Effects of artificial warming on different soil organic carbon and nitrogen pools in a subtropical plantation

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\textbf{A R T I C L E I N F O}

\textbf{A B S T R A C T}

Uncertainty about the effects of climate warming on belowground processes of tropical and subtropical forests limits our ability to predict the response and feedback of such ecosystems to future climate change. Few field experiments in the tropics and subtropics have been conducted on the effects of manipulating warming on microbial community, enzyme activities and soil organic carbon (C) decomposition of forest ecosystems. Here, using buried cable techniques, we conducted a microcosm warming experiment to investigate extractable and acid resistant C and nitrogen (N) pools, microbial community composition, and enzyme activity after about 1.5 years of soil warming (+5 °C) in a subtropical plantation in southeastern China. The microbial community structure was quantified with phospholipid fatty acid (PLFA) analysis. Soil extractable and acid resistant C and N fractions were determined using a two-step sulfuric acid hydrolysis. We found that warming increased soil extractable C by 28% and acid resistant N decomposition by 20%. Soil warming decreased soil microbial N use efficiency by 31% but did not alter microbial C use efficiency. Warming differentially affected bacteria, fungi and enzymes activities. Our results suggest that climate warming can alter microbial community structure and enzyme activity and consequently lead to a serious imbalance between soil N and C decomposition in subtropical tree plantations.

1. Introduction

According to the RCP 8.5 scenario, global terrestrial surface temperatures will likely increase 2.6–4.8 °C by the end of this century (IPCC, 2014). Increasing temperature could have a significant impact on global carbon (C) cycles (Koven et al., 2017; Wang et al., 2015; Crowther et al., 2016). To mitigate the negative impact of global warming on terrestrial ecosystems, numerous studies have been conducted on sequestering more C into soils for a longer period of time without causing significant detrimental effects. One key factor that must be considered in a warming manipulative study is the composition and partitioning of different soil organic matter (SOM) pools with contrasting decomposability and residence time in soils. However, few studies have examined warming effects on different SOM pools and the underlying mechanisms, particularly in tropical and subtropical forest ecosystems (Cox et al., 2013; Cavalieri et al., 2015).

Measurement of the total C and nitrogen (N) of SOM does not often allow to detect their subtle changes with altered environmental factors, particularly in short-term experimental studies. Fractionating SOM into extractable and acid resistant pools based on their decomposibility and nutritional availability and quantifying these pools could be more valuable for better understanding the impact of altered environmental factors on SOM dynamics (Schmidt et al., 2011; Xu et al., 2015). For example, Zak et al. (1996) and Trumbore and Zheng (1996) found that the labile pool was very sensitive to temperature change. In contrast, Davidson and Janssens (2006) reported that acid resistant SOC was more sensitive to temperature increase with a higher Q10 value. Ziegler et al. (2013) and Seo et al. (2015) suggested that warming destabilized acid resistant SOC pool and caused a significant loss of soil C in temperate and boreal regions. Other studies suggested that elevated temperature decreased labile C due to enhanced microbial activity and increased acid resistant C due to C-to-N ratio changes (Cheng et al.,...
2007; Zhou et al., 2013a). The highly inconsistent findings on the warming effects on the labile and stable fractions of SOM calls for more studies, especially in the tropical and subtropical regions with much less information available.

Microbes play a key role in soil C decomposition and N mineralization (Zhang et al., 2005; Rudrappa et al., 2006). Soil microbial biomass C and N pools are considered to be vital components of SOM in forest ecosystems and sensitive to environmental perturbation. The ratios of soil microbial biomass C (SMBC) to extractable pool C (LP-C) and soil microbial biomass N (SMBN) to extractable pool N (LP-N) have been used as parameters to determine nutrient use efficiency of the microbes and substrate availability in soils (Belay-Tedla et al., 2009). Warming could affect microbial community composition and SMBC and consequently affect soil extractable C and N pools depending on site conditions (Zhou et al., 2013a, b; Garcia-Palacios et al., 2015; Tischer et al., 2015).

A variety of extracellular enzymes produced by different microbial groups, such as Gram + and Gram− bacteria, actinomycetes and mycorrhizal fungi, mediate nearly every step of SOM decomposition in different ways depending on the forms of SOM (Zumsteg et al., 2013; Cenini et al., 2016). Different forms of SOM with varying components such as cellulose, lignin, and chitin targeted by a wide range of enzymes may have different temperature sensitivities (Tischer et al., 2015). Acid resistant C sources such as wood containing much lignin and tannin were often broken down by oxidative enzymes produced by fungi and gram + bacteria, while extractable C sources were broken down mostly by hydrolytic enzymes produced by another group of bacteria (Ma et al., 2014; Xu et al., 2015). Many studies reported that C decomposition and N mineralization were closely associated with enzyme activity (e.g. Cenini et al., 2016). Hydrolytic enzymes such as β-1,4-glucosidase (βG), Cellobiohydrolase (CBH), β-1,4-acetylglucosaminidase (NAG) degraded mostly extractable SOC and tended to increase with mineral N concentration, while oxidative enzymes, Phenol Oxidase (PhOx) and Peroxidase (PerOx) generally degraded acid resistant SOC and were often suppressed with N additions (Acosta-Martinez et al., 2007; Cusack et al., 2011; Wallenstein and Burns, 2011; Ma et al., 2014). Although previous studies demonstrated that interactions between microbial resource demands and SOM quality determined the direction and magnitude of soil C responses to temperature change (e.g., Billings and Ballentine, 2013) and SOM-decaying enzymes changes with temperature (Billings et al., 2016), data was scarce on the interactions of enzymes and decomposition of various SOM fractions under field experimental warming conditions, especially in the tropics and subtropics.

The dominant species of China plantations is Chinese fir (Cunninghamia lanceolata), which accounts for most commercial plantations with respect to acreage and timber production (Lu et al., 2014). We used Chinese fir planting as a model system to understand the C and N response to the effects of artificial soil warming by conducting a heating cable approach. A thorough understanding of the responses of extractable and acid resistant C and N of Chinese fir plantation to warming is critical for accurately predicting belowground C dynamics of subtropical plantations with future climate change.

In the present study, by using soil warming manipulation, C and N fractionation and phospholipid fatty acid (PLFA) techniques, we examined the effects of soil warming on different C and N pools, soil microbial community structure, enzyme activities and SOC decomposition rates in a subtropical young plantation. Specifically, we aimed to answer the following questions: (1) How will soil warming affect extractable and acid resistant soil organic C and N decomposition in a Chinese fir plantation? and (2) How will soil microbial community structure and enzyme activity change with soil warming and consequently affect the processes of soil C and N decomposition?

2. Materials and methods

2.1. Site and experimental design

This research was conducted at Saming experimental site of the Research Station of Forest Ecosystems and Global Change of Fujian Province, South China (26°19 N, 117°36 E). The study area has a typical subtropical monsoon climate with a mean annual temperature of 19.1 °C, a mean annual precipitation of 1670 mm, and an average humidity of 80%. The mean annual precipitation is approximately 1656 mm, > 70% of the precipitation occurring from March to August. The mean annual air temperature is 19.1 °C and the relative humidity averages 80%. The soil is classified as red soil based on the China’s soil classification systems, equivalent to Oxisols in the USDA Soil Taxonomy. Overtstory tree species are dominated by castanopsis carlesii, Castanopsis fissa, Schima superba, Lithocarpus glaber, Symlocos caudate, Machilus velatina. During the past several decades, the majority of the broadleaf evergreen natural forests in this region were converted to plantations of Chinese fir (Cunninghamia lanceolata) to meet the growing demand for timber, fuel material, and other non-timber forest products (Guo et al., 2016). The total area of the Chinese fir plantations was over 9.11 million ha. The present study was carried out in a young Chinese fir plantation established in 2013.

The experiment included five warming and five control plots. Each plot had a size of 2 × 2 m. The experiment was established in August 2013. Heating cables (TXLP/1, Nexans, Norway) were buried in both control and warming soils at a depth of 10 cm with a horizontal interval of 20 cm but the cables in the control plots were not heated. Data were not collected after 6 months to minimize the difference between the disturbed and undisturbed controls. Temperature sensors (T109, Campbell Scientific Inc. Logan, USA) were placed between cables at a depth of 10 cm, three in each warming plot and two in each control plot. Soil temperature in the warming plots was continuously maintained at 5 °C above the temperature in the control plots. A datalogger maintained this differential temperature by switching the cables on and off on a 2-min cycle.

2.2. Soil sampling

In November 2015, soil was sampled with a 3.5-cm soil sampler to a depth of 0–10 cm. Six cores were randomly taken in each plot. All soil samples were immediately transferred to the laboratory and stored in a refrigerator at 4 °C until chemical analyses occurred within a week. Subsamples for soil water content were oven-dried at 105 °C for 24 h. Subsamples for analysis of soil pH, total organic C and N, and inorganic N were air-dried for 2–4 days, ground and passed through a 2-mm sieve. Subsamples for PLFA and enzyme analyses were stored at a temperature of −20 °C. Soil pH was determined using a pH meter at a soil:water ratio of 1:2.5. Total soil C and N were determined by using an elemental analyzer (Elementar Vario MAX, EA Consumables, Inc. USA). For nitrate and ammonium analyses, five grams of fresh soil from each sample were extracted with a 2-mol L−1 KCl solution. The extractant was shaken for 40 min and then filtered for nitrate and ammonium determination using a Continuous Flow Analyzer (SKALAR san++, Holland).

2.3. Enzyme analysis

Enzyme analysis was conducted by following a procedure as described in Saiya-Cork et al. (2002) and Sinsabaugh et al. (1992). Enzymes assayed in this study included acid phosphatase, beta glucosidase, cellobiohydrolase, β-1,4-N-acetylglucosaminidase, phenol oxidase and peroxidase. Suspensions of 1 g soil to 125 ml of acetate buffer at a concentration of 50 mol L−1 were prepared for each sample and agitated for 1 min using a Brinkmann Polytron PT 3000 homogenizer. The sample suspensions were continuously mixed with a magnetic stir plate
during which 200 ml of the suspensions was portioned into 96-well microplates at 16 replicate wells per sample per assay. Fluorometric assays were conducted for all enzymes except phenol oxidase and peroxidase. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filter. After a correction for quenching and negative controls all activity units were presented as nmol h$^{-1}$ g$^{-1}$ dry weight. 4-dihydroxyphenylalanine (DOPA) was used as the substrate for spectrophotometrical analysis for phenol oxidase and peroxidase activity. Each sample well had 50-μl solution of 25 mM DOPA and peroxidase assays had 10-μl of 0.3% H$_2$O$_2$. Blank wells had 200-μl of the sample suspension and 50 μl of acetate buffer. A 50-μl solution of DOPA and 200 μl of acetate buffer were added to negative control wells for phenol oxidase. Negative control and blank wells for peroxidase had 10 μl of H$_2$O$_2$. This produced 8 replicate wells for blanks and controls, and 16 replicate sample wells for each enzyme assay. Microplates were incubated at 20 °C for 18 h in the dark. Activity was considered as a measure of absorbance at 450 nm by using a microplate spectrophotometer.

2.4. PLFA analysis

The microbial community structure was determined using the phospholipid fatty acid (PLFA) analysis as described by White et al. (1979) and Bardgett et al. (1996). In summary an extracted mixture of chloroform, methanol and citrate buffer with a volume ratio of 1:2:0.8 from a 10-g dry sieved soil was used for PLFA analysis. The extraction process included two phases: the chloroform phase and the citrate buffer phase. The lipid materials were recovered and evaporated under nitrogen gas during the chloroform phase. These lipids were then re-suspended in chloroform and then fractionated on silicic chromatography acid columns (CWN Bohydro, 500 mg, 3 ml). Neutral lipids, glycolipids and phospholipids were eluted with 5 ml of chloroform, acetone and methanol respectively. Nitrogen gas was used to dry the phospholipid fraction before a mild alkaline methanolysis was conducted to prepare for fatty acid methyl esters. For alkaline methanolysis, one ml of methyl alcohol and methylbenzene with a ratio of 1:1 in volume and then 1.0 ml of methanolic KOH were added to each sample. After swirling to mix, the samples were sealed and placed in bath at 37 °C for 30 min. Afterwards, 2.0 ml of hexane was added to each sample and after swirling 0.2 ml of 1.0 M acetic acid was added to each sample. Two ml of deionized H$_2$O was added to each sample to break phase and vortex samples for 30 s and then the samples were centrifuged for 2 min. The top phase was transferred to clean labeled 10-ml vials with a short Pasteur pipette. Afterwards, 2.0 ml of hexane was added to each sample and then swirled. Nitrogen gas was also used to evaporate these methyl esters and stored at −20 °C until running gas chromatography analysis (GC). For the GC process, 200-μl HPLC-grade ethyl acetate was used to dissolve and separate the individual methyl esters. They were then run on GC with SGE 25QC3 BP-5 25 m × 0.32 μm film in thickness. A chromatographic retention time comparison to bacterial methyl esters was used to identify and quantify the separated fatty acids (Supelco Bacterial Acid Methyl Esters CP Mix 47080-U). Relative smoles per gram of dry soil was used to express the abundance of individual fatty acids using a standard nomenclature (Tunlid et al., 1989; Frostegård et al., 1993a, b). PLFAs that represented gram-positive bacteria (GP) were considered to be i-14:0, i-15:0, a-15:0, i-16:0, i-17:0, and a-17:0, and the PLFAs that represented gram-negative bacteria (GN) were 16:1 w9c, 16:1 w7c, cy-17:0 w7c, 18:1 w7c, cy-19:0 w7c. PLFAs that represented fungal species (fungi) were 18:2 w6c and 18:1 w9c. PLFAs for actinomycetes (ACT) were 16:0 10-methyl, 17:0 10-methyl, 18:0 10-methyl, 17:1 w7c 10-methyl, and 18:1 w7c 10-methyl. The PLFA for arbuscular mycorrhizae and (VAM) anaerobes (DMA) were 16:1 w5c and 15:0 respectively. For the total bacteria (Tbacteria) determination the PLFAs for gram+ and gram- 14:0, 15:0, 17:0 and 18:0 were included. The ratio of fungal to bacterial PLFAs (F:B) was used to estimate of the relative importance of the bacterial and fungal metabolic presence in the community.

2.5. Carbon and nitrogen pools analysis

To determine extractable and acid resistant concentrations of SOC and SON, 20 ml of 5N H$_2$SO$_4$ was added to 500 mg dry soil, and the sample was hydrolyzed for 30 min at 105 °C in a fenton tube. The hydrolysate was centrifuged for 20 min and then passed through a 0.45-μm filter paper. This initially extracted hydrolysate included extractable pool I carbon (LPI-C) and extractable pool I nitrogen (LPI-N). The residue from this process was flushed repeatedly with deionized water and then oven-dried at 60 °C. The samples were hydrolyzed with 2 ml of 13 mol L$^{-1}$ H$_2$SO$_4$. The samples were continuously shaken for at least 10 h at room temperature. Acid concentration was reduced to 1 mol L$^{-1}$ with de-ionized water and then hydrolyzed at 105 °C for 3 h. The hydrolysate was then centrifuged at 4500 r min$^{-1}$ and passed through a 0.45-μm filter paper. This hydrolysate included extractable pool II carbon (LPII-C) and nitrogen (LPII-N). Remaining residue was taken out and washed with deionized water repeatedly and then oven-dried at 60 °C. Acid resistant pool carbon (RP-C) and nitrogen (RP-N) were determined using a C-N analyzer (ElementarVario, MAX, Germany).

2.6. Microbial biomass C and N

Soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N) were determined using the chloroform (CHCl$_3$) fumigation and potassium sulfate (K$_2$SO$_4$) extraction techniques as described by Vance et al. (1987) and Xu et al. (2015). In summary, CHCl$_3$ was used to fumigate 5-g fresh soil for 24 h in dark plastic bags paired with unfumigated controls. A 20-ml solution of 0.5 mol L$^{-1}$ K$_2$SO$_4$ were used and the samples were fumigated and then shaken for 30 min at a rate of 250 r min$^{-1}$. Samples were centrifuged for 10 min at 4000 r min$^{-1}$ and then passed through a 0.45-μm glass fabric filter paper.

2.7. Dissolved organic C and N, ammonium and mineral nitrogen

Dissolved organic carbon (DOC) and nitrogen (DON) was extracted with deionized water. Ten grams of dry sieved soil and 40 ml of deionized water were used to make a solution at a ratio of 1:4 and then was shaken for 30 min at 250 r min$^{-1}$. Samples were centrifuged for 10 min at a rate of 4000 r min$^{-1}$ and then passed through a 0.45-μm glass fabric filter paper.

Soil ammonium (NH$_4^+$) and nitrate nitrogen (NO$_3^-$) was extracted using potassium chloride (KCl). A 5-g soil sample was added to 20 ml of KCl at a concentration of 2 mol L$^{-1}$ to form a mixture which was shaken for 30 min at 250 r min$^{-1}$, centrifuged for 10 min at a rate of 4000 r min$^{-1}$ and then filtered using a 0.45-μm filter paper. The extractants were then used for NH$_4^+$ and NO$_3^-$ analysis.

2.8. Soil pH, SOC and SON

Soil pH was determined using 10-g dry soil and 25 ml CO$_2$-free water at a soil:water ratio of 1:2.5. The mixture was stirred for 20 min and then settled for 30 min at room temperature. pH was measured using a pH meter. The dry sieved soil was analyzed for SOC and SON using a C-N analyzer (ElementarVario, MAX, Germany).

2.9. Statistical analysis

All statistical analyses were done using the IBM SPSS Statistics 22 version 19.0 software (SPSS Inc. Chicago, IL), OriginPro 9.0 (OriginLab Corporation, Northampton, Mass) and CANOCO 5.0 software (Microcomputer Power Inc. Ithaca, NY). An independent-sample t-test was used to determine the effects of warming on soil microbial enzyme concentrations, PLFA biomarkers, LPI-C, LPII-C, RP-C, LPI-N, LPII-N, and RP-N values, MBC, MBN, DON, DOC, SOC, SON, pH, ammonium,
and nitrate. Analysis of similarities (ANOSIM) based on Bray-Curtis distances was used to test the significance of microbial communities between the treatments (Oksanen et al., 2007). Redundancy analysis (RDA) was used to test the treatment effects on soil microbial community structure and environmental variables.

3. Results

3.1. Extractable and acid resistant pools of soil C and N

Soil warming did not affect the size of total organic C pool but significantly reduced extractable C pool I (LPI-C) and pool II (LPII-C) in the top 10 cm of the soils (Fig. 1a). The content of LPI-C was 5.53 (± 0.69) and 7.42 (± 1.18) g kg⁻¹ soil for the warming treatment and the control respectively and the content of LPII-C was 1.64 (± 0.11) and 2.55 (± 0.16) g kg⁻¹ soil for the warming and the control respectively. We did not find a significant difference in acid resistant C pool (RP-C) between the warming treatment and the control though there was an increasing trend in the warming treatment (Fig. 1a). The content of RP-C was 4.88 (± 0.89) and 3.99 (± 0.40) g kg⁻¹ soil for the warming and control, respectively.

There was a significant difference in all N pools with lower values for the three pools in the warming treatment (Fig. 1b). The content of extractable N pool I (LPI-N) was 0.25 (± 0.016) and 0.32 (± 0.056) g kg⁻¹ soil for the warming and the control respectively. The content of extractable N pool II (LPII-N) was 0.043 (± 0.006) and 0.057 (± 0.004) g kg⁻¹ soil for the warming and the control respectively. The content of acid resistant N pool (RP-N) was 0.599 (± 0.067) and 0.753 (± 0.052) g kg⁻¹ soil for the warming and the control, respectively (Fig. 1b).

3.2. DOC, DON and soil microbial C and N

Soil warming significantly (p < 0.05) reduced soil microbial biomass C (SMB-C) and microbial biomass N (SMB-N) (Table 1). Warming did not significantly affect the ratio of SMB-C:SMB-N, soil NH₄⁺-N and soil pH. Warming increased soil dissolved organic C (DOC) and NO₃⁻-N but decreased dissolved organic N (DON) (Table 1).

The ratio of SMB to extractable pool (LP) for both C and N was also significantly decreased by warming (Fig. 2). Warming significantly decreased the SMB-N:LPN ratio (p < 0.05) but did not significantly affect the SMB-C:LP.

3.3. Soil enzyme activities and microbial PLFAs

Warming altered both the C- and N-degrading enzyme activities (Table 2). Warming increased the C-degrading enzymes βG (p < 0.01) and CBH (p < 0.01) but decreased PHO (p < 0.01). Warming decreased N-degrading enzyme AP (p < 0.01) but had no significant effect on P-degrading enzyme AP (Table 2). In addition, the ratio of N-degrading to P-degrading enzymes was significant lower in the warming treatment than the control (tₜ = −4.24, p = 0.003; data not shown).

Warming significantly affected the microbial community composition (Fig. 3). All the PLFA biomarkers showed a decreasing trend. The PLFA of total microbes, total bacteria, gram-negative bacteria, un-specific bacteria, actinomycete, fungus and anaerobe significantly (p < 0.05) decreased with warming compared to the control. However, warming did not significantly affect the PLFA of gram-positive bacteria and arbuscular mycorrhizal fungi although there was a

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Warming</th>
<th>Independent t-test tₜ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMB-C (mg kg⁻¹)</td>
<td>295.24 ± 59.98</td>
<td>212.71 ± 12.76</td>
<td>tₜ = −3.01 (0.017)</td>
</tr>
<tr>
<td>SMB-N (mg kg⁻¹)</td>
<td>24.99 ± 5.19</td>
<td>15.21 ± 1.46</td>
<td>tₜ = −4.05 (0.004)</td>
</tr>
<tr>
<td>SMB-C:SMB-N</td>
<td>12.11 ± 2.99</td>
<td>14.09 ± 1.54</td>
<td>tₜ = 1.31 (0.226)</td>
</tr>
<tr>
<td>DOC (mg kg⁻¹)</td>
<td>4.96 ± 1.28</td>
<td>6.98 ± 0.83</td>
<td>tₜ = 2.96 (0.018)</td>
</tr>
<tr>
<td>DON (mg kg⁻¹)</td>
<td>7.52 ± 0.56</td>
<td>6.49 ± 0.49</td>
<td>tₜ = −3.09 (0.015)</td>
</tr>
<tr>
<td>NH₄⁺-N (mg kg⁻¹)</td>
<td>1.94 ± 0.64</td>
<td>2.08 ± 0.62</td>
<td>tₜ = 0.35 (0.739)</td>
</tr>
<tr>
<td>NO₃⁻-N (mg kg⁻¹)</td>
<td>4.19 ± 0.39</td>
<td>6.57 ± 1.07</td>
<td>tₜ = 4.68 (0.002)</td>
</tr>
<tr>
<td>Soil pH</td>
<td>4.53 ± 0.10</td>
<td>4.66 ± 0.12</td>
<td>tₜ = 1.86 (0.101)</td>
</tr>
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Fig. 2. Soil microbial biomass to labile pool ratio (SMBC: LPC and SMBN: LPN) under experimental warming. Values are mean ± SD (n = 5). ns, no significance; *, p < 0.05.

Fig. 1. Amount of C and N pool fractions, including (a) labile C pool I (LPI-C, mainly polysaccharides) and II (LPII-C, mostly cellulose), and recalcitrant C pool (RP-C), and total organic C pool sizes, and (b) labile N pool I (LPI-N, mainly polysaccharides) and II (LPII-N, mostly cellulose), and recalcitrant N pool (RN-N), and total organic N pool sizes in control and warmed plots. Values are mean ± SD (n = 5). Different letters between control and warming treatment means significant difference at P < 0.05 level.
3.4. Correlation between soil PLFAs and physicochemical variables

Based on redundancy analysis (RDA), there was a significant difference in microbial community composition between the warming treatment and the control (ANOSIM, R = 0.636, p = 0.005). Warming explained 59.9% of the variance (Fig. 4). The RDA showed that DOC, DON and LPII-C accounted for 37.4%, 34.1% and 29.8% of the variance respectively (Table 3). Soil microbial community composition was significantly correlated with LPI-C and LPII-N, which explained 30.6 and 28.8% of the variance respectively (p < 0.1). The first axis was positively correlated with DON, LPI-N, LPI and LPI-C but negatively correlated with DOC. The second axis was positively correlated with LPI-C and all variables explained 73.2% of the variance of microbial composition in the warming treatment (59.2%) and the control (14.0%) (Fig. 4).

4. Discussion

Prediction of climate change largely depends on effects of warming on SOM decomposition and the understanding of temperature sensitivities of different SOM fractions (Davidson and Janssens, 2006; Luo, 2007; Belay-Tedla et al., 2009). This is especially so for tropical forests, which accounts for a third of global soil C and remains an important source of uncertainty in climate model projections (Jobbagy and Jackson, 2000; Cox et al., 2013; Cavalieri et al., 2015). Belay-Tedla et al. (2009) reported that warming increased extractable C content in soils due to warming-induced increase of above- and below-ground biomass. Several long-term studies reported that extractable soil C was positively correlated with the litter input (Cambardella and Elliott, 1992; Janzen et al., 1992; Wieder et al., 2015). However, Peterjohn et al. (1993) and Xu et al. (2015) found that labile pool size of SOM decreased with warming as a result of increased soil respiration and SOM decomposition. In our study we found that warming significantly reduced...
extractable C pool I (LPI-C) and pool II (LPII-C) in the top 10 cm of the soils. This finding indicates that the onset of warming increases the extractable C decomposition rate and thus reduces extractable C in the soils with little litter input at the early stage of the plantation.

Previous studies reported that the decomposition of extractable C rather than acid resistant C may be accelerated under a warming condition (Giardina and Ryan, 2000; Bradford et al., 2008; Xu et al., 2010, 2015). Our result that short-term warming did not significantly change soil acid resistant C but had an increasing trend indicates that long-term warming may increase acid resistant C in subtropical tree plantations.

Previous studies found that warming could increase net N mineralization and nitrification rate by 52.2 and 32.2% respectively (Bai et al., 2013), leading to N loss from ecosystems. Dawes et al. (2016) and Melillo et al. (2011) reported that experimental soil warming in temperate forests stimulated soil organic N mineralization. We found in the present study that soil warming reduced both extractable and acid resistant organic N pools but increased mineral N, which indicates warming may enhance mineralization of organic N in subtropical forest soils.

Our result that both extractable C and N were reduced in the warming treatment implies that warming enhances soil microbial activity in the subtropical plantation. In the present study SMB-C and SMB-N were significantly reduced in the warming plots compared to control (Table 1). Soil warming might lead to higher (Belay-Tedla et al., 2009), lower (Frey et al., 2008) or unvarying (Biasi et al., 2008) microbial biomass. Microbial C use efficiency has long been the focus of many studies in soil biomegeochemistry, while microbial N use efficiency refers to the partitioning of organic N taken up between growth and the release of inorganic N to the environment (that is, N mineralization), has relatively less been studied (Mooshammer et al., 2014). SMB/LP ratio can be considered as a measure of nutrient use efficiency by microbes (Belay-Tedla et al., 2009). The finding in the present study that warming decreased SMB-N/LP-N ratios but did not change SMB-C/LP-C (Fig. 2) could not be explained with the data obtained in this experiment. Probably multifactorial experiments with both N and warming as treatments are needed in such forest plantations.

Soil microorganisms drive soil biomegeochemical processes and soil microbial community directly affects organic matter degradation and its response to temperature change (Ostle and Ward, 2012). An important variable in determining how warming will affect SOC decomposition is the relative dominance of bacteria and fungi. Fungi are associated with a slow energy channel (slow turnover of more acid resistant and N-poor substrates, leading to high soil-C accumulation), whereas bacteria are associated with a fast energy channel (fast turnover of extractable and N-rich substrates), leading to low soil-C accumulation (Clemmensen et al., 2013; Bardgett and van der Putten, 2014). Our result that warming significantly increased the ratio of gram-positive bacteria to gram-negative bacteria and fungal to bacteria (Fig. 3) implies a possible shift from bacterial-to-fungal-dominated microbial communities. This change may be associated with a reduction in SOC as found in previous studies (Clemmensen et al., 2013; Bardgett and van der Putten, 2014). The RDA analysis showed that the variations in the soil microbial community structure were associated with extractable C and N pools and dissolved organic C and N in the soils. This finding suggests that warming may affect the soil microbial community structure by mediating the quantity of microbial substrates and the availability of C and N. The DOC concentration was negatively correlated with the first axis, indicating an association with the gram-positive bacteria and fungi biomass, which supported the previous study that DOC in soils was a product of decomposition process (Schimel and Bennett, 2004). The increase in DOC and decrease in DON under warming likely resulted from the lower N use efficiency under warming treatment as suggested by Weintraub et al. (2007), Chen and Xu (2008) and Carrera et al. (2015). Therefore, a potential shift in the soil microbial community structure could primarily be regulated by soil substrate availability and nutrient use efficiency under climate warming.

Our study showed that warming stimulated the extractable C-degrading enzymes (β-glucosidase and cellobiohydrolase) but not the acid resistant C-degrading enzymes (phenol oxidase and peroxidase) activities. This result could be a direct explanation for the decrease in soil extractable C pools. The change in enzyme activity can be attributed to the changes in microbial community structure as well as its sensitivity to warming. The lower ratio of N-degrading to P-degrading enzymes in this study implies lower N use efficiency with warming treatment as reported by Nottingham et al. (2015) and Cenini et al. (2016). The result on enzyme activity in the present study indicates that warming can differentially affect the extractable C- and acid resistant C-degrading enzymes and consequently change the SOC decomposition and sequestration. This field warming experiment conducted in China is one of pioneer studies in the subtropics. However, due to limited resource (high demand for power) and topography constraints we used 2 × 2 measuring plots, which is too small for the research results to be extrapolated to the entire subtropical forest ecosystems.

In conclusion, soil warming enhanced extractable SOC decomposition and did not change acid resistant SOC. Warming reduced extractable C and acid resistant N and soil microbial N use efficiency. Fungal and bacterial phospholipid fatty acid biomarker content significantly decreased under warming condition. Extractable C-degrading enzymes were significantly increased with warming treatment. Our results suggest that climate warming can remarkably alter microbial community structure and enzyme activity and thus consequently lead to a serious imbalance between soil N and C decomposition in subtropical forests.

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