Contents lists available at ScienceDirect

## Applied Soil Ecology



journal homepage: www.elsevier.com/locate/apsoil

## Initial soil responses to experimental warming in two contrasting forest ecosystems, Eastern Tibetan Plateau, China: Nutrient availabilities, microbial properties and enzyme activities

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#### ARTICLE INFO

Article history: Received 14 March 2010 Received in revised form 29 June 2010 Accepted 9 July 2010

Keywords: Experimental warming Microbial biomass Dissolved organic matter Soil enzyme Extractable nitrogen Eastern Tibetan Plateau Coniferous forest

#### ABSTRACT

In order to understand the effects of projected global warming on soils in different land-use types, we compared the impacts of warming on soils in two contrasting forest ecosystems (a dragon spruce plantation and a natural forest) using the open-top chamber (OTC) method in the Eastern Tibetan Plateau of China. The OTC on average enhanced daily mean soil temperatures by 0.61 °C (plantation) and by 0.55 °C (natural forest) throughout the growing season, respectively. Conversely, soil volumetric moisture declined by 4.10% in the plantation and by 2.55% in the natural forest, respectively. Warming did not affect dissolved organic C (DOC) and N (DON) in the plantation but significantly increased them in the natural forest. Elevated temperature significantly increased net N mineralization rates and extractable inorganic N pools in both sites. Warming had no effects on microbial biomass C (MBC) and N (MBN) and their ratios (MBC/MBN) in the plantation and significantly increased MBC and MBN only late in the growing season in the natural forest. Warming did not affect basal respiration in the plantation but significantly increased it in the natural forest. No clear change was observed in metabolic quotient between warming regimes for both forest types. Experimental warming tended to increase invertase and urease in both forest soils. Measured pools related to N turnover generally showed significant interactions in warming, forest type and sampling date. Taken together, our results indicate that responses of soils to experimental warming depend strongly on forest managements and seasons.

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#### 1. Introduction

Global air temperatures are predicted to increase 1.8–4.0 °C over this century, with a greater warming occurring in the higher latitudinal and altitudinal ecosystems (IPCC, 2007). Temperature is a key factor that regulates almost all biochemical processes of terrestrial ecosystems, such as soil respiration, soil net N mineralization and soil enzyme activity (Rustad et al., 2001). Warmer air temperatures would likely result in warmer soil temperatures which could, in turn, largely affect the biogeochemical processes of soils in these ecosystems (Kirschbaum, 2004). Over last two decades, considerable studies have demonstrated that experimental warming had pronounced effects on soil dissolved organic matter (DOM), soil N turnover and transformation, soil microbial activities and structures, soil enzyme activities and soil respiration, etc. (Rustad et al., 2001; Rillig et al., 2002; Schmidt et al., 2002; Jonasson et al., 2004; Staddon et al., 2003; Zhang et al., 2005; Sardans et al., 2006, 2007, 2008; Biasi et al., 2008; Allison and Treseder, 2008). However, ecosystem responses differed largely among ecosystem types.

Ecosystem response could depend strongly on the initial conditions of ecosystems, such as stocks and initial turnover rates of soil organic matter, the relative size of the plant and soil C pools and the chemical composition and turnover rates of plant residues (Shaver et al., 2000). Additionally, soil responses to warming are likely to be complicated by land-use change (Striegl and Wichland, 1998; Zhang et al., 2005). Therefore, it is very important to synchronously investigate soil properties under different land-use types in a warming experiment. Comparing responses of soils under different land-uses to warming could more accurately predict the impacts of global warming on terrestrial ecosystems.

The sub-alpine and alpine forest ecosystems in the Eastern Tibetan Plateau located at the transitive zone from Qinghai-Tibet plateau to Sichuan basin could be very sensitive to global climate change with important consequences for the global C and N balance (Wang et al., 2003). The magnitude of warming on the Tibetan Plateau is projected to be large relative to many other regions



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<sup>0929-1393/\$ -</sup> see front matter © 2010 Elsevier B.V. All rights reserved. doi:10.1016/j.apsoil.2010.07.005

SOM (Xu and Ding, 2003; Jiang et al., 2009). Thus, soil nutrient turnover processes of alpine forest soils in this region could be more pronounced than in other ecosystems under future warmer conditions. Last century, natural coniferous forests in southwestern China were deforested and reforested with dragon spruce (Picea asperata Mast.). Currently, there are over one million hectares of dragon spruce plantation in Western Sichuan, accounting for approximately 50% of the forest area in this region. Reforestation may induce great changes in soil biochemical properties, and further affect the responses of forest soils to projected global warming. Hence, in this study, we conducted an experiment in two contrasting forest ecosystems (dragon spruce plantation versus spruce-fir dominated natural forest) to assess the effects of experimental warming on nutrient availabilities, microbial properties and soil enzyme activities. Specifically, we hypothesized that (1) an increase in temperature will stimulate soil net mineralization rate and benefit microbial growth; (2) soil warming will increase soil enzyme activities in two experimental sites; and (3) reforestation will influence the responses of forest soils to warming.

#### 2. Materials and methods

#### 2.1. Experimental site

The study was conducted on two sites that were within approx. 300 m distance of each other. One site was in a dragon spruce plantation (65-year old) and the other was in a spruce-fir-dominated natural forest (200-year old). There are some mosses and grasses (e.g., Carex capilliformis, Deveuxia arundinacea, Festuca ovina) growing under the plantation. Conversely, the understory is dominated by mosses, woody trees (e.g., Betula albo-sinensis, Acer mono, Lonicera spp.) and grasses (e.g., Carex capilliformis, Anemone rivularis) in the natural forest. Both experimental sites are located at the Miyaluo Experimental Forest of Lixian County, Eastern Tibetan Plateau (31°35′N; 102°35′ E; 3150 m a.s.l.). This spruce plantation was originated from the clear-cutting land 1950s. According to the local Forestry Bureau, not any management practice (e.g., artificial fertilizer, irrigation) was added in this plantation. For basic climate conditions in the study region see Yang et al. (2008). Soils at two sites are classified as the mountain brown soil series (Chinese taxonomy). Basic soil properties in both ecosystems were shown in Table 1.

In order to increase the soil temperature, in late September 2008, six OTCs were set up in each forest type. One control plot was randomly established in the vicinity of each OTC. The OTC used in this study was hexagonal and 80 cm high, made of solar transmittance with  $2.45 \text{ m}^2$  in the ground area and  $1.64 \text{ m}^2$  in open-top area. It was expected that all of the selected plots were similar in microhabitat characteristics. The OTC installations were completed in late September 2008 and observations were initiated from late April 2009, providing roughly 8 months equilibrium period to minimize disturbance effects.

#### 2.2. Microclimates monitoring

To quantify the environmental factors affected by the OTC, two automatic recording systems were set up in both experimental sites, respectively. Air temperature at 30 cm high and soil temperature 5 cm below the soil surface were measured by the sensors (DS1921G-F5, Maxim Integrated Products, Dallas Semiconductor Inc., Sunnyvale, CA, USA) connected to a dataloger (Campbell AR5, Avalon, USA). Data were taken at 60 min intervals with the automatic recording system from early May to late October in 2009. Temperature monitoring was carried out in three OTCs and three control plots. Soil volumetric moisture 10 cm below the soil surface was measured with a hand-held probe (IMKO, Germany) at about 1-week interval.

#### 2.3. Soil sampling

Soil samples were collected from the topsoil (0–15 cm) early (May 2nd), mid (July 16th) and late (September 29th) in the growing season of 2009. Growing season was estimated via the phenological events of dominant species (dragon spruce). Five cores (3 cm in diameter, 0–15 cm deep) were randomly taken at each plot. The five soil cores from each plot were mixed to get one composite sample and delivered immediately to the laboratory for routine biological analysis. Each composite sample was passed through a sieve (4mm diameter), and any visible living plant material was manually removed from the sieved soil. The sieved soil was kept in the refrigerator at 4°C (less than 1 week) for microbial properties, available nutrients, dissolved organic matter and enzyme activity.

#### 2.4. Soil analysis

Available N (ammonium and nitrate) was extracted with a 2 M KCl extracting water solution. Ammonium and nitrate in extract was measured by colorimetry. Soil microbial biomass C (MBC) and N (MBN) were determined using the fumigation-extraction method (Vance et al., 1987). Soil extractable organic C and total N in the K<sub>2</sub>SO<sub>4</sub> extracts before and after the fumigation were quantified using a total C/N analyzer (Multi-N/C 2100, Analytik Jena AG, Germany). The released C and N were converted to MBC and MBN, respectively, using Kec: 0.45 and Ken: 0.45. Soil basal respiration late in the growing season was estimated using the methods described by Chen et al. (2000). The metabolic quotient was calculated by dividing the hourly basal respiration rate by the corresponding MBC.

Soil N mineralization rates were measured from in situ incubations using the buried bag technique (Adams et al., 1989). The incubations were performed using perforated PVC tubes (15 cm in height and 5 cm in diameter). Parafilm covered the top of each tube to avoid leaching of nitrate. The technique prevents plant uptake of mineralized nutrients but allows uptake by the microorganisms. The seasonal net N mineralization was expressed as the difference in inorganic N (nitrate and ammonium) in the soil before and after incubation in the bags.

Dissolved organic C (DOC) and total dissolved N (TDN) were extracted by the method of Jones and Willett (2006). In brief, 20 g fresh soil was extracted with 100 ml ultra-pure water in a centrifuge tube by shaking the mixture for 1 h on a reciprocal shaker, and then centrifuging it at 13,000 rpm for 30 min at 4 °C. The supernatant was filtered through a 0.45 µm glass fiber filter. The C and N in the extracts were also measured using a C/N analyzer (Multi-N/C

#### Table 1

Soil organic C (SOC), total N, P and K, C/N, and pH for soils in the plantation and in the natural forest.

Forest type	$TOC(gkg^{-1})$	Total N $(g kg^{-1})$	Total $P(gkg^{-1})$	Total K (g kg $^{-1}$ )	C/N	pH(H <sub>2</sub> O)
Plantation Natural forest	$\begin{array}{c} 50.24 \pm 3.83 \\ 145.02 \pm 14.87 \end{array}$	$\begin{array}{c} 2.95 \pm 0.44 \\ 9.56 \pm 1.19 \end{array}$	$\begin{array}{c} 0.65  \pm  0.03 \\ 0.67  \pm  0.07 \end{array}$	$\begin{array}{c} 14.16 \pm 0.39 \\ 11.31 \pm 0.51 \end{array}$	$\begin{array}{c} 17.03  \pm  1.05 \\ 15.16  \pm  0.87 \end{array}$	$\begin{array}{c} 6.19 \pm 0.47 \\ 5.85 \pm 0.65 \end{array}$

Data are means  $\pm$  SD, n = 4.

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