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# Effects of mycorrhiza and hyphae on the response of soil microbial community to warming in eastern Tibetan Plateau

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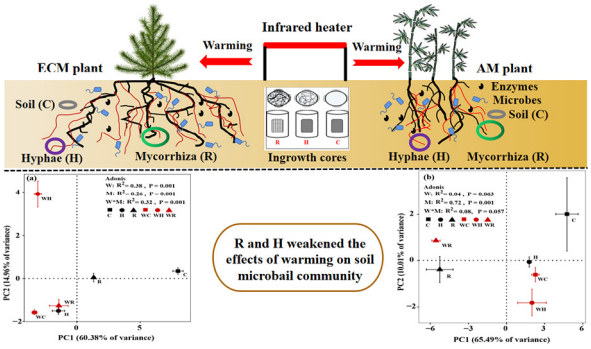
## HIGHLIGHTS

- Both ECM and AM mycorrhiza/hyphae increased the biomass of soil microbes.
- The response of microbes to warming was altered with mycorrhiza/hyphae presence.
- Soil microbes in *P. asperata* plots were more sensitive to warming than *F. nitida*.

## GRAPHICAL ABSTRACT

Mycorrhiza and hyphae can regulate the response of soil microbial community to warming.

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## ABSTRACT

The effects of mycorrhiza and its external hyphae on the response of soil microbes to global warming remain unclear. This study investigates the role of mycorrhiza and its hyphae in regulating soil microbial community under warming by examining the microbial biomass and composition in the ingrowth cores of arbuscular mycorrhiza (AM) plant, *Fargesia nitida*, and ectomycorrhiza (ECM) plant, *Picea asperata*, with/without mycorrhiza/hyphae and experimental warming. The results showed that warming significantly increased the biomass of all soil microbes (by 19.89%–137.48%) and altered the microbial composition in both plant plots without mycorrhiza/hyphae. However, this effect was weakened in the presence of mycorrhiza or hyphae. In *F. nitida* plots, warming did not significantly affect biomass and composition of most soil microbial groups when mycorrhiza or hyphae were present. In *P. asperata* plots, warming significantly increased the total and ECM fungi (ECMF) biomass in the presence of hyphae ($p < 0.05$) and the total, Gn, and AM fungi (AMF) biomass in the presence of mycorrhiza ($p < 0.05$). Meanwhile, the response of enzyme activities to warming was also altered with mycorrhiza or hyphae. Additionally, soil microbial community composition was mainly influenced by soil available phosphorus (avaP), while enzyme activities depended on soil avaP, dissolved organic carbon (DOC), and nitrate concentrations. Our results indicate that mycorrhiza and its hyphae are essential in regulating the response of microbes to warming.

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## 1. Introduction

Soil microbial community plays an essential role for the biogeochemical cycle (Wagg et al., 2014) as these microbes drive nutrient transformation of terrestrial ecosystems, such as soil carbon, nitrogen, and phosphorus transformation. To derive substrate for building biomass, microbes secrete extracellular enzymes that transform carbon, nitrogen, and phosphorus to decompose soil organic matter (Moore et al., 2015). These microorganisms are sensitive to environmental changes, such as soil moisture, nutrient composition, and temperature (Zhang et al., 2014; Kang et al., 2021; Liu et al., 2022). Rise in temperatures due to global warming is more apparent at higher-latitude and higher-altitude regions (Jing et al., 2016). Studying the response of soil microbial community and enzyme activities to warming enables better understanding of soil biochemical processes under global warming (Wang et al., 2020).

Reports have shown that the mycorrhiza, which constitutes the entire symbiotic system including root and external hyphae, and its hyphae affect the soil microbial community through their root-derived or hyphae-derived nutrient input, which is an energy source for microbe growth (de Graaff et al., 2010; Finzi et al., 2015; Keiluweit et al., 2015; Meier et al., 2015). Host plants with mycorrhiza, including ectomycorrhiza (ECM) and arbuscular mycorrhiza (AM), acquire water and nutrient differently from plants without mycorrhiza (Phillips et al., 2013; Wen et al., 2022), resulting in differences in soil carbon and nitrogen pools, and/or the C/N ratio, and nutrient cycling (Lin et al., 2017; Zhu et al., 2018). For instance, studies predict that the soil around ECM plants stores more carbon than AM soil does (Averill and Hawkes, 2016). Studies also indicate that the mycorrhiza and hyphae of AM plants have higher nitrogen content and turnover rates compared with ECM. Soil dominated with AM had higher soil nutrient availability and organic input quality than ECM soil (Veresoglou et al., 2012; Lin et al., 2017). Specifically, AM plants are better at enhancing soil nitrogen input and microbe growth and influence soil microbial community composition and function (Kallenbach et al., 2016; Craig et al., 2018). Additionally, AM and ECM plants have different effects on soil properties, e.g., pH, soil carbon sink, and extracellular enzymes, which indirectly affects the soil microbes (Craig et al., 2018; Wen et al., 2022). The morphology of ECM roots consists of a thick mantle with layers of narrow, thick-walled hyphae. However, AM roots have no mantle and broad, thin-walled hyphae (Phillips and Fahey, 2006; Wang et al., 2020; Wen et al., 2022). Although a large number of tree species are colonized by AM or ECM fungi (Soudzilovskaia et al., 2020), their roles, especially their effect, on the response of soil microbes to warming are still unclear. The chemical properties and turnover rates induced by mycorrhiza and hyphae also vary (Cairney, 2012). Previous studies have overlooked the roles of hyphae on the roots as it is invisible to the naked eye (Pollierer et al., 2007; Wallander et al., 2013). Therefore, the individual effects of hyphae on soil microbes are poorly understood.

Rise in global temperatures alter the production of extramatrical hyphae (Staddon et al., 2002; Kernaghan, 2005). Previous studies have shown that warming positively influenced the ECM hyphae production (Clemmensen et al., 2006; Deslippe et al., 2011; Leppälammi-Kujansuu et al., 2013), increased ECM hyphal density, and stimulated the percentage of mycorrhiza length colonized by mycorrhizal fungi (Staddon et al., 2004). Meanwhile, reports have shown that warming can change biomass and composition of ECM fungi (Domisch et al., 2002; Allison and Treseder, 2008). Studies have indicated that AM fungi are more tolerant to warming than other microbes (Zhang et al., 2020) and that AM fungi colonization were not affected by warming in an alpine meadow in northeastern Qinghai-Tibetan Plateau (Yang et al., 2013). Additionally, reports have shown that a temperature rise of 5 °C decreased AM fungi colonization in grass mycorrhiza (Olsrud et al., 2010). In Iceland, AM fungal biomass, measured using phospholipid fatty acid (PLFA) of 16.1ω5, decreased with increase in temperature (Leblans, 2016). Although ECM and AM fungi are known to respond differently to warming, the response of soil microbial community biomass, composition, and functions to warming in mycorrhiza and hyphae soil with ECM and AM plants is poorly understood.

The subalpine forest ecosystem in the eastern Tibetan Plateau of China is highly sensitive to global warming (Xu et al., 2010; Zhao et al., 2014; Liu et al., 2021). *Picea asperata* and *Fargesia nitida* are the dominant tree and shrub species, respectively, in this coniferous forest. Studies have shown that experimental warming (4-year-long night warming using infrared heaters) slightly affected the biomass of microbial groups in the rhizospheric soil of *P. asperata*, an ECM plant (Sun et al., 2016; Zhao et al., 2014, 2022). Four-year-long warming period with open-top chambers reduced soil microbial biomass at 0- to 10-cm depth during the non-growing season but increased the biomass during the growing season. However, these studies only focused on the response of microbial community to warming in rhizospheric soil but overlooked microbes in hyphospheric soil. Moreover, studies have explored the effect of mycelium and root-derived carbon on soil organic carbon pools and nitrogen cycling of *P. asperata* (Zhang et al., 2018c, 2019). However, studies comparing soil microbes in rhizospheric and hyphospheric soil, especially in the context of warming, are scarce. Additionally, the mycorrhiza/extramatrical hyphae of *F. nitida*, an AM plant, and their effects on soil microbial community under warming condition are rarely studied. This study aims at assessing the effects of experimental warming on soil microbial community biomass, composition, and function in bulk, rhizospheric, and hyphospheric soil, and further investigating the impact of different mycorrhiza and hyphal types on these effects.

Based on previous studies, we hypothesized that (1) the existence of mycorrhiza and its hyphae would have different effects on soil microbial community; and (2) the ECM tree *P. asperata* and AM shrub *F. nitida* would have different effects on the responses of soil microbial community biomass, composition, and activities to warming.

## 2. Material and methods

### 2.1. Experimental design

The study was conducted in Maoxian Mountain Ecosystem Research Station of the Chinese Academy of Science in Sichuan Province (103°53′, 31°41′), where the altitude, mean annual precipitation, evaporation, and temperature are 1826 m, 919.5 mm, 795.8 mm, and 8.9 °C, respectively. This research site in the eastern Tibetan Plateau of China is vulnerable to global warming. *P. asperata* and *F. nitida* are typical ECM and AM plants, respectively, and widely distributed in this area.

Four pairs of plots (4 warming and 4 control) of 2 m × 2 m × 0.5 m (length × width × depth) were set. Soil pH, total carbon, soil organic carbon, and total nitrogen were 5.88, 21.48 g $kg^{-1}$, 17.76 g $kg^{-1}$, and 2.68 g $kg^{-1}$, respectively. The warming and control plots were separated by 5 m. Each plot was divided into two 2 m × 1 m × 0.5 m subplots. Each subplot was cultivated with uniform 3-year-old *P. asperata* or 2-year-old *F. nitida* seedlings based on plant height and stem perimeter in July 2018. Double-layer plastic film was buried at 50-cm depth around each subplot to avoid the impact of plant roots on the surroundings. Meanwhile, based on a device by Booth (2004) and Phillips et al. (2012), we modified and made three types of ingrowth cores (soil cores (C), hypha cores (H) and mycorrhiza cores (R)), which were buried in each subplot, made using PVC pipes with length, internal, and external diameter of 15 cm, 5 cm, and approximately 6 cm, respectively. The wall and bottom of C, H, and R cores were covered tightly in a mesh of three different pore sizes (1, 48, and 1000 μm). The 1-μm mesh was used to prevent the ingrowth of mycorrhiza and hyphae while allowing changes in water and nutrients. The 48- and 1000-μm meshes would allow the entry of hypha and mycorrhiza, respectively (Zhang et al., 2018c). The soil passed through a 5-mm mesh was placed into the ingrowth cores.

We used 165 cm × 15 cm infrared heaters (Kalgo Electronics, Inc., Bethlehem, PA, USA) to simulate warming. Each warmed plot was heated by an infrared heater hanging 1.8 m above the plot center. In the control plot, a "dummy" heater was included to simulate the shading effects of the infrared heater. Experimental warming was started from October 2018. The warmed plots were heated for 12 h per day from 7:00 pm to

7:00 am, and all the plots were routinely monitored. Soil temperatures (5 cm depth) were measured using DS1923G temperature/humidity iButton data loggers (Maxim/Dallas Semiconductor Inc., USA). Experimental warming elevated the monthly soil temperature of the warmed plots by 3.55 °C (Fig. 1).

### 2.2. Soil sampling

Soil samples were separately collected from the three ingrowth cores (C, H, and R) in late November 2019. Four similar ingrowth cores from each subplot were mixed to obtain one composite sample resulting in four composite samples for C, H, and R for each treatment. Each composite sample was passed through a 2-mm mesh to remove visible plant material and stones, stored in an icebox at 4 °C and then immediately delivered to the laboratory for further analysis. A composite sample was divided into two parts, one was used to analyze PLFA profiles and enzyme activities, while the other was used to analyze soil physiochemical factors.

PLFA was analyzed based on Bossio and Scow (1998). Here, 14:0 iso, 15:0 iso, 15:0 anteiso, 16:0 iso, 17:0 iso, and 17:0 anteiso were chosen as gram-positive bacteria (Gp) markers (Kaiser et al., 2010; Willers et al., 2015). Then, 16:1 ω11c, 16:1 ω7c, 16:1 2OH, 17:1 ω8c, 17:0 cyclo, 18:1 ω7c, 18:1 ω5c, and 19:0 cyclo ω8c were used as indicators of gram-negative bacteria (Gn) (Parker et al., 1982; Robie and White, 1989; Frostegård et al., 1993; Zelles, 1997; Fierer et al., 2003). Among the bacterial (B) markers for Gp and Gn bacteria, 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl represented actinomycetes (ACT) (Robie and White, 1989; Zelles, 1997). For fungal determination, 16:1 ω5c was considered as AM fungi (AMF) (Frostegård et al., 1993); 18:2 ω 6,9c and 18:1 ω9c were used to identify fungi (F); and 18:2 ω 6,9c was used to identify ECM fungi (Frostegård et al., 1993). Total biomass included B, ACT, AMF, F, ECMF, and unspecific biomass, i.e., 15:1 iso G, 16:0, 18:0, 18:1 ω7c 11-methyl, and 19:0.

Activities of 4-β-*N*-acetylglucosaminidase (NAG) and acid phosphatase (AP) were determined by fluorometric methods (Saiya-Cork et al., 2002) at 365-nm excitation and 450-nm emission. For these analyses, soil suspensions were prepared separately by adding 2–3 g of fresh soil from each sample to 100 ml of 50 mM acetate buffer (pH 5) and homogenizing for 1 min using a magnetic stirrer. The resultant suspensions were continuously stirred using a magnetic stirrer while 200-μl aliquots of each suspension were dispensed into 96-well microplates. Substrates were 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide and 4-methylumbelliferyl phosphate, respectively, for both enzymes. The microplates were incubated in the dark at 20 °C for 2 h. A full-wavelength multi-function reading instrument (Variokan flash, Thermo Scientific, USA) was used for fluorescence reading. Urease (Ure) activity was assayed as described by Kandeler and Gerber (1988), in which 5 g of soil was incubated for 2 h at 37 °C with 2.5 ml of 80 mM urea and 20 ml of 0.1 M borate buffer (pH 10). After incubation, 30 ml of 2 M KCl was added to the solution, and absorbance was determined at 690 nm after shaking for 30 min.

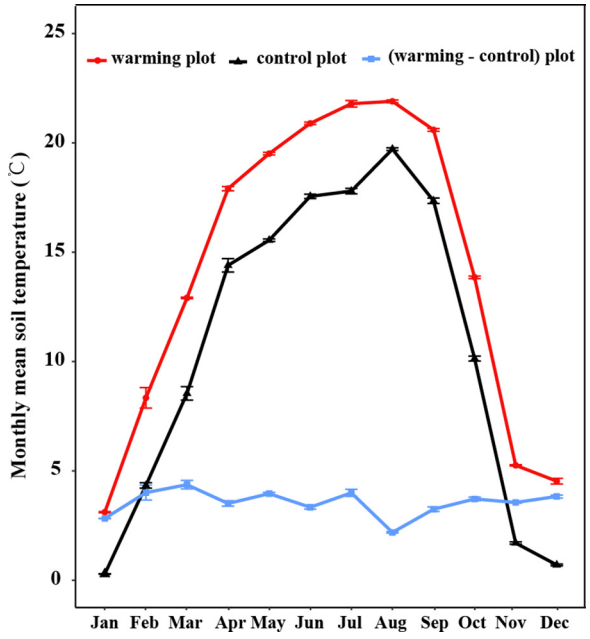



**Fig. 1.** Monthly mean soil temperature in control and warming plots in 2019.

Soil total carbon (TC), total nitrogen (TN), and dissolved organic carbon (DOC) in the extracts (1:5, soil: deionized water) were analyzed using an elemental analyzer (MACRO cube, Elementar, Germany). Soil ammonium- and nitrate-N were assayed using colorimetric methods. Total phosphorus (TP) was determined by $H_2SO_4$-$HClO_4$ acid dissolution-Mo-Sb colorimetry. The content of available phosphorus (avaP) in soil was determined by $NaHCO_3$-Mo-Sb colorimetry.

### 2.3. Statistical analysis

We used three-way analysis of variance (ANOVA) to examine the effects of warming, mycorrhiza/hyphae, plant species, and their interactions on all indexes. Individual treatment means comparisons within each species were conducted using the least significant difference method (LSD), which is used to identify significant difference at $p = 0.05$. Microbial community composition was analyzed using principal component analysis (PCA) of the individual fatty acid content for each species. Permutational multivariate ANOVA (Adonis) was used to test the individual fatty acids variation for each species between different treatments. Redundancy analysis (RDA) was used visualize the relationship between the response variable values (PLFAs), enzyme activities, and the explanatory values (soil physicochemical properties). Forward selection was done to identify the key factors that best explained the variations of soil microbial community composition and enzymes based on the RDA (Li et al., 2022). Hierarchical partitioning (HP) was used to calculate the interpretation rate of each soil physicochemical property (Chevan and Sutherland, 1991). Additionally, correlations among soil properties, microbial community biomass, and enzyme activities were determined using the Spearman correlation analysis. We tested normality and homogeneity of variance of all data with Shapiro-Wilk normality and Bartlett test, respectively. All statistical analyses conducted in R3.4.3. PCA, RDA, forward selection, and HP were performed using functions in the "vegan," "adespatial," and "hier.part" packages in R project.

## 3. Results

### 3.1. Biomass and composition of soil microbial community

The biomass of total microbes, Gp, Gn, B, and AMF are significant affected by global warming, plant species, mycorrhiza/hyphae, and their interactions (Table 1). While the biomass of most microbial groups of H and R cores were significantly higher (22.68%–140.73%) than that of C cores in both the *P. asperata* plots and the *F. nitida* plots under non-warming conditions (Fig. 2). However, H and R cores had a significantly lower biomass of most microbial groups than C cores in *P. asperata* plots under warming condition. Compared to C cores, the biomass of all microbial groups in R cores were significantly higher (26.68%–154.40%) than that in C cores, but that of H and C cores were similar under warming conditions in *F. nitida* plots.

Experimental warming significantly increased the microbial biomass of C cores in both *P. asperata* and *F. nitida* plots (except ECMF in *F. nitida* plots) ($p < 0.05$) (Fig. 2). In the presence of mycorrhiza or hyphae, warming did not significantly alter the biomass of any microbial groups except ECMF in *F. nitida* plots. In *P. asperata* plots, warming also had no significant effects on the soil microbe biomass except the total and ECMF biomass of H cores, but it significantly increased the biomass of total, Gn, and AMF of R cores by 0.97%, 13.02%, and 60.42%, respectively (Fig. 2).

Evaluation of microbial community composition (Fig. 3a and b) using PCA indicated that variations in PLFAs of *P. asperate* and *F. nitida* plots

**Table 1**
Effects of warming, plant species, and mycorrhiza/hyphae on soil microbial community, enzyme activities, and environmental factors (F values of three-way ANOVA).

| Soil trait | Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | P | W | M | P ∗ W | P ∗ M | W ∗ M | P ∗ W ∗ M |
| Total | 62.46*** | 85.80*** | 76.64*** | 59.13*** | 53.07*** | 27.02*** | 6.75** |
| Gp | 30.25*** | 36.58*** | 71.77*** | 30.65*** | 76.22*** | 45.52*** | 10.91*** |
| Gn | 43.46*** | 49.62*** | 68.44*** | 16.84*** | 37.92*** | 29.35*** | 11.47*** |
| B | 44.27*** | 51.54*** | 78.98*** | 25.03*** | 58.98*** | 40.39*** | 12.63*** |
| ACT | 32.23*** | 14.54*** | 25.19*** | 2.53 | 25.47*** | 25.21*** | 5.06* |
| AMF | 43.46*** | 58.68*** | 59.67*** | 24.33*** | 11.99*** | 17.90*** | 4.41* |
| ECMF | 157.01*** | 204.68*** | 105.19*** | 3.51 | 109.63*** | 5.89** | 65.23*** |
| F | 18.95*** | 34.99*** | 53.20*** | 3.90 | 54.10*** | 31.71*** | 18.58*** |
| NAG | 102.51*** | 55.19*** | 48.82*** | 4.20 | 47.44*** | 41.71*** | 0.37 |
| Ure | 35.03*** | 25.81*** | 12.43*** | 5.23* | 12.47*** | 21.20*** | 3.70* |
| AP | 1.09 | 77.45*** | 13.95*** | 6.78* | 62.81*** | 31.16*** | 15.76*** |
| TC | 7.11* | 24.19*** | 39.00*** | 0.03 | 4.11* | 0.13 | 0.93 |
| DOC | 0.54 | 0.03 | 14.49*** | 0.14 | 5.52 | 12.41*** | 8.33** |
| TN | 3.46 | 0.08 | 0.55 | 0.59 | 0.46 | 0.82 | 2.48 |
| TP | 2.10 | 0.45 | 1.23 | 0.33 | 0.27 | 1.16 | 0.39 |
| $NO_3^-$ | 9.12** | 162.10*** | 11.52*** | 0.02 | 5.36* | 4.57* | 1.59 |
| $NH_4^+$ | 5.93* | 1.34 | 3.12 | 9.24** | 0.10 | 0.16 | 3.73* |
| avaP | 28.02*** | 0.05 | 57.02*** | 3.36 | 4.33* | 13.30*** | 9.38*** |

P, effects of plant species; W, effects of warming; M, effects of mycorrhiza/hyphae; P ∗ W, the interactions between plant species and warming; P ∗ M, the interactions between plant species and mycorrhiza/hyphae; W ∗ M, the interactions between warming and mycorrhiza/hyphae; P ∗ W ∗ M, the interactions between plant species, warming and mycorrhiza/hyphae. Total, total PLFAs; Gp, gram-positive bacterial PLFAs; Gn, gram-negative bacterial PLFAs; B, bacterial PLFAs; ACT, actinobacterial PLFAs; AMF, arbuscular mycorrhiza fungal PLFAs; ECMF, ectomycorrhiza fungal PLFAs; F, fungal PLFAs; NAG, *N*-acetylglucosaminidase; Ure, urease; AP, acid phosphatase; TC, soil total carbon concentration; DOC, soil dissolved organic carbon concentration; TN, soil total nitrogen concentration; TP, soil total phosphorus concentration; $NO_3^-$, nitrate concentration, $NH_4^+$, ammonium concentration; avaP, available phosphorus concentration.

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

were explained by the first principal component (PC1) as 60.38% and 65.49% and the second principal component (PC2) as 4.96% and 10.01%, respectively. Permutational multivariate ANOVA (Adonis) showed that warming ($p = 0.01$), mycorrhiza/hyphae ($p = 0.01$) and their interactions ($p = 0.01$) in *P. asperate* plots and mycorrhiza/hyphae ($p = 0.01$) in *F. nitida* plots significantly impacted the microbial community composition.

### 3.2. Soil enzyme activities

Soil enzyme activities were significantly affected by warming, plant species, mycorrhiza/hyphae, and their interactions (Table 1). Under non-warming conditions, the activities of Ure in the H cores and AP in H and R cores were significantly lower than that in C cores ($p < 0.05$) of *P. asperate* plots. In *F. nitida* plots, H cores had significantly higher NAG activities than C cores ($p < 0.05$). Under warming conditions, R cores had significantly higher NAG and Ure activities than C cores in both plots ($p < 0.05$).

Experimental warming significantly affected soil Ure activities in C cores, resulting in 30.54% and 36.73% reduction in *P. asperate* and *F. nitida* plots, respectively (Fig. 4). However, in the presence of hyphae and mycorrhiza, warming significantly decreased Ure but increased AP activities in H cores and significantly increased NAG (58.70%) and AP (45.61%) activities in R cores of *P. asperate* plots. In *F. nitida* plots, warming significantly increased NAG (79.41%), Ure (24.08%), and AP (104.54%) activities in the presence of mycorrhiza.

### 3.3. Soil physicochemical properties

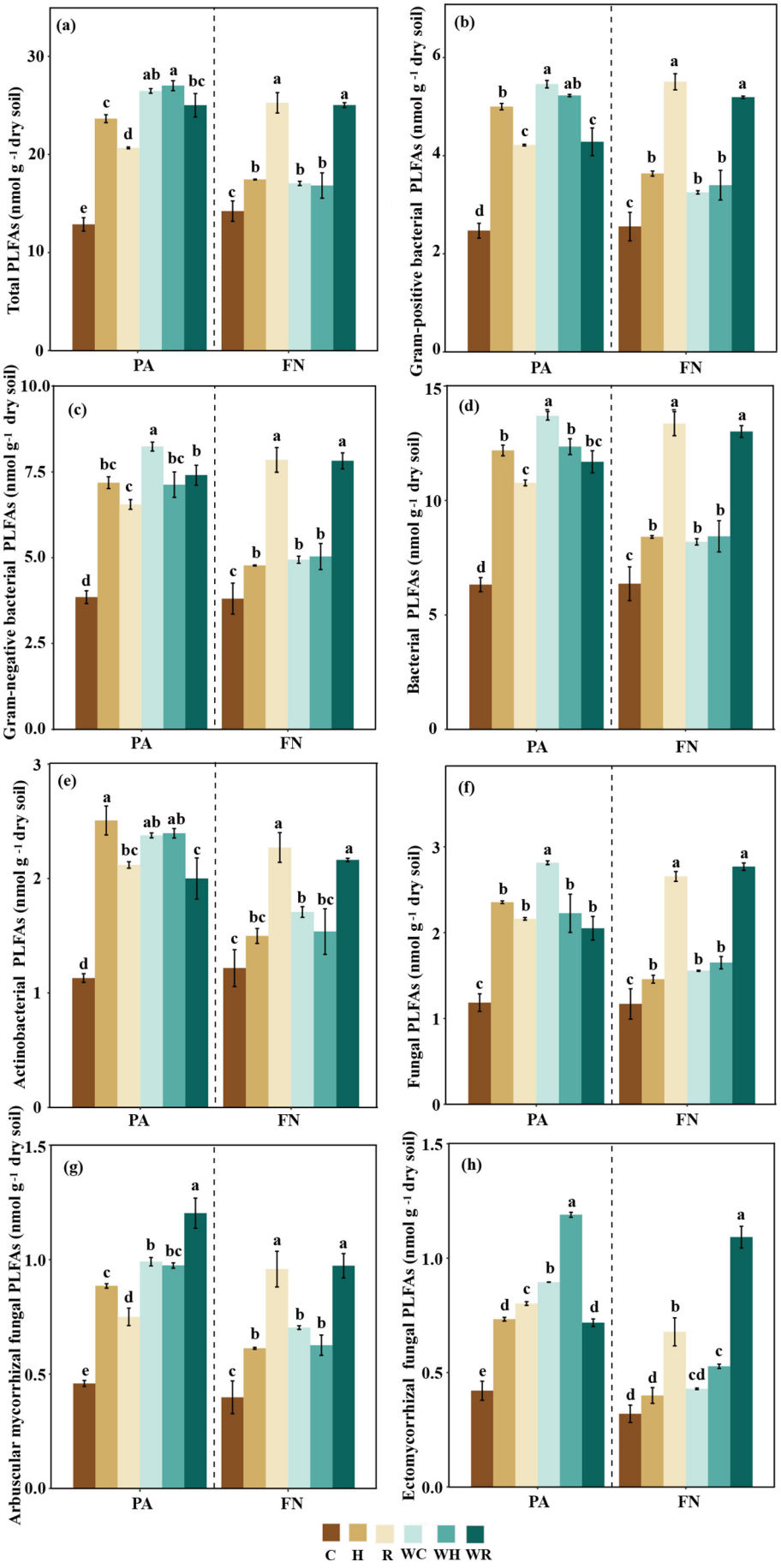
Soil TC, nitrate, and avaP concentrations were significantly affected by warming, plant species, mycorrhiza/hyphae, and their interactions (Table 1). Under non-warming conditions, ammonium concentrations (2.51 and 2.69 $mg{\cdot}kg^{-1}$) and avaP (19.13 and 21.58 $mg{\cdot}kg^{-1}$) in H and R cores were lower than those in C cores (3.19 and 36.48 $mg{\cdot}kg^{-1}$) in *P. asperate* (Table 2). For *F. nitida*, H and R cores had significantly higher TC (22.46 and 24.56 $g{\cdot}kg^{-1}$) and lower avaP (28.62 and 27.44 $mg{\cdot}kg^{-1}$) concentrations compared with C cores (21.12 and 43.60 $mg{\cdot}kg^{-1}$). Under warming conditions, compared with C cores, H and R cores had significantly lower avaP, and higher DOC but lower nitrate concentrations for *F. nitida* and *P. asperate*, respectively.

Experimental warming significantly increased the concentrations of nitrate in all cores ($p < 0.05$) but decreased ammonium (18.18%) and avaP (10.28%) concentrations in C cores. For *P. asperate*, avaP was increased in the presence of hyphae, while with mycorrhiza, TN was reduced (Table 2). For *F. nitida*, warming significantly increased the concentrations of nitrate and ammonium but decreased the concentrations of soil DOC and avaP in C cores ($p < 0.05$). In H cores, it increased nitrate but decreased TC and avaP concentrations ($p < 0.05$). In R cores, it significantly enhanced DOC, nitrate, and avaP, but it reduced TC concentrations ($p < 0.05$).

### 3.4. Correlation

RDA results showed that the first two axes explained 45.35% and 0.34% of the variation for the relationship between soil microbial community composition and soil environmental factors (Fig. 5a). Forward selection indicated that soil avaP was the key factor related to variations in microbial community biomass (Table 3). HP showed that avaP contributed to 26.43% of the variation in microbial community composition (Fig. 6a). Spearman correlation results also showed that all microbes except F and ECMF were negatively corrected with avaP (Table S1).

**Fig. 2.** Amount of (a) total phospholipid fatty acids (PLFAs), (b) Gram-positive bacterial PLFAs, (c) Gram-negative bacterial PLFAs, (d) bacterial PLFAs, (e) actinobacterial PLFAs, (f) fungal PLFAs, (g) arbuscular mycorrhizal fungal PLFAs, and (h) ectomycorrhizal fungal PLFAs across three ingrowth cores in *P. asperate* and *F. nitida* plots under non-warming/warming conditions. Data presented are means ± SE ($n = 4$). Means with different letters are significantly different based on the least significant difference method (LSD) among different treatments for each species ($p < 0.05$). PA, *P. asperate* plots; FN, *F. nitida* plots; C/WC, control/warming soil cores; H/WH, control/warming hyphae cores; R/WR, control/warming mycorrhizal cores.


(a)
Total PLFAs (nmol g -1 dry soil)
(b)
Gram-positive bacterial PLFAs (nmol g -1 dry soil)
(c)
Gram-negative bacterial PLFAs (nmol g -1 dry soil)
(d)
Bacterial PLFAs (nmol g -1 dry soil)
(e)
Actinobacterial PLFAs (nmol g -1 dry soil)
(f)
Fungal PLFAs (nmol g -1 dry soil)
(g)
Arbuscular mycorrhizal fungal PLFAs (nmol g -1 dry soil)
(h)
Ectomycorrhizal fungal PLFAs (nmol g -1 dry soil)
PA
FN
C H R WC WH WR

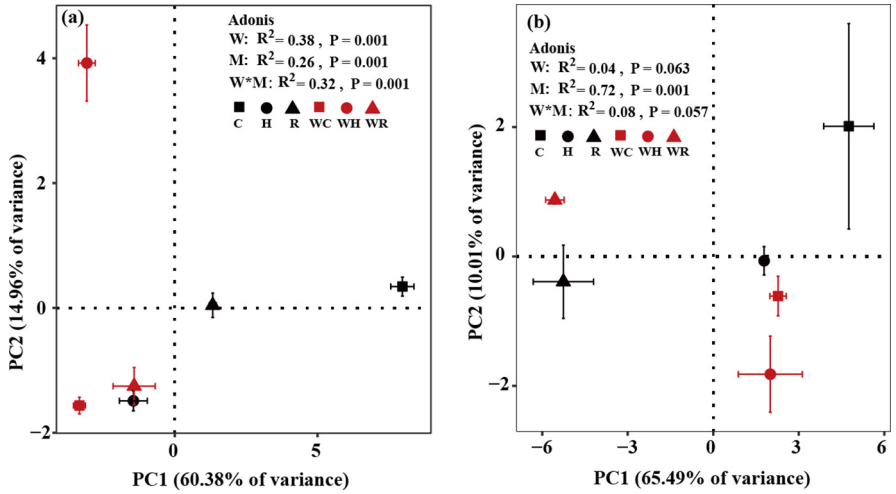



**Fig. 3.** Principal component analysis (PCA) ordination diagram of microbial phospholipids fatty acids content in *P. asperate* (a) and *F. nitida* (b) plots. Data presented are means ± SE ($n$ = 4). W, effects of warming; M, effects of mycorrhiza/hyphae; W ∗ M, the interactions between warming and mycorrhiza/hyphae. See Fig. 2 for abbreviations and explanations.

The first and second axes denote 42.74% and 10.49% of the variations in the three enzyme activities, respectively (Fig. 5b). Soil avaP, DOC, and nitrate concentrations were obviously correlated with soil enzyme activities (Table 3). They explained 46.32%, 20.68%, and 11.98% of the variance in soil enzyme activities, respectively (Fig. 6b). In addition, soil avaP, DOC, and TC were significantly positively correlated with AP, NAG, and Ure, respectively. However, soil nitrate was significantly negative correlated with Ure (Table S1). The biomass of F and ECMF was positively correlated with AP, and the total, Gn, AMF, and ECMF biomass was positively correlated with NAG, while the total, ACT, and ECMF biomass was negatively correlated with Ure (Table S2).

## 4. Discussion

### *4.1. Mycorrhiza and hyphae weaken the response of microbial biomass and composition to warming*

Experimental warming significantly increased soil microbial biomass and changed its composition in C cores in both *P. asperate* and *F. nitida* plots. This is consistent with previous studies showing increased soil microbial biomass due to warming (Zhang et al., 2014; Zhao et al., 2016; Li et al., 2018) but differs from studies that showed experimental warming had no or negative effects on soil microbial community (Zhang et al., 2013; Hu et al., 2020). The variations among these studies might be due to the differences in treatments (warming with/without precipitation gradients) and in the ecosystems (semi-arid grassland, alpine steppe, semi-arid steppe, forest). The response of microbiomes to warming is possibly determined by the interactions between climatic conditions (location), vegetation types, and soil (biotic and abiotic) traits, as in the case of microbial biogeography (Malard et al., 2019; Zeng et al., 2019; Trivedi et al., 2020). Previous studies have shown that soil microbes in cold conditions favor growth temperatures above the field temperature, which explains the rapid increase of soil microbial biomass under elevated temperatures (Margesin et al., 2009; Rousk and Bååth, 2011; Yuan et al., 2014). Our sampling time was at the end of November when the average temperature of topsoil was approximately 1.7 °C, which might have inhibited the growth and activity of soil microbes. Hence, warming might have a direct positive impact on soil microbial biomass in cold season or alpine regions (Zhang et al., 2014; Jansson and Hofmockel, 2020).

Additionally, phosphorus is a vital nutrient for microbes, and the concentration of avaP is crucial for soil microbial growth (Tian et al., 2020; Luo et al., 2021). Soil microbes require elements such as phosphorus for energy and growth (Bardgett, 2005), and some of them could increase the immobilization of nutrients, such as transforming avaP to microbial biomass phosphorus (Zhang et al., 2018b). Our results indicated that soil avaP was the key factor affecting microbial community composition variation. It negatively correlated with microbial biomass except F and ECMF (Table 3; Table S1), probably due to increased microbial biomass in soil in the absence of mycorrhiza or hyphae after warming, and microbes uptake more avaP for their growth demand temporarily although the soil of research area was not reported to be phosphorus limited (Bünemann et al., 2012; Jin et al., 2014; Jiang et al., 2021). Consistent with our study, reports have shown that as the biomass of 16:0 10-methyl, 17:0 10-methyl, and 18:0 10-methyl increased, avaP declined (Bünemann et al., 2004; Allison et al., 2007).

Experimental warming was observed to have reducing effect on microbial community in the presence of mycorrhiza and hyphae (Fig. 2; Fig. 3), specifically in *F. nitida* plots. This result indicated that the mycorrhizal symbiosis could weaken the response of the associated soil microbiome to warming, and this effect varies according to the plant species or mycorrhiza type. For example, in the presence of hyphae and mycorrhiza, warming only increased the total and ECMF biomass and total Gn and AMF biomass, respectively, in *P. asperate* plots. These results are consistent with a previous study that showed experimental warming did not induce significant changes in soil microbes in the presence of roots in *P. asperate* plots (Zhao et al., 2014). Previous studies showed that the growth and activity of soil microbial community is limited by the availability of substrate, especially carbon (Sinsabaugh et al., 2013). Root-derived low-molecular-weight carbon compounds such as sugar and amino acids are easily assimilated by microorganisms; therefore, mycorrhizal roots play an essential role in regulating microbial community dynamics (Bais et al., 2006). Mycorrhizal plants also transfer their photosynthetic products into soil via hyphae. AM and ECM fungal symbiotic plants have been estimated to invest 10–20% and 20–50% of the photosynthetically fixed carbon in their fungal partner, respectively (Johnson et al., 2002; Hobbie and Hobbie, 2008). The hyphal residues and carbon-containing compounds secreted by the hyphae are vital substrates for soil microbial community and regulate microbe composition and activities (Pollierer et al., 2007; Johansson et al., 2009; Cairney, 2012). Host plants probably regulate the growth and physiology of mycorrhiza and hypha by altering the resource supply to soil microorganisms under warming conditions, and consequently weaken the response of the microbial community to warming. Studies have also found that carbon allocation

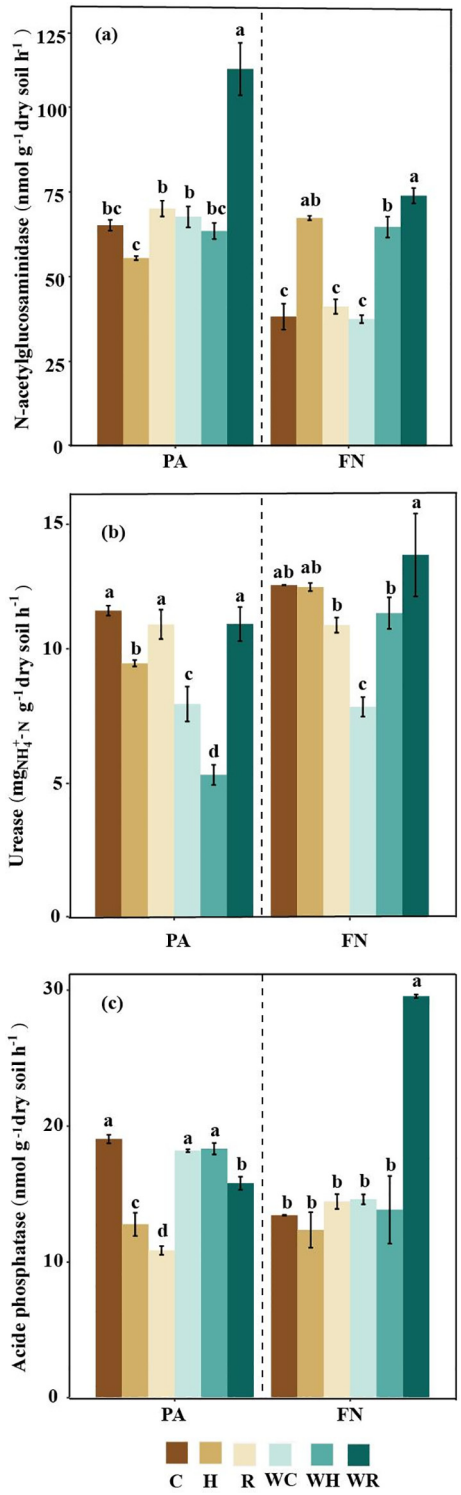



**Fig. 4.** Soil enzyme activities of (a) *N*-acetylglucosaminidase, (b) urease, and (c) acid phosphatase, across three ingrowth cores in *P. asperate* and *F. nitida* plots under non-warming and warming conditions. Data presented are means ± SE (n = 4). Means with different letters are significantly different based on the least significant difference method (LSD) among different treatments for each species ($p < 0.05$). See Fig. 2 for abbreviations and explanations.

patterns and mycorrhiza exudation quantity and quality of plants are affected by warming and indirectly regulate the response of soil microbes to warming (Bragazza et al., 2015; Ward et al., 2015).

### 4.2. Microbial biomass and composition were more sensitive to warming in *P. asperate* plots compared with *F. nitida*

Warming significantly affected the total, Gn, AMF, and ECMF biomass and microbial community composition in the presence of mycorrhiza or hyphae in *P. asperate* plots, but only significantly influenced the ECMF biomass in *F. nitida* (Figs. 2; 3). This difference reflects the effects of plant and/or the corresponding mycorrhizal type on mycorrhizal microbiome. Previous studies showed that the effect on rhizosphere microbiome was related to the presence of symbiotic mycorrhiza fungal hyphae (Kourtev et al., 2002; Yin et al., 2021). The mycorrhiza and hyphae properties of *P. asperate* and *F. nitida* are different, resulting in differences between ECM and AM plants (Phillips and Fahey, 2006; Wen et al., 2022). Specifically, the roots and hyphae of AM plants have higher N content and turnover rates than those of ECM, which promotes microbial community growth (Anderson and Cairney, 2007; Lin et al., 2017). Kleber et al. (2015) reported that AM soil with higher-quality organic inputs enhanced the production of microbial compounds such as microbial biomass carbon/nitrogen/phosphorus, which are essential for microbial growth. In this case, the effects of environmental changes on soil microbial community may be neutralized by AM roots and hyphae. Therefore, plants are more important regulators of rhizospheric microorganisms than slight environment changes (Selmants et al., 2016; Zhang et al., 2018a).

Additionally, the quantity and quality of available resources might influence effects of plant on soil microbes (Zhang et al., 2017). Studies indicated that ECM associations possess stronger carbon sink and exhibit greater quantity and more abundant root exudates (sugars, amino acid, etc.) compared to AM species (Phillips et al., 2013; Wen et al., 2022). Thus, the differences in nutrient condition of rhizosphere and hyphosphere between *F. nitida* and *P. asperate* might contribute to difference in sensitivity of soil microbial community biomass and composition to warming.

### 4.3. Mycorrhiza and hyphae significantly influenced the response of soil microbial community function to warming

Warming potentially affects soil nutrient cycles by altering not only soil microbial community biomass and composition but also its function (e.g., enzyme activities) (Baldrian et al., 2010; Souza et al., 2017; Li et al., 2018). In this study, warming significantly decreased soil Ure activities but had no effect on NAG and AP activities in both plots in the absence of the mycorrhiza or hyphae (Fig. 4). Consistent with our study, Jing et al. (2014) also reported that a rise in temperature of 1.9 °C does not have any significant impact on the activities of extracellular enzymes. However, studies have shown that experimental warming increased of soil enzyme activities (Xu et al., 2010; Gong et al., 2015; Zi et al., 2018). These diverse responses may be caused by different warming magnitudes, methods, and ecosystem types (Meng et al., 2020).

In addition, mycorrhiza and hyphae significantly influenced the response of soil enzyme activities to warming in both plots (Fig. 4), consistent with the response of microbial biomass and composition to warming (Figs. 2; 3) as most extracellular enzymes are expressed and secreted into the soil via microbes (Nannipieri et al., 2002; Raiesi and Salek-Gilani, 2018). Moreover, soil enzyme activities are highly related to soil physicochemical properties (Zhang et al., 2015). In this study, activities of enzymes related to nitrogen and phosphorus transformation in the soil were mainly affected by soil avaP, DOC, and nitrate concentrations (Fig. 5). TC and DOC were significantly positively correlated with Ure and NAG activities (Table S1). Consistent with our study, another study also indicated that soil factors related to nutrient availability might explain most of the variations in enzyme activities, and positive correlation exists between N-cycling enzyme activities and C availability (Bowles et al., 2014). This phenomenon might be related to the effects of C/N ratio on microbial activities. Bowles et al. (2014) believed that increased diverse C sources could result in N limitation for microbe growth, which might stimulate the nitrogen-fixing microbes and enhance the production of N-mineralizing enzymes. Additionally, Ure activity was found to be negatively correlated with soil

**Table 2**
Effects of experimental warming and mycorrhiza/hyphae on soil environmental factors in *P. asperate* and *F. nitida* plots.

| Plant | Treatment | TC (g·kg$^{-1}$) | DOC (g·kg$^{-1}$) | TN (g·kg$^{-1}$) | TP (mg·kg$^{-1}$) | $NO_3^-$-N (mg·kg$^{-1}$) | $NH_4^+$-N (mg·kg$^{-1}$) | avaP (mg·kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| *Picea asperata* | C | 21.77 ± 058ab | 15.69 ± 0.10c | 2.62 ± 0.17b | 676.55 ± 28.11a | 7.77 ± 0.14b | 3.19 ± 0.07a | 36.48 ± 1.59a |
| | H | 21.49 ± 0.84ab | 16.03 ± 0.56bc | 2.60 ± 0.07b | 646.53 ± 41.53a | 7.78 ± 0.77b | 2.51 ± 0.22b | 19.13 ± 1.83d |
| | R | 22.95 ± 0.52a | 18.34 ± 0.19ab | 3.01 ± 0.09a | 595.43 ± 22.45a | 7.31 ± 119b | 2.69 ± 0.02b | 21.58 ± 0.88 cd |
| | WC | 20.38 ± 0.18b | 16.19 ± 0.77bc | 2.67 ± 0.04b | 629.14 ± 42.39a | 15.49 ± 0.92a | 2.61 ± 0.17b | 32.73 ± 1.27b |
| | WH | 20.41 ± 0.16b | 14.16 ± 1.38c | 2.71 ± 0.08b | 646.82 ± 16.44a | 13.62 ± 1.16a | 2.53 ± 0.15b | 25.16 ± 0.08c |
| | WR | 22.21 ± 0.35a | 20.44 ± 0.89a | 2.60 ± 0.04b | 638.76 ± 7.90a | 14.32 ± 0.62a | 2.60 ± 0.10b | 24.42 ± 0.62c |
| *Fargesia nitida* | C | 21.12 ± 0.01c | 18.09 ± 0.85ab | 2.61 ± 0.28a | 650.06 ± 31.06a | 10.42 ± 0.62 cd | 2.63 ± 0.21b | 43.6 ± 1.20a |
| | H | 22.46 ± 0.41b | 16.23 ± 0.57bc | 2.52 ± 0.24a | 613.86 ± 27.02a | 8.15 ± 0.52d | 2.77 ± 0.31b | 28.62 ± 0.04c |
| | R | 24.56 ± 0.38a | 15.26 ± 0.06 cd | 2.46 ± 0.40a | 613.45 ± 24.68a | 9.33 ± 1.38d | 2.78 ± 0.16b | 27.44 ± 2.99 cd |
| | WC | 20.51 ± 0.06c | 13.17 ± 0.09d | 2.39 ± 0.07a | 613.71 ± 18.12a | 20.68 ± 1.47a | 3.60 ± 0.30a | 34.48 ± 3.50bc |
| | WH | 21.01 ± 0.01c | 16.89 ± 1.49bc | 2.66 ± 0.03a | 603.85 ± 10.15a | 12.66 ± 0.92bc | 2.84 ± 0.14b | 20.56 ± 1.84d |
| | WR | 23.18 ± 0.16b | 19.26 ± 0.33a | 2.65 ± 0.09a | 601.54 ± 30.39a | 14.71 ± 0.26b | 3.19 ± 0.29ab | 38.14 ± 2.14ab |

Data presented are means ± SE ($n = 4$). Means with different letters are significantly different based on the least significant difference method (LSD) among different treatments for each species ($p < 0.05$). C/WC, control/warming soil cores; H/WH, control/warming hyphae cores; R/WR, control/warming mycorrhiza cores. Lowercase letters represent significant differences among three ingrowth cores under control and warming conditions at $p < 0.05$. Refer to Table 1 for abbreviations and explanations of environmental factors.

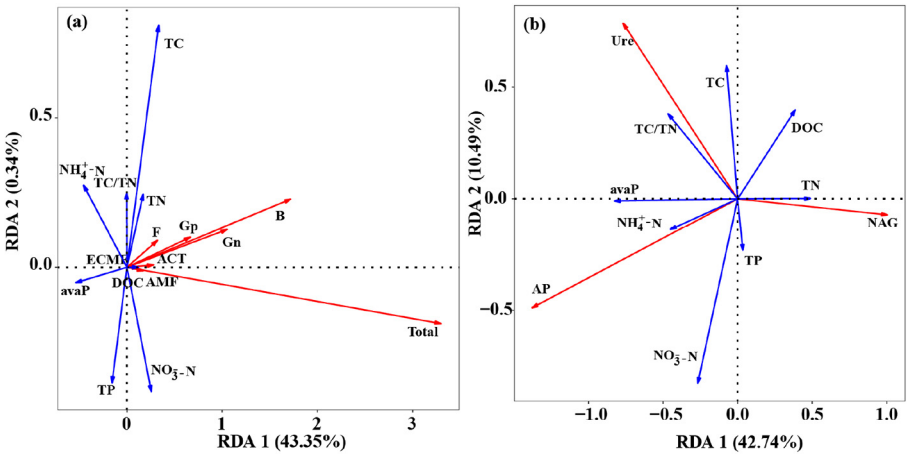



**Fig. 5.** Redundancy analysis (RDA) on soil microbial PLFAs (a) and enzyme activities (b). See Table 1 for abbreviations and explanations.

**Table 3**
Key factors selected for the variation of microbial community PLFAs and of enzyme activities using forward selection.

| Variation | Factor | $R^2$ | F value | *p* value |
|---|---|---|---|---|
| PLFAs | avaP | 0.126 | 4.893 | 0.032 |
| | avaP | 0.293 | 14.067 | 0.001 |
| Enzyme activities | DOC | 0.084 | 4.521 | 0.015 |
| | $NO_3^-$ | 0.066 | 3.790 | 0.026 |

Refer to Table 1 for abbreviations and explanations of environmental factors.

nitrate content (Table S1), possibly due to the inhibition of N mineralization by higher N availability (Osburn et al., 2018). We also found that AP activity positively correlated with soil avaP content, consistent with study by Olander and Vitousek (2000), who reported that in plots with high phosphorus content, phosphatase activity was also the highest. However, soil phosphatase activity increased with decreased in soil phosphorus (Zalamea et al., 2016; Guilbeault-Mayers et al., 2020), and no clear correlation existed between AP activity and soil phosphorus content (Kitayama, 2013; Batterman et al., 2018). These contradictory results may be due to

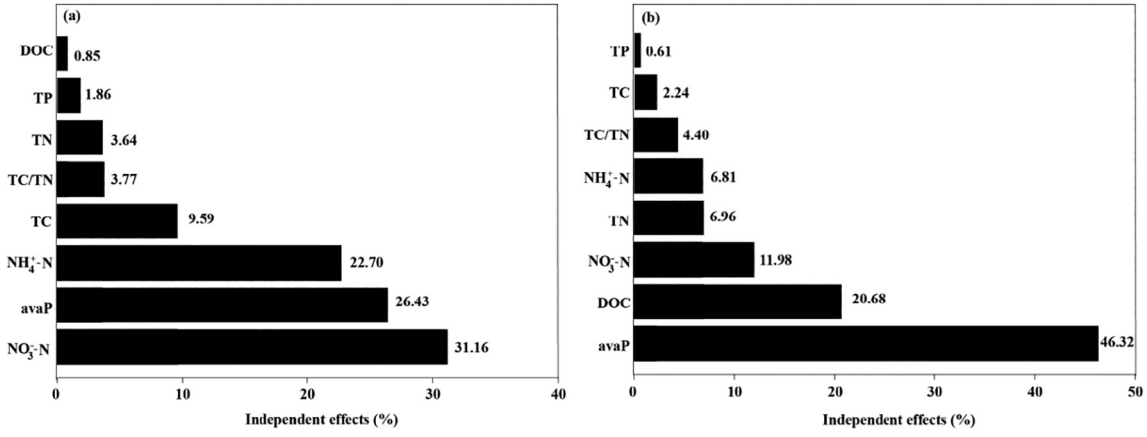



**Fig. 6.** Hierarchical partitioning (HP) showed the independent effects of soil properties on soil microbial community PLFAs (a) and enzyme activities (b). See Table 1 for abbreviations and explanations.

the variations in the study sites or to the soil physiochemical features (Kivlin and Treseder, 2014; Jian et al., 2016).

## 5. Conclusion

We observed that the presence of mycorrhiza and its hyphae might increase the biomass of soil microbial groups under non-warming conditions, while experimental warming significantly enhanced soil microbe biomass and altered microbial community composition. These effects were weakened in the presence of mycorrhiza or hyphae. Microbial biomass and community composition were more sensitive to warming in *P. asperate* plots than were those in *F. nitida* plots, indicating that the differences in regulation of microbial community are closely related to plant mycorrhiza types. We also found mycorrhiza and hyphae significantly influenced the response of enzyme activities to warming. These findings evidenced that mycorrhizal symbiosis might enhance the resistance of host-related soil microbiome to warming.

## CRediT authorship contribution statement

**Lin Luo:** Investigation, Formal analysis, Writing. **Min Guo:** Investigation, Formal analysis. **Entao Wang:** Supervision, Writing - Review & Editing. **Chunying Yin:** Writing - Review & Editing, Funding acquisition. **Yanjie Wang:** Writing - Review & Editing. **Heliang He:** Formal analysis. **Chunzhang Zhao:** Supervision, Writing - Review & Editing, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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