

# Effects of elevated CO<sub>2</sub> on organic matter decomposition capacities and community structure of sulfate-reducing bacteria in salt marsh sediment

Soohyun Jung<sup>1</sup>, Seung-Hoon Lee<sup>2</sup>, Seok-Soon Park<sup>1</sup> and Hojeong Kang<sup>2,\*</sup>

<sup>1</sup>Department of Environmental Engineering and Science, Ewha Womans University, Seoul 120-750, Korea

<sup>2</sup>School of Civil and Environmental Engineering, Yonsei University, Seoul 120-749, Korea

Increasing atmospheric CO<sub>2</sub> affects the soil carbon cycle by influencing microbial activity and the carbon pool. In this study, the effects of elevated CO<sub>2</sub> on extracellular enzyme activities (EEA; β-glucosidase, N-acetylglucosaminidase, aminopeptidase) in salt marsh sediment vegetated with *Suaeda japonica* were assessed under ambient atmospheric CO<sub>2</sub> concentration (380 ppm) or elevated CO<sub>2</sub> concentration (760 ppm) conditions. Additionally, the community structure of sulfate-reducing bacteria (SRB) was analyzed via terminal restriction fragments length polymorphism (T-RFLP). Sediment with *S. japonica* samples were collected from the Hwangsando intertidal flat in May 2005, and placed in small pots (diameter 6 cm, height 10 cm). The pots were incubated for 60 days in a growth chamber under two different CO<sub>2</sub> concentration conditions. Sediment samples for all measurements were subdivided into two parts: surface (0-2 cm) and rhizome (4-6 cm) soils. No significant differences were detected in EEA with different CO<sub>2</sub> treatments in the surface and rhizome soils. However, the ratio of β-glucosidase activity to N-acetylglucosaminidase activity in rhizome soil was significantly lower ( $P < 0.01$ ) at 760 ppm CO<sub>2</sub> than at 380 ppm CO<sub>2</sub>, thereby suggesting that the contribution of fungi to the decomposition of soil organic matter might in some cases prove larger than that of bacteria. Community structures of SRB were separated according to different CO<sub>2</sub> treatments, suggesting that elevated CO<sub>2</sub> may affect the carbon and sulfur cycle in salt marshes.

**Key words:** extracellular enzyme activity, salt marsh sediment, sulfate reducing bacteria, T-RFLP

## INTRODUCTION

Atmospheric CO<sub>2</sub> increases partially as the result of all anthropogenic activities, most notably the usage of fossil fuels (Moore and Bolin 1986) and deforestation (Detwiler and Hall 1988); atmospheric CO<sub>2</sub> is expected by some to increase to 760 ppm within the next few decades. The impact of this increasing concentration on ecosystems has been an issue of some controversy among environmentalists for several decades (IPCC 2007).

The effects of increased CO<sub>2</sub> have been extensively studied in terrestrial ecosystems (Mooney et al. 1991),

usually with a focus on primary production and changes in the physiological characteristics of plants (DeLucia et al. 1999). Generally, increases in plant biomass and primary production have been observed as the result of increased CO<sub>2</sub>, and were seen in C<sub>3</sub> plants rather than C<sub>4</sub> plants. The C:N ratio in green leaves was also increased with rising CO<sub>2</sub> concentrations, because C components like starch were increased and N contents were reduced as the result of elevated CO<sub>2</sub> levels (Cotrufo and Ineson 1996). However, litter chemistry did not change with in-

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Received 22 April 2010, Accepted 21 June 2010

\*Corresponding Author

E-mail: hj\_kang@yonsei.ac.kr  
Tel: +82-2-2123-5803

creases in CO<sub>2</sub> levels (Hirschel et al. 1997). Soil environments associated with plant roots were also reported to be affected by elevated CO<sub>2</sub>. For example, root biomass and growth were also shown to be increased (Cheng and Johnson 1998) in C<sub>3</sub> plants. In addition to the observed changes in the physiological characteristics of plants, Carney et al. (2007) previously demonstrated that elevated CO<sub>2</sub> could alter the microbial community in forest soil, such that the amount of carbon in the soil could be reduced as the result of enhanced fungal abundance and activity.

Salt marshes are vegetated coastal habitats, and have been recognized as one of the most productive types of ecosystem. Salt marshes contribute to the nutrient balance of adjacent ecosystems by regulating the flux of material between sediment and overlying water (Neubauer and Anderson 2003, Sousa et al. 2008), a process in which microorganisms perform a key role. For instance, despite its small area, accounting for < 2% of the area of the ocean (Duarte and Cebrián 1996), net ecosystem production in salt marshes can support secondary production or respiration of microorganisms in adjacent ecosystems (Duarte et al. 2005). Therefore, the changes in primary production in these vegetated coastal habitats that may eventually be induced by elevated atmospheric CO<sub>2</sub> are expected to affect not only these ecosystems themselves, but also the marine ecosystems adjacent to the salt marshes.

It has been previously reported that elevated CO<sub>2</sub> increases photosynthesis, shoot density, above ground biomass, and C:N ratios in green leaves in salt marshes in which C<sub>3</sub> plants predominate (e.g. *Scirpus olneyi*), whereas no such effects were noted in C<sub>4</sub> communities (e.g. *Spartina patens*) (Arp et al. 1993, Gray and Mogg 2001). This was controlled by other environmental factors, including salinity and flooding (Lenssen et al. 1993, Erickson et al. 2007). For example, as salinity decreased, biomass and C:N ratio were increased. Other physiological characteristics associated with water, including water use efficiency and evapotranspiration, responded to elevated CO<sub>2</sub> in the same fashion (Drake 1989) for both C<sub>3</sub> and C<sub>4</sub> plant communities, i.e. water use efficiency increased and evapotranspiration decreased. This was recently corroborated by Li et al. (2010), who identified salinity as an important factor controlling the extent of influence of elevated CO<sub>2</sub> on evapotranspiration in salt marshes. Elevated CO<sub>2</sub> also affects underground carbon pools in salt marshes. Marsh et al. (2005) previously demonstrated that elevated CO<sub>2</sub> induced an increase in dissolved inorganic carbon (DIC) in porewater in a brackish

marsh, and attributed this increase to enhanced root biomass and root respiration.

Increased CO<sub>2</sub> could affect not only plant physiology and geochemistry in soil, but also physical characteristics in the soil of salt marshes. Enhanced root productivities in salt marsh as the result of elevated CO<sub>2</sub> were recently determined to affect the physical stability of the soil in those ecosystems (Langley et al. 2009). Enhanced root productivities were implicated in an increase in soil elevation gains, and thus it may prove possible to compensate for the extent of the threat posed by future rises in sea level to salt marshes.

Responses of microbes to elevated CO<sub>2</sub>, most notably changes in activity and community structure, have been fairly thoroughly studied in terrestrial ecosystems (Mooney et al. 1991); the results indicated that elevated CO<sub>2</sub> levels could alter nutrient cycling and organic matter decomposition capacities. Extracellular enzymes decompose particulate organic matter into smaller organic matter, thereby allowing microorganisms to use it directly. Therefore, decomposition by extracellular enzymes can be considered a rate-limiting step in the decomposition of organic matter (Meyer-Reil 1991, Azam 1998). Elevated CO<sub>2</sub> was reported to influence the activities of phenol oxidase, which is related with recalcitrant carbon (Carney et al. 2007) in terrestrial ecosystems. Sulfate-reducing bacteria are important to the biogeochemistry of salt marsh sediments, as sulfate reduction is the principal process relevant to the decomposition of organic matter (Goldhaber and Kaplan 1975, Howarth and Hobbie 1982), one of the hallmark ecosystem functions of salt marsh sediment.

In this study, the impacts of elevated CO<sub>2</sub> on extracellular enzyme activities and changes in the community structure of sulfate-reducing bacteria in salt marsh sediment were analyzed in order to determine the effects of elevated CO<sub>2</sub> on organic matter decomposition capacity in salt marsh sediments.

## MATERIALS AND METHODS

### Sampling and setting growth chamber

Sampling was conducted in May 2005, in a salt marsh located at Hwangsan-do, where *Suaeda japonica* predominates. Sediment samples were acquired using small pots (diameter: 6 cm). Samples were transported to the lab and incubated in growth chambers. Two growth chambers were operated under two CO<sub>2</sub> concentration

conditions - 380 ppm for the ambient condition and 760 ppm for the elevated condition. 40 pots were placed in each chamber. Artificial seawater (30‰; Sigma, St. Louis, MO, USA) was supplied to each pot to prevent desiccation of sediment every 3 days. Table 1 shows the operating conditions of the growth chambers. Incubations lasted for 46 days and analyses were conducted four times, including an analysis of field samples for controls. All measurements were investigated for both surface sediment (0-2 cm) and root concentrating sediment (4-6 cm).

**Table 1.** Operating conditions of growth chambers

CO <sub>2</sub> (ppm)	380	760
Humidity (%)	65	65
Temperature (°C) (minimum/maximum)	12/20	12/20
Intensity of illumination (lux) (dark/light cycle)	0/13000	0/13000

## Analysis

Sediment samples for analyzing each measurement were obtained from surface (depth 0-1 cm) and rhizosphere sediment (4-5 cm) at approximately 2-week intervals. Water content in the sediments were measured after 24 hours of drying at 103°C, and organic matter content in the sediment was measured after combusting the dried samples for 24 hours at 600°C (loss on ignition).

Extracellular enzyme activities were measured via the methylumbelliferyl-substrate method for β-glucosidase and N-acetylglucosaminidase, and the methylcoumain-substrate method for aminopeptidase (Hoppe 1983, 1993). Substrate solutions for β-glucosidase, N-acetylglucosaminidase, and aminopeptidase were prepared using 4-methyl-β-glucoside (400 μM; Sigma), 4-methyl-N-acetylglucosamine (400 μM), L-leucine-7-amido-4-methyl-coumarin (1,000 μM), respectively. Fluorescence was analyzed via a fluorometer (TD 700; Turner designs, Sunnyvale, CA, USA) with an excitation/emission wavelength of 360 nm/430 nm for β-glucosidase and N-acetylglucosaminidase and 380 nm/440 nm for aminopeptidase.

DNA in 0.5 g of sediment was extracted using an UltraClean soil DNA kit (MoBio, Solano Beach, CA, USA) following the manufacturer's protocols. In order to analyze the community structure of sulfate-reducing bac-

teria, the *dsrAB* gene encoding for dissimilatory sulfite reductase was used as the marker gene. Primers DSR1F (5'-ACSCACTGGAAGCACG-3', labeled at the 5' end with 6-carboxyfluorescein; Perkin-Elmer Life Sciences Inc., Boston, MA, USA) and DSR4R (5'-GTGTAGCAGTTACCGCA-3') were used to amplify the *dsrAB* gene. Each PCR contained 2 μL (100-200 ng) of template DNA, 1 mM of dNTPs, 1 μM of DSR1F primer, 1 μM of DSR4R primer, 1.5 mM of MgCl<sub>2</sub>, 1.5 mM of bovine serum albumin (Sigma) 4 μg, 1.25 U of Taq (Promega, Madison, WI, USA), and 3 μL of 10× PCR buffer (500 mM KCl, 200 mM Tris-HCl [pH 8.4], 0.1% Triton-X), and the final reaction volume was adjusted to 30 μL with sterilized distilled water. Amplification was performed using a thermal cycler (PTC-100; MJ Research, Waltham, MA, USA) with the following conditions: 1 cycle of 94°C for 5 min, followed by 35 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1.5 min, with a final 10-minute extension step at 72°C. After amplification, a band of the expected size (ca. 1.9 kb) was separated via electrophoresis using 0.5× TBE buffer after visualization with ethidium bromide of 3 μL of each *dsrAB* amplicon. Triplicate PCR amplifications of *dsrAB* genes were performed under identical conditions.

The digested amplicons were then precipitated with 35 μL of 95% ethanol and centrifuged for 15 min at 16,000× g. The DNA pellet was rinsed with 70% ethanol, dried, and resuspended in a mixture of 14.5 μL of deionized formamide and 0.5 μL of DNA fragment length internal standard (TAMRA 500; Perkin-Elmer Life Sciences Inc.). Following electrophoresis, restriction enzyme reactions were conducted with the restriction enzyme *Sau3A* I (DCC-BIONET, Seoul, Korea) after selective retrieval and purification of the target fragment using a DNA purification kit (MoBio). The purification reaction was conducted for 6 hours with 10 μL of PCR purification products, 5 U of restriction enzyme and 1.5 μL of 10× reaction buffer with the final volume adjusted to 15 μL at 37°C. The digested amplicons were concentrated and purified via ethanol precipitation to enhance the sensitivity of terminal restriction fragment length polymorphism (T-RFLP) reaction. After the T-RF results were acquired with a DNA sequencer, a single peak was established as an individual phylotype and the diversity indices of the phlotypes were calculated. For similarity analysis of the community structure of each sample, the composition ratios of phlotypes were calculated via the ratio of the area of the single peak to the area of the total peak, and Jacquard's coefficients were determined. Changes in the community structure of sulfate-reducing bacteria were visualized by dendrograms generated with the UPGMA algorithm.

## Statistical analysis

Mann-Whitney analysis was conducted to determine whether there were any significant differences between surface and rhizome soils and between the 380 ppm CO<sub>2</sub> treatment and 760 ppm CO<sub>2</sub> treatments (SPSS ver. 12.0; SPSS Inc., Chicago, IL, USA ).

## RESULTS

### pH, water content and organic matter content

The results of pH, water content and organic matter content were listed in Table 2. pH in surface soil was analyzed to be 7.12 and 7.14 on average under 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively. In rhizome soil, it was measured to be 7.29 and 7.22 on average under 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively. Both in surface and in rhizome soil, pH was not significantly different by CO<sub>2</sub> treatments.

Water content in surface soil was analyzed to be 25.52% and 23.59% on average under 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively, which were not significantly different. However, in rhizome soil, water content of 22.07% on average under 380 ppm CO<sub>2</sub> was reported to be significantly higher than that of 20.05% on average under 760 ppm CO<sub>2</sub> (Mann-Whitney U test, *N* = 7, *P* < 0.01).

Organic matter contents in surface soil were reported to be 4.49% and 4.71% (on average) in the 380 ppm CO<sub>2</sub>

and 760 ppm CO<sub>2</sub> treatment groups, respectively. In rhizome soil, the organic matter contents were measured to be 4.75% and 3.41% (on average) in the 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub> treatment groups, respectively. Similarly, no significant differences in pH were detected between the CO<sub>2</sub> treatment groups.

### Extracellular enzyme activities

No effects of CO<sub>2</sub> on extracellular enzyme activities were noted in surface soils during the total incubation period (Fig. 1a). However, β-glucosidase activities in rhizome soils differed significantly between the CO<sub>2</sub> treatment groups (Mann-Whitney U test, *N* = 7, *P* < 0.01) (Table 3 and Fig 1b), but these differences persisted only until the 38<sup>th</sup> day of incubation (i.e. third sampling day).

Aminopeptidase activities in surface soil evidenced no consistent pattern associated with CO<sub>2</sub> treatment, whereas in the rhizome soil, aminopeptidase activities were higher at a CO<sub>2</sub> concentration of 380 ppm than at a CO<sub>2</sub> concentration of 760 ppm, although this effect was not significant and was also only observed until day 38 of incubation (Fig. 1a and 1b). As with aminopeptidase, no specific pattern of β-glucosidase activities was noted in the surface soil, but significantly lower activities were noted in the rhizome soil at a CO<sub>2</sub> concentration of 760 ppm relative to the 380 ppm concentration (Mann-Whitney U test, *N* = 7, *P* < 0.01) (Table 3, Fig. 1a and 1b). N-acetylglucosaminidase evidenced patterns similar to those of aminopeptidase activities (Fig. 1a and 1b).

**Table 2.** Summarized data for pH, water content and organic matter content

CO <sub>2</sub> (ppm)	pH		Water content (%)		Organic matter content (%)	
	380	760	380	760	380	760
Surface	7.12 ± 0.17	7.14 ± 0.21	25.52 ± 8.97	23.59 ± 9.27	4.49 ± 0.75	4.71 ± 2.42
Rhizome	7.29 ± 0.22	7.22 ± 0.13	22.07 ± 5.23 <sup>a</sup>	20.05 ± 4.28 <sup>b</sup>	4.75 ± 0.98	3.41 ± 0.65

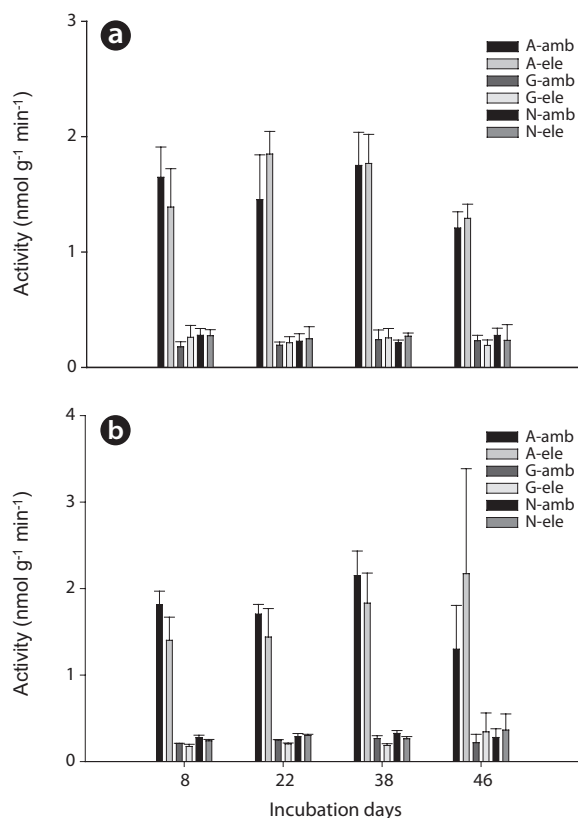
Superscripts a and b indicated differences between CO<sub>2</sub> treatments which were tested at *P* = 0.05.

**Table 3.** Summarized *P*-values of Mann-Whitney analysis

		A	G	N	A/G	A/N	G/N
Effects of CO <sub>2</sub> concentration by same soil position	Surface	0.90	0.41	0.27	0.36	0.32	0.57
	Rhizome	0.15	0.02*	0.31	0.32	0.57	0.00**
Effects of soil position by same CO <sub>2</sub> concentration	380 ppm	0.60	0.31	0.09	0.47	0.36	0.89
	760 ppm	0.28	0.08	0.95	0.49	0.57	0.00**

\* *P* < 0.05, \*\* *P* < 0.01.

A, aminopeptidase activity; G, β-glucosidase activity; N, N-acetylglucosaminidase activity.



**Fig. 1.** Change in extracellular enzyme activities in surface (a) and rhizome soil (b). Error bar indicates standard deviation ( $N = 2$  or  $3$ ). A, aminopeptidase; G,  $\beta$ -glucosidase; N, N-acetylglucosaminidase; amb, 380 ppm CO<sub>2</sub>; ele, 760 ppm CO<sub>2</sub>.

### The ratio of enzyme activities

The ratio of aminopeptidase activity to  $\beta$ -glucosidase activity was reported to be 7.17 and 7.24 on average at 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively. In rhizome soil, the ratios were (on average) 7.14 at 380 ppm CO<sub>2</sub> and 7.74 at 760 ppm CO<sub>2</sub> (Table 4). The ratio of aminopeptidase activity to N-acetylglucosaminidase activity in surface soil was measured at (on average) 6.39 at 380 ppm CO<sub>2</sub> and 8.33 at 760 ppm CO<sub>2</sub>. In the rhizome soil, the ratios were (average) 5.87 at 380 ppm CO<sub>2</sub> and 5.86 at 760

ppm CO<sub>2</sub> (Table 4). The ratio of  $\beta$ -glucosidase activity to N-acetylglucosaminidase activity was 0.92 and 1.09 (on average) at 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively. In rhizome soil, the ratios were 0.82 and 0.77 at 380 ppm CO<sub>2</sub> and 760 ppm CO<sub>2</sub>, respectively (Table 4).

No differences in the ratios of extracellular enzyme activities were noted between the surface and rhizome soils at 380 ppm CO<sub>2</sub>. At 760 ppm CO<sub>2</sub>, however, only the ratio of  $\beta$ -glucosidase activity to N-acetylglucosaminidase activity differed significantly between the surface and rhizome soils (Mann-Whitney U test,  $N = 7$ ,  $P < 0.01$ ) (Table 3), although this difference persisted only until the 38<sup>th</sup> day of incubation. Additionally, we noted the effects of elevated CO<sub>2</sub> on the ratio of  $\beta$ -glucosidase activity to N-acetylglucosaminidase activity in rhizome soils, which were significantly lower at 760 ppm CO<sub>2</sub> than at 380 ppm CO<sub>2</sub> (Mann-Whitney U test,  $N = 7$ ,  $P < 0.01$ ) (Table 3).

### Community structure of sulfate-reducing bacteria

Amplification of the *dsrAB* gene was noted in 38 of the 41 total samples. According to the results of T-RFLP analysis of the amplified *dsrAB* genes, significant T-RFs (T-RFs > 10) were obtained from 24 samples. Therefore, the results from only 24 samples were employed for the analysis of the Shannon-Weiner diversity index and similarity. The dominant species appeared to be unaltered during incubation. In surface soil, the Shannon-Weiner diversity index increased at the beginning of incubation and declined with advancing incubation time. This was much more apparent at 760 ppm CO<sub>2</sub> than at 380 ppm CO<sub>2</sub> (Fig. 2). The diversity index in rhizome soil at 760 ppm CO<sub>2</sub> increased at an early stage of incubation as well; however, it was sustained at a certain level. At CO<sub>2</sub> concentrations of less than 380 ppm, it increased at the beginning and then declined to initial levels (Fig. 3).

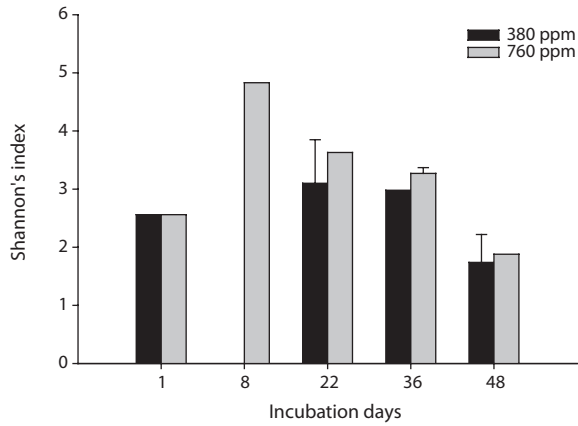
The results of our similarity analysis of sulfate-reducing bacteria showed the effects of atmospheric CO<sub>2</sub> concentration on the community structure of sulfate-reducing

**Table 4.** Descriptive statistics of enzyme ratios

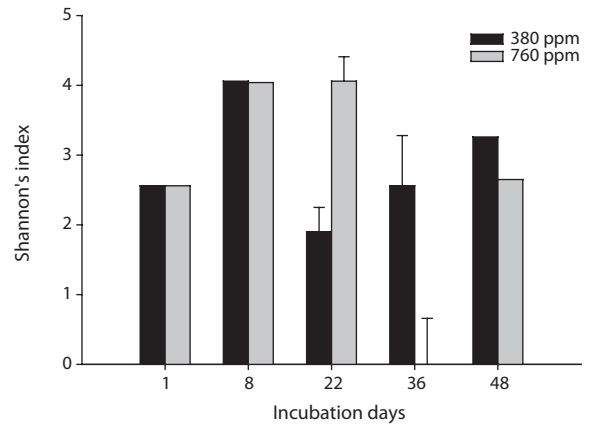
Soil part	CO <sub>2</sub> concentration (ppm)	A/G	A/N	G/N
Surface	380	7.17 ± 1.99	6.39 ± 2.17	0.92 ± 0.32
	760	7.24 ± 1.98	8.33 ± 8.27	1.09 ± 0.76
Rhizome	380	7.14 ± 1.13	5.87 ± 1.12	0.82 ± 0.09
	760	7.74 ± 1.60	5.86 ± 0.96	0.77 ± 0.14

Average ± standard deviation ( $N = 9-16$ ).

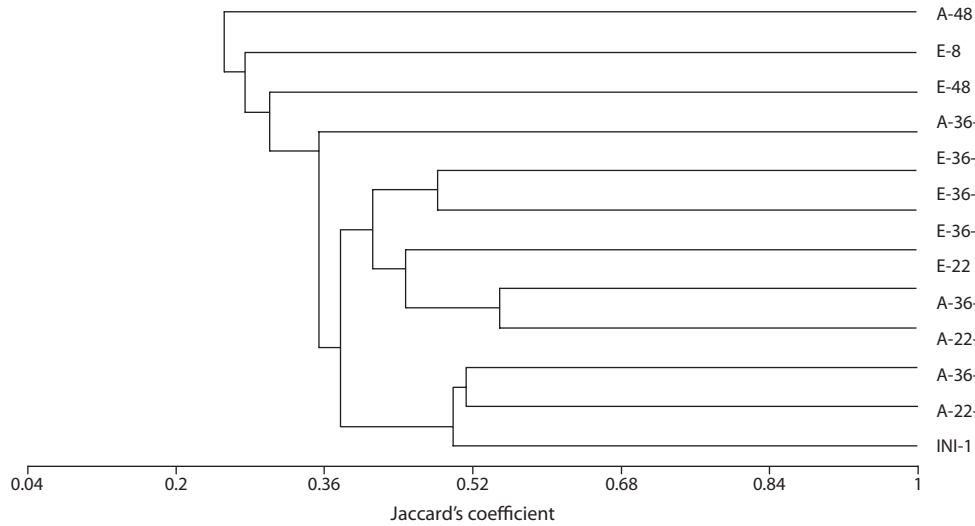
A, aminopeptidase activity; G,  $\beta$ -glucosidase activity; N, N-acetylglucosaminidase activity.



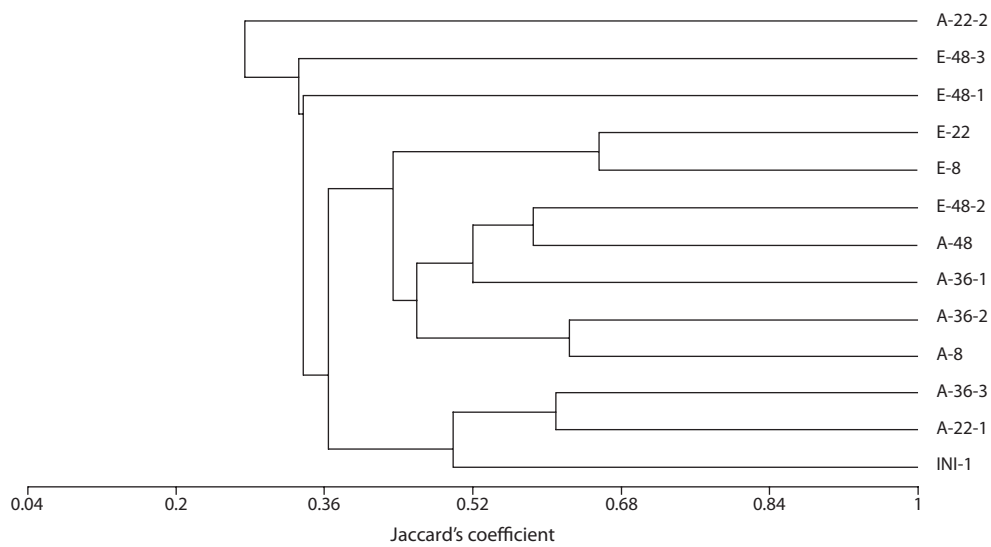
**Fig. 2.** Shanon-Wiener diversity index of community structure of sulfate-reducing bacteria in surface soil.



**Fig. 3.** Shanon-Wiener diversity index of community structure of sulfate-reducing bacteria in rhizome soil.



**Fig. 4.** Similarity of community structure of sulfate-reducing bacteria in surface soil. INI, initial soil; A, ambient (380 ppm CO<sub>2</sub>); E, elevated (760 ppm CO<sub>2</sub>). Each label consists of CO<sub>2</sub> condition-sampling date-replicates.



**Fig. 5.** Similarity of community structure of sulfate-reducing bacteria in rhizome soil. INI, initial soil; A, ambient (380 ppm CO<sub>2</sub>); E, elevated (760 ppm CO<sub>2</sub>). Each label consists of CO<sub>2</sub> condition-sampling date-replicates.



bacteria more apparently than the diversity index analysis. Both in surface and rhizome soils, the separation of community structure was observed at 380 ppm CO<sub>2</sub> and at 760 ppm CO<sub>2</sub>. Additionally, as incubation continued, the community structure of sulfate-reducing bacteria was increasingly altered (Figs. 4 and 5). It was noted that the patterns of these changes in the community structure of sulfate-reducing bacteria appeared to differ according to the position in the soil from which samples were acquired. In other words, as incubation continued, the separation in the community structure of sulfate-reducing bacteria in surface soil by atmospheric CO<sub>2</sub> concentration appeared to be weaker than that in the rhizome soil.

## DISCUSSION

Generally, more labile carbon accumulated in the rhizosphere than in bulk soil, because the products of photosynthesis are partially transported to the rhizosphere (Domanski et al. 2001). Many previous studies have shown that elevated atmospheric CO<sub>2</sub> increased labile carbon in the rhizosphere, through which products of stimulated photosynthesis are transported under elevated CO<sub>2</sub> levels (Cheng and Johnson 1998), even though this differed according to plant type (C<sub>3</sub> or C<sub>4</sub>) (Paterson et al. 1996). C<sub>3</sub> plant growth and root biomass were enhanced under elevated CO<sub>2</sub> concentration conditions, whereas C<sub>4</sub> plant growth evidenced declining patterns or no effects (Curtis et al. 1990, Arp et al. 1993, Gray and Mogg 2001). Elevated CO<sub>2</sub> also affects the quantity of labile carbon in pore water in wetland soils. Elevated atmospheric CO<sub>2</sub> has been shown to induce an increase in dissolved organic carbon (DOC) in wetland soils by increasing root exudates (Freeman et al. 2004) or enhancing the decomposition of soil organic matter (Wolf et al. 2007). According to the study of Keller et al. (2009), DOC in brackish marsh in which C<sub>3</sub>-sedge predominated was reported to increase under-elevated CO<sub>2</sub>.

Soil microbial activity can be stimulated by available carbon supply, because soil microorganisms are generally carbon-limited. Higher microbial activities are generally reported in the rhizosphere relative to that detected in other parts of soil (Zak et al. 2000). However, no significant changes in soil enzyme activities by CO<sub>2</sub> treatment were detected in surface soil or in rhizome soil. The responses of soil microbial enzyme activities to elevated atmospheric CO<sub>2</sub> were generally enhancements (Dhillon et al. 1996, Finzi et al. 2006, Drissner et al. 2007), although the extent of enhancement differed by season (Drissner

et al. 2007).

Results from the surface soil were consistent with the results from bulk soil evaluated in previous studies (Zak et al. 2000), while somewhat different patterns were noted in rhizome soil. In rhizome soil, extracellular enzyme activities did not differ by CO<sub>2</sub> treatment group; this was attributed to the lack of changes in DOC (data not shown). Increased available carbon in rhizome soil via the enhanced translocation of photosynthetic products was one of the most important factors in increasing microbial activities in rhizome soils (Hungate et al. 2000). However, DOC in rhizome soil did not increase as the result of CO<sub>2</sub> treatment in this study, and this would be considered a primary factor in the observed lack of significant changes in extracellular enzyme activities. Nitrogen depletion may be another factor in the lack of significant differences in extracellular enzyme activities by CO<sub>2</sub> treatments, as nitrogen is a key factor in regulating the responses of the soil carbon pool to elevated atmospheric CO<sub>2</sub> (Hungate et al. 2009). Considering that the pronounced effects of enhanced atmospheric CO<sub>2</sub> on soil carbon pool were mainly associated with C<sub>3</sub> plants (Long et al. 2004), *S. japonica* may not truly be a C<sub>3</sub> plant; this would support the findings of this study regarding the quantity and quality of DOC.

Elevated CO<sub>2</sub> could also affect alterations in the relative contribution of microbial communities to organic matter decomposition in the rhizosphere. The ratio of β-glucosidase activity to N-acetylglucosaminidase activity in rhizome soil was significantly lower at 760 CO<sub>2</sub> ppm than at 380 CO<sub>2</sub> ppm, demonstrating that the contribution of fungi to soil organic matter decomposition under elevated CO<sub>2</sub> concentrations might prove larger than that of bacteria (Rillig et al. 1998, Treseder and Allen 2000). Previous studies have reported increased fungal biomass and activities in the rhizosphere under elevated CO<sub>2</sub> conditions (Rillig et al. 1998), and Chung et al. (2007) previously demonstrated that, even in bulk soil where no plant root effects were detected, fungal activities were increased under elevated atmospheric CO<sub>2</sub> conditions. It has also been frequently observed that the increase in specific soil enzyme activities occurs concomitantly with enhanced fungal biomass and abundance. Carney et al. (2007) also corroborated that the proportion of fungi to bacteria rose as the result of elevated CO<sub>2</sub> concentrations, which were accompanied by an increase of phenol oxidase activities partly produced by fungi (Hammel 1997). Fungi decompose recalcitrant organic matter more voraciously even than bacteria; therefore, enhanced fungal activity might be expected to affect substantially the car-

bon cycles in salt marshes, by reducing carbon storage in sediment.

Successful amplification of *dsrAB* genes from sediment samples confirmed that sulfate-reducing bacteria were abundant and performed a critical role in the decomposition of organic matter in marine sediment. Because the incubation period was so short (ca. 50 days), no sudden changes were noted in the variables analyzed in this study. However, the overall patterns demonstrated that elevated CO<sub>2</sub> concentrations may affect the community structures of sulfate-reducing bacteria. Diversity indices were altered by elevated CO<sub>2</sub> concentration in both surface and rhizome soils. Additionally, unlike extracellular enzyme activities, the community structure of sulfate-reducing bacteria was separated according to CO<sub>2</sub> treatment groups. Previously, shifts in bacterial community structures by CO<sub>2</sub> treatment were noted in studies conducted for periods longer than the one in this study (e.g., 5 years in Janus et al. 2005). However, even though the incubations in this study were conducted for a relatively short period, separation of the community structure was noted. This was because the responses of bacteria to environmental changes on the community level might be more sensitive than at the process level (Kennedy and Smith 1995).

Although the physicochemical properties of sediment were not determined to be significantly affected by CO<sub>2</sub> concentration, the separation of sulfate-reducing bacteria by elevated CO<sub>2</sub> was detected. This might be explained by changes in the quality of available carbon sources for sulfate-reducing bacteria. In this study, specific ultra violet absorbance (SUVA), an indicator of recalcitrant DOC with aromatic rings, did not differ significantly by CO<sub>2</sub> treatment group (data not shown). Therefore, elevated atmospheric CO<sub>2</sub> was not likely to provide carbon sources to hamper microbial activities. Instead, organic acids, such as propionate and lactate, which are major carbon sources in regulating changes in the community structure of sulfate-reducing bacteria (Kleikemper et al. 2002), might be influenced by CO<sub>2</sub> treatments. DOC is composed of various carbon sources, and is one of the main factors controlling the processes of organic matter decomposition processes, such as sulfate reduction, in marine sediment (Leloup et al. 2005). Organic acid is another important component, and the rates of production and consumption of organic acids were frequently sufficiently different to determine whether the net flux of organic acids is reflective of production or consumption (McMahon and Chapelle 1991). Similarly, organic acids in sediment might be consumed too rapidly to be detect-

ed in the changes in DOC evaluated in this study.

Even though sulfate reduction rates were not measured in this study, changes in the community structures of sulfate-reducing bacteria indicated that sulfate reduction might be affected by elevated CO<sub>2</sub>. Keller et al. (2009) demonstrated that sulfate reduction rates in brackish marshes were enhanced by elevated CO<sub>2</sub>. However, in this study, factors that enhanced sulfate reduction rates, such as DOC, were unaffected by CO<sub>2</sub> treatments. Thus, elevated CO<sub>2</sub> was not considered likely to enhance sulfate reduction rates in this study.

According to the results observed herein, elevated CO<sub>2</sub> appeared not to affect the capacity of organic matter decomposition in salt marsh sediment vegetated with *S. japonica*, as extracellular enzyme activities were not altered significantly by CO<sub>2</sub> treatments. However, alterations in microbial communities as the result of CO<sub>2</sub> treatments were observed, particularly in rhizome soil. It is worth mentioning that the changes in microbial communities were not linked to changes in ecosystem function, such as organic matter decomposition. For example, the role of fungi in organic matter decomposition (relative to the role of bacteria) appeared to be greater under elevated CO<sub>2</sub> conditions than under ambient CO<sub>2</sub> conditions; however, the activity of N-acetylglucosaminidase which is produced principally by fungi, did not increase significantly under elevated CO<sub>2</sub> conditions. Generally, changes in microbial communities were accompanied by changes in ecosystem function (Carney et al. 2007). Little is currently known regarding the responses of sulfate-reducing bacteria in salt marsh sediment to elevated atmospheric CO<sub>2</sub>. In this study, factors that regulate sulfate reduction, such as DOC, were unaffected by elevated CO<sub>2</sub>; however, the community structures of sulfate-reducing bacteria were unaltered by elevated CO<sub>2</sub> concentrations. This finding indicates that elevated atmospheric CO<sub>2</sub> may affect microbial diversity rather than microbial activity, or that the response of microbial diversity to elevated CO<sub>2</sub> may be faster than that of microbial activity. These possibilities should be carefully considered in future studies of the relationship between microbial diversity and ecosystem functions.

## CONCLUSION

In this study, extracellular enzyme activities in salt marsh sediment were found not to be affected by elevated CO<sub>2</sub>. However, the relative contributions of fungi and bacteria to organic matter decomposition were appar-



ently altered under elevated CO<sub>2</sub> conditions. Additionally, the community structure of sulfate-reducing bacteria was also altered by elevated CO<sub>2</sub>. Therefore, elevated atmospheric CO<sub>2</sub> may affect microbial diversity rather than microbial activity; alternatively, the response of microbial diversity to elevated CO<sub>2</sub> may be more rapid than that of microbial activity.

## ACKNOWLEDGMENTS

This study was supported by NRF (2009-0092795) and the Center for Aquatic Ecosystem Restoration (CAER) of Eco-STAR project from Ministry of Environment (MOE), Republic of Korea.

## LITERATURE CITED

- Arp WJ, Drake BG, Pockman WT, Curtis PS, Whigham DE. 1993. Interactions between C<sub>3</sub> and C<sub>4</sub> salt marsh plant species during four years of exposure to elevated atmospheric CO<sub>2</sub>. *Vegetatio* 104/105: 133-143.
- Azam F. 1998. Microbial control of oceanic carbon flux: the plot thickens. *Science* 280: 694-696.
- Carney KM, Hungate BA, Drake BG, Megonigal JP. 2007. Altered soil microbial community at elevated CO<sub>2</sub> leads to loss of soil carbon. *Proc Natl Acad Sci USA* 104: 4990-4995.
- Cheng W, Johnson DW. 1998. Elevated CO<sub>2</sub>, rhizosphere processes, and soil organic matter decomposition. *Plant Soil* 202: 167-174.
- Chung H, Zak DR, Reich PB, Ellsworth DS. 2007. Plant species richness, elevated CO<sub>2</sub>, and atmospheric nitrogen deposition alter soil microbial community composition and function. *Global Change Biol* 13: 980-989.
- Cotrufo ME, Ineson P. 1996. Elevated CO<sub>2</sub> reduces field decomposition rates of *Betula pendula* (Roth) leaf litter. *Oecologia* 106: 525-530.
- Curtis PS, Balduman LM, Drake BG, Whigham DE. 1990. Elevated atmospheric CO<sub>2</sub> effects on belowground processes in C<sub>3</sub> and C<sub>4</sub> estuarine marsh communities. *Ecology* 71: 2001-2006.
- DeLucia EH, Hamilton JG, Naidu SL, Thomas RB, Andrews JA, Finzi A, Lavine M, Matamala R, Mohan JE, Hendrey GR, Schlesinger WH. 1999. Net primary production of a forest ecosystem with experimental CO<sub>2</sub> enrichment. *Science* 284: 1177-1179.
- Detwiler RP, Hall CAS. 1988. Tropical forests and the global carbon cycle. *Science* 239: 42-47.
- Dhillon SS, Roy J, Abrams M. 1996. Assessing the impact of elevated CO<sub>2</sub> on soil microbial activity in a Mediterranean model ecosystem. *Plant Soil* 187: 333-342.
- Domanski G, Kuzyakov Y, Siniakina SV, Stahr K. 2001. Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). *J Plant Nutr Soil Sci* 164: 381-387.
- Drake BG. 1989. Effects of Elevated Carbon Dioxide on Chesapeake Bay Wetlands. IV. Ecosystem and Whole Plant Responses April-November, 1988. US DOE/CO<sub>2</sub> Greenbook 051- Atmospheric and Climate Research Division, ER-76, US Department of Energy, Washington, DC.
- Drissner D, Blum H, Tscherko D, Kandeler E. 2007. Nine years of enriched CO<sub>2</sub> changes the function and structural diversity of soil microorganisms in a grassland. *Eur J Soil Sci* 58: 260-269.
- Duarte CM, Cebrián J. 1996. The fate of marine autotrophic production. *Limnol Oceanogr* 41: 1758-1766.
- Duarte CM, Middelburg JJ, Caraco N. 2005. Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences* 2: 1-8.
- Erickson JE, Megonigal JP, Peresta G, Drake BG. 2007. Salinity and sea level mediate elevated CO<sub>2</sub> effects on C<sub>3</sub>-C<sub>4</sub> plant interactions and tissue nitrogen in a Chesapeake Bay tidal wetland. *Global Change Biol* 13: 202-215.
- Finzi AC, Sinsabaugh RL, Long TM, Osgood MP. 2006. Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems* 9: 215-226.
- Freeman C, Fenner N, Ostle NJ, Kang H, Dowrick DJ, Reynolds B, Lock MA, Sleep D, Hughes S, Hudson J. 2004. Export of dissolved organic carbon from peatlands under elevated carbon dioxide levels. *Nature* 430: 195-198.
- Goldhaber MB, Kaplan IR. 1975. Controls and consequences of sulfate reduction rates in recent marine sediments. *Soil Sci* 119: 42-55.
- Gray AJ, Mogg RJ. 2001. Climate impacts on pioneer salt-marsh plants. *Clim Res* 18: 105-112.
- Hammel KE. 1997. Fungal degradation of lignin. In: *Driven by Nature: Plant Litter Quality and Decomposition* (Cadish G, Giller KE, eds). CAB International, Wallingford.
- Hirschel G, Korner C, Arnone JA 3rd. 1997. Will rising atmospheric CO<sub>2</sub> affect leaf litter quality and in situ decomposition rates in native plant communities? *Oecologia* 110: 387-392.
- Hoppe HG. 1983. Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbelliferyl-substrates. *Mar Ecol Prog Ser* 11: 299-308.
- Hoppe HG. 1993. Use of fluorogenic model substrates for

- extracellular enzyme activity (EEA) measurement of bacteria. In: Handbook of Methods in Aquatic Microbial Ecology (Kemp PF, Sherr BF, Sherr EB, Cole JJ, eds). Lewis Publishers, Boca Raton, FL, pp 423-432.
- Howarth RW, Hobbie JE. 1982. The regulation of decomposition and heterotrophic microbial activity in salt marsh soils: a review. In: Estuarine Comparisons (Kennedy VS, ed). Academic Press, New York, NY, pp 103-127.
- Hungate BA, Jaeger CH, Gamara G, Chapin FS, Field CB. 2000. Soil microbiota in two annual grasslands: responses to elevated atmospheric CO<sub>2</sub>. *Oecologia* 124: 589-598.
- Hungate BA, van Groenigen KJ, Six J, Jastrow JD, Lue Y, de Graaff MA, van Kessel C, Osenberg CW. 2009. Assessing the effect of elevated carbon dioxide on soil carbon: a comparison on four meta-analyses. *Global Change Biol* 15: 2020-2034.
- IPCC. 2007. Summary for policymakers. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL eds). Cambridge University Press, Cambridge and New York, NY.
- Janus LR, Angeloni NL, McCormack J, Rier ST, Tuchman NC, Kelly JJ. 2005. Elevated atmospheric CO<sub>2</sub> alters soil microbial communities associated with trembling aspen (*Populus tremuloides*) roots. *Microb Ecol* 50: 102-109.
- Keller JK, Wolf AA, Weisenhorn PB, Drake BG, Megonigal JP. 2009. Elevated CO<sub>2</sub> affects porewater chemistry in a brackish marsh. *Biogeochemistry* 96: 101-117.
- Kennedy AC, Smith KL. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant Soil* 170: 75-86.
- Kleikemper J, Pelz O, Schroth MH, Zeyer J. 2002. Sulfate-reducing bacterial community response to carbon source amendments in contaminated aquifer microcosms. *FEMS Microbiol Ecol* 42: 109-118.
- Langley JA, McKee KL, Cahoon DR, Cherry JA, Megonigal JP. 2009. Elevated CO<sub>2</sub> stimulates marsh elevation gain, counterbalancing sea-level rise. *Proc Natl Acad Sci USA* 106: 6182-6186.
- Leloup J, Petit F, Boust D, Deloffre J, Bally G, Clarisse O, Quillet L. 2005. Dynamics of sulfate-reducing microorganisms (*dsrAB* genes) in two contrasting mudflats of the Seine estuary (France). *Microb Ecol* 50: 307-314.
- Lenssen GM, Lamers J, Stroetenga M, Rozema J. 1993. Interactive effects of atmospheric CO<sub>2</sub> enrichment, salinity and flooding on growth of C<sub>3</sub> (*Elymus athericus*) and C<sub>4</sub> (*Spartina anglica*) salt marsh species. *Vegetatio* 104: 379-388.
- Li JH, Erickson JE, Peresta G, Drake BG. 2010. Evapotranspiration and water use efficiency in a Chesapeake Bay wetland under carbon dioxide enrichment. *Global Change Biol* 16: 234-245.
- Long SP, Ainsworth EA, Rogers A, Ort DR. 2004. Rising atmospheric carbon dioxide: plants face the future. *Annu Rev Plant Biol* 55: 591-628.
- Marsh AS, Rasse DP, Drake BG, Megonigal JP. 2005. Effect of elevated CO<sub>2</sub> on carbon pools and fluxes in a brackish marsh. *Estuaries* 28: 694-704.
- McMahon PB, Chapelle FH. 1991. Microbial production of organic acids in aquitard sediments and its role in aquifer geochemistry. *Nature* 349: 233-235.
- Meyer-Reil LA. 1991. Ecological aspects of enzymatic activity in marine sediments. In: *Microbial Enzymes in Aquatic Environments* (Chróst RJ, ed). Springer-Verlag, Berlin, pp 84-95.
- Mooney HA, Drake BG, Luxmoore RJ, Oechel WC, Pitelka LF. 1991. Predicting ecosystem responses to elevated CO<sub>2</sub> concentrations. *BioScience* 41: 96-104.
- Moore B 3rd, Bolin B. 1986. The oceans, carbon dioxide, and global climate change. *Oceanus* 29: 9-15.
- Neubauer SC, Anderson IC. 2003. Transport of dissolved inorganic carbon from a tidal freshwater marsh to the York River estuary. *Limnol Oceanogr* 48: 299-307.
- Paterson E, Rattray EAS, Killham K. 1996. Effect of elevated atmospheric CO<sub>2</sub> concentration on C-partitioning and rhizosphere C-flow for three plant species. *Soil Biol Biochem* 28: 195-201.
- Rillig MC, Allen ME, Klironomos JN, Chiariello NR, Field CB. 1998. Plant species-specific changes in root-inhabiting fungi in a California annual grassland: responses to elevated CO<sub>2</sub> and nutrients. *Oecologia* 113: 252-259.
- Sousa AI, Lillebø AI, Caçador I, Pardal MA. 2008. Contribution of *Spartina maritima* to the reduction of eutrophication in estuarine systems. *Environ Pollut* 156: 628-635.
- Treseder KK, Allen ME. 2000. Black boxes and missing sinks: fungi in global change research. *Mycol Res* 104: 1281-1283.
- Wolf AA, Drake BG, Erickson JE, Megonigal JP. 2007. An oxygen-mediated positive feedback between elevated carbon dioxide and soil organic matter decomposition in a simulated anaerobic wetland. *Global Change Biol* 13: 2036-2044.
- Zak DR, Pregitzer KS, Curtis PS, Holmes WE. 2000. Atmospheric CO<sub>2</sub> and the composition and function of soil microbial communities. *Ecol Appl* 10: 47-59.