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Effects of simulated climate change on soil microbial biomass and enzyme activities in young Chinese fir (*Cunninghamia lanceolata*) in subtropical China

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ABSTRACT

Global warming and nitrogen deposition have been responsible for numerous environmental disturbances, and have attracted much attention from researchers, government agencies and international community. Recent studies indicate that the trend of global warming and nitrogen deposition will continue over the next few decades. These changes not only affect the growth of aboveground vegetation, but also change the belowground soil environment, and thus directly or indirectly affect the microbial process. The microbial biomass and soil enzymes play significant roles in terrestrial environments, particularly through the decomposition of soil organic matter, dynamic fluctuation between carbon sink and source, and the transformation of soil nutrient. However, little is known about that how global warming and nitrogen deposition will affect the soil microbial and soil enzymes in the subtropical zone.

In the present study, we aim to evaluate the responses of the microbial biomass and soil enzyme activity to short-term simulated warming and nitrogen deposition in young Chinese fir (*Cunninghamia lanceolata*) in Sanming Fujian province in subtropical China. The results showed that soil warming increased microbial biomass carbon content and improved the activity of acid phosphatase and lignin enzymes significantly ($P < 0.05$). Additionally, microbial biomass carbon content was significantly higher than that of the control after the application of nitrogen fertilizer. Besides, nitrogen addition also significantly raised the C/N ratio of microbial biomass. It also reduced the activity of lignin and cellulose hydrolysis. The combination of warming and nitrogen treatment was more effective than individual warming and nitrogen treatments, increasing the content of soil microbial biomass carbon and nitrogen, decreasing the activity of lignin hydrolytic enzymes and chitinase, and leading to further acidification of the soil. Redundancy analysis (RDA) showed that moisture and pH are the major determinants of soil enzyme activity at the 0–10 cm depth. However, at the 10–20 cm depth, the major determiner is microbial biomass. In summary, the simulated warming and nitrogen deposition affected soil microbial biomass and enzyme activity significantly in the short-term, and the interaction of the two factors was significant. That suggests that the climate change could have a profound effect on soil microbial processes. Therefore, the effects of simulated warming and nitrogen deposition on microbial biomass and soil enzyme activity and the mechanism of their interaction with soil, microorganisms and plants need to be studied further, in order to reveal the responses and feedback mechanisms of Chinese fir plantations to global climate change in subtropical China.

1. Introduction

Climate warming and nitrogen deposition are the two main features of the global climate change, thus provoking a series of environmental problems to the terrestrial ecosystem, which has become a concern of traitor, research scientists, government agencies and the international community [1]. According to climate models, IPCC (2013) predicted that global surface will mean increase temperature 1.8–4.0 °C at the end of the 21st century [2]. Nitrogen deposition will still continue to increase within the coming decades in the worldwide areas under the influence of human activities [3,4]. Nitrogen deposition increase and the warming will undoubtedly directly or indirectly impact soil microbial biomass and soil enzyme activity. Moreover, soil microbial biomass and soil enzyme activity exert an important role, such as fixation and

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decomposition of organic matter, carbon source and sink, soil nutrient cycling and transformation process, which cannot be replaced [5]. And due to the complexity and heterogeneity of the soil ecosystem, global climate change on soil microbial biomass and soil enzyme activity ring is still in great uncertainty [6]. Currently, the field warming control experiment is primarily concentrated in the middle and high latitude area grassland, farmland, tundra and forest ecosystem [7,8]. In the tropical and subtropical regions of the south, there is no field soil warming experiment, nor the interaction between temperature and climate change factors [9]. There is no denying the fact that simulating the interaction of climate experiment to understand individual effect of each factor and interaction effects can be integrated to reflect forest ecosystem soil microbial transformation and nutrient cycling process of global climate change response and feedback situation.

The humid subtropical in China is inclusive at the same latitude with rare oasis distribution area of the world's largest and most typical evergreen broad-leaved forest, which is the global subtropical biodiversity center. Because the heroic endeavor to create artificial forest, the area contributed to the forest carbon sink capacity of 65%. The Chinese fir forest is one of the most important plantations in the area, accounting for the area of plantation in the world 6.5% [10], accounting for 19% of the area of artificial forest in China with the volume of 25% in the forestry production and carbon sequestration in our country, which has a pivotal position. However, this region is confronted with rapid global climate change and the change of soil microbial response to environmental changes will inevitably exert an important influence on the growth of Chinese fir plantations. But researches on the region under the background of global change on Chinese fir plantation soil microbial research is still scarce, especially that the combined effects of climate on the Chinese fir plantation soil microbial biomass and soil enzyme activity influence research traitor are reported rarely. Therefore, research on early on humid subtropical Chinese fir plantation ecosystems is based on related working and on the soil of Chinese fir plantation seedlings to simulate to increase moderate nitrogen deposition [11]. It combined processing of field control experiment to research traitor in Chinese fir plantation soil microbial quantity and soil enzyme activity of elevated temperature and nitrogen increased response in order to provide scientific basis for soil nutrient cycling response and feedback of artificial forest under the background of global change.

2. Materials and methods

2.1. Study site

The experiment was conducted at the Fujian Normal University's Forest Ecosystem and Global Change Research Station in Chenda Town, Sanming City in the Fujian Province of China (26° 19′ N,117° 36′ E). This site is situated in a latosol soil; the regional climate is subtropical monsoon. The average annual precipitation, temperature, and evaporation are 1749 mm, 19.1 °C and 1585 mm respectively. The elevation is 300 m above sea level.

2.2. Experimental design and sampling

The experiment was a randomized complete block factorial design, with warming and N fertilization as fixed factors. There were four treatments (five replicates) in this study: (1) control (CT); (2) nitrogen addition (N); (3) warming (W); and (4) warming + nitrogen addition (WN). There were 20 2 m × 2 m mini-plots, and indigenous soil in the plots was replaced, to a depth of 60 cm, with sieved topsoil from a same forest area. PVC pipes (200 cm width, 60 cm depth) were buried vertically in each plot for planting four Chinese fir seedlings together. In November 2013, 120 healthy, uniform Chinese fir seedlings were selected based on plant basal diameter, height, and fresh weight. Four seedlings were randomly transplanted into each mini-plot.

Beginning in March 2014, artificial warming and nitrogen addition were conducted. Heating cables were used to generate a warmed environment and were buried in a spiral pattern 10 cm below the ground. Soil temperature was measured in each plot using temperature sensors (T109; Campbell Scientific Inc., Logan, UT, USA) buried continuously between heating cables. The warming cables significantly increased (5 °C) the soil temperature in the warmed plots at the 10 cm depth, and the effects of the cables over the soil surface were equal to the control. The control plot and unwarmed plots had a "dummy" heater the same shape and size as the cables in order to simulate the shading effects of the heater. Detail of the experimental warming system design has been reported previously [11]. During this same period, two N levels were applied (0 and 80 kg N ha^{-1} $year^{-1}$, added as ammonium nitrate (NH_4NO_3)). Nitrogen was added twelve times each year.

After one year, soil (0–10 cm) was collected from five random points in each plot using 5 cm soil cores. Soil samples were immediately transported to the laboratory and stored at 4 °C until the analyses. The soil was cleared of roots and all organic debris, air-dried and analyzed for soil pH, soil organic carbon (SOC), total nitrogen (N) and available phosphorus (P). Air-dried soil samples were ground and passed through a 2 mm sieve. Soil pH was determined using a pH meter with a soil:water ratio of 1:2.5. Soil organic C and soil total N was measured in a single analysis using a CN auto analyzer (Elementar Vario MAX, Germany). A 3 g air-dried soil sample was mixed with 30 mL of the Mehlich-3 extracting solution [12], shaken immediately for 5 min, and centrifuged for 5 min at 8000 rpm. The supernatant was used to determine the available P (Skalar san++). Stand characteristics and soil properties are provided in Table 1.

2.3. Soil microbial biomass analysis

Soil microbial biomass C (MBC), N (MBN) and P (MBP) were measured by the chloroform fumigation–extraction method as described previously [13]. Briefly, two portions of 10 g field moist soil samples were weighed, and one portion of them was fumigated with chloroform for 24 h and extracted with 0.5 mol·L^{-1} K_2SO_4 in an end-to-end shaker for 1 h, then the supernatants were filtered through a Whatman no. 42 paper. The other proportion of the soil was directly extracted as above. The amounts of total C in the fumigated and un-fumigated soil extracts were determined using a TOC-VCPH/CPN analyzer. MBP was extracted with 0.5 mol·L^{-1} $NaHCO_3$ and rinsed in deionized water and the phosphate recovered by shaking for 1 h in 50 mL of 0.25 mol·L H_2SO_4. The amounts of total N and P was detection by Skalar san++. MBC,MBN and MBP were calculated as the difference between the fumigated and unfumigated samples and corrected for unrecovered biomass using k_C, k_N and k_P factor of 0.38, 0.45 and 0.4 respectively [14].

2.4. Measurements of soil enzyme activity

We used a procedure adapted from Saiya-Cork and Sinsabaugh [15] (2002) to analyze soil enzyme. Activities of soil enzymes involved in C, N and P cycling were measured. These enzymes included four hydrolytic enzymes: β-glucosidase (βG), cellobiohydrolase (CBH), *N*-acetylglucosaminidase (NAG), and acid phosphomonoesterase (AP) and two oxidase enzymes: phenol oxidase (PHO) and peroxidase (PEO). We marked with 4-methylumbelliferone (MUB) as substrate labeled hydrolytic enzymes activity and the activity of lignin enzyme was estimated using L-dihydroxyphenylalanine (L-DOPA) as substrate. Briefly, 1 g fresh soil sieved at 2 mm was mixed with 125 ml 50 mmol·L^{-1} acetate buffer (pH = 5.0), then used a magnetic stirrer stirring 5 min to homogenize and took 200 μL to a 96 well plate by pipettor. The mixture was then incubated in the dark for 4 h (hydrolytic enzymes) and 18 h (oxidase enzymes) at 20 °C. Following incubation, we measured sample fluorescence using 365-nm excitation and 450-nm emission filters (hydrolase enzymes) or absorbance at 450 nm (lignin enzymes) on a SpectraMax M5 Microplate Reader (MDS Analytical Technologies,