



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

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ABSTRACT

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 decomposability 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.,

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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 (SMB-C) to extractable pool C (LP-C) and soil microbial biomass N (SMB-N) 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 SMB-C 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 (β G), Cellobiohydrolase (CBH), β -1,4-N-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 Ballentyne, 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. Overstory tree species are dominated by *castanopsis carlesii*, *Castanopsis fissa*, *Schima superba*, *Lithocarpus glaber*, *Symplocos 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

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