Divergent effects of short-term warming on microbial resource limitation between topsoil and subsoil in a young subtropical Chinese fir forest

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Abstract

Purpose The effect of warming on microbial resource limitations, especially in resource-poor subsoils, remains unclear. This study investigated the effects of warming on microbial resource [carbon (C), nitrogen (N) and phosphorus (P)] limitation and explored their relationships with soil properties and microbial community structure in topsoil (0-10 cm) and subsoil (40-60 cm).

Methods In 2014, ten 2 m×2 m plots were established and assigned to the warming and control treatments at a Chinese fir (*Cunninghamia lanceolata*) plantation in southern China. Microbial resource limitation with warming treatment was accessed via vector analysis of soil extracellular enzymatic stoichiometry after two years.

Results Warming aggravated microbial C limitation in the topsoil but alleviated microbial C limitation in the subsoil, while it shifted the microbial nutrient limitation from P limitation to N limitation in the subsoil. Soil microbial C limitation was explained by soil properties (specifically, ammonium nitrogen) in the topsoil while by microbial community composition in the subsoil based on variance partition analysis. The soil microbial nutrient limitation was explained by soil properties in the topsoil and subsoil. The decrease in actinomycetes abundance in the warming treatment may have led to a decreased microbial C limitation in the subsoil.

Conclusion Our study highlighted the differences in warming effects between the topsoil and subsoil. We argue that the microbial resource demand of the subsoil should be further implemented in soil biogeochemistry to improve the prediction of the impact of climate warming on soil C dynamics.

1. Introduction

The effect of climate warming on soil organic matter decomposition has major implications for soil carbon sequestration and nutrient circulation (Fontaine et al., 2007; Liang et al., 2019; Meyer et al., 2018). Soil microorganisms, such as soil organic matter decomposers, synthesize various extracellular enzymes to depolymerize macromolecular soil organic matter into absorbable substrates to fulfill their demands (e.g., C, N, and P) for growth and metabolism (Sinsabaugh et al., 2008, Allison et al., 2011). Extracellular enzymes thus reflect microbial resource constraints (Cui et al., 2021; Sinsabaugh et al., 2008) and are widely used to assess resource limitations (Chen et al., 2018; Cui et al. 2019; Guan et al., 2020; Jing et al., 2020; Moorhead et al., 2016; Peng et al., 2016; Waring et al., 2014). Although warming has been shown to affect numerous ecological processes, its effects on microbial resource limitations remain unclear.

The soil extracellular enzyme stoichiometric theory combines ecological metabolism theory with the stoichiometric theory of ecology to evaluate the resource limitation of microbial metabolism through the ratio of various enzyme activities (Chen et al., 2018 a, b; Cui et al., 2021; Cui et al., 2018; Sinsabaugh et al., 2008, 2012; Yuan et al., 2019; Zhou et al., 2020). Thus, enzyme stoichiometry is regarded as an effective tool for assessing the environmental drivers and resource constraints of microbial metabolism; therefore, it has been widely used to explore biogeochemical cycles (Nottingham et al., 2015; DeForest

and Moorhead, 2020). The enzymes commonly used in enzymatic stoichiometry are mainly involved in the microbial acquisition of C (β-1,4-glucosidase, BG), N (β-1,4-N-acetylglucosaminidase, NAG), and P (acid phosphatase, AP, Sinsabaugh et al., 2008). Moorhead et al. (2013) proposed a vector model to identify the relative resource limitation of microorganisms by using the vector length and angle calculated from the enzymatic C:P versus C:N acquisition ratio. Using this approach, Moorhead et al. (2016) showed that soil microbial communities are more limited by P in montane tropical forests than in lowland tropical forests. Zheng et al. (2020) reported that warming reduced microbial C limitation in the mineral soil layer and increased P limitation in the organic layer in an alpine shrubland ecosystem. Lie et al. (2019) found that warming mitigated P limitation and increased N consumption in tropical forests by increasing the soil P availability. However, these studies have mainly focused on the 0–20 cm soil depth topsoil, and the knowledge of the effects of warming on microbial resource limitation below the 20 cm soil depth subsoil remains limited.

Subsoil accounts for over 60% of the soil C stock in the top 1 m of soil (Jobbágy and Jackson, 2000). The subsoil and topsoil differ in many crucial physical and chemical properties. Subsoil tends to have a lower temperature (Zogg et al., 1997) and oxygen concentration (DeAngelis et al., 2010) and higher soil moisture (Serna-Chavezet al., 2013) and pH (Fierer and Jackson, 2006) than topsoil. These physical and chemical properties play relevant roles in the regulation of extracellular enzyme activity by modifying the diffusion and adsorption of available substrates (Deng et al., 2019; Feng et al., 2019). Thus, the resource limitation for soil microorganisms in subsoil can substantially differ from that in topsoil. For example, fresh C input is more limited in subsoil than topsoil such that the subsoil may have greater C limitation than topsoil. Several recent studies have shown that warming decreases topsoil soil moisture, deepening root growth; therefore, C input via root exudation increases and C limitation decreases in subsoil (Querejeta et al., 2021; Zheng et al., 2020). The relief of C limitation in subsoil has been suggested to increase soil organic matter (SOM) decay (Shahzad et al., 2018; Sullivan et al., 2020). However, the understanding of how warming affects C and nutrient limitations in subsoil relative to topsoil is incomplete because few studies have focused on subsoil. Considering the subsoil C storage, slight changes in subsoil carbon may have significant effects on the global carbon cycle. Therefore, clarifying the impact of warming on microbial resource limitations in the subsoil is necessary.

Microbial community composition, recognized as context-dependent (e.g., climate and soil properties; Fierer and Jackson, 2006), has been shown to be a major factor regulating enzyme production (McGuire and Treseder, 2010; Schnecker et al., 2014; Stone et al., 2014; Strickland et al., 2009, Li et al., 2019) and may have a reciprocal influence on soil microbial resource limitations. Gram-positive (GP) and gram-negative (GN) bacteria are oligotrophic and copiotrophic bacteria respectively (Fierer et al., 2007; Fanin et al., 2018). GP uses recalcitrant carbon (C) compounds, exhibiting a higher C limitation than GN. Comparatively, GN uses labile C compounds (Fanin et al., 2018; Naylor and Coleman-Derr, 2018), showing a lower C limitation than GP. In addition, warming has been shown to increase, decrease, or have no effect on the abundances of GP and GN (Feng and Simpson, 2009; Frey et al., 2008; Karhu et al., 2010; Rinnan et al., 2008; Rinnan et al., 2009; Vanhala et al., 2011). Moreover, the impact of warming on the soil microbial community composition is depth-dependent (Dove et al., 2021). Although many studies have

focused on the effects of warming on the microbial community structure, the relationship between microbial communities and microbial resource limitation has rarely been examined. Studying the relationship between microbial community structure and resource limitations would advance the understanding of the effects of warming on C, N, and P cycles.

To illustrate the outlined issues outlined above, we investigated microbial resource limitation and explored its relationships with microbial communities and soil properties in the topsoil and subsoil in a field manipulation experiment after 2 years of warming treatment in a subtropical Chinese fir plantation. Based on the commonly reported differences in abiotic factors and microbial communities between topsoil and subsoil, we hypothesized that warming would have different effects on microbial resource limitation between topsoil and subsoil (H_1). Because of the critical role of microbial enzymes in nutrient acquisition, we also hypothesized that the microbial community structure plays a primary role in regulating microbial resource restriction (H_2).

2. Materials And Methods

2.1. Study site and experimental design

The study site was located at the Chenda Research Station (300 m above sea level) of Fujian Normal University, Fujian Province, southeast China (26°19N, 117°36E). The site has a subtropical monsoon climate, with a mean annual air temperature of 19.1 ℃, annual precipitation of 1750 mm, and annual evaporation of 1585 mm. The soil at the study site was classified as red soil on the basis of China's soil classification systems, equivalent to Oxisols in the United States Department of Agriculture Soil Taxonomy.

Ten 2 m×2 m plots were established and randomly and evenly assigned to the warming and control treatments. Within each warmed plot, resistance-heating cables (Nexans-type TXLP, Oslo, Norway) were installed 10 cm into the soil at a 20 cm interval. Heating cables were also installed in the unwarmed (control) plots but were not heated, to account for the effect associated with the installation of the heating cables. The temperature difference between the control and warmed plots was maintained at 5°C at a soil depth of 10 cm. The details of the experimental setup are in Liu et al. (2017).

Each 2 m×2 m plot was divided into four 1 m×1 m subplots, and Chinese fir seedlings were planted in each subplot. The soil moisture of each plot was measured year-round at 10 cm and 60 cm depths with two ECH_2O-5 soil moisture probes (Decagon, Pullman, Washington, 138 USA). The soil temperature was measured using temperature sensors (T109; Campbell Scientific Inc., Logan, UT, USA) placed between the cables. Three temperature sensors were installed at 10 cm soil depth and three at 60 cm soil depth in each warmed plot, and two were installed at each of the two soil depths in the control plot. The soil temperature and moisture were recorded every 30 min using a computer-based control system.

2.2. Soil sampling and biogeochemical analyses

Soil samples of topsoil and subsoil were collected with a 3.5 cm soil corer on April 20, 2016. Upon sampling, six soil cores in each plot were randomly collected between heating cable lines to ensure that all samples received similar heat input. Soil samples from each depth in the same plot were mixed to form a composite sample. The soil samples were stored in a cooling box and immediately transported to the laboratory at the site. The soil was cleared of visible living plant materials and stones. Before further analysis, soil water content was gravimetrically determined (105°C for 24 h). A subsample of approximately 5 g from each soil sample was frozen at -20°C for enzyme activity assessment. The remaining sample was sieved through a 2 mm mesh and divided into two subsamples, one freeze-dried for phospholipid fatty acid (PLFA) analysis and the other air-dried for chemical analyses. All data are expressed on a dry soil weight basis.

Soil pH was determined using a pH meter with a soil:water ratio of 1:2.5. Before measurements of total C and N, a subsample of air-dried soil was ground (< 500 mm) using a mortar and pestle. Soil organic C (SOC) and total N (STN) were determined using a CN Autoanalyzer (Elementar Vario MAX, Germany). For soil total P (STP) analysis, 0.25 g air-dried soil was digested with 2 mL $HClO_4$ and 3 mL H_2SO_4 for 180 min at 120–130°C and then diluted with deionized water to 100 mL. After overnight stratification of the digestion liquid, 5 mL supernatant liquid was added to 5 mL of molybdenum antimony reagent, and water was added to 50 mL. The total P content of the solution was measured using an ultraviolet spectrophotometer (Hitachi UV2300) at 700 nm. For soil active P (SAP) analysis, 3 g of dry soil was added to 30 mL of M3 extraction solution (soil: solution ratio 1:10), shaken for 5 min (120 oscillations min^{-1}), and centrifuged for 10 min. The supernatant was filtered through a 2.5 μm Whatman no. 42 filter paper. The SAP concentration in the filtrate was determined using a Skalar SAN plus Segmented Flow Analyzer. Soil NH_4^+-N and nitrate nitrogen (NO_3^--N) were determined in the KCl extract (Zhou et al., 2012). Briefly, each fresh soil sample (5 g) was added to a 50 mL tube with 20 mL 2 M KCl, shaken for 0.5 h, and then centrifuged for 10 min. The supernatant was filtered through a 2.5 μm Whatman no. 42 filter paper. The concentrations of inorganic N in the supernatants were measured using a continuous flow analyzer (Skalar san++, Netherlands). Dissolved organic carbon (DOC) and DON were measured using K_2SO_4 extract. Briefly, 5 g of a soil sample was extracted with 0.5 M K_2SO_4 solution (1:4, w/v, soil/extractant ratio), shaken for 0.5 h and centrifuged for 10 min, and then filtered using a 0.45 mm nitrocellulose filter. The organic C concentration in the supernatant was measured using a TOC analyzer (TOC-VCPH/CPN, Shimadzu, Japan) as the DOC concentration. Mineral nitrogen (NH_4^+-N, NO_3^--N) and total N concentration in the supernatant were measured using a continuous flow analyzer (Skalar san++, Netherlands). The organic N concentration in the supernatant was derived from the difference between the total N concentration and mineral N concentration as the DON concentration.

2.3. Potential enzyme activities analysis

The activities of four enzymes, BG (EC 3.2.1.21), NAG (EC 3.1.6.1), AP (EC 3.1.3.2), and peroxidase (PER, EC 1.11.1.7), were measured following using the methods of Saiya-Cork et al. (2011), a modified method of Saiya-Cork et al. (2002). BG, NAG, and AP are related to microbial C, N, and P acquisition, respectively (Sinsabaugh et al. 2009), and PER is related to the decomposition of recalcitrant organic C (Sinsabaugh,

2010). Briefly, 1 g fresh soil was homogenized in 125 mL sodium acetate buffer (pH 5.5) by using a magnetic blender. Fifty μL of fluorescent substrate proxies specific to each enzyme (BG: 4-MUB-β-D-glucoside, NAG: 4-MUB-N-acetyl-β-D-glucosaminide, AP: 4-MUB-phosphate, PER: L-DOPA) were added to eight replicate assay wells at optimal concentrations to measure the total potential activity (optimal concentrations were determined before the experiment). Assays were performed using two standard columns containing soil homogenates and methylumbelliferone (MUB). Each assay microplate also contained blank substrate columns containing 50 μL substrate and 200 μL sodium acetate buffer. Soil homogenate blanks were measured simultaneously. Assays for BG, NAG, and AP were incubated at 20°C for 4 h (optimal duration of assay determined before the experiment), and activity was measured fluorimetrically (excitation 365 nm and emission 450 nm). Plates for PER activity measurements were incubated for 18 h at 20°C; next, 10 μL 1 M NaOH was added to each well to terminate the enzyme activity. Within 1 min of NaOH addition (DeForest, 2009), fluorescence was measured (absorbance 450 nm).

2.4. Phospholipid fatty acid analysis

Extraction and measurement of PLFAs followed the procedure in Tunlid et al. (1989), with modifications to maximize the extraction of fatty acids from the soil. PLFAs were extracted from 9 g of soil by using a chloroform–methanol–phosphate buffer (1:2:0.8) and purified on silica acid columns (LC-Si SPE, Supleco, Bellefonte, PA, USA) using chloroform, acetone, and methanol. After the addition of the internal standard (methylnonadecanoate), PLFAs were converted to fatty acid methyl esters (FAMEs) by mild alkaline methanolysis. Samples were analyzed using a gas chromatograph (Hewlett Packard 5890 GC) equipped with a 6890 series injector, a flame ionization detector, and an Ultra 2 capillary column (25 m×0.2 mm, 0.33 μm film thickness). Individual FAMEs were identified based on their retention times in combination with the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE). The markers 18:1ω9 and 18:2ω6,9 were used as markers for fungi; i14:0 i15:0, a15:0, i16:0, i17:0, and a17:0 were used to indicate GP bacteria (Denef et al., 2009; Landesman and Dighton, 2010); 16:1ω7, 16:1ω9, cy17:0ω7, cy19:0ω7, 18:1ω7, 18:1ω5c, and 15:0 were used to indicate GN bacteria (Frostegård et al., 2011; Ushio et al., 2008); actinomycetes (ACT) was measured as the sum of 10Me16:0, 10 Me17:0 and 10 Me18:0.

2.5. Vector analysis for measurement of resource limitation

To measure the extent of soil microbial C and nutrient limitation, we followed the vector analysis of soil enzymatic stoichiometry proposed by Moorhead et al. (2013). Vector lengths and angles were measured by plotting the proportional enzyme C:P to C:N ratios, which were calculated to illustrate the potential and relative resource use limitations for soil microorganisms. Vector length represents the relative C and nutrient investment (Deng et al., 2019), and the vector angle represents the relative P and N investment (Fanin et al., 2016; Moorhead et al., 2016). We calculated the vector length and vector degree as follows:

$$\text{Vector Length} = \sqrt{(\mathrm{BG}/\mathrm{AP})^2 + (\mathrm{BG}/\mathrm{NAG})^2}$$

$$\text{Vector Angle} = \operatorname{atan2}(\mathrm{BG}/\mathrm{AP},\ \mathrm{BG}/\mathrm{NAG}) \times 180/\pi$$

where atan2 is a trigonometric function that provides the angles in radians between the x-axis and the vector from the plot origin to point (x, y), and 180/π is used to convert the angles in radians into degrees.

2.6. Statistical analysis

All results are reported as the mean ± standard error (SE) for each replicate. We used two-way analysis of variance followed by Tukey HSD tests to examine differences in measured items between the topsoil and subsoil and between warmed and control treatments. Differences between the groups were considered statistically significant at $P < 0.05$. Before analysis, the data were log transformed or rank normalized to fulfill the normal distribution assumption. Statistical analyses were performed using the vegan package (Oksanen et al., 2019).

To examine the relative importance of soil physiochemical properties and microbial communities on regulating soil microbial C, using vector length as the indicator, and nutrient limitations, using vector angle as the indicator, we performed multiple regression analysis followed by variation partitioning analysis between the two explanatory variables (Borcard et al., 1992). Before the multiple regression analysis, we conducted principal component analyses for a) physicochemical properties and b) microbial community composition separately for the topsoil and subsoils. The first two principal components (PCs) of the soil variables (explained 65% of the variation in the soil physicochemical properties in the topsoil [Fig. S1] and 64% in the subsoil [Fig. S2]), and the first two PCs of the microbial communities explained 95% of the variation in microbial community composition in the topsoil (Fig. S3) and 94% in the subsoil (Fig. S4). The first two PCs were used for regression analysis. The R^2 of each multiple linear regression model was then used for variation partitioning analysis to estimate the relative importance of the two types of variables in regulating soil microbial C and nutrient limitations.

We also conducted multiple regression analysis to examine the relationships among soil microbial C, nutrient limitation, and soil physiochemical properties (all measured soil variables except SOC, STN, and STP, which were conjugated to other soil variables) and the five soil microbial communities. To evaluate potential collinearity from relationships among model covariates (Schmidt-Nielsen, 1984), we calculated variance–inflation factors (VIFs) for each covariate in each model and excluded the covariates with a VIF higher than 10 (Dormann et al., 2013; Schmidt-Nielsen, 1984). A VIF was calculated by using the function “vif” from the package “car” in R. Next, a model selection process was used to select the best explanatory variable based on corrected Akaike’s information criterion (AICc; $\Delta AICc < 4$; Burnham and Anderson, 2002). The procedures were conducted for the topsoil and subsoil separately (Table S1-S4), using the function “dredge” in the R package “MuMIn” (Bartoń, 2020). Model averaging was performed based on the AICc weights when multiple models were selected. All predictors and response variables were standardized before analysis by using Z scores to interpret parameter estimates on a comparable scale. Predictors were log transformed when necessary before the analysis to fulfill the assumptions of the tests used. All statistical analyses were conducted using R 3.4.1 (R Development Core Team, 2017).

3. Results

3.1. Soil physiochemical properties

Warming increased topsoil DOC content; decreased topsoil NH_4^+-N content; and decreased subsoil DOC and DON content; but had no significant effects on SOC, STN, STP, and pH in either soil layer (Table 1).

Table 1
Soil physicochemical property, microbial biomass and ecoenzyme activities in topsoil and subsoil in control (CT) and warming (W) treatment.

	Topsoil		**Subsoil**	
	CT	W	CT	W
SOC	12.79 ± 1.64	12.33 ± 1.59	2.96 ± 0.32	3.12 ± 0.06
STN	1.06 ± 0.12	1.02 ± 0.11	0.52 ± 0.04	0.51 ± 0.06
STP	0.28 ± 0.01	0.29 ± 0.01	0.34 ± 0.00	0.34 ± 0.01
DOC	156.64 ± 2.40	**188.13 ± 9.46*** **	50.53 ± 7.09	**41.06 ± 4.55***
DON	5.82 ± 0.89	6.57 ± 1.14	5.48 ± 0.67	**3.15 ± 0.69*** **
NH_4^+-N	5.05 ± 0.90	**3.65 ± 0.23****	2.55 ± 0.24	2.16 ± 0.54
NO_3^--N	1.52 ± 0.17	1.40 ± 0.31	0.57 ± 0.04	0.43 ± 0.29
SAP	1.22 ± 0.04	**1.47 ± 0.22****	1.17 ± 0.12	**1.39 ± 0.12***
pH	4.31 ± 0.15	4.27 ± 0.09	5.02 ± 0.01	**4.99 ± 0.02***
T	19.78 ± 0.51	**24.72 ± 0.34*** **	17.67 ± 0.28	**20.58 ± 0.44*** **
M	0.27 ± 0.02	**0.25 ± 0.02****	0.28 ± 0.00	0.27 ± 0.00
BG	39.27 ± 4.03	**49.64 ± 3.50****	53.78 ± 1.31	**38.93 ± 0.60*** **
NAG	53.31 ± 1.97	**59.97 ± 5.82***	30.67 ± 3.31	34.34 ± 2.57
AP	161.29 ± 11.59	79.75 ± 13.36	41.81 ± 4.10	**22.91 ± 1.36*** **
PER	24.32 ± 1.58	**28.74 ± 2.12****	38.41 ± 2.10	38.59 ± 2.28
BG/NAG	1.39 ± 0.51	1.49 ± 0.27	1.78 ± 0.28	**1.08 ± 0.02*** **
BG/AP	0.25 ± 0.04	**0.63 ± 0.08*** **	1.29 ± 0.13	**1.70 ± 0.12*** **
NAG/AP	0.19 ± 0.06	**0.44 ± 0.11****	0.74 ± 0.11	**1.59 ± 0.11*** **

Values (means ± SE, n = 5). M: soil moisture. Different asterisks indicate significant differences between CT and W at the same depth. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

3.2. Microbial C and nutrient limitation

Warming increased microbial C limitation in the topsoil (i.e., increased vector length), but decreased it in the subsoil (i.e., no significant effect on vector length, Fig. 1a). Warming alleviated microbial P limitation (i.e., decreased vector angle) in the topsoil and subsoil (Fig. 1b). The vector angle of enzyme activities was smaller than 45° in the warmed subsoil, indicating a greater N than P limitation for subsoil microbes (Fig. 1b). Across the control and warmed plots, vectors were longer in the subsoil than in the topsoil, while the vector angle was smaller in the subsoil than in the topsoil in the control and warming treatments (Fig. 1), indicating that microbial C limitation was higher in the subsoil than in the topsoil, and microbial P limitation was lower in the subsoil than in the topsoil.

Warming significantly increased topsoil BG (26%, $P = 0.002$) and NAG (12%, $P = 0.041$) activity while decreased topsoil AP activity (-50%, $P = 0.005$) (Table 1). However, warming did not affect subsoil NAG activity and decreased subsoil BG activity by 13% ($P = 0.037$) and AP activity by 45% ($P < 0.001$) (Table 1). Warming increased the ratios of BG:AP and NAG:AP in the topsoil and subsoil, decreased the ratio of BG:NAG in the subsoil, and had no effect on the BG:NAG ratio in the topsoil (Table 1).

3.3. Microbial community composition

Warming did not affect the microbial community composition in the topsoil but decreased the abundance of ACT in the subsoil. (Fig. 2).

3.4. Variation partitioning on vector analysis

Soil physicochemical properties and microbial community composition accounted for 87% of the variation in soil microbial C limitation (i.e., variance in vector length) and 87.0% of nutrient limitation (i.e., variance in vector angle) in the topsoil. Variance decomposition showed that topsoil microbial C and nutrient limitations were primarily explained by soil physicochemical properties. The soil physicochemical properties accounted for 54% of the variation in microbial C limitation (Fig. 3a) and 45.4% of the variation in microbial nutrient (P vs. N) limitation (Fig. 3b) in the topsoil. By contrast, microbial communities only accounted for ~ 1% of the variation in microbial C relative to the nutrient limitation and P relative to the N limitation in the topsoil.

Soil physicochemical properties and microbial community composition accounted for 72% of the soil microbial C limitation (Fig. 3c) and 85% of the nutrient (P vs. N) limitation (Fig. 3d) in the subsoil. By contrast, the majority of the variation in microbial C limitation (64%) was explained by microbial communities, and 8% was explained by soil physicochemical properties in the subsoil (Fig. 3c). Approximately 59% of the variation in microbial nutrient limitation was explained by soil physicochemical properties, and 12% by microbial communities in the subsoil (Fig. 3d).

In the topsoil, microbial C limitation was negatively related to NH_4^+-N content (Fig. 4a), while microbial nutrient limitation was positively related to NH_4^+-N content (Fig. 4b). The subsoil microbial C limitation was positively related to ACT (Fig. 4c), and microbial nutrient limitation was negatively related to SAP content but positively related to DON content (Fig. 4d).

4. Discussion

4.1. Effects of warming on microbial resource limitation

Recent studies have shown that microbial growth is primarily limited by C, whereas nutrients play a secondary role (Soong et al., 2020). The increase in microbial C limitation in topsoil and the decrease in subsoil after the warming treatment in our study (Fig. 1a) contrasted with decreased microbial C limitation after the warming treatment reported for an alpine timberline of the eastern Tibetan Plateau (Zheng et al., 2020). The response of microbial C limitation to warming is likely to be region- or ecosystem-dependent. In boreal forests, plant growth is largely limited by low temperatures, such that warming increases C input to the soil by enhancing plant growth, which alleviates microbial C limitation (Zheng et al., 2020). By contrast, although subtropical forests store smaller quantities of soil C than temperate forests and boreal forests (Jing et al., 2017), the temperature is generally not limiting in subtropical forests; thus, the facilitative effects of warming on mitigating C limitation by increasing C input to soil were not observed.

Several studies have shown that warming decreases soil labile C in subtropical ecosystems because labile C is preferentially decomposed (Li et al., 2018; Melillo et al., 2019). The increased PER activity (Table 1), which is notable for decomposing recalcitrant C, such as lignin and humus (Sinsabaugh et al., 2008), in topsoil under the warming treatment relative to the control supports that labile SOC was largely depleted by warming. Thus, warming is more likely to aggravate than mitigate microbial C limitation in the topsoil in subtropical ecosystems. Additionally, the decrease in microbial C limitation under warming in the subsoil probably resulted from the enhanced root growth in the subsoil associated with warming-induced decreased soil moisture, which increased C input to the subsoil (Giardina et al., 2014; Melillo et al., 2002).

The greater P than N limitation for microbial communities in the studied subtropical ecosystem (Fig. 1b) is consistent with the findings of many studies that point to the P limitation in soil microbial communities in tropical and subtropical ecosystems (Camenzind et al., 2018; Sinsabaugh et al., 2008; Xu et al., 2017). The observed warming alleviated microbial P limitation in the topsoil is in agreement with a translocation warming experiment in tropical China (Lie et al., 2019). The alleviation of microbial P limitation probably resulted from increased SOC decomposition under the warming treatments. This inference is supported by the increased BG activity under warming because BG is a crucial enzyme involved in the hydrolysis of disaccharides to glucose (Bell et al., 2010). Moreover, the increase in SAP content under warming indicated an increased degradation of organic phosphorus, supporting that warming increased SOM degradation.

Our results showed that warming shifted the microbial nutrient limitation from P to N in the subsoil. In general, soil N is more abundant than P in subtropical ecosystems (Gorham et al., 1979; Jing et al., 2020; Meyer et al., 2018). Due to limited plant input to the subsoil, dissolved organic matter is the main form of organic matter and is retained in mineral soil by adsorption (Rumpel and Kögel-Knabner, 2011). Because

of the relatively limited source of N input in the subsoil, decreased DON under warming may induce microbial N limitation. A 5-year warming experiment by Lie et al. (2019) also found that warming improved soil P availability and reduced the available N supply in tropical forests.

The greater soil microbial C limitation in the subsoil than in the topsoil in our study may have resulted from strong organo–mineral associations in the subsoil (Dungait et al., 2012; Fontaine et al., 2007; SalomÃ et al., 2010). Rumpel et al. (2008) has reported that SOM in subsoil is adsorbed on unsaturated mineral surfaces, which restricts microbe accessibility.

The smaller decrease in soil microbial P limitation in the subsoil than in the topsoil (based on changes in vector angle) can be explained by the similar total and available P between the topsoil and subsoil but lower total N and DON in the subsoil than in the topsoil (Table. 1). Imai et al. (2010) has suggested that trees compete with microbes for P in the topsoil in P-limited tropical forests. Enzymes are proteins with high N content; therefore, decreases in N availability limit the synthesis of enzymes. Thus, decreased subsoil extracellular enzyme activity under warming also supports warming-induced microbial N limitation in the subsoil.

4.2. Soil properties and microbial communities in relation to microbial C and nutrient limitation

Warming has major effects on the physical and chemical properties of soil, such as increasing soil temperature and decreasing soil moisture. We found that soil physicochemical properties accounted for ~ 90% of the variation in microbial C limitation in the topsoil (Fig. 3a), suggesting that changes in soil physicochemical properties could have major impacts on the microbial C limitation of topsoil. By contrast, the result that soil microbial community composition accounted for ~ 64% of the variation in subsoil microbial C limitation (Fig. 3c) implies that warming-induced changes in the microbial community (Biasi et al., 2005; Creamer et al., 2015; Schindlbacher et al., 2011) probably affect subsoil C limitation. Notably, soil physicochemical properties accounted for the majority of microbial nutrient limitation in the topsoil and subsoil, highlighting the potential effects of warming on microbial nutrient limitation by altering soil physicochemical properties. These results only partially rejected H_2, which predicts that community structure plays a primary role in regulating microbial resource restriction.

Based on the regression models, NH_4^+-N explained the majority of warming-induced increases in microbial C and nutrient limitation in the topsoil under warming (Fig. 4a). Microbial C limitation has been suggested to be affected by soil N availability due to stoichiometrically coupled C and N cycling (Moorhead et al., 2016; Sinsabaugh et al., 2008). Thus, the increase in microbial C limitation under warming may have resulted from the reduced NH_4^+-N under warming. Based on the response of microbial C limitation with resource availability, the co-occurrence of warming-enhanced microbial C limitation and the increases in DOC in our study were unexpected. One possible explanation is that DOC in soils is a product of decomposition (Schimel and Bennett, 2004), and its increase is the result of soil organic matter degradation.

In the subsoil, microbial C limitation was positively correlated with ACT abundance (Fig. 4c). Because warming decreased the abundance of ACT, it alleviated the microbial C limitation. Notably, the shift in microbial nutrient limitation from P to N may have resulted from increased SAP and decreased DON caused by warming (Fig. 4d).

5. Conclusions

Our study provides empirical evidence that the microbial C and nutrient limitation of topsoil and subsoil responded differently to warming in subtropical Chinese fir plantations. Warming aggravated the microbial C limitation in the topsoil and mitigated it in the subsoil, which accelerated soil organic matter decomposition in the subsoil. In addition, warming shifted the subsoil nutrient limitation from P- to N-limited soil. Furthermore, we found that microbial C limitation was more related to soil physicochemical properties than microbial community in the topsoil, for example, NH_4^+-N and DON, but closely related to microbial community composition in the subsoil. Soil properties explained most of the variation in microbial nutrient limitation in the topsoil and subsoil.

In summary, our study provides insights into soil C and nutrient limitations of microbial metabolism in subtropical forests and highlights that the main regulatory factors of microbial C and nutrient limitation differ between topsoil and subsoil. These findings advance the understanding of soil microbial resource limitations and the potential effects of warming on soil C sequestration and nutrient cycles in subtropical forest ecosystems.

Declarations

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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.

Author contributions Zhijie Yang, Weisheng Lin and Yusheng Yang designed and conceived of the study; Weisheng Lin, Shidong Chen, Xiaofei Liu, Decheng Xiong and Chao Xu maintained the study plots; Wei Zheng, Jiacong Zhou and Yong Zheng performed field sampling and laboratory analysis; Wei Zheng, Wei Lin, and Yuexin Fan analyzed the data and drew the figures; Wei Zheng wrote the first draft of the manuscript. Zhijie Yang, Yiqing Li, Yuexin Fan and Yusheng Yang commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability The datasets generated during the current study are available from the corresponding author on request.

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Figures

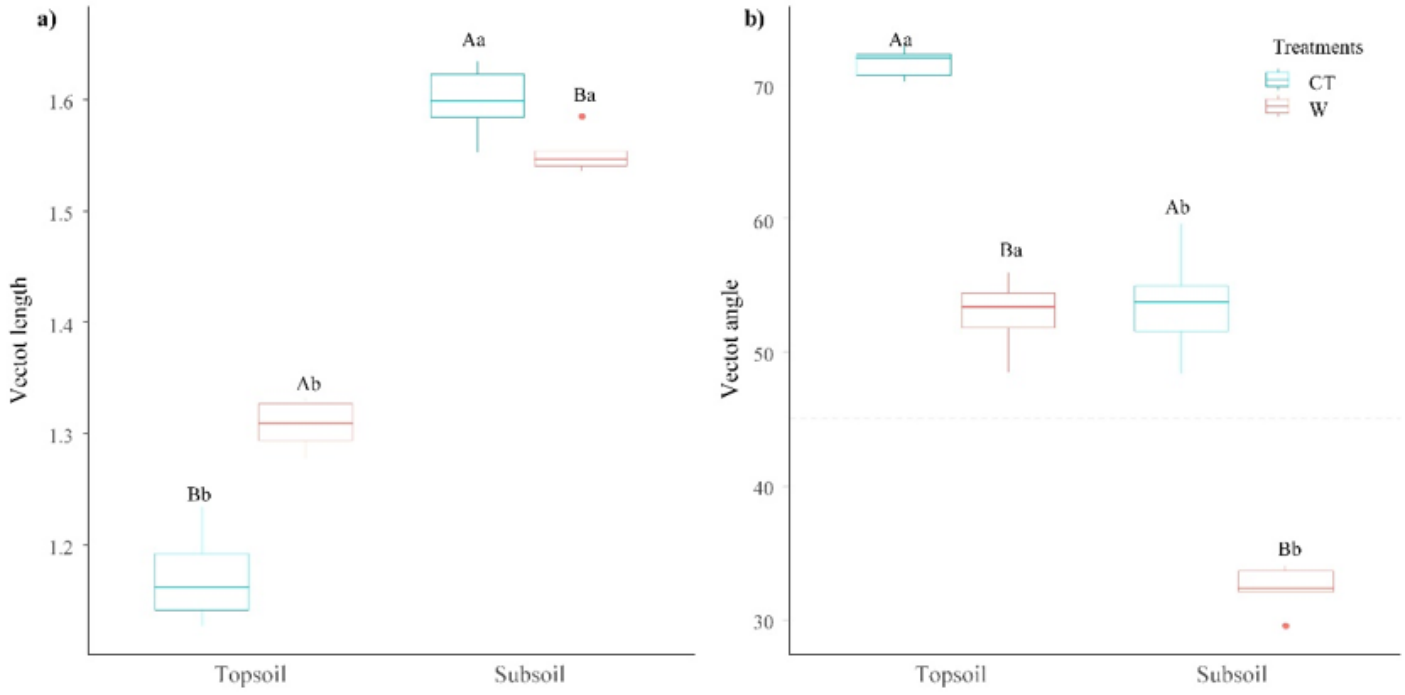

Figure 1

Vector lengths and angle in topsoil and subsoil in CT and W treatments. a) vector lengths; b) vector angle. Values (means ± SE, n=5). Different uppercase letters and lowercase letters indicate significant differences between CT and W at the same depth and between two depths in the same treatment.

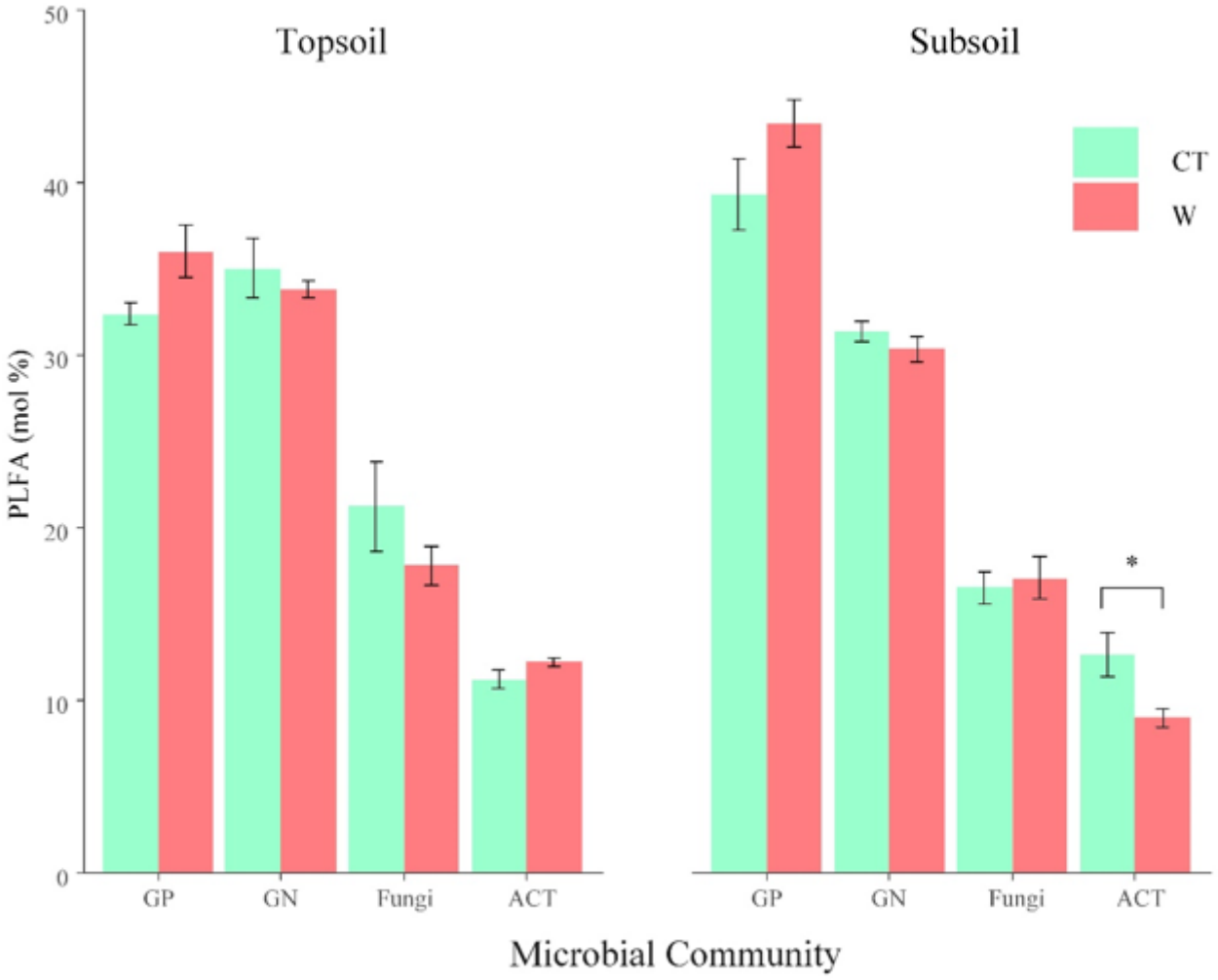

Figure 2

Proportional mol % of microbial communities' PLFA in topsoil and subsoil in CT and W treatment. Values (means ± SE, n=5). Different asterisks indicate significant differences between CT and W at the same depth.

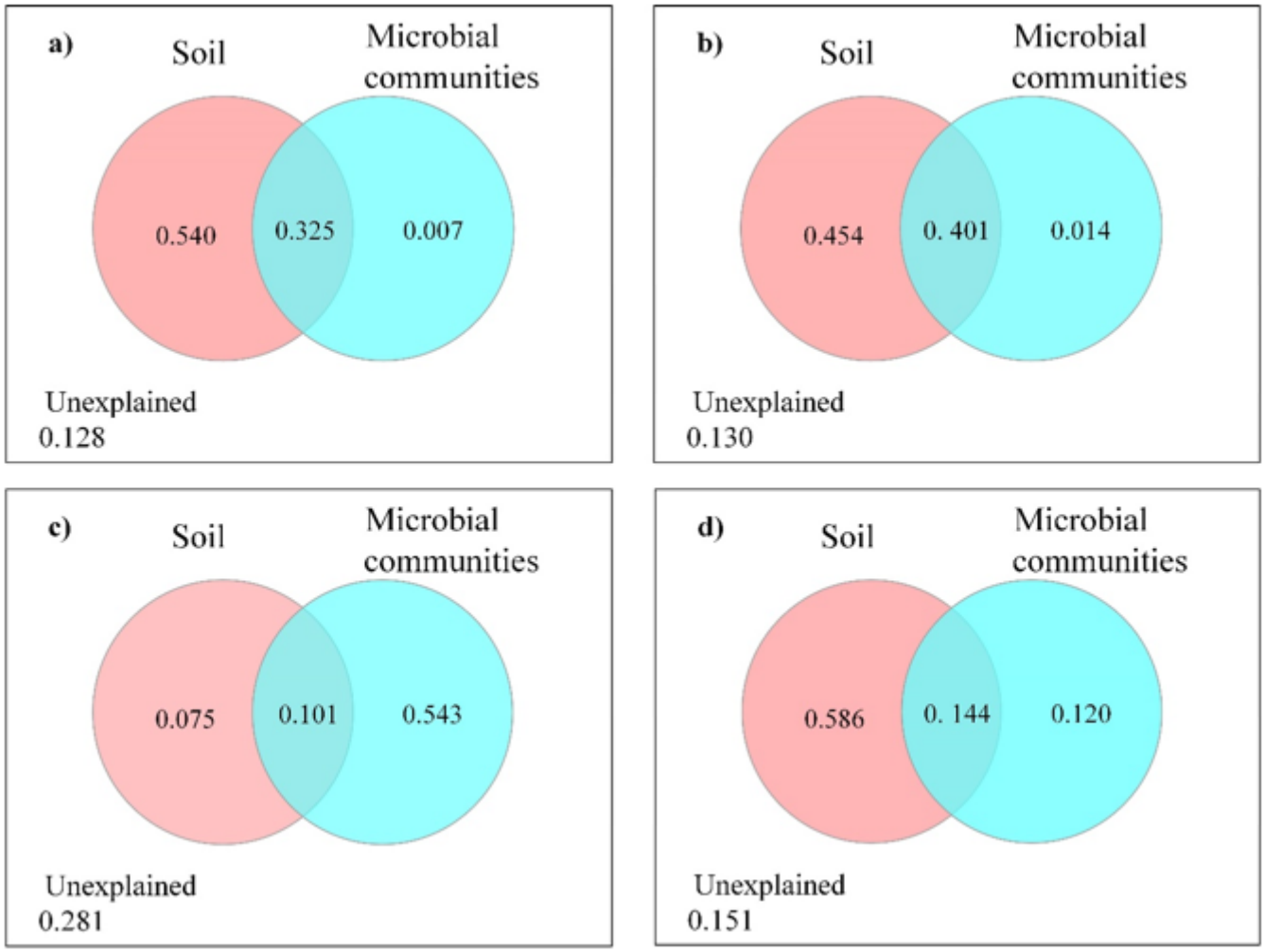

Figure 3

Venn diagram showing variation partitioning in soil microbial C and nutrient limitations in two sets of explanatory variables (soil physicochemical properties and microbial communities). a) Soil microbial C limitation in topsoil; b) soil microbial P vs. N limitation in topsoil. c) Soil microbial C limitation in subsoil; d) soil microbial P vs. N limitation in subsoil. Values denote the proportion of variance accounted for by each of the explanatory variables.

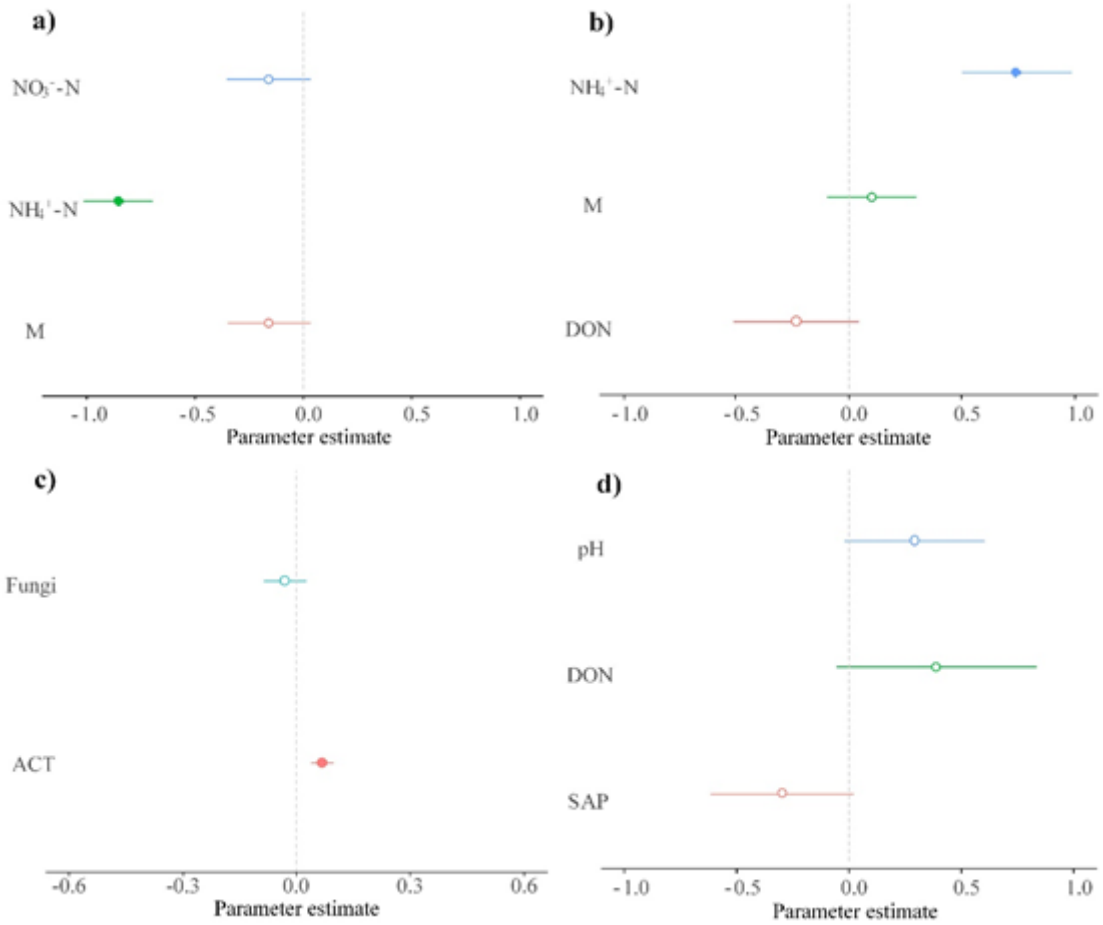

Figure 4

Model-averaged effect size of the predictors on microbial C and P vs. N limitation (based on Z scores with linear mixed-effects models). a) Soil microbial C limitation in topsoil; b) soil microbial P vs. N limitation in topsoil. c) Soil microbial C limitation in subsoil; d) soil microbial P vs. N limitation in subsoil. M: soil moisture.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.pdf