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Response of Soil Microbial Biomass and Enzyme Activities Under Three Temperate Tree Species to Elevated CO_2 in Changbai Mountain, Northeastern China

Jun Qiang Zheng, Ying Wang and Shi Jie Han

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1. Introduction

Owing to fossil fuel combustion, deforestation, and intense agriculture, the concentrations of atmospheric CO_2 [CO_2] has risen by 100ppm since the mid 1800s [1], and it has been predicted to double until the end of this century compared to the pre-industrial value [2]. Numerous studies have shown a greater biomass gain of plants, higher fine root and leaf litter C/N in some species under elevated CO_2 condition [3–7]. Moreover, the rising CO_2 also could alter litter chemistry (e.g., total N, lignin and starch content) and fine root turnover. Because microbial growth is limited by the type and amount of organic substrates entering the soil [8, 9], the changes in above- and below-ground plant input under elevated CO_2 could potentially alter both the substrate availability and microbial activity. Although the effect of elevated CO_2 via plants on soil microorganisms has been few studies investigated [10–12], the detailed plant-mediated effects still are unclear because of the complexity of microbial processes.

Soil microorganisms play an important role in nutrient cycling, CO_2 emission and in formation of soil total organic carbon (TOC) pool. Therefore, any effect of the rising [CO_2] on soil microorganisms might in turn feedback on the response of terrestrial ecosystem to atmospheric CO_2 and the sequestration of extra carbon [9]. Soil enzymes drive soil organic matter decomposition and nutrient transformations. Soil enzyme also was considered as a sensitive indicator, which could be significantly affected by temporal variability [13]. It is evident that the seasonal patterns of temperature and moisture of north temperate ecosystems can affect the activity of soil enzymes [14]. Although several studies have investigated the effects of increased CO_2 on the soil microbial biomass and activity, to our knowledge, only relative

few studies have measured the seasonal fluctuations of microbial biomass and soil enzyme activity under higher CO_2 levels [10,11]. At the Oak Ridge FACE site, Sinsabaugh *et al.* (2003) found that soil extracellular enzyme activities, substrate utilization, gross N mineralization and denitrification were all not responsive to CO_2 enrichment [10]. In contrast in an agroforestry ecosystem with typical Mediterranean climate, Moscatelli *et al.* (2005) observed that all soil biochemical properties were significantly affected by the temporal variability, and the interaction between time and CO_2 level significantly influenced β-glucosidase activity and microbial respiration [11]. These studies have produced many conflicting results, with few common themes. Furthermore, the specific seasonal patterns of soil enzyme activities vary with soil properties and types of aboveground vegetations.

Temperate forest ecosystems, which occupy much of the earth's terrestrial surface area, have been considered as the most important C sink for sequestering the increasing atmospheric CO_2 [15]. To understand elevated CO_2 effects on temperate forest ecosystems, a growing number of free air CO_2 enrichment (FACE) and open-top chamber (OTC) research project have been initiated throughout the world. In 1998, we began a long term CO_2-enrichment experiment using open top chamber (OTC) growing three species of trees, Korean pine (*Pinus koraiensis* Sieb. *et* Zucc), Changbai pine (*Pinus sylvestriformis* (Takenouchi) T. Wang ex Cheng) and oak (*Quercus mongolicus* Fisch) in Changbai mountain, China [16]. This mountain ecosystem belongs to the temperate continental climate influenced by monsoon and has the prominent characteristics of mid-latitudinal upland climate. The soil type of this area is upland dark-brown forest volcanic soil [17]. The area has four obvious different seasons as windy spring, hot and rainy summer, cool autumn and cold winter. Our previous studies in this OTC site demonstrated that elevated CO_2 increase aboveground biomass and photosynthesis of the trees (Zhou and Han, unpublished data) and decrease total soil respiration [18]. These results were consistent with other reports from temperate forest ecosystems, e.g., Duke temperate forest FACE site [19]. To date, however, there is still a poor understanding of effects of elevated CO_2 on microbial activity of soil under different tree species.

The objectives of this study were (a) to investigate effects of elevated CO_2 on microbial biomass C/N and the variations in activities of various enzymes throughout the growing season, and (b) to compare the CO_2 response of activities of C, N and P cycling related enzymes in the soils under three different tree species.

2. Materials and methods

Experimental site, design and sampling

The experimental fields were located at Changbai Mountain in Jilin province, northeastern China (42º24′N, 128º06′E, and 738 m elevation). The soil is a dark-brown soil developed from volcanic ash. The topography is basaltic mesa, and the parent rock is loose volcanic ash sand. The mechanical composition of the soil is approximately 29% sand (20 μm − 2 mm), 40% silt (2 − 20 μm) and 31% clay (< 2 μm). Soil organic carbon in 0-10 cm layer is approximately 8.5%, and pH is 5.7 (1:2.5, soil: water). The ecosystem is temperate

with a mean annual temperature of 5°C and annual average precipitation of 967.3-1400 mm. A randomized complete block design of ambient and elevated CO_2 was established in an open-top chamber facility at the research station of Changbai mountain forest ecosystems, Chinese Academy of Sciences, in the spring of 1999. Eighteen open-top chambers (each 4.2m in diameter with hexagon and 4 m in height enclosed with a clear glass open-top chamber) were utilized to control CO_2 levels. Korean pine (*P. koraiensis Sieb. et Zucc*) and Changbai pine (*P. sylvestriformis*) seeds were prepared and sowed in May, 1999. CO_2 fumigation treatments began after seeds germination in May 1999. For oak (*Q. mongolica*) experiment, five-year old seedlings were transplanted into open top chambers in autumn 2004, and CO_2 enrichment started at the end of April, 2005. In each year, the exposure started at the end of April and stopped at the end of October (the whole growing season). Half of the chambers were maintained at ambient atmospheric CO_2 concentrations (ca. 350 ppm), others were maintained at elevated levels (ca. 500 ppm) by dispensing 100% CO_2 into the blower fans only in the daytime. Elevated CO_2 concentrations were maintained by continuously monitoring CO_2 concentrations in elevated and ambient-level chambers with an infrared gas analyzer (A-SENSE-D, SenseAir, Sweden) by a computer control system that recorded 10-second averages of CO_2 concentration every 3 minutes and then periodically adjusting the flow of 100% CO_2 into the chambers.

Soil samples were collected seven times: May, June, August and September in 2006, and May, July and September in 2007. At each sampling date, five soil cores (3 cm in diameter and 0–10 cm at deep) were collected within each chamber. The pooled samples were homogenized and roots removed by passing the soil through a 2-mm sieve. The samples used for measurements of soil enzyme activity were kept frozen (-70°C) and microbial biomass were kept cool (4°C) until analysis within 1 week after sampling.

Soil microbial biomass analysis

Subsamples of soil were dried at 105°C for 12 h to determine gravimetric water content. Soil pH was measured in solutions of 50 ml water and 10 g air-dry soil. Microbial biomasses C (C_{min}) and N (N_{mic}) were measured by fumigation-extraction method [20]. Subsamples of sieved soil were fumigated with alcohol-free $CHCl_3$ for 24 h, and then extracted with 0.5 M K_2SO_4 solution. The K_2SO_4 soil extract was analysed for total dissolved organic C (DOC) and total dissolved N (TDN) using a Total Organic Carbon Analyzer (multi N/C 3000, Jena, Germany). Microbial biomass C and N were calculated as differences in extractable DOC and TDN between fumigated and unfumigated soils using a correction factor (Kc) of 0.38 for C_{min} and 0.54 for N_{mic} [20, 21].

Soil enzyme activity analysis

Enzyme activities were determined following Freeman *et al*. (1995) and Kang & Freeman (1999) [22, 23]. We used fluorogenic substrates, 4-methylumbelliferyl-linked (MUB) compounds as substrate analogues (Table 1). Briefly, approximately 0.1 g fresh mass, were homogenized in 1.8 ml of 50 mM tris-maleate buffer (pH 5.0) and 0.2 ml of substrate solution were combined in a 5 ml polypropylene tube. For each sample, there were four analytical replicates plus negative controls (sample plus 4-MUB and buffer, separately) for both sam-

ple and substrate color. The tubes were tumbled for 1 h at 30°C, except for phosphatase, which was incubated for 30min. The assay was terminated by adding 2 ml ice cold 96% ethanol. The tubes were centrifuged for 5 min at 4°C (6000 r.p.m.). Fluorescence was determined with a HITACHI 650-60 Fluorescence spectrophotometer at 446 nm emission and 377 nm excitation wavelength. The increase of fluorescence was converted to enzymatic activity with a standard curve was drawn using a series of known concentrations of 4-methylumbellife. Enzyme activities are expressed in units of nmol or µmol of substrate converted per hour per g soil dry mass (nmol h^{-1} g^{-1} or µmol h^{-1} g^{-1}).

Enzyme	Abbreviation	EC	Substrate
1,4-β-glucosidase	bG	3.2.1.21	4-MUB-β-D-glucoside
1,4-α-glucosidase	aG	3.2.1.20	4-MUB-α-D-glucoside
1,4-β-Acetylglucosaminidase	NAG (1,4-β-NAG)	3.1.6.1	4-MUB-N-acetyl-β-D-glucosaminide
Cellobiohydrolase	CBH	3.2.1.91	4-MUB-β-D-cellobioside
1,4-β-xylosidase	bX	3.2.1.37	4-MUB-β-D-xyloside
Phosphatase	PA	3.1.3.2	4-MUB-phosphate
Phenol oxidase	PPO	1.10.3.2	L-3,4-Dihydroxyphenylalanine
Peroxidase	PO	1.11.17	L-3,4-Dihydroxyphenylalanine

EC: enzyme commission number; MUB: 4-methylumbelliferyl; Abbreviation: used in this article

Table 1. Enzyme assays conducted on soil samples collected from the open top chamber in this study.

For the phenol oxidase and peroxidase activities assay, l-3,4-dihydroxyphenylalanine (L-DOPA) was used as substrate [24]. We prepared soil slurry solutions of 5.0 g soil in 100 ml of 50 mmol l^{-1} acetate buffer (pH 5.0.) The reaction mixture for the phenol oxidase assay, containing 2 ml, 5 mM l-3,4-dihydroxyphenylalanine (L-DOPA) solution and 2 ml of soil slurry in 5 ml tube, was vortexed for exactly 60 min at 20°C in a shaking incubator and was centrifuged for 5 min at 4°C (6000 r.p.m.). The absorbance of the filtrate was read at 460 nm. For peroxidase activities assay, at the beginning of the incubation, were processed in the same way as phenol oxidase, with L-DOPA substrate and the addition of 200 µl of 0.3 % H_2O_2 [25]. Phenol oxidase activity was subtracted from peroxidase activity to calculate the net peroxidase activities.

Statistical analysis

Data were analyzed using repeated measures analysis of variance (RM-ANOVA) with CO_2 treatment, plant species and their interaction as explaining variables. Prior to analysis, the data was checked for heterogeneity of variance, and when necessary, the variable was transformed to improve normality. RM-ANOVA analyses were performed with SPSS 13.0 statistical software package (SPSS Inc.). Correlation analysis was used to test for correlations between microbial biomass C, N and soil enzyme evaluated as significant at $P<0.05$. Correla-

tions among enzyme activities and microbial biomass as well as dissolve organic C and N were explored using a principal components analysis on the correlation matrix. Principal component analyses (PCA) and canonical discriminate analysis were performed with the STATISTICA 6.0 software package (StatSoft Inc.).

3. Results

Elevated CO_2 significantly decreased the activities of 1,4-β-NAG, 1,4-β-xylosidase, phosphatase, 1,4-β-glucosedase, and phenol oxidase in soil under Changbai pine in 2007 (Fig. 1). On all sampling dates, there was no CO_2 main effect on soil enzyme activity across three tree species (Table 2). Time was a significant factor affecting the activities of hydrolotic enzymes and the two oxidase enzymes in soil (Fig. 1, Table 2). The activity of 1,4-β-NAG and phenol oxidase over the two years for all sample dates showed an interaction between tree species and CO_2 ($P < 0.01$), suggesting the responses of these two enzymes to CO_2 enrichment were dependent on aboveground vegetation. Interactions between CO_2 treatment and sampling time were significant for CBH, peroxidase and phenol oxidase activities.

	βG	αG	βNAG	CBH	βX	PA	PPO	PO
Tests of Between-Subjects Effects								
CO_2	2.69[NS]	0.70[NS]	4.06[NS]	0.63[NS]	0.06[NS]	2.59[NS]	1.75[NS]	<0.01[NS]
Species	10.97**	4.03[NS]	35.05***	2.45[NS]	120.13***	32.09***	88.93***	410.14***
Species ×CO_2	1.90[NS]	1.04[NS]	5.28*	2.10[NS]	0.39[NS]	2.94[NS]	6.69*	3.72[NS]
Tests of Within-Subjects Effects								
Time	8.89***	2.92*	1.90[NS]	4.11**	15.19***	5.85***	12.65***	37.86***
Time × Species	7.43***	2.97*	5.84***	2.97**	7.33***	5.06***	10.88***	33.40***
Time × CO_2	1.99[NS]	0.85[NS]	2.02[NS]	2.53*	0.82[NS]	1.52[NS]	6.72***	6.02***
Time ×species × CO_2	5.69***	1.16[NS]	2.66*	3.21**	0.60[NS]	2.19*	1.91*	5.03***

Note: *F* values are displayed and *, ** and *** represent significane at *P*< 0.05, 0.01 and 0.001 level, respectively. NS represents no significant effect.

Table 2. *F*-values from RM-ANOVA showing effects of elevated CO_2 and time on soil enzyme activity.
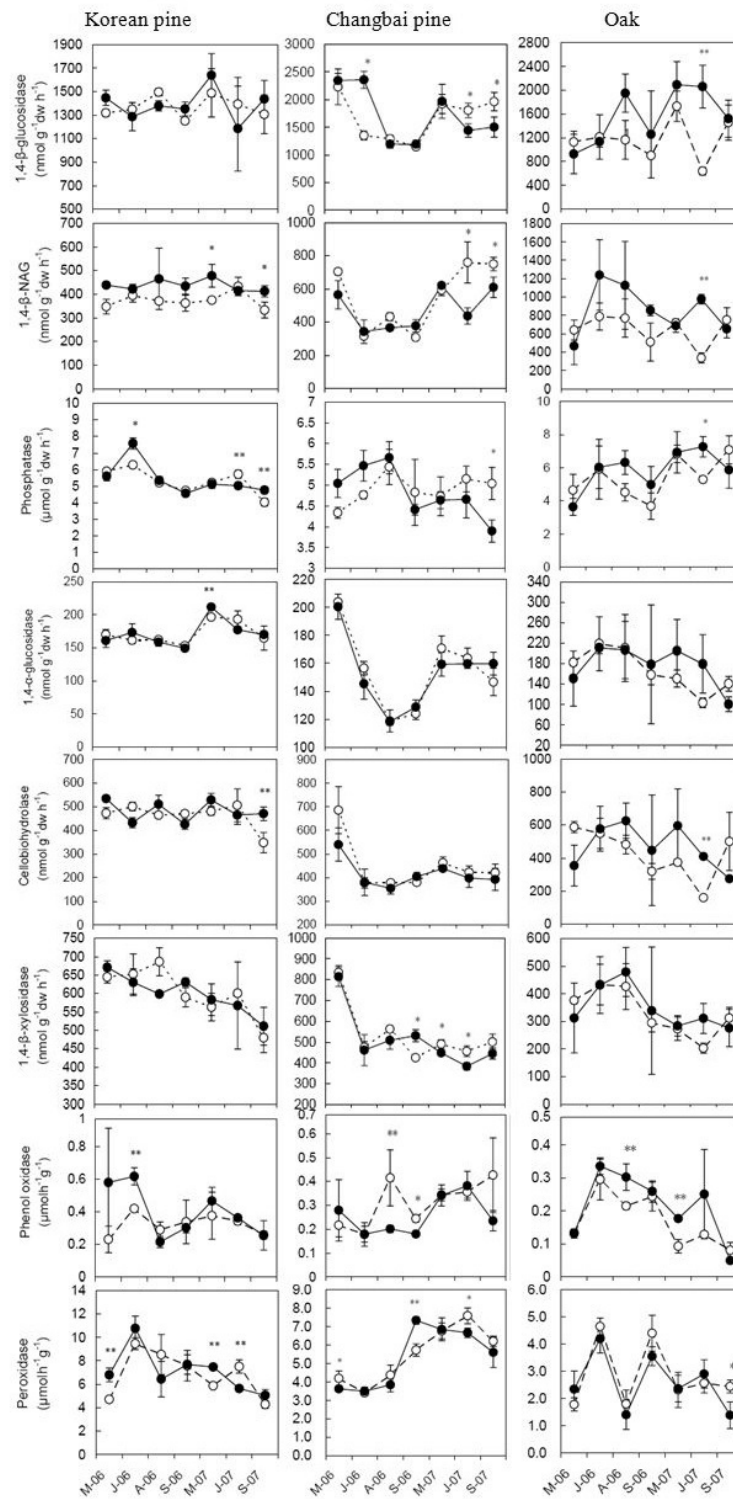
Figure 1. Variations in activities of soil enzymes under Korean pine, Changpai Scotch pine and oak after exposure to ambient (open symbols) and elevated (solid symbols) CO_2 concentration. Error bars represent standard errors of the means; asterisks represent significant differences, e.g. * $p < 0.05$, ** $p < 0.01$.

Correlation analysis indicated that soil moisture was positively correlated with 1,4-α-glucosidase. The TDN content under Korean pine was negatively correlated with DOC (r = -0.6166, P< 0.01) and positively correlated with phosphatase (r = 0.5300, P< 0.01) and peroxidase (r = 0.6559, P< 0.01). TDN from Changbai pine was negatively correlated with DOC (r = -0.7242, P< 0.01), peroxidase (r = -0.7274, P< 0.01) and phenol oxidase (r = -0.5289, P< 0.01), positively correlated with xylosidase (r = 0.6849, P< 0.01).

Mean enzyme activity decreased from highest to lowest in the following order: PPO > AP > βG > βX > CBH > NAG > PO > αG in the Korean pine soil. Mean enzyme activity in the Changbai pine soil decreased from highest to lowest in a very similar order with Korean pine: PPO > AP > βG > βX > NAG > CBH > PO > αG, therefore, tree species is a important factor influencing soil enzyme activities, there were significant interactive effects between species and sampling time.
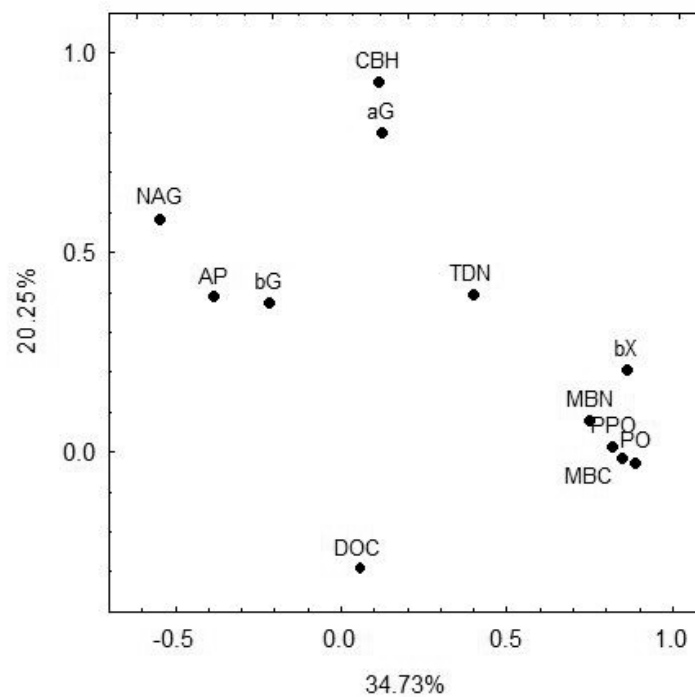

Figure 2. Factor plot from a principal components analysis of soil enzyme activity data for Korean red pine, Changpai Scotch pine and oak exposed to two levels of atmospheric CO$_2$. PO: peroxidase, PPO: phenol oxidase, AP: acid phosphatase, αG: 1,4-α-glucosidase, CBH: cellobiohydrolase, bX: 1,4-β-xylosidase, NAG: 1,4-β-acetylglucosaminidase, bG: 1,4-β-glucosidase, MBC: microbial biomass C, MBN: microbial biomass N, DOC: dissolve organic C, TDN: total dissolve N.

Soil pH was slightly acidic (5.4–6.1) and did not vary significantly between treatment or sampling dates, and varied among species. Multivariate statistics were used to assess the functional diversity and temporal vary of the soil microbial enzyme. In the principal component analysis of the data of all eight enzyme activities, PC1 and PC2 explained 20.25% and 34.73% of the total variance, respectively (Fig. 2). The first factor (PC1) appears to be associated with labile nutrient acquisition, the second factor (PC2) appears to be associated with

lignocelluloses degradation. For the canonical discriminant analysis of the soils under three trees sampled in 2006 and 2007, the following variables were determined: 1,4-β-xylosidase, 1,4-α-glucosidase, 1,4-β-NAG, cellobiohydrolase, 1,4-β-xylosidase, phosphatase, phenol oxidase, peroxidase. Canonical discriminant analysis also showed that the effect of the tree species on soil enzymes during the two experimental years (Fig. 3). Root 1 seems to discriminate mostly between pines and oak (means of the canonical variables: -2.005 and 4.010, respectively). In the vertical direction (Root 2), seems to discriminate the two pines (means of the canonical variables: Korean pine -1.005, Changbai pine 1.264). Correlation analysis indicated that soil moisture was positively correlated strongly with 1,4-α-glucosidase, microbial biomass C and N across three species of tree (Table 3). Peroxidase activity showed strong correlations with N_{mic} (r = 0.54, $P < 0.01$) and C_{mic} (r = 0.81, $P < 0.01$), phenol oxidase activity showed a high correlation with N_{mic} (r = 0.48, $P < 0.01$) and C_{mic} (r = 0.63, $P < 0.01$).
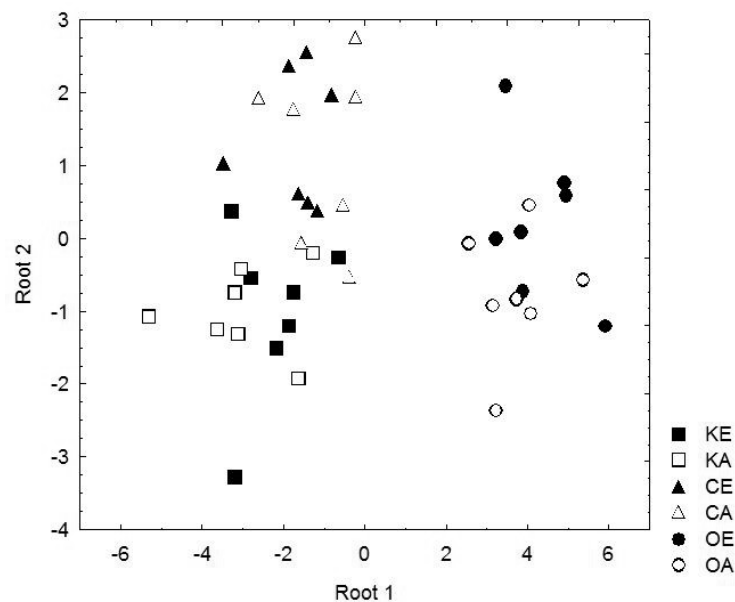

Figure 3. Canonical variates of the microbial properties in the soils under Korean pine, Changpai Scotch pine and oak. KE, Korean pine under elevated CO_2; KA, Korean pine under ambient CO_2; CE, Changpai Scotch pine under elevated CO_2; CA, Changpai Scotch pine under ambient CO_2 ;OE, oak under elevated CO_2; OA, oak under ambient CO_2.

4. Discussioin

Effect of CO_2 concentrations on soil enzyme activities

Elevated atmospheric CO_2 can lead to an increase in the size of the substrate pool utilized by soil microbes and to stimulate the activities of soil enzyme [26]. For example, Larson *et al.* (2002) and Lipson *et al.* (2005) found a significant stimulation of soil extracellular enzyme activities under elevated CO_2 [27, 28]. However, our results, along with other studies [29, 10] have not detected significant increase in C-cycling enzymes across the course of experiment, and only significant at specific time was observed with a higher mean value under elevated

CO$_2$. Furthermore, the significantly different sampling time point was independent among three types of trees. These results suggest that response of soil enzymes to long-term CO$_2$ fumigation may be dependent on soil environment and aboveground vegetation type.

1,4-β-NAG is one of the enzymes regulating nitrogen availability in soil, the activity is often used as an indicator of N demand by microbes. Its enhancement under elevated CO$_2$ (av. +18%) in our study of Korean pine reflected a microbial demand for N nutrient. It is interesting to note that the activities of cellobiohydrolase, 1,4-β-NAG, 1,4-β-xylosidase, phenol oxidase and microbial biomass were decreased significantly in soil of Changbai pine under elevated CO$_2$ in summer 2007, which can be observed also from mean activity of the enzymes and microbial biomass in 2007. Ebersberger *et al*. (2004) also found a significant reduce in proportion of fungi indicator of PLFA at elevated CO$_2$ in comparison to ambient CO$_2$ [30]. Finzi *et al*. (2006) proposed that there is increasing evidence that microbial function is progressively N limited under elevated CO$_2$ as duration elongation of CO$_2$ enrichment [31]. The lower microbial activity in the Changbai pine exposed to elevated CO$_2$ may be attributed to the greater competition for N nutrient by rapidly growth of the tree [32].

Tree species effects on soil enzyme under elevated CO$_2$

The ecophysiological responses of the three species to elevated CO$_2$ were significant different (Zhou and Han, unpublished data). The fast-growing Changbai pine tends to show larger growth increases under elevated CO$_2$ than the slow-growing Korean pine, but the biomass was stored in stem and branch. Many studies showed that above-ground cover plant could determine the composition of the soil microbial community structure and function [33 –35]. In our study, discrimination between the pines and the oak was observed (Fig. 3), but these results must be careful explained, because of variation in duration of elevated CO$_2$ between the pines and oak. Changbai pine tends to possess characteristics promoting rapid growth generally associated with high competitive ability. This can explain why elevated CO$_2$ caused a decrease in microbial biomass and extracellular enzyme activity under Changbai pine (Fig. 1), due to the increase in competition for mineral nutrients with microorganisms. We speculate that fast-growing plant may be strongly affect soil N and P cycling compared to the slow-growing plant under elevated CO$_2$ condition, contributing disadvantage to soil microbial community as well as significant declining the activities of microbial enzyme. These results also imply that tree species might also differ in their influence on soil microbial activity that in turn affects soil properties under elevated atmospheric CO$_2$ concentration.

Due to Korean pine has a slower growth rate and related physiological characteristics, the tree shows a narrower response to resource levels; whereas Changbai pine has a faster growth rate related to Korean pine shows a broad response to N and P nutrient. Hungate *et al*. (1997) observed a species-specific growth response of species to elevated atmospheric CO$_2$, that could either increase or decrease soil-N availability [36]. Schimel and Bennett (2004) reported fast-growing species grew significantly better and took up more of the available N and P in the elevated CO$_2$ condition [32]. Fast-growing Changbai pine required more canopy space and more soil volume than the Korean pine and showed a relative higher de-

mand for soil N, as the high-to-low order of 1,4-β-NAG and CBH shown, compared with CBH-to-1,4-β-NAG order in Korean pine.

Seasonal and temporal dynamic of the activities of soil enzymes under elevated CO_2

Temporal fluctuation of soil moisture, soil temperature, and C input from tree roots, rhizosphere products (e.g., root exudates), and tree residues can have large effects on soil microbial biomass and activity [38]. Most of the enzymes measured in our study (table 2), were significantly affected by the temporal variability, and the interaction between time and CO_2 level significantly influenced CHB, phenol oxidase and peroxidase activities across the three species. Our data also revealed a strong positive correlation between soil moisture and microbial biomass or soil enzyme activity (Table 3), especially the activity of 1,4-α-glucosidase that correlated with soil moisture independent on tree species. These results are consistent with Devi and Yadava (2006), who reported that microbial biomass showed a positive significant correlation with soil moisture [39]. Soil moisture may be a master variable controlling microbial biomass and mineralization of starch (catalyzed by 1,4-α-glucosidase) in this temperate volcanic soil (Table 3).

	Korean pine	Changbai pine	Oak
1,4-β-glucosidase	0.0993	**0.6063***	0.3779
1,4-α-glucosidase	**0.5620***	**0.6385***	**0.6419***
1,4-β-NAGase	0.0551	0.3233	0.5305
Cellobiohydrolase	0.4239	0.3061	**0.5417***
1,4-β-xylosidase	0.2733	0.2320	**0.5856***
Phosphatase	**0.6050***	0.1812	0.3672
Phenol oxidase	0.4411	0.2139	0.2976
Peroxidase	0.2568	-0.2690	-0.1960
Microbial biomass C	0.5843	**0.7042***	0.5018
Microbial biomass N	0.4207	**0.9528****	0.6033

Levels of significance of the coefficients are indicated for $P < 0.05$* or $P < 0.01$**.

Table 3. Correlation analysis of extracellular enzyme and moisture of the soil under Korean pine, Changbai pine and oak after fumigation with elevated CO_2 concentration in 2006 and 2007.

Tree growth often stimulates an increase in the size of microbial biomass during the growing season [40, 41]. The seasonal variations in 1,4-β-glucosidase of Changbai pine we observed were consistent with the seasonal variations in 1,4-α-glucosidase, 1,4-β-NAG, and cellobiohydrodase activity (e.g., greatest activity in spring), which were in agreement with a report of study in a oak forest in USA [42]. Seasonal fluctuations of these enzymes might be strongly influenced by life cycle of Changbai pine. Because spring and early summer is the fast-growing period of the pine, the developing root system could provide enough of easily

mineralizable substrate to microorganisms and exhibited a positive effect on their activities. So for Changbai pine, the peak activity of soil microbial community was detected in spring. Seasonal patterns of soil enzyme were controlled by growth also observed from oak, which growth began from mid of May to end of October. This indicates that the activity of soil enzyme may be concomitantly controlled by plant seasonal growth.

In addition, differently seasonal pattern of identical enzyme from elevated and ambient CO_2 was also found, e.g., cellobiohydrolase from Korean pine, 1,4-β-NAG from Changbai pine, 1,4-β-glucosidase and 1,4-β-NAG from oak. This may be due to soil extracellular enzymes can be either induced by the substrate [43], which are often altered by elevated CO_2 [44], and influenced by soil moisture, or controlled by combine effect of there factors and/or other environmental factors [13], e.g., tree species, duration of CO_2 enrichment. Differences between the two coniferous pine and the broadleaf oak results from OTC may be best accounted for by the different seasonal patterns of soil microbial biomass and enzyme in respond to tree species and elevated CO_2. So our results confirm the importance of taking into account the seasonal variation of biochemical parameters when these are used as indicators of soil ecosystem in response to elevated CO_2.

In conclusion, seasonal variations are the factor mostly affecting soil biological properties and nutrients availability in Changbai mountain forest ecosystem. Long-term exposure of elevated CO_2 can alter microbial biomass and the production of enzymes, but the effects are always detectable at specific times and are closely linked to plant processes, soil moisture and aboveground vegetations. Hence, a single sampling of the soil may not fully reveal its response to prolonged elevated atmospheric CO_2. We proposed that it is imperative to assess microbial function for soil ecosystem with one or two year-round sampling regime.

Acknowledgements

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