



Changes in soil microbial biomass carbon and enzyme activities under elevated CO₂ affect fine root decomposition processes in a Mongolian oak ecosystem

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ABSTRACT

The relationships between soil microbial properties and fine root decomposition processes under elevated CO₂ are poorly understood. To address this question, we determined soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N), enzymes related to soil carbon (C) and nitrogen (N) cycling, the abundance of cultivable N-fixing bacteria and cellulolytic fungi, fine root organic matter, lignin and holocellulose decomposition, and N mineralization from 2006 to 2007 in a Mongolian oak (*Quercus mongolica* Fischer ex Ledebour) ecosystem in northeastern China. The experiment consisted of three treatments: elevated CO₂ chambers, ambient CO₂ chambers, and chamberless plots. Fine roots had significantly greater organic matter decomposition rates under elevated CO₂. This corresponded with significantly greater SMB-C. Changes in the activities of protease and phenol oxidase under elevated CO₂ could not explain the changes in fine root N release and lignin decomposition rates, respectively, while holocellulose decomposition rate had the same response to experimental treatments as did cellulase activity. Changes in cultivable N-fixing bacterial and cellulolytic fungal abundances in response to experimental treatments were identical to those of N mineralization and lignin decomposition rates, respectively, suggesting that the two indices were closely related to fine root N mineralization and lignin decomposition. Our results showed that the increased fine root organic matter, lignin and holocellulose decomposition, and N mineralization rates under elevated CO₂ could be explained by shifts in SMB-C and the abundance of cellulolytic fungi and N-fixing bacteria. Enzyme activities are not reliable for the assessment of fine root decomposition and more attention should be given to the measurement of specific bacterial and fungal communities.

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1. Introduction

Fine roots of plants hold a key position in terrestrial carbon (C) cycling, as they account for one third of global annual net primary production and have relatively short longevity and fast decomposition rates (Jackson et al., 1997). During the last decade, an increasing number of studies have begun to examine the responses of fine root production and mortality to elevated CO₂ (Matamala and Schlesinger, 2000; Lukac et al., 2003; Handa et al., 2008). However, fine root decomposition draws less attention although it regulates the processes of soil organic matter formation, nutrient release rates, and CO₂ emission (Dilustro et al., 2001). Thus, understanding fine root decomposition patterns would be important for understanding the belowground C cycling processes.

Elevated CO₂ concentration can stimulate the production of rhizodeposition which affects soil microbial activity and therefore fine root decomposition processes (Cheng, 1999; van Groenigen et al., 2005; Allard et al., 2006). Previous studies showed that fine root decomposition did not have consistent responses to elevated CO₂ because of the variabilities in ecosystems, experimental designs, and lengths of study (Gorissen et al., 1995; Dilustro et al., 2001; King et al., 2005). However, most published studies on the effect of elevated CO₂ on decomposition of plant material have been undertaken using the microcosm incubation method (Cotrufo and Ineson, 1995; van Ginkel et al., 2000; Ross et al., 2002; van Groenigen et al., 2005), and their results may not be extrapolated to field situations. Field studies usually did not include measurement of soil microbial activity (Dilustro et al., 2001; Allard et al., 2004), thus, integrated approaches may improve our understanding of mechanism by which elevated CO₂ might influence fine root decomposition processes.

Soil enzymes play a key role in controlling the decomposition of soil organic matter and have been widely accepted as indicators of

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changes in belowground processes (Sinsabaugh et al., 1993, 2002). Elevated CO₂ influences the soil enzyme activities through altering the quantity and quality of plant-derived substrates entering the soil (Rogers et al., 1994; Zak et al., 2000). The responses of soil enzyme activities to elevated CO₂ have been explored in a number of studies (Larson et al., 2002; Finzi et al., 2006; Kandeler et al., 2006), but it remains unclear whether the changes in the activities of the soil enzymes responsible for the depolymerization of organic nitrogen (N) and labile and recalcitrant C compounds will cause corresponding changes in fine root decomposition processes. In addition, relatively few studies have explicitly considered the roles of soil bacteria and fungi in regulating fine root decomposition which are also important to understanding elevated CO₂ effect.

Mongolian oak (*Quercus mongolica* Fischer ex Ledebour) is a widely distributed tree species in northeastern China. It often forms large areas of pure Mongolian oak forests on semi-humid and infertile lands where the previous natural forests were heavily exploited (Li et al., 2001). We conducted an open-top chamber (OTC) experiment on the Mongolian oak seedlings to assess the effect of elevated CO₂ on fine root decomposition processes and soil microbial activities. Air temperature is higher in ambient CO₂ chambers than in chamberless plots, resulting in a chamber effect. We observed in previous studies in this ecosystem that soil heterotrophic respiration, fine root biomass, and rhizodeposition were greatly increased by elevated CO₂ and the chamber effect (unpublished data).

In this study, we used live fine roots collected from the chamberless plots as substrates in a decomposition experiment to 1) assess the impacts of elevated CO₂ and chamber on fine root organic matter mass loss, N mineralization rate, and lignin and holocellulose decay rates; 2) to determine the responses of soil microbial biomass carbon (SMB-C) and nitrogen (SMB-N), activities of enzymes related to the N mineralization and decomposition of lignin and holocellulose, cultivable N-fixing bacterial and cellulolytic fungal abundances to the elevated CO₂ and chamber effect; and 3) to test whether changes in these soil microbial parameters will result in corresponding changes in fine root mass loss, holocellulose and lignin decay, and N mineralization rates in the Mongolian oak seedling ecosystem.

2. Methods

2.1. Experimental site

The study was conducted at an OTC facility located at the Changbai Mountain Research Station, Chinese Academy of Sciences (42° 25' N, 128° 05' E). The elevation is 738 m above sea level, and the mean annual temperature and precipitation are 3.5 °C and 700 mm, respectively. In the spring of 2004, 10 hexagonal open-top chambers (OTC; diameter 4.4 m and height 4 m) were established at the experimental garden. Two year-old Mongolian oak seedlings were planted in each of 10 chambers and three chamberless plots established nearby immediately following establishment. The seedlings were from the same nursery and had the same genotype. The soil in the experimental garden is loamy sand. Each OTC facility has a centrally located intake line that monitored ambient atmospheric CO₂ concentration and a CO₂ or air dispensing line. An automatic control system (A-SENSE-D, SenseAir, 147 Sweden) recorded 3-min averages of CO₂ concentration every 30 min over a 24-h period and adjusted it to the desired CO₂ concentrations in each elevated CO₂ chamber (500 μmol) and ambient chamber (atmospheric CO₂ concentration) by regulating the influx rate of CO₂ or air. Chambers were fumigated continuously since installed in 2004. Air temperature (HMP45C, Vaisala, Helsinki, Finland) and

soil water content (TDR-CS616, Campbell, U.S.A) at 5 cm depth in each chamber or control plot were recorded at a 10 min intervals.

Treatments were chamberless plots, ambient CO₂ chambers, and elevated CO₂ chambers. Each treatment was replicated three times using a randomized complete block design (the other 4 chambers were not involved in this experiment). We examined the influence of elevated CO₂ by comparing elevated CO₂ chambers with ambient CO₂ chambers, while the chamber effect was assessed by comparing ambient CO₂ chambers with chamberless plots.

2.2. Fine root decomposition

Fine roots growing in the chamberless plots were used in the decomposition experiment. In May 2006, 45 Mongolian oak seedlings were dug out of the chamberless plots. The seedling roots were soaked in basins for 5 h to loose the clay adhered and then rinsed with water to remove the soil. Fine roots were defined as < 2.0 mm diameter. The data presented in this study are for live roots only, which were separated from dead roots based on color and consistency (e.g. intact, succulent cortex). Selected fine roots from chamberless plots were rinsed with distilled water and dried at air temperature to constant weight. Subsamples were taken to determine the air-dry to oven-dry factor (55 °C) and then reserved for chemical analysis. Approximately 1.00 g of air-dried fine roots was put into a 0.05-mm polyethylene mesh bag. The bag size was about 5 × 5 cm². In May 2006, 50 bags were inserted at circa 5 cm depth of the soils in each chamber and chamberless plot, yielding 450 bags in total. Bags were collected in July 2006, September 2006, May 2007, July 2007, and November 2007, corresponding to 61, 122, 365, 426, and 559 days following the start of the experiment. On each sampling day, 5 or more bags were collected from each chamber or chamberless plot. Soil particles adhering to the bags were carefully removed. The fine roots were dried at 55 °C to constant weight and weighed.

Dried samples were milled to pass through a 0.1 mm screen using plant material mill (LG-04A, Ruian Company, China) prior to chemical analysis. The concentrations of C and N were determined using element analyzer (Vario EL III, Elementar, Germany). Lignin and holocellulose concentrations in the samples were determined by sequential extraction and gravimetric analysis based on Chinese National Standard (GB/T 2677.8–1994; GB/T 2677.10–1995). Briefly, samples for the lignin assay were extracted with an ethanol: phenol solution. Then the samples were treated with 72% H₂SO₄ solution and digested at 100 °C. The supernatant was filtrated and the residues were rinsed with distilled water until there was no turbidity generated in the BaCl₂ solution. For holocellulose assay, samples were extracted with an ethanol: phenol solution. Extracts were treated with the mixture of distilled water, glacial acetic acid, and NaClO₂ and heated at 75 °C until they turned white. The supernatant was filtrated and the residues were rinsed with acetone solution three times. All the residues were dried at 105 °C to constant weight and weighed. Ash content was quantified using a muffle furnace. C, N, lignin, and holocellulose concentrations were calculated on an ash-free, dry-mass base.

2.3. Cultivable N-fixing bacteria and cellulolytic fungal abundances and enzyme assays

Six 3.5 by 10 cm soil cores were collected at random locations between stems from each of chambers or chamberless plots. The six samples were combined into one and sieved through a 2-mm mesh to remove roots and gravel, stored on ice, and transported to laboratory for analysis. Soils were sampled in late May 2006, July 2006, September 2006, June 2007, and August 2007.

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