Research Paper
J. Ecol. Field Biol. 33(2): 133-139, 2010
DOI: 10.5141/JEFB.2010.33.2.133

JEFB Journal of Ecology and Field Biology

Effects of elevated CO_2 on growth of *Pinus densiflora* seedling and enzyme activities in soil

Sunghyun Kim[1], Soohyun Jung[2], Hojung Kang[3] and Insook Lee[1,*]

[1]Division of EcoScience, Ewha Womans University, Seoul 120-750, Korea
[2]Department of Environmental Science and Engineering, Ewha Womans University, Seoul 120-750, Korea
[3]Department of Civil and Environmental Engineering, Yonsei University, Seoul 120-750, Korea

Atmospheric CO_2 concentrations have increased exponentially over the last century and, if continued, are expected to have significant effects on plants and soil. In this study, we investigated the effects of elevated CO_2 on the growth of *Pinus densiflora* seedling and microbial activity in soil. Three-year-old pine seedlings were exposed to ambient as well as elevated levels of CO_2 (380 and 760 ppmv, respectively). Growth rates and C:N ratios of the pine seedlings were also determined. Dissolved organic carbon content, phenolic compound content, and microbial activity were measured in bulk soil and rhizosphere soil. The results show that elevated CO_2 significantly increased the root dry weight of pine seedling. In addition, overall N content decreased, which increased the C:N ratio in pine needles. Elevated CO_2 decreased soil moisture, nitrate concentration, and the concentration of soil phenolic compounds. In contrast, soil enzymatic activities were increased in rhizosphere soil, including β-glucosidase, N-acetylglucosaminidase and phosphatase enzyme activities. In conclusion, elevated CO_2 concentrations caused distinct changes in soil chemistry and microbiology.

Key words: elevated CO_2, enzyme activity, *Pinus densiflora,* soil

INTRODUCTION

At their current rate of increase, CO_2 levels are expected to double to 750 ppm by the end of this century. Elevated CO_2 significantly influences both soil nutrient availability and soil microbes that are associated plants (Janus et al. 2005). Numerous studies that have investigated the effects of elevated CO_2 on plants found that elevated CO_2 increases the growth rate of plants (Gifford 1994, Naidu et al. 1998) while significantly affecting physiological function (Melillo et al. 1990). In addition, elevated atmospheric CO_2 causes an increase in the C:N ratios of plants by reducing the N concentration (Berntson and Bazzaz 1996). Such results were due to changes in the level of Rubisco or respiratory proteins, or to the dilution of nitrogen resulting from the accumulation of non-structural carbohydrates. Sims et al. (1998) recently reported that nitrogen levels are higher in plants under elevated CO_2. However, Saxe et al. (1998) found that elevated CO_2 had no effect on the nitrogen or carbon content of oak and beech seedlings. These results suggest the level of nitrogen in plant tissue exposed to elevated CO_2 is species-dependent.

Enzymatic changes under elevated CO_2 can alter the microbial demand for N and therefore the flow of N between soil microorganisms and plant roots (Zak et al. 2000). This in turn alters the overall chemical composition of plants as well as the types of organic substrates available for microbial metabolism (Finzi et al. 2006). Elevated CO_2 can also increase microbial activity in the soil (Hungate et al. 1996) mainly by providing extra C sources for rhizospheric microorganisms (Zak et al. 2000). Soil

Received 17 November, 2009, **Accepted** 5 April, 2010
***Corresponding Author**
E-mail: islee@ewha.ac.kr
Tel: + 82-2-3277-2375

microbial activity can be measured in terms of CO_2 production or based on the activities of major metabolic enzymes (Klose et al. 2003).

The responses of plants and microorganisms to elevated CO_2 vary according to the plant species (Cotrufo et al. 1998). While much information is available on the effect of elevated CO_2 on trees, little is known about how pine seedlings are affected. Since pine trees are a dominant tree species in eastern Asia, we believe that such information would be highly valuable.

The purpose of this study was to investigate the growth of pine seedling as well as soil microbial activity in response to elevated CO_2 in a growth chamber. We report on the impact of increased CO_2 on the root and shoot growth, biomass (dry weight) and C:N ratio of *Pinus densiflora*, emphasizing the interdependency of soil chemistry and microbiology, soil moisture and plant growth.

MATERIALS AND METHODS

Experimental design

Natural soil was sampled from the pine forest on the Ewha Woman's University campus in Seoul, Korea. Soil samples (1 kg/pot, diameter 10 cm) were used for the planting of three-year-old pine seedlings (*P. densiflora*), which were obtained from the Korean forest service. The plants were incubated for 12 months in growth chambers (Dasol scientific Co., Hwaseong, Korea) under 380 or 760 ppmv CO_2. The growth chamber was controlled at 25°C and 60% humidity, then subjected to a 16 h light/8 h dark cycle. The CO_2 concentrations were chosen based on Intergovernmental Panel on Climate Change (IPCC) reports (2007) that found the concentration of CO_2 in the atmosphere during the 21st century will rise to 700 ppm, which is double the current concentration. Further, 30 mL of water and 20 mL of 1/2 nutrient solution (KNO_3 606.6 6 mg/L, $Ca(NO_3)_2 \cdot 4H_2O$ 944.60 mg/L, $NH_4H_2PO_4$ 115.02 mg/L, $MgSO_4$ 492.94 mg/L, $FeCl_2 \cdot 7H_2O$ 492.94 mg/L, $MnCl_2 \cdot 4H_2O$ 1.78 mg/L, H_3BO_3 2.84 mg/L, $ZnSO_4 \cdot 7H_2O$ 0.23 mg/L, $CuSO_4 \cdot 5H_2O$ 0.075 mg/L) were added to the soil one time per week. Every 4 months, all the aboveground and underground parts were harvested. The shoot was separated and the roots were collected from the soil by washing. All plant parts were oven-dried before measuring dry matter at 80°C for 12 hours. Then, the shoot, root dry weight, and C, N content were measured. After incubation for 12 months, dissolved organic carbon (DOC) and enzyme activities were measured after bulk and rhizosphere soil were divided. The bulk soil was that remaining after the roots were picked from the pot. The rhizosphere soil was that which still adhered to the roots after gentle shaking.

Pine seedling growth and C:N ratio analysis

The pine seedlings were planted in a growth chamber at 25°C and 60% humidity, and were subjected to a 16 h light/8 h dark cycle. Shoot, root length, and biomass (dry-weight) were measured every four months. All tests were performed in triplicate. Percent dry weight of N and C content were estimated from leaf and root powder using a Flash EA 1112 Analyzer (Thermo Electron Corporation, Waltham, MA, USA).

Soil characteristics

Soil pH was determined by adding soil to water at a ratio of 1:5 (w:v). Soil moisture was determined gravimetrically by drying at 105°C for 24 hours, and organic matter content was determined by loss on ignition at 700°C (MAS 7000 oven; CEM, Mattews, NC, USA). Soil cation-exchange capacity was determined according to EPA 9081 methods (US Environmental Protection Agency 1986). Soil nitrate (NO_3^-) content was determined by extracting soil with deionized water and then measuring NO_3^- content in the liquid phase using an NO_3^- electrode (Gelderman and Beegle 1998).

Analysis of dissolved organic carbon and phenolic compounds in soil

To measure the concentration of DOC, soil was added to water at a ratio of 1:10 (w/v) after which the DOC content was measured using a TOC 5000 (Shimadzu Co., Kyoto, Japan) meter. Specific UV absorbance (SUVA) reveals the nature or quality of DOC in a given sample and is used as a surrogate measurement of DOC aromaticity (Chin et al. 1994). SUVA was measured at 254 nm ($SUVA_{254}$) due to the strong absorption of natural organic matter at this wavelength. This value correlates strongly with the aromatic carbon content of organic matter (Chin et al. 1994). Phenolic compound content was assayed using Folin-Ciocalteau phenol reagent (Box 1983). One milliliter of sample was added to 1.5 mL of Na_2CO_3 solution (50 g/L). Then, 0.5 mL of Folin-Ciocalteau solution (diluted 1/4 with deionized water; 0.5 N) was added, followed by incubation of the mixture for 2 hours at room temperature. A standard curve was prepared by applying

the same chemicals to a series of 0 to 2 mg/L phenol solutions. The color change of reactants was measured spectrophotometrically at 750 nm. Once out of range of the standard curve, the samples were diluted with distilled water and the procedure was repeated. Phenol oxidase activity was determined using 10 mM L-dihydroxyphenylalanine solution as a substrate, according to Pind et al. (1994).

Analysis of microbial activity in soil

The activities of four extracellular enzymes (β-glucosid ase, N-acetylglucosaminidase, phosphatase, and arylsulfatase) were measured by the MUF-substrate method (Freeman et al. 1996). The concentrations of the MUF-β-glucoside, MUF-N-acetylglucosamine, and MUF-arylsulfate substrate solutions were 400 μM (Sigma, St. Louis, MO, USA) while the concentration of the MUF-phosphate substrate solution was 800 μM (Sigma). Enzyme activities in a slurry containing soil and substrate solution (1:5 w/v) were measured using a fluorimeter. Dehydrogenase activity was measured by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) assay (Tabatabai 1982). Mixtures of soil (3 g fresh soil) and substrate solution were incubated for 24 hours at 37°C after which the reaction products were detected using a spectrophotometer (DR/3000 Spectrophotometer; HACH, Mount Holly, NJ, USA) at 485 nm.

Statistical analysis

Data were analyzed by one-way ANOVA using SPSS ver. 9.0 (SPSS Inc., Chicago, IL, USA). Tukey's test after one-way ANOVA was used to determine significance differences in soil parameters and soil enzyme activities in each sample. Growth, biomass, DOC, SUVA, phenolic compounds and phenol oxidase activity were tested for significance by a t-test between ambient and elevated CO_2 ($P < 0.05$).

RESULTS

The effects of elevated CO_2 on the biomass and C:N ratios of pine

Although root and shoot elongation were not significantly affected by elevated CO_2, the root dry weight of pine under elevated CO_2 was increased ($P < 0.05$) (Fig. 1). C:N ratios of pine needles were increased under elevated CO_2 (Table 1) as well. However, no difference in C:N ratios were observed in roots.

Comparison of soil physical and chemical characteristics

The physicochemical parameters of the soils are listed in Table 2. Under elevated CO_2, both NO_3^- and water content were decreased in soil. The soil pH was found to be mildly acidic (6.3-7.1) while the organic matter content ranged from 2.3 g/kg to 3.5 g/kg.

Various levels of dissolved organic carbon, aromatic and phenolic compounds in soil

Elevated CO_2 had no effect on DOC concentration in soil (Fig. 2a). However, the composition of DOC did appear to be influenced. Under elevated CO_2, the proportion of aromatic material in DOC (estimated by $SUVA_{254}$) was increased in bulk soil but decreased in rhizosphere soil (Fig. 2b). In contrast, phenolic compound content was decreased in both rhizosphere and bulk soil (Fig. 2c).

Table 1. Carbon concentration, Nitrogen concentration, and the C:N ratio of *Pinus densiflora* grown under ambient or elevated atmospheric CO_2

Soil sample	Root				Leaf			
	C (%)	N (%)	C:N	Difference	C (%)	N (%)	C:N	Difference
C - 4M	48.6	1.2	40.5	NS	51.4	1.3	39.5	**
E - 4M	47.5	1.2	39.6		50.1	1.1	45.5	
C - 8M	46.9	1.2	39.1	NS	50.7	1.3	39.0	**
E - 8M	47.0	1.2	39.2		51.7	1.0	51.7	
C - 12M	47.7	1.2	39.8	NS	50.3	1.5	33.5	**
E - 12M	45.5	1.2	37.9		50.6	0.9	56.2	

Significant t-test compared with ambient and elevated CO_2 concentration.
C, CO_2 380 ppmv; E, CO_2 760 ppmv; 4M, after 4 months; 8M, after 8 months; 12M, after 12 months; NS, $P > 0.05$.
** $P < 0.01$.

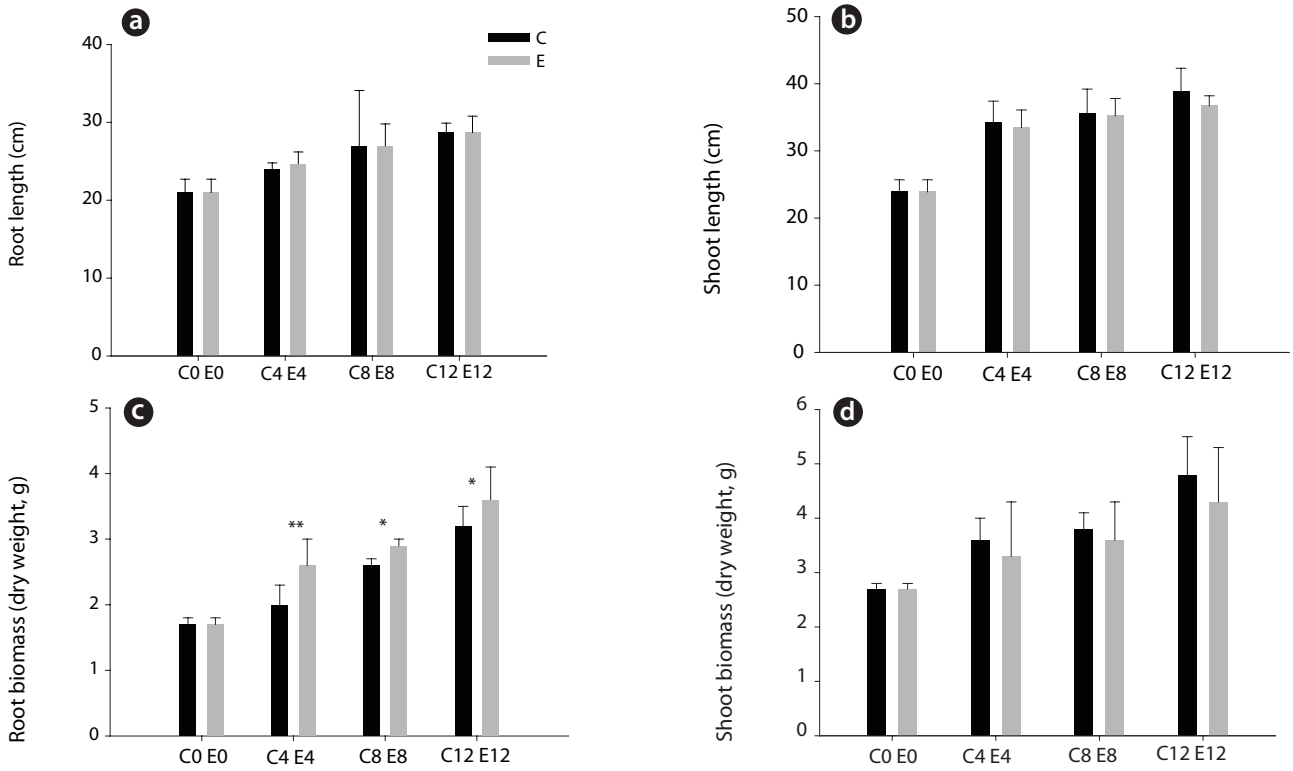

Fig. 1. Root, shoot length and biomass of pine seedling under ambient and elevated CO_2. (a) Root length, (b) shoot length, and (c) biomass. C, CO_2 380 ppmv; E, CO_2 760 ppmv; 4M, after 4 months; 8M, after 8 months; 12M, after 12 months.

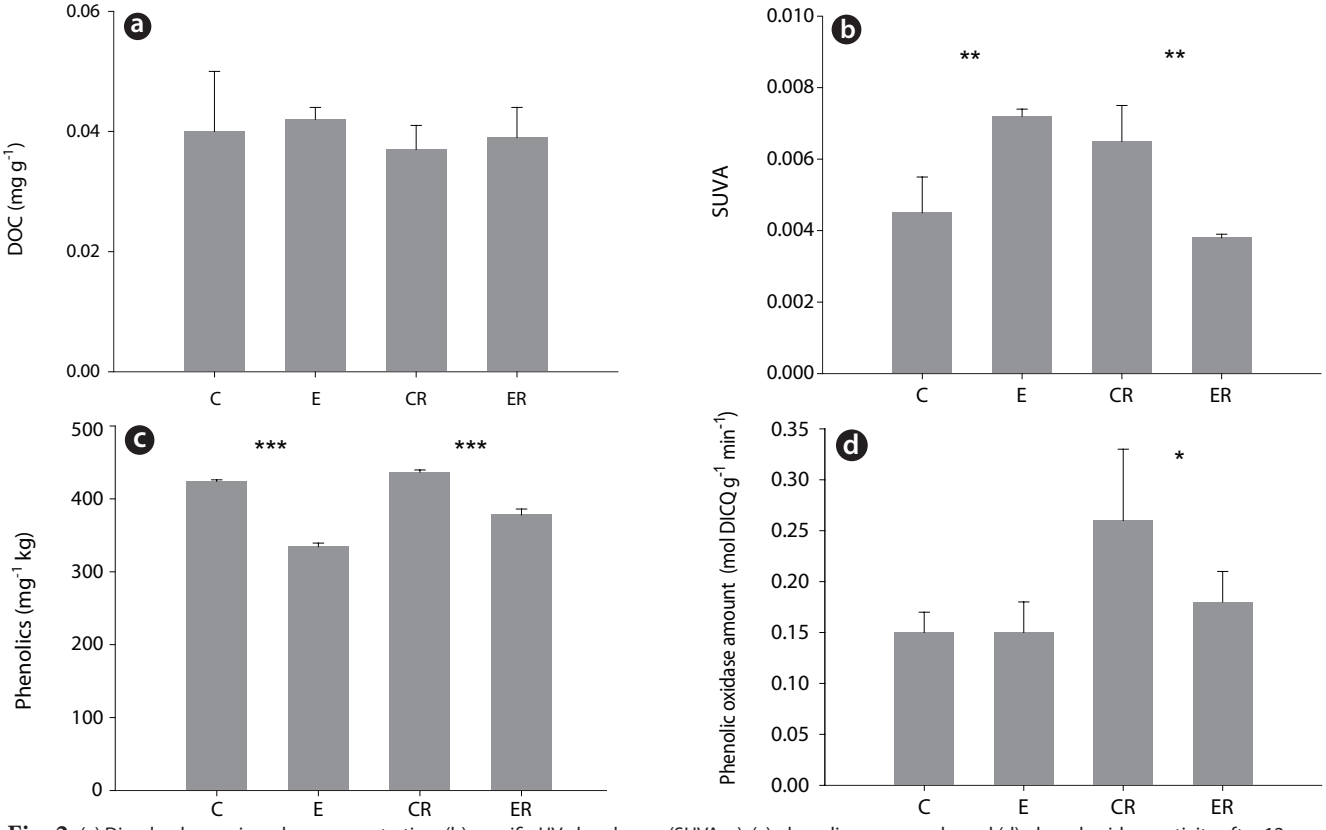

Fig. 2. (a) Dissolved organic carbon concentration, (b) specific UV absorbance ($SUVA_{254}$), (c) phenolic compounds, and (d) phenol oxidase activity after 12 months. C, 380 ppmv, bulk soil; CR, 380 ppmv, rhizosphere soil; E, 760 ppmv, bulk soil; ER, 760 ppmv, rhizosphere soil.

Interestingly, phenol oxidase activity was decreased only in rhizosphere soil (Fig. 2d).

Soil enzyme activity

Elevated CO_2 increased dehydrogenase activity (a measure of microbial intracellular activity) in bulk soil only (Table 3). β-glucosidase, N-acetylglucosaminidase, and phosphatase activities under elevated CO_2 were increased in soils, especially rhizosphere soil (Table 3).

DISCUSSION

Elevated CO_2 affected the C:N ratio of pine seedling as well as soil enzyme activity and N content. However, plant growth and soil DOC content were unaffected. Interestingly, elevated CO_2 increased the C:N ratio of plants as well as microbial activities. It has been suggested that increased CO_2 decreases the level of nitrate in soil. Elevated CO_2 could likely decrease N into the soil, both of which are necessary for plant and microbial decomposition function. These decreases in soil nitrate levels are extremely important because net primary productivity is nitrogen dependent (Janus et al. 2005). Thus, as the amount of available N for plant uptake is decreased, the C:N ratios in pine needles are increased. Gifford et al. (2000) also similarly reported that the C:N ratio of pine seedling is increased 15% due to a 21% decrease in the N content of needles under elevated CO_2.

It was observed that the root dry weight of pine seedling was increased under elevated CO_2, although root and shoot elongation were unaffected (Fig. 1). Many studies show that the overall biomass is more affected than either root or shoot growth under elevated CO_2. Pushnik et al. (1999) reported that the root biomass of *Pinus ponderosa* is significantly increased at 500 and 700 ppmv CO_2. Further, King et al (2001) reported that the root biomass of pine is increased by 96% under elevated CO_2. It has been hypothesized that elevated CO_2 will increase biomass partitioning to fine roots (Curtis et al. 1994), thereby increasing the total root surface area. The increase in root biomass would be expected to favor the growth of microbial fungi in soil due to altered soil chemical composition.

The microbial activities of β-glucosidase, N-acetylglu-

Table 2. Soil physical and chemical parameters

Soil sample	pH	Moisture content (g/kg)	Organic matter (g/kg)	CEC ($cmol_c$/kg)	Nitrate (mg/kg)
Initial	7.1 ± 0.04	8.3 ± 0.7	2.3 ± 0.1	14.7 ± 1.1	12 ± 0.5
C - 4M	6.6 ± 0.4	10.4 ± 1.2^{a*}	2.8 ± 0.7	13.9 ± 1.6	15 ± 2^{a}
E - 4M	7.0 ± 0.1	13.3 ± 0.1^{b***}	2.6 ± 0.6	13.9 ± 1.3	9 ± 0.3^{b}
C - 8M	6.5 ± 0.01	11.5 ± 1.3^{a**}	2.9 ± 0.02	10.8 ± 0.4**	17 ± 3^{a*}
E - 8M	6.5 ± 0.7	9.3 ± 0.1^{b}	3.3 ± 0.005	10.0 ± 2.1***	9 ± 0.7^{b}
C - 12M	6.3 ± 0.05*	9.2 ± 0.3^{a}	3.5 ± 0.01	9.9 ± 0.2***	18.7 ± 3^{a**}
E - 12M	6.5 ± 0.02	5.5 ± 0.1^{b**}	3.5 ± 0.1	10.4 ± 0.02***	6 ± 0.6^{b**}

Values are means ± SD. Significant t-test compared to initial soil at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Values in the ambient CO_2 and elevated CO_2 columns followed by the same letter do not differ significantly from one another (as determined by ANOVA/Tukey analysis).

CEC, cation exchange capacity; C, CO_2 380 ppmv; E, CO_2 760 ppmv; 4M, after 4 months; 8M, after 8 months; 12M, after 12 months.

Table 3. Enzyme activities in soil under ambient and elevated CO_2

Soil sample	Phosphatase	β-glucosidase	N-acetylglucosaminidase	Arylsulfatase	Dehydrogenase
	($nmol\ g^{-1}\ min^{-1}$)				
C	105.7 ± 16^{a}	37.5 ± 4^{a}	38.2 ± 2^{a}	4.6 ± 0.3^{a}	8 ± 0.9^{a}
E	147.2 ± 15^{a}	51.4 ± 3^{a}	60 ± 6^{b}	6.5 ± 0.3^{b}	13.3 ± 1.2^{b}
CR	122 ± 18^{a}	60 ± 7.5^{b}	76 ± 6^{c}	6.6 ± 0.3^{b}	17 ± 5^{b}
ER	202.8 ± 15^{b}	85.8 ± 7^{c}	94.9 ± 4^{d}	7.3 ± 0.2^{bc}	20 ± 3.6^{b}

Values are means ± SD. Values in the ambient CO_2 and elevated CO_2 columns followed by the same letter do not differ significantly from one another (as determined by ANOVA/Tukey analysis).

C, 380 ppmv, bulk soil; E, 760 ppmv, bulk soil; CR, 380 ppmv, rhizosphere soil; ER, 760 ppmv, rhizosphere soil.

cosaminidase, and phosphatase were increased under elevated CO_2 (Table 2), particularly in rhizosphere soil ($P < 0.05$). These increases in enzyme activities may be related to an increase in root exudates and rhizosphere microbe activity (Fig. 1). Several rhizospheric bacterial species are known to produce compounds such as phytohormones, antifungal molecules or siderophores that assist the plant through atmospheric nitrogen fixation (Rillig et al. 1997). Increases in the activities of N-acetylglucosaminidase and phosphatase often occur in response to nitrogen (Gifford et al. 2000). It has been shown that under elevated CO_2, β-glucosidase releases more C from organic matter into soil (Larson et al. 2002, Henry et al. 2005) and that both C and N in general, which are important for microbial metabolism, are released.

Elevated CO_2 did not influence the concentration of dissolved organic matter in soil, but the concentrations of phenolic compounds in soil and aromatic compounds in rhizosphere soil were decreased (Fig. 2). Phenolic compounds are resistant to the nitrification of microbial decomposition activity. Therefore, elevated CO_2 could promote their use as carbon sources by microbes (Rouhier and Read 1998). This notion is supported by our finding that phenol oxidase activity in soil was reduced under elevated CO_2.

In this study, changes in the level of nitrogen in soil could explain the effects of elevated CO_2 on microbial activities and pine seedling growth. However, as our work is limited to the growth chamber, further investigation is needed study.

The results of this study demonstrated that elevated CO_2 had significant effects on the growth of pine seedling as well as soil microbial activity. These findings suggest that rising levels of atmospheric CO_2 cause a reduction in pine seedling biomass as well as distinct changes in soil chemistry and microbiology.

ACKNOWLEDGMENTS

This study was conducted with the support of the Forest Science & Technology Projects (S120507L0201104), provided by Korea Forest Service, and the 2008 KOSEF-DAAD Summer Institute Program, provided by Korea Science and Engineering Foundation.

LITERATURE CITED

Berntson GM, Bazzaz FA. 1996. Belowground positive and negative feedbacks on CO_2 growth enhancement. Plant Soil 187: 119-131.

Box JD. 1983. Investigation of the Folin-Ciocalteau Phenol reagent for the determination of polyphenolic substances in natural waters. Water Res 17: 511-525.

Chin YP, Aiken G, O'Loughlin E. 1994. Molecular weight, polydispersity, and spectroscopic properties of aquatic humic substances. Environ Sci Technol 28: 1853-1858.

Cotrufo MF, Ineson P, Scott A. 1998. Elevated CO_2 reduces the nitrogen concentration of plant tissues. Global Change Biol 4: 43-54.

Curtis PS, O'Neill EG, Teeri JA, Zak DR, Pregitzer KS. 1994. Belowground responses to rising atmospheric CO_2: implications for plants soil biota and ecosystem processes. Plant Soil 165: 1-6.

Finzi AC, Moore DJP, DeLucia EH, Lichter J, Hofmockel KS, Jackson RB, Kim HS, Matamala R, McCarthy HR, Oren R, Pippen JS, Schlesinger WH. 2006. Progressive nitrogen limitation of ecosystem processes under elevated CO_2 in a warm-temperate forest. Ecology 87: 15-25.

Freeman C, Liska G, Ostle NJ, Lock MA, Reynolds B, Hudson J. 1996. Microbial activity and enzymic decomposition processes following peatland water table drawdown. Plant Soil 180: 121-127.

Gelderman RH, Beegle D. 1998. Nitrate-nitrogen. In Recommended Chemical Soil Test Procedures for the North Central Region (Brown JR, ed). University of Missouri-Columbia, Columbia, MO, pp 17-20.

Gifford RM. 1994. The global carbon cycle: a viewpoint on the missing sink. Aust J Plant Physiol 21: 1-15.

Gifford RM, Barrett DJ, Lutze JL. 2000. The effects of elevated [CO_2] on the C : N and C : P mass ratios of plant tissues. Plant Soil 224: 1-14.

Henry HAL, Juarez JD, Field CB, Vitousek PM. 2005. Interactive effects of elevated CO_2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. Global Change Biol 11: 1808-1815.

Hungate BA, Jackson RB, Field CB, Chapin FS. 1996. Detecting changes in soil carbon in CO_2 enrichment experiments. Plant Soil 187: 135-145.

Intergovernmental Panel on Climate Change. 2007. The Physical Science Basis: Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Janus LR, Angeloni NL, McCormack J, Rier ST, Tuchman NC, Kelly JJ. 2005. Elevated atmospheric CO_2 alters soil microbial communities associated with trembling aspen

(*Populus tremuloides*). Microb Ecol 50: 102-109.

King JW, Mohamed A, Taylor SL, Mebrahtu T, Paul C. 2001. Supercritical fluid extraction of *Vernonia galmensis* seeds. Ind Crops Prod 14: 241-249.

Klose S, Wernecke KD, Makeschin F. 2003. Microbial biomass and enzyme activities in coniferous forest soils as affected by lignite-derived deposition. Biol Fertil Soils 38: 32-44.

Larson JL, Zak DR, Sinsabaugh RL. 2002. Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. Soil Sci Soc Am J 66: 1848-1856.

Rillig MC, Scow KM, Klironomos JN, Allen MF. 1997. Microbial carbon-substrate utilization in the Rhizosphere of *Gutierrezia Sarothrae* grown in elevated atmospheric carbon dioxide. Soil Biol Biochem 29**:** 1387-1394.

Melillo JM, Callaghan TV, Woodward FI, Salati E, Sinha SK. 1990. Effects on ecosystems. In Climate Change (Houghton JT, Jenkins GJ, Ephraums JJ, eds). Cambridge University Press, Cambridge, pp 285-310.

Naidu SL, DeLucia EH, Thomas RB. 1998. Contrasting patterns of biomass allocation in dominant and suppressed loblolly pine. Can J For Res 28: 1116-1124.

Pind A, Freeman C, Lock MA. 1994. Enzymic degradation of phenolic materials in peatlands: measurement of phenol oxidase activity. Plant Soil 159: 227-231.

Pushnik JC, Garcia-Ibilcieta D, Bauer S, Anderson PD, Bell J, Houpis JLJ. 1999. Biochemical responses and altered genetic expression patterns in ponderosa Pine (*Pinus ponderosa* Doug ex P. Laws) grown under elevated CO_2. Water Air Soil Pollut 116: 413-422.

Rouhier H, Read DJ. 1998. Plant and fungal responses to elevated atmospheric carbon dioxide in mycorrhizal seedling of *Pinus sylvestris*. Environ Exp Bot 40: 237-246.

Saxe H, Ellsworth DS, Heath J. 1998. Tree and forest functioning in an enriched CO_2 atmosphere. New Phytol 139: 395-436.

Sims DA, Luo Y, Seemann JR. 1998. Comparison of photosynthetic acclimation to elevated CO_2 and limited nitrogen supply in soybean. Plant Cell Environ 21: 945-952.

Tabatabai MA. 1982. Soil enzymes. In Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. Agronomy Monograph (Page AL, ed). American Society of Agronomy, Madison, WI, pp 903-904.

US Environmental Protection Agency. 1986. Test Methods for Evaluating Solid Waste. SW-846, Method 9081. US Environmental Protection Agency, Washington, DC.

Zak DR, Pregitzer KS, King JS, Holmes WE. 2000. Elevated atmospheric CO_2, fine roots and the response of soil microorganisms: a review and hypothesis. New Phytol 147: 201-222.