduced the Shannon-Wiener and Simpson indices of the whole plant community and AMF diversity within individual plant roots, probably because the diversity and species composition of plant communities are closely related to their symbiotic AM mycorrhizal communities, and warming changes the plant community composition and thus affects AMF host selectivity (Shi et al. 2017; Veresoglou and Rillig 2014).

We then analyzed the environmental factors affecting AMF communities, and previous studies have shown that soil conditions are an important factor influencing AMF communities. Warming can increase the rate of soil nutrient cycling processes and increase the content of effective soil nutrients by promoting the activity of soil microorganisms or changing community composition (Bai et al. 2013; Salazar et al. 2020). Experimental results showed that an increase in phosphorus availability was detected under warming conditions (Table 1), which could be due to the positive effect of warming on microbial phosphorus mineralization and phosphorus solubilization (Barrett et al. 2014). In addition, soil effective N and P contents were consistent with changes in the relative abundance of soil *Glomeraceae*, in contrast to *Paraglomeraceae*, suggesting that warming-induced changes in nutrient effectiveness may affect different AMF taxa (Xu et al. 2022). It was also found that warming significantly increased soil organic carbon content (Zhang et al. 2020). However, some studies found that warming significantly reduced soil organic carbon or had no effect on soil organic carbon (Wang et al. 2014; Zhang et al. 2015). It may be that the difference in the effect of warming on soil organic carbon may be related to the key controlling factors of carbon cycling in different grassland ecosystems (Xu et al. 2012). In contrast, warming did not affect soil nitrogen transformations, but instead reduced whole nitrogen content in plants, due to the fact that nitrogen enrichment had the least positive effect on graminoids at elevated temperatures (Huang et al. 2021). The decrease in the relative abundance of *Glomeraceae* and *Paraglomeraceae* in plant roots may be due to the indirect suppression of AMF by warming through increased microbial mineralization and nutrient availability (more effective phosphorus) in plants on the one hand (Dai et al. 2020; Rustad et al. 2001), and reducing plant dependence on AMF. On the other hand, plants select AMF with the best phosphorus uptake, such as *Gigasporaceae*, and allocate less carbon to *Glomeraceae* and *Paraglomeraceae* resulting in a reduction in their relative abundance (Hart and Reader, 2002).

Soil nutrient availability is one of the main environmental filters that contribute to AMF community aggregation (Vályi et al. 2016; Camenzind et al. 2014)). Based on the CCA and Mantel tests (Fig. 3), we found that soil AMF communities were significantly associated with TN and SOC, and $\text{NH}_4^+\text{-N}$ and SMC significantly affected AMF communities within roots (Govindarajulu et al. 2005). Previous studies showed that N availability is strongly correlated with AMF abundance in N-limited environments, and N enrichment reduced plant dependence on AMF partners due to the increase of soil available N, thereby reducing AMF abundance (Van Diepen et al. 2011; Dean et al. 2014). For example, high N addition treatments resulted in a reduction of *Glomeraceae* (Cao et al. 2020). Mycorrhizal fungi also have an impact on SOC storage, with AMF significantly contributing to SOC formation (Clemmensen et al. 2013; Orwin et al. 2011). For example, Zhang et al. (2020) found that moderate soil warming can increase AMF biomass, which can affect subsurface carbon allocation.

Conclusion

This study reveals the different effects of long-term warming on soil and dominant plant AMF communities in alpine meadows of the Tibetan Plateau. The soil AMF community composition was different from that of the plant, with higher richness. Long-term warming significantly affected soil AMF communities and increased α -diversity, but had less effect on individual plant AMF communities. We also found that the AMF community was strongly influenced by N, and the soil AMF community was closely related to SOC, which provided a new idea to explain the response of AMF to temperature increase.

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Figures

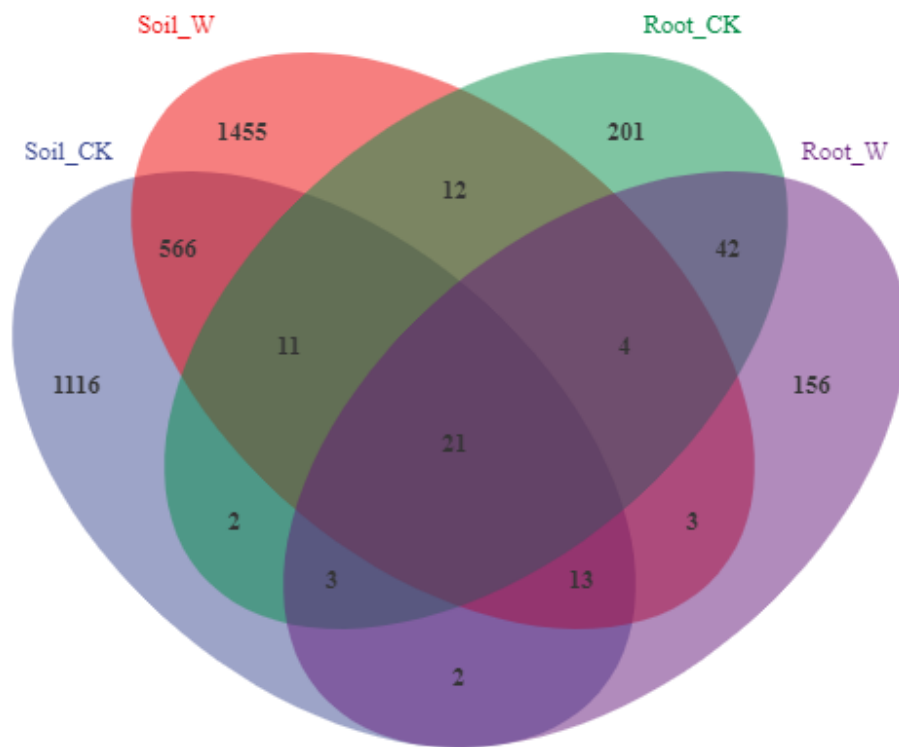


Figure 1

Venn diagram analyses of AMF communities in the soil and root samples. Venn diagram demonstrated the numbers of shared and unique observed ASVs among treatments. Soil_CK represents soil sample in control treatment; Soil_W represents soil sample in warming treatment; Root_CK represents root sample in control treatment; Root_W represents root sample in warming treatment.

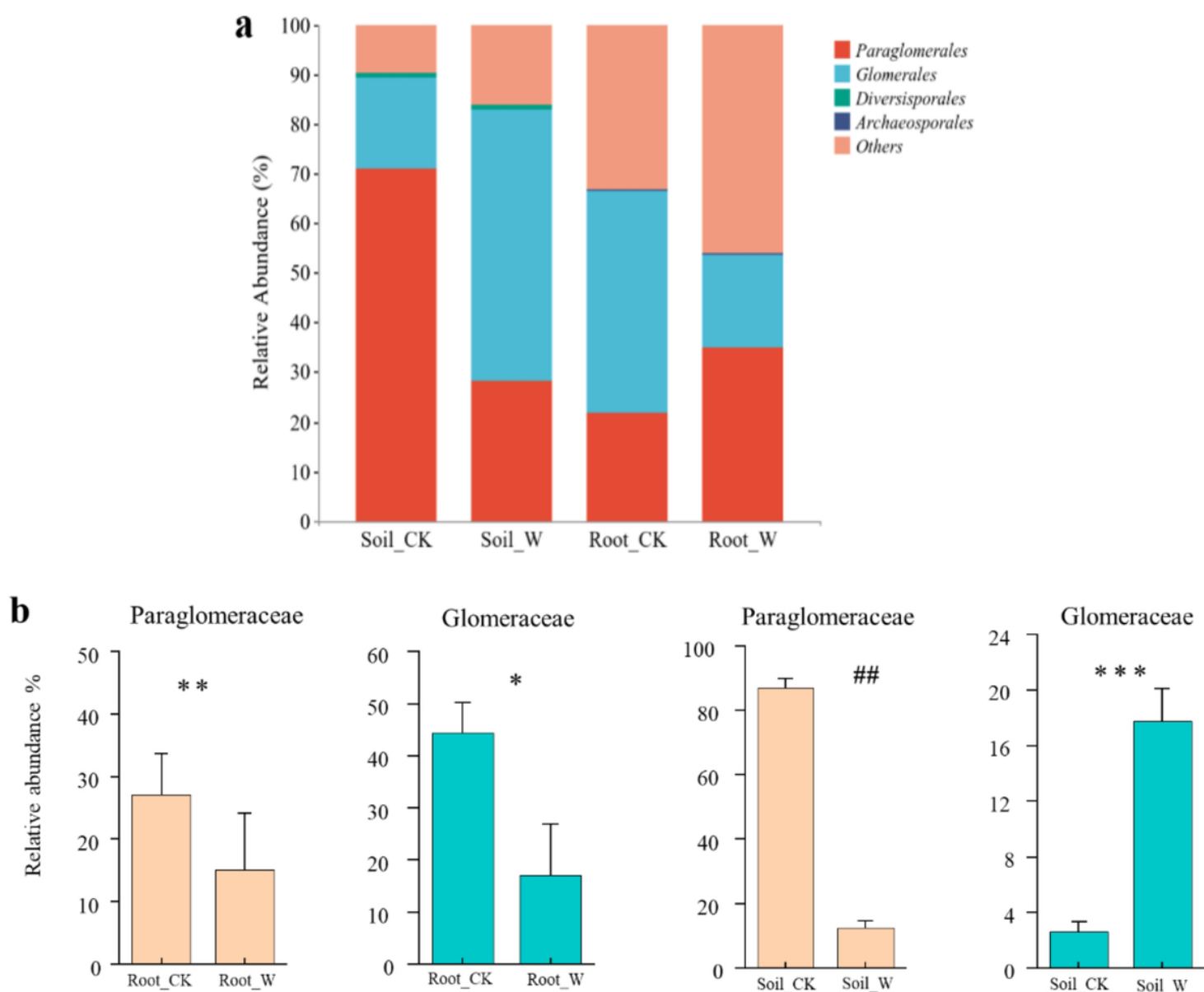


Figure 2

Taxonomic composition of soil and root AMF community at the order (a) and dominant family (b) levels under different treatments. Data are means $\pm$ SE ($n = 4$). Soil_CK and Root_CK represent control, Soil_W and Root_W represent warming, respectively. The differences between the control and warming were tested by two-tailed paired t-tests, indicated by *** when $P < 0.01$, ** when $P < 0.05$ or * when $P < 0.10$. The differences for some parameters were also tested with Wilcoxon-test as indicated by ## when $P < 0.05$ and # when $P < 0.10$.

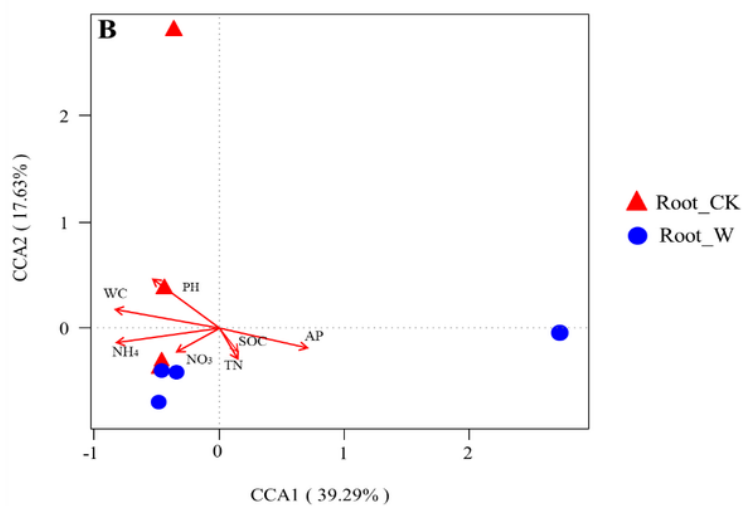
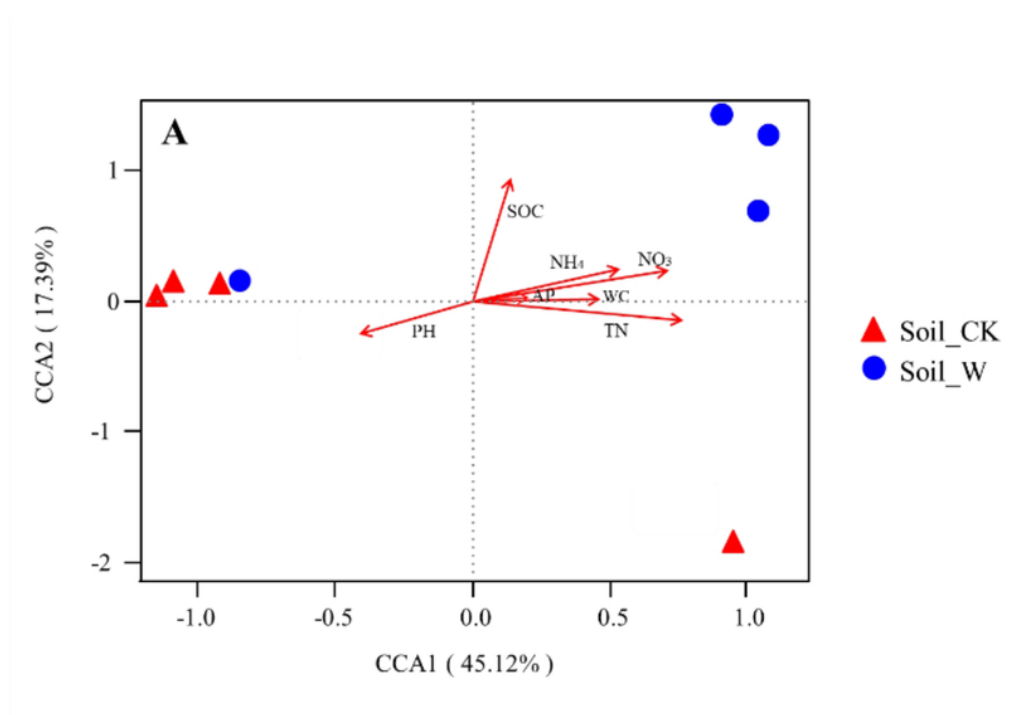


Figure 3

Canonical correspondence analysis (CCA) showing the relationship between soil characteristics and AMF community in soil (a) and roots (b) samples.

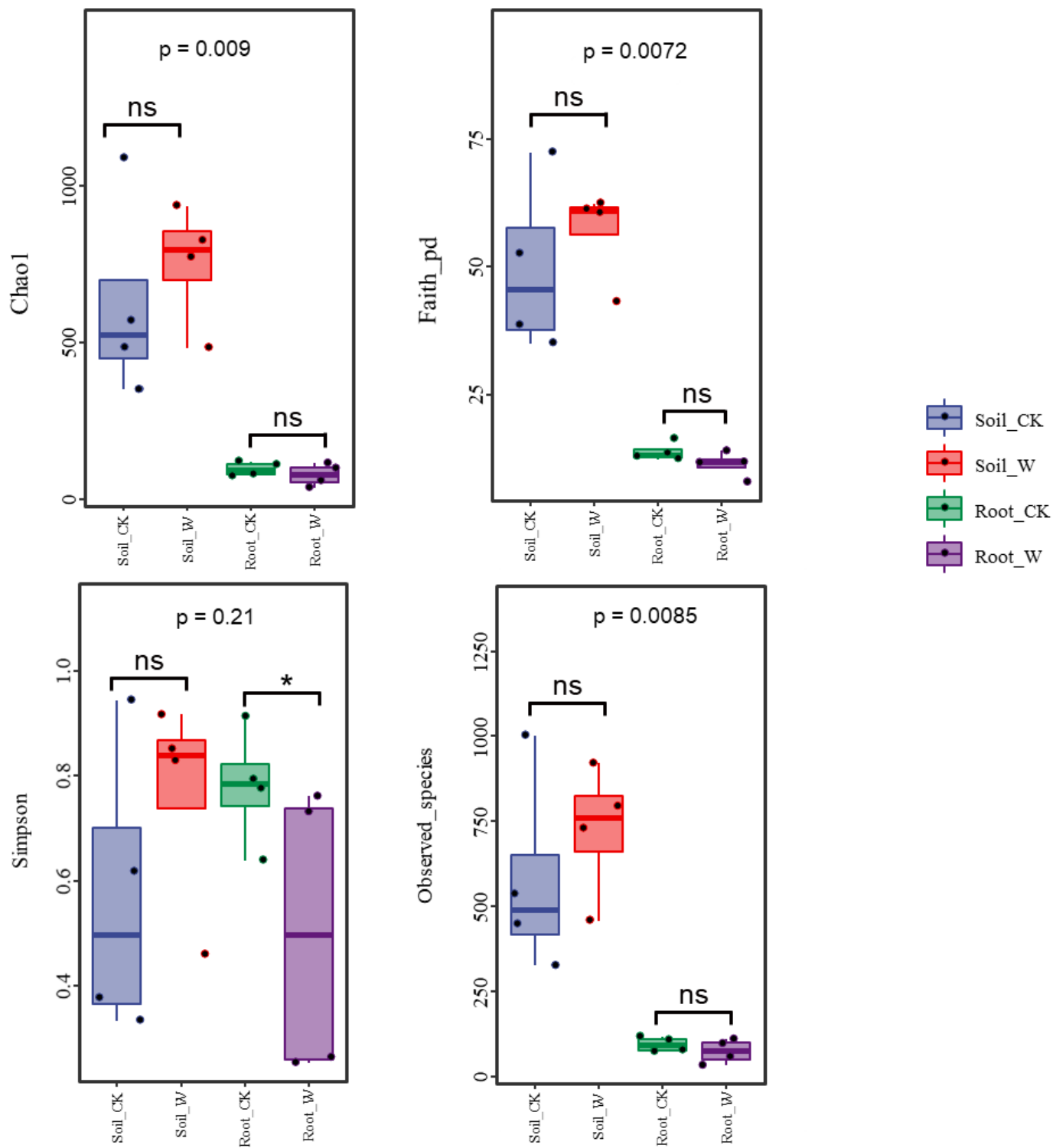


Figure 4

Alpha diversity indices of AMF communities in soil and root samples, including Chao1, Faith_pd, Simpson and observed_species. The box plot shows median (thick line), first quartile–third quartile percentiles (box range) and 1.5×the interquartile range. P value was obtained by Kruskal-Wallis test. The differences between the control and warming were tested by Dunn's test, indicated by ** when $P < 0.05$ or * when $P < 0.10$. There were four independent replicates of each treatment.

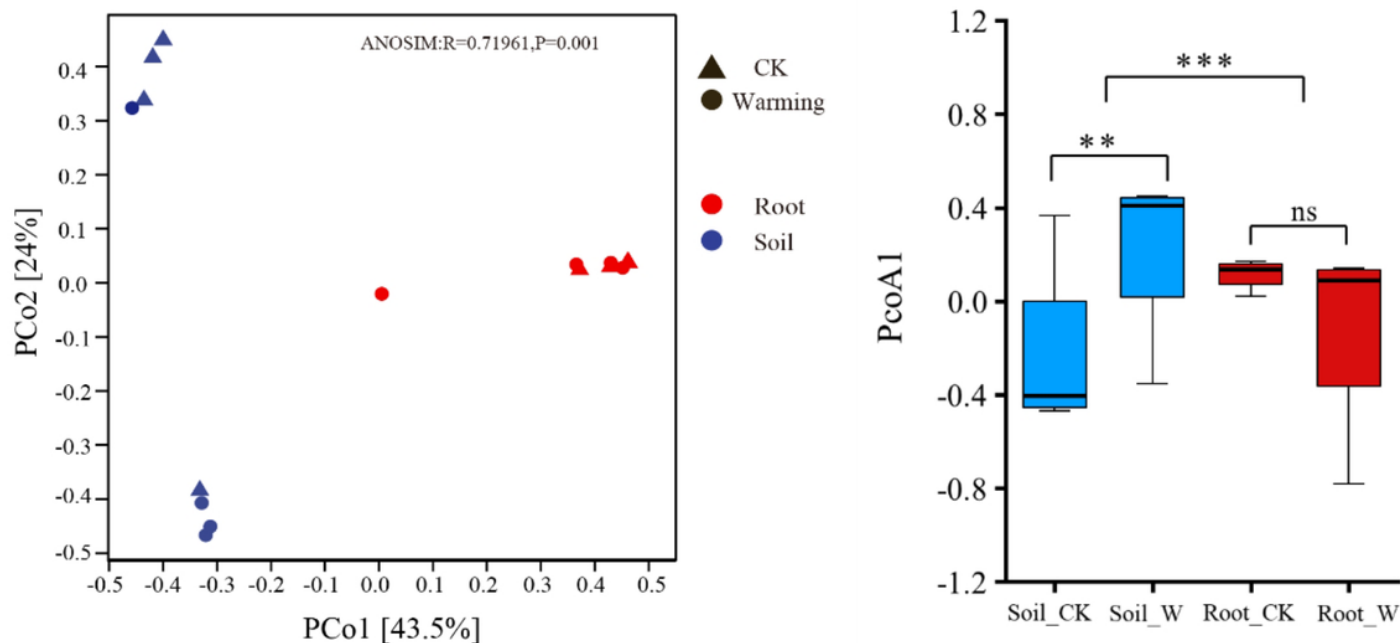


Figure 5

PCoA analyses of AMF communities in the soil and root samples. The PCoA plot was based on the Bray-Curtis distances ordination of variation in community structure of the AMF community.

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