The Journal of Microbiology (2012) Vol. 50, No. 6, pp. 895–901
DOI 10.1007/s12275-012-2207-1

Effects of Elevated CO_2 and Pb on the Microbial Community in the Rhizosphere of *Pinus densiflora*

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(Received April 17, 2012 / Accepted July 23, 2012)

Rising levels of atmospheric CO_2 may stimulate forest productivity in the future, resulting in increased carbon storage in terrestrial ecosystems. However, heavy metal contamination may interfere with this, though the response is not yet known. In this study, we investigated the effect of elevated CO_2 and Pb contamination on microorganisms and decomposition in pine tree forest soil. Three-year old pine trees (*Pinus densiflora*) were planted in Pb contaminated soils (500 mg/kg-soil) and uncontaminated soils and cultivated for three months in a growth chamber where the CO_2 concentration was controlled at 380 or 760 mg/kg. Structures of the microbial community were comparatively analyzed in bulk and in rhizosphere soil samples using community-level physiological profiling (CLPP) and 16S rRNA gene PCR-DGGE (denaturing gradient gel electrophoresis). Additionally, microbial activity in rhizospheric soil, growth and the C/N ratio of the pine trees were measured. Elevated CO_2 significantly increased microbial activities and diversity in Pb contaminated soils due to the increase in carbon sources, and this increase was more distinctive in rhizospheric soil than in bulk soils. In addition, increased plant growth and C/N ratios of pine needles at elevated CO_2 resulted in an increase in cation exchange capacity (CEC) and dissolved organic carbon (DOC) of the rhizosphere in Pb contaminated soil. Taken together, these findings indicate that elevated CO_2 levels and heavy metals can affect the soil carbon cycle by changing the microbial community and plant metabolism.

Keywords: elevated CO_2, *Pinus densiflora*, enzyme activities, lead contamination, microbial community

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Introduction

The wide-ranging impacts of elevated carbon dioxide (CO_2) concentrations and the rising prevalence of metal-contaminated soils are serious problems in forest ecosystems. The main effects of elevated CO_2 on plants are increases in the photosynthetic rate and water-use efficiency, and higher translocation of photosynthate to the root and rhizosphere (Nowak *et al.*, 2004; Norby, 2005). Plant growth changes as a result of CO_2 fertilization can change the soil microbial community (Matthias *et al.*, 1997). For example, substrate utilization by the microbial community of *Gutierrezia sarothrae* roots significantly changed with elevated atmospheric CO_2 (Matthias *et al.*, 1997). Elevated CO_2 levels had significant effects on both soil nutrient availability and the community composition of soil microbes associated with *Populus tremuloides* (Lori *et al.*, 2005).

Heavy metal contamination can also have dramatic effects because they are toxic at high levels in both natural and man-made environment ecosystems. Among heavy metals, lead (Pb), which is commonly associated with soil pollution, is considered particularly toxic and is responsible for significant decreases in biological activities in soil. In soil, Pb is typically present at concentrations ranging from 10–100 mg/kg, but the Pb content in polluted soils can be greater than 500 mg/kg (Glazovskaya, 1994). The addition of Pb to soil inhibits soil microbial activity. High concentrations of Pb in soil can adversely affect soil microbes via population loss, changes in population structure, and decreases in physiological activity (Akmal *et al.*, 2005).

Due to industrial activities, extensive areas of soil have been contaminated with heavy metals in addition to increases in CO_2 concentrations in the atmosphere. Recently, the effect of elevated CO_2 and metal contamination on microorganisms has received special attention because microorganisms are key components of the C cycle and nutrient recycling (Gadd, 2004; Kim and Kang, 2011). Microorganisms regulate and influence many ecosystem processes such as nutrient transformation, litter decomposition, transformation of organic matter into soil and maintenance of plant health (Cahyani *et al.*, 2003). Therefore, soil microbial community structure and function are commonly used as indicators of soil quality and fertility. Gaining a better understanding of the relationship between structure and function is currently an important topic in research on soil ecosystems (Yao *et al.*, 2003). However, most studies have only separately examined the effect of elevated CO_2 or metal toxicity on microorganisms.

In this study we examined the effects of both Pb contamination and elevated CO_2 on soil chemistry and microbial properties. The objectives of this study were to (1) investigate

the interactive effect of elevated CO_2 and Pb on soil microbial diversity and (2) determine the relate soil microbial changes with organic decomposition influenced by elevated CO_2 concentrations and in Pb contaminated soils.

Materials and Methods

Experimental design

This study employed mesocosm experiments based on previous results on phytoextraction and enzyme activity caused by elevated CO_2 and Pb contamination (Kim and Kang, 2011). Natural soil was sampled at a depth of 5 to 15 cm from the pine forest of Korea, transported to the lab, and then passed through a 2-mm sieve. To determine microorganism changes due to both heavy metals and elevated CO_2, we conducted a microcosm study with four groups at different CO_2 concentrations (380, 760 μl/L) and Pb contamination levels (0, 500 mg/kg-soil). For each experiment, aliquots (1 kg each in 1.5 L plastic cylinders, diameter 10 cm) of soil were artificially contaminated with 500 mg Pb/kg. Test pots, containing 1 kg of contaminated soil were planted with three-year-old pine seedlings (*Pinus densiflora*), which were obtained from the Korean forest service, then placed in a growth chamber (Dasol Scientific Co. Korea). The CO_2 content in the growth chamber was maintained at 380 μl/L or 760 μl/L at 25°C, 60% humidity, and subjected to a 16 h light/8 h dark cycle for three months. Soil characteristics and microbial activities were compared in the four different soil samples (Control: CO_2 380 μl/L + Pb 0 mg/kg; Pb: CO_2 380 μl/L + Pb 500 mg/kg; Ele. CO_2: CO_2 760 μl/L + Pb 0 mg/kg; Pb+Ele. CO_2: CO_2 760 μl/L + Pb 500 mg/kg). Microbial diversity was measured by dividing the soil into bulk and rhizosphere soil. Bulk soil was the soil that remained after the roots were removed from the pot. The rhizosphere soil was the soil that remained adhered to the roots after gentle shaking. All tests were performed in triplicate. Thirty milliliters of water and 20 ml of 1/2 Hoagland solution (Hoagland and Arnon, 1950) were added to the soil once per week.

Soil microbial community determination

Community-level physiological profiling (CLPP): After incubating for three months, the soil was divided into bulk and rhizosphere soil samples. Three grams of each soil sample was mixed with 27 ml of sterilized water and shaken for 10 min at 200 rpm. After settling for 1 h, the resulting suspension was inoculated onto Eco-plates (Biolog, USA) and the plates were incubated at 20°C for 14 days. Color development was measured based on at 595 nm absorbance using an automated microplate reader (Multiskan Ascent, Thermo Lab Systems, Finland). For Biolog data analysis, plates were read daily, and the average well color development (AWCD) over time for all carbon sources was calculated as a measure of total microbial activity. Data were analyzed using the following equation:

Average Well Color Development = $\sum(C - R)/n$

C: color production within each well (OD_{595nm})

R: OD value of the no-substrate control well of each plate

n: number of substrate utilization (n=31)

Diversity was calculated using the following formula: $H = - \sum P_i \ln P_i$, where H is the Shannon index, P_i is the ratio of activity on a particular substrate to the sum of activities on all substrates (Ian and Peter, 2003).

PCR-DGGE: Denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments was used to analyze the microbial community structure in the rhizosphere and bulk soil samples. Genomic DNA was extracted from fresh 0.5 g soil using the BIO101 FastDNA SPIN Kit for Soil (Q-BIOgene, USA). The PCR was used to amplify a 560 base pair portion of the 16S rRNA gene using primers 341fGC (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GCC TAC GGG AGG CAG CAG-3′) and 907r (5′-CCC CGT CAA TTC ATT TGA GTT-3′) (Muyzer *et al.*, 1993). The PCR was conducted by subjecting the samples to 45 sec of denaturation at 94°C, 45 sec of annealing, and 90 sec of extension at 72°C. During an initial touch-down cycle, the annealing temperature was lowered from 63°C to 51°C in intervals of two for two cycles, after which 20 additional annealing cycles were conducted at 45°C. The samples were also subjected to a final extension at 72°C for 10 min. DGGE was conducted using a D-Code 16/16 cm gel system with a gel width of 0.7 mm (Bio-Rad, USA), which contained 6% polyacrylamide:bisacrylamide (37.5:1) and were poured with a urea and formamide gradient of 35% to 55%. Gels were run at 60 V and 60°C for 15 h, after which they were stained with ethidium bromide (0.5 mg/L) and then destained twice in 1×TAE buffer for 15 min each. The strong DGGE bands were excised using a razor blade and then soaked in 20 μl of purified water overnight. The products were then purified and sequenced using an ABI-Prism model 377 automatic sequencer (Applied Biosystems, USA). The DGGE image was translated into the microbial community structure by comparison using Gel ComparII (Applied Maths, USA) to perform the unweighted pair group method with arithmetic mean (UPGMA) clustering using the Jaccard coefficient based on band position (Ian and Peter, 2003). Diversity statistics were calculated from the DGGE profile of the microbial community. Microbial diversity distribution treatments by using the number and intensity of bands in each DGGE profile as representations of the number and relative abundance of different phylotypes in each gel. The relative intensities (pi) of each band were used as the variables, and no band was treated as zero.

The Shannon-Weiner diversity index was calculated from following equation:

$H= -\sum (pi)(\log_2 Pi)$

where pi the proportion of an individual band intensity relative to the sum of all band intensities (Margalef, 1958).

Analysis of soil microbial activities

The activity of four extracellular enzymes (β-glucosidase, N-acetylglucosaminidase, phosphatase, and arylsulfatase) was measured using the MUF-substrate method (Freeman *et al.,* 1996). The concentrations of each substrate solution were 400 μM (Sigma; MUF-β-glucoside, MUF-N-acetylglucosamine, MUF-arylsulfate) except for phosphate (Sigma; MUF-phosphate, 800 μM). Enzyme activities were measured from the slurry of soil and substrate solutions (1:5) using a fluorometer (TD 700; Turner designs, USA).

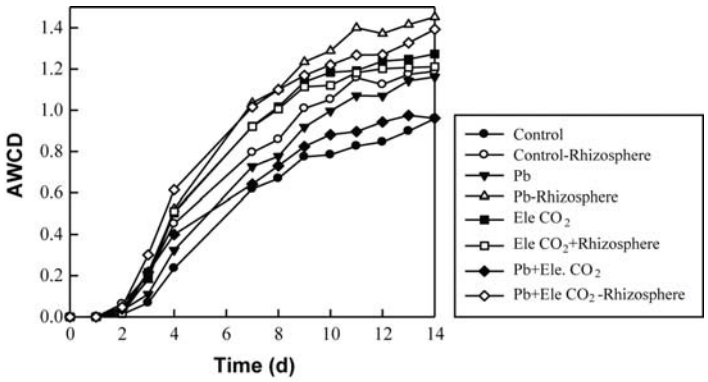

Fig. 1. Variation in the average well color development over time in Eco-plates.

Dehydrogenase activity and intracellular activity were measured using the INT assay (Tabatabai, 1982). The substrate used for these assays was 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT). Reaction products were detected using a spectrophotometer (DR/3000 Spectrophotometer, HACH, USA) at 485 nm.

Soil characteristics

Soil pH was determined by adding soil to water at a ratio of 1:5 (w:v). Soil moisture content was determined gravimetrically by drying for 24 h at 105°C, and the organic matter content was determined by loss on ignition at 600°C in a furnace (MAS 7000, CEM, USA). The soil cation-exchange capacity (CEC) was determined using the EPA 9081 methods (EPA US, 1986). Soil nitrate content was determined by extraction with deionized water and measured using a nitrate electrode (Gelderman and Beegle, 1998). Additionally, NH_4^+ was measured using indophenol blue methods (Dorich and Nelson, 1983). Soil DOC (dissolved organic carbon) was determined by adding soil to distilled water at a ratio of 1:10 w/v to form a slurry form. Extractable DOC was measured by filtering through a 0.45-mm filter, and then analyzed by a TOC analyzer (TOC-VCHP, Japan).

Plant biomass and C:N ratio

After three months of growth, the plants were carefully harvested and the length and biomass of shoots and roots were measured. Carbon and nitrogen concentrations contained in the shoots and roots were analyzed using a Flash EA 1112 model analyzer (Thermo Electron Corporation, USA).

Statistical analysis

Relationships among different samples on the basis of the raw-difference data were evaluated using principal compo-

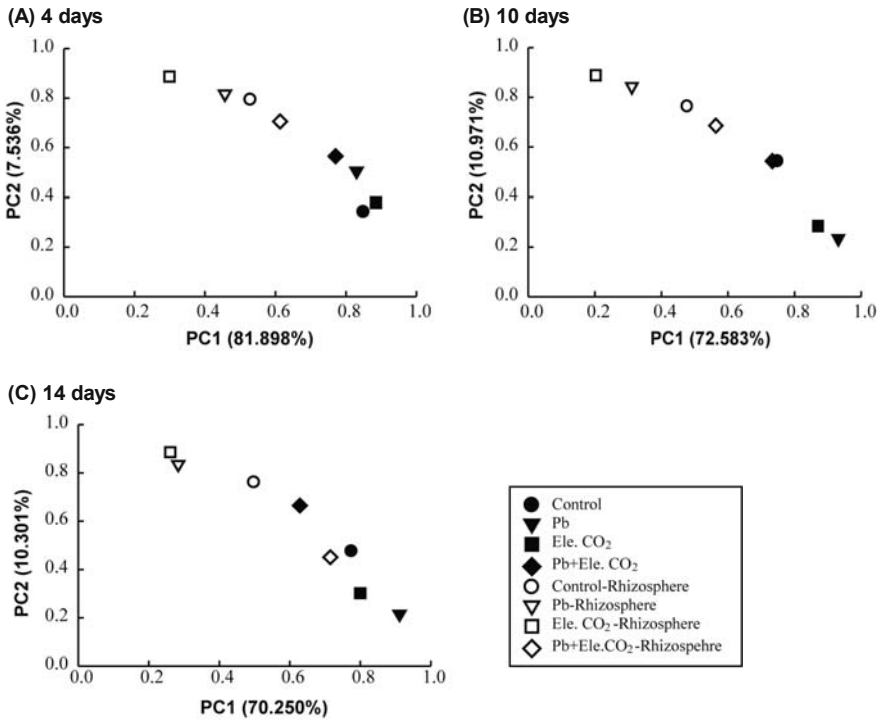

Fig. 2. Principal component analysis of average well color development for 14 days.

Table 1. Similarity based on DGGE fingerprint

	Initial	Initial-R	Con	Pb	Ele.	Pb+Ele.	Con-R	Pb-R	Ele.-R	Pb+ Ele.-R
Initial	100									
Initial-R	60	100								
Con	43.8	40	100							
Pb	41.2	37.5	40	100						
Ele.	33.3	37.5	36	81.8	100					
Pb+Ele.	47.1	43.8	38	76.9	50	100				
Con-R	38.9	43.8	38	43.8	40	50	100			
Pb-R	46.7	42.9	36	53.8	50	50	50	100		
Ele.-R	46.7	53.8	36	53.8	50	50	62	64	100	
Pb+Ele.-R	43.8	50	43	50	46.2	57	57	58	73	100

R, Rhizosphere; Con, Control; Ele., Elevated CO_2

nent analysis (PCA) conducted using SPSS 12.0 (SPSS, Inc., USA). Two-way ANOVA was carried out to compare the soil physico-chemical parameters, soil enzyme activity, and DOC values between the groups. Tukey's test was then carried out on the parameters, including root and shoot lengths, C/N ratio, and Shannon index. Standard deviations are shown as numerals in the tables and as error bars in the figures.

Results

Carbon substrate utilization patterns

The effect of elevated CO_2 and heavy metal on the microbial communities in rhizosphere and non-rhizosphere soils of *P. densiflora* was investigated using CLPP. The rate of color intensity on the Biolog plates over time was determined by calculating the AWCD on each plate at each reading time (Fig. 1). The AWCD in the Biolog EcoPlate assay varied for different soil samples. The AWCD value was the highest in the Pb contaminated soil for rhizosphere soil. AWCD value was high in Pb+Ele. CO_2 treatment for both the rhizosphere and bulk soil, and was the lowest in the control soil.

Principle component analysis demonstrated that there were significant differences in the carbon substrate utilization patterns of bacterial communities between different treatments (P<0.001) (Fig. 2). Soil samples separated along the first principal component (PC1) axis for all three reading times (4, 10, and 14 d). The effect of the rhizosphere separated according to PC1 explained 81.9% of the variation over 4 days, 72.6% over 10 days, and 70.2% over 14 days.

Soil microbial communities studied by DGGE

To compare the microbial community structure among treatments, DGGE analysis was conducted (Fig. 3). Clustering of the profiles showed that there were very large differences among the profiles of the soil samples. In bulk soil, similarity between elevated CO_2 and Pb was the highest with 81.8% (Table 1). Profiles of Pb and Pb+elevated CO_2 showed approximately 76.9% similarity with respect to clustering. Profiles of the other samples showed a similarity below 40%. These data indicate that bacterial communities in the soil near the Pb contamination changed substantially. In rhizosphere soil, the greatest similarity was 72.7% between elevated CO_2-R and Pb+elevated CO_2-R soil samples. Therefore, the bulk soils differed considerably due to Pb contamination, but the rhizosphere soils were most influenced by treatment with an elevated CO_2 concentration.

Soil diversity index

The differences in the microbial diversity among treatments were confirmed by the Shannon-Weaver diversity index (H′) values calculated from the AWCD and DGGE profiles (Table 2). Although the diversity index of bulk soil was not significantly affected by Pb or elevated CO_2, the diversity

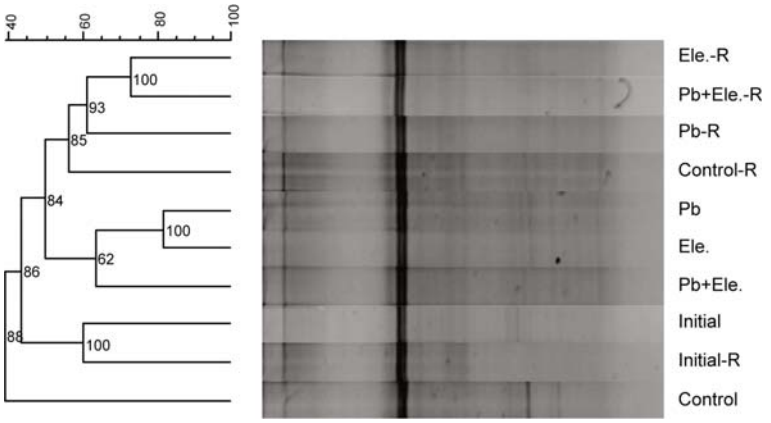

Fig. 3. DGGE profiles of 16S rRNA gene fragments amplified from the soil samples (R: rhizosphere).

Table 2. Shannon index calculated from the AWCD and DGGE of soil microbial community

Treatment	Shannon index from Biolog		Shannon index from DGGE	
	Bulk	Rhizosphere	Bulk	Rhizosphere
Control	2.1[a]	2.3[a]	3.1[a]	3.8[c]
Pb	3.2[b]	3.4[b]	2.9[a]	3.1[a]
Ele.	3.2[b]	2.5[a]	2.9[a]	3.4[b]
Pb+Ele.	3.2[b]	3.0[c]	3.0[a]	3.6[b]

Values followed by the same letter in each column do not differ significantly (two-way ANOVA followed by Tukey's post-hoc test).

index of rhizosphere was affected by Pb and CO_2 X Pb interaction (p<0.001). The diversity indices of rhizosphere soils from AWCD treated with only Pb (H'=3.4±0.06) and with Pb+elevated CO_2 (H'=3.0±0.06) were significantly greater (P<0.001) than those of the control soil (H'=2.3±0.1) and elevated CO_2 (H'=2.5±0.1) soils.

Visual comparison of the overall bacterial DGGE profiles and the Shannon–Weaver diversity index (H') calculated for each treatment in rhizophere soil indicated that the band for soil treated with Pb+elevated CO_2 (H'=3.6±0.01) were significantly more intense (P<0.05) than the band for soil treated with only Pb (H'=3.1±0.1) or with only elevated CO_2 (H'=3.4±0.1).

Soil microbial activities and soil characteristics

Table 3 showed two-way ANOVA results from enzyme activity and soil characteristics for Pb treatments with elevated CO_2. β-Glucosidase and arylsulfatase activities were greatly influenced by both Pb and elevated CO_2 levels. Additionally, CO_2 X Pb interactions were found to be statistically significant. Activities of N-acetyl-glucosaminidase and dehydrogenase markedly increased by elevated CO_2 and Pb+elevated CO_2, while the activity of phosphatase was influenced by only Pb. The pH and nitrate level were greatly influenced by both Pb and elevated CO_2. The contents of DOC and CEC were highly influenced by elevated CO_2.

Effects on the biomass and C/N ratios of the pine seedlings

Results of these experiments showed that root elongation of pine seedlings increased under elevated CO_2 (Fig. 4A). Although the shoot elongation of the pine seedlings grown in Pb-contaminated soil was not significantly affected by elevated CO_2, the total dry weights of plants grown in contaminated soils under elevated CO_2 were significantly greater than those grown under ambient CO_2 (p<0.05).

Figure 4B shows the C/N ratios of the roots and leaves of pine seedlings after three months, by which chemical changes in pine seedlings caused by elevated CO_2 and Pb contamination were measured. The C/N ratios in the leaves increased slightly with elevated CO_2, indicating that the carbon concentrations increased while the nitrogen concentrations decreased with elevated CO_2. However, the C/N ratios in the roots were not affected by elevated CO_2 or Pb contamination.

Discussion

Effect of microbial diversity and activity in soil treated with elevated CO_2 and Pb

In this study, microbial diversity was influenced by Pb contamination and CO_2 X Pb interaction (p<0.01). Although the Shannon index from the AWCD values was highest in Pb-contaminated soil, the Shannon index from DGGE was lowest in Pb-contaminated soil. The activation of microbial populations in the rhizosphere may be a mechanism of plant defense against Pb contaminants. Microbial adaptation might result in the selection of a few abundant species capable of utilizing a broad spectrum of carbon sources via root exudation when a limited amount of carbon is added to the soil through root turnover (Farrar *et al.*, 2003). Specially, the main C-sources for microorganisms included D-malic acid, D-glucosaminic acid, and α-D-Lactose (data not shown) from pine root exudate under elevated CO_2 in Pb contaminated soil in this study. Wu *et al.* (2009) also reported that elevated CO_2 increased the actinomycete population in the

Table 3. Two-way ANOVA for elevated CO_2 and Pb treatments. Variables include: physic-chemical characteristics and soil enzyme activities

Variable	CO_2		Pb		CO_2 X Pb	
	F	P	F	P	F	P
pH	490.95	**0.000**	9.298	**0.006**	30.124	**0.000**
Moisture content	0.468	0.502	4.320	0.051	3.613	0.072
Organic matter	1.439	0.244	3.840	0.064	1.184	0.290
CEC	49.116	**0.000**	0.136	0.716	0.136	0.716
DOC	166.192	**0.000**	0.118	0.735	1.271	0.273
Nitrate	9.284	**0.006**	9.346	**0.006**	9.284	**0.006**
NH_4^+	3.848	0.064	1.963	0.176	0.707	0.410
Dehydrogenase	48.161	**0.000**	0.991	0.331	9.951	**0.005**
Phosphatase	1.769	0.198	6.505	**0.019**	2.29	0.146
β-Glucosidase	149.9	**0.000**	74.617	**0.000**	6.889	**0.016**
N-Acetylglucosaminidase	391.367	**0.000**	0.515	0.481	128.46	**0.000**
Arylsulfatase	137.858	**0.000**	20.084	**0.000**	116.77	**0.000**

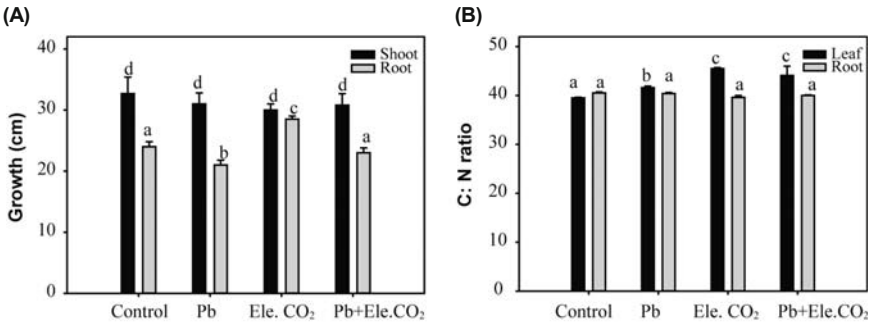

Fig. 4. (A) Comparison of shoot and root length of pine seedlings after three months. (B) C:N ratio of pine seedlings grown under ambient or elevated atmospheric CO_2. Values are means± SDs of three replicates. Values followed by the same letter do not differ significantly (two-way ANOVA followed by Tukey's post-hoc test).

rhizosphere. Denton *et al.* (2007) observed an increase in the bacterial population in plants treated with elevated CO_2, which suggests that microbes alleviate metal phytotoxicity.

Microbial activity was affected by elevated CO_2 and CO_2 X Pb interaction except that of phosphatase. In addition, microbial diversity and activity were higher in the rhizosphere than in the bulk soil. It appears that elevated CO_2 levels stimulated bacterial and fungal growth and increased the activity of these microorganisms due to an altered rhizospheric environment. It is plausible that the effect of elevated CO_2 on the rhizosphere in Pb-contaminated soil occurred because microbial communities have adapted to polluted soils under elevated CO_2, which would result in an increase in several specific populations of soil microorganisms.

In this study, the root elongation and biomass of pine seedlings increased under elevated CO_2 concentrations due to the decreased toxicity of Pb (Fig. 4). Although root growth is inhibited by Pb, the toxicity should countervail because elevated CO_2 ensures root growth. These results may be related to the fact that microbial diversity and activities increased as a result of an increasing input of C into the soil by the pine tree roots. Under elevated CO_2 conditions in the short-term, assimilated carbon can exist more readily than labile carbon in the soil. Kim *et al.* (2011) reported that the amount of root exudates increased after Pb contamination. About 15–25% of C allocated below-ground was reported to be exuded from roots into soil (Kuzyakov, 2002). These exuded organic substances can induce fast C turnover in the vicinity of the roots. The rhizosphere is characterized by very intensive C turnover forced by microorganisms. Rhizosphere microorganisms utilize these substances as easily available C and energy sources to facilitate rapid growth and reproduction. Exudation of organic substances during rapid root growth phases may lead to strong decomposition of organic matter and nutrient mobilization.

Effect of organic decomposition via microbial changes in soil treated with elevated CO_2 and Pb

In this study, the CEC and dissolved organic carbon (DOC) in rhizosphere soil were influenced by elevated CO_2 (Table 3). These results indicate that the soil characteristics and microbial activities were more affected by elevated CO_2 than by Pb contamination. In other words, elevated CO_2 can alter soil decomposition through changes in the distribution of particular microbial population. Numerous studies have investigated the effects of elevated CO_2 on terrestrial ecosystems (Naumburg *et al.*, 2003; Bunce, 2004; Marchi *et al.*, 2004). Among those effects, elevated CO_2 increased carbon in the rhizosphere, through which products of stimulated photosynthates were transported under elevated CO_2 (Cheng and Johnson, 1998). Ross *et al.* (2002) suggested that an increase in organic carbon in soil exposed to high CO_2 levels might have been caused by the decomposition of easily decomposable soil carbon. This was explained by a greater production of microbial activity in response to an increased C input. Under elevated CO_2, β-glucosidase releases more C from organic matter into the soil (Larson *et al.*, 2002; Henry *et al.*, 2005). In this study, β-glucosidase and N-acetylglucoamidase were also increased by elevated CO_2 in uncontaminated and Pb contaminated soils.

Alteration in the relative availability of C and N under elevated CO_2 conditions may significantly influence microbial N transformation. In this study, nitrate (NO_3^-) content was influenced by CO_2, Pb and CO_2 X Pb (Table 3). Nitrate content decreased under elevated CO_2 conditions in Pb-contaminated soils. These results indicate that elevated CO_2 levels may increase plant N uptake, thereby reducing NH_4^+ availability for nitrifiers in Pb-contaminated soil. Increased plant N uptake can reduce soil extractable N (NH_4^+ and NO_3^-). Therefore, the C surplus and N deficit in the rhizosphere under elevated CO_2 conditions in Pb-contaminated soil may be more strongly pronounced than in uncontaminated soil. However, these experiments were conducted in the short-term and did not consider N dynamics when elevated CO_2 resulting in N mineralization and subsequent transformation may lead to N losses. Elevated CO_2 could likely drive N mineralization in the soil and both are necessary for microbial decomposition functions in contaminated soil.

The present study provides evidence of major changes in the chemical and physical properties of soils following treatment with elevated CO_2 and Pb contamination, which could have lasting impacts on the microbial communities in plant rhizospheres. Elevated CO_2 and Pb concentrations increased the microbial activities and diversity of the rhizosphere by increasing C. In addition, chemical metabolism in pine tissue and organic decomposition processes were more strongly affected by elevated CO_2 than by Pb contamination. The results of this study demonstrate that elevated CO_2 can increase the soil decomposition rate of microbes in metal-contaminated soil. However, more research needs to be conducted to clarify the mechanism of interaction between microbes and plants in the presence of elevated CO_2 and metal contamination.

Acknowledgements

H. Kang is grateful to NRF (20110029802) and S/ERC (20110030843) for financial support.

References

Akmal, M., Xu, J., Li, Z., Wang, H., and Yao, H. 2005. Effects of lead and cadmium nitrate on biomass and substrate utilization pattern of soil microbial communities. *Chemosphere* **60**, 508–514.

Bunce, J.A. 2004. Carbon dioxide effects on stomatal responses to the environment and water use by crops under field conditions. *Oecologia* **140**, 1–10.

Cahyani, V.R., Matsuya, K., Asakawa, S., and Kimura, M. 2003. Succession and phylogenetic composition of bacterial communities responsible for the composting process of rice straw estimated by PCR-DGGE analysis. *Soil Sci. Plant Nutr.* **49**, 619–630.

Cheng, W. and Johnson, D.W. 1998. Elevated CO_2, rhizosphere processes, and soil organic matter decomposition. *Plant Soil* **202**, 167–174.

Denton, B. 2007. Advances in phytoremediation of heavy metals using plant growth promoting bacteria and fungi, MMG445. *Basic Biotechnol. Ej.* **3**, 1–5.

Dorich, R.A. and Nelson, D.W. 1983. Direct measurement of ammonium in potassium chloride extracts of soils. *Soil Sci. Soc. Am. J.* **47**, 833–836.

E.P.A. 1986 Test methods for evaluating soilid waste. SW-846, method 9081. Washington, D.C., USA.

Farrar, J., Hawes, M., Jones, D.L., and Lindow, S. 2003. How roots control the flux of carbon to the rhizosphere. *Ecology* **84**, 827–837.

Freeman, C., Liska, G., Ostle, N.J., Lock, M.A., Reynolds, B., and Hudson, J. 1996. Microbial activity and enzymic decomposition processes following peatland water table drawdown. *Plant Soil* **180**, 121–127.

Gadd, G.M. 2004. Microbial influence on metal mobility and application for bioremediation. *Geoderma* **122**, 109–119.

Gelderman, R.H. and Beegle, D. 1998. Nitrate-nitrogen. Recommended chemical soil test procedures for the North Central Region. North Central Regional Research Publication No. 221 (Revised). Columbia: Missouri Agricultural Experiment Station.

Glazovskaya, M.A. 1994. Criteria for classification of soils according to lead-pollution risk. *Eurasian Soil Sci.* **26**, 58–74.

Henry, H., Juarez, J.D., Field, C.B., and Vitousek, P.M. 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.

Hoagland, D.R. and Arnon, D.I. 1950. The water culture method for growing plants without soil, University of California, Agricultural Experiment Station, Berkley, USA.

Ian, F.S. and Peter, J.P. 2003. A tribute to claude Shannon (1916~2001) and a plea for more rigorous use of species richness, species diversity and the shannon-wiener index. *Global Ecol. Biogeo.* **12**, 177–179.

Kim, S. and Kang, H. 2011. Effects of elevated CO_2 and Pb on phytoextraction and enzyme activity. *Water Air Soil Poll.* **219**, 365–375.

Kim, S., Lee, I., and Kang, H. 2011. Effects of *Pinus densiflora* on soil chemical and microbial properties in Pb-contaminated forest soil. *J. Ecol. Field Biol.* **34**, 1–8.

Kuzyakov, Y. 2002. Review: Factors affecting rhizosphere priming effects. *J. Plant Nutr. Soil Sci.* **165**, 382–396.

Larson, J.L., Zak, D.R., and Sinsabaugh, R.L. 2002. Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. *Soil Biol. Biochem.* **66**, 1848–1856.

Lori, R.J., Angeloni, N.L., McCormack, J., Rier, S.T., Tuchman, N.C., and Kellym, J.J. 2005. Elevated atmospheric CO_2 alters soil microbial communities associated with trembling aspen (populus tremuloides) roots. *Microb. Ecol.* **50**, 102–109.

Marchi, S., Tognetti, R., Vaccari, F.P., Lanini, M., Kaligaric, M., Miglietta, F., and Raschi, A. 2004. Physiological and morphological responses of grassland species to elevated atmospheric CO_2 concentrations in FACE-systems and natural CO_2 springs. *Functional Plant Biol.* **31**, 181–194.

Margalef, R. 1958. Information theory in ecology. *Gen. Syst.* **3**, 36-71.

Matthias, C.R., Kate, M.S., Jhon, N.K., and Michael, F.A. 1997. Microbial carbon-substrate utilization in the rhizosphere of *Gutierrezia sarothrae* grown in elevated atmospheric carbon dioxide. *Soil Biol. Biochem.* **29**, 1387–1394.

Muyzer, G., deWaal, E.C., and Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electropores is analysis of polymerase chain reaction genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **59**, 695–700.

Naumburg, E., Housman, D.C., Huxman, T.E., Charlet, T.N., Loik, M.E., and Smith, S.D. 2003. Photosynthetic responses of mojave desert shrubs to free air CO_2 enrichment are greatest during wet years. *Global Change Biol.* **9**, 276–285.

Norby, R.J. 2005. Forest response to elevated CO_2 is conserved across a broad range of productivity. *Proc. Natl. Acad. Sci. USA* **102**, 18052–18056.

Nowak, R.S., Ellsworth, D.S., and Smith, S.D. 2004. Functional responses of plants to elevated atmospheric CO_2: do photosynthetic and productivity data from FACE experiments support early predictions? *New Phytol.* **162**, 253–280.

Ross, D.J., Tate, K.R., Newton, P.C.D., and Clark, H. 2002. Decomposability of C3 and C4 grass litter samples under different concentration of atmospheric carbon dioxide at a natural CO_2 spring. *Plant Soil* **240**, 275–286.

Tabatabai, M.A. 1982. Soil enzymes, *In* Page, A.L. (ed.), Methods of soil analysis, part Agronimy monograph, vol. 9, pp. 903–904, American Society of Agronomy, Madison, Wisconsin, USA.

Yao, H., Xu, J., and Huang, C. 2003. Substrate utilisation pattern, biomass and activity of microbial communities in a sequence of heavy metal polluted paddy soils. *Geoderma* **115**, 139–148.

Wu, H.B., Tang, S.R., Zhang, X.M., Guo, J.K., Song, Z.G., Tian, S., and Smith, D. 2009. Using elevated CO_2 to increase the biomass of a *Sorghum vulgare* × *Sorghum vulgare var. sudanense* hybrid and *Trifolium pratense* L. and to trigger hyperaccumulation of cesium. *J. Hazard. Mater.* **170**, 861–870.