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Effects of elevated CO_2 on organic matter decomposition capacities and community structure of sulfate-reducing bacteria in salt marsh sediment

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Increasing atmospheric CO_2 affects the soil carbon cycle by influencing microbial activity and the carbon pool. In this study, the effects of elevated CO_2 on extracellular enzyme activities (EEA; β-glucosidase, N-acetylglucosaminidase, aminopeptidase) in salt marsh sediment vegetated with *Suaeda japonica* were assessed under ambient atmospheric CO_2 concentration (380 ppm) or elevated CO_2 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_2 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_2 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_2 than at 380 ppm CO_2, 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_2 treatments, suggesting that elevated CO_2 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_2 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_2 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_2 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_2, and were seen in C_3 plants rather than C_4 plants. The C:N ratio in green leaves was also increased with rising CO_2 concentrations, because C components like starch were increased and N contents were reduced as the result of elevated CO_2 levels (Cotrufo and Ineson 1996). However, litter chemistry did not change with in-

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creases in CO_2 levels (Hirschel et al. 1997). Soil environments associated with plant roots were also reported to be affected by elevated CO_2. For example, root biomass and growth were also shown to be increased (Cheng and Johnson 1998) in C_3 plants. In addition to the observed changes in the physiological characteristics of plants, Carney et al. (2007) previously demonstrated that elevated CO_2 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_2 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_2 increases photosynthesis, shoot density, above ground biomass, and C:N ratios in green leaves in salt marshes in which C_3 plants predominate (e.g. *Scirpus olneyi*), whereas no such effects were noted in C_4 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_2 in the same fashion (Drake 1989) for both C_3 and C_4 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_2 on evapotranspiration in salt marshes. Elevated CO_2 also affects underground carbon pools in salt marshes. Marsh et al. (2005) previously demonstrated that elevated CO_2 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_2 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_2 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_2, 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_2 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_2 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_2 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_2 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_2 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_2 (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 bacteria, the *dsr*AB 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 *dsr*AB 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 $MgCl_2$, 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 *dsr*AB amplicon. Triplicate PCR amplifications of *dsr*AB 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 *Sau*3A 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 phylotypes were calculated. For similarity analysis of the community structure of each sample, the composition ratios of phylotypes 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_2 treatment and 760 ppm CO_2 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_2 and 760 ppm CO_2, respectively. In rhizome soil, it was measured to be 7.29 and 7.22 on average under 380 ppm CO_2 and 760 ppm CO_2, respectively. Both in surface and in rhizome soil, pH was not significantly different by CO_2 treatments.

Water content in surface soil was analyzed to be 25.52% and 23.59% on average under 380 ppm CO_2 and 760 ppm CO_2, respectively, which were not significantly different. However, in rhizome soil, water content of 22.07% on average under 380 ppm CO_2 was reported to be significantly higher than that of 20.05% on average under 760 ppm CO_2 (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_2 and 760 ppm CO_2 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_2 and 760 ppm CO_2 treatment groups, respectively. Similarly, no significant differences in pH were detected between the CO_2 treatment groups.

Extracellular enzyme activities

No effects of CO_2 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_2 treatment groups (Mann-Whitney U test, $N = 7$, $P < 0.01$) (Table 3 and Fig 1b), but these differences persisted only until the 38th day of incubation (i.e. third sampling day).

Aminopeptidase activities in surface soil evidenced no consistent pattern associated with CO_2 treatment, whereas in the rhizome soil, aminopeptidase activities were higher at a CO_2 concentration of 380 ppm than at a CO_2 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_2 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

	pH		Water content (%)		Organic matter content (%)	
CO_2 (ppm)	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[a]	20.05 ± 4.28[b]	4.75 ± 0.98	3.41 ± 0.65

Superscripts a and b indicated differences between CO_2 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_2 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_2 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.

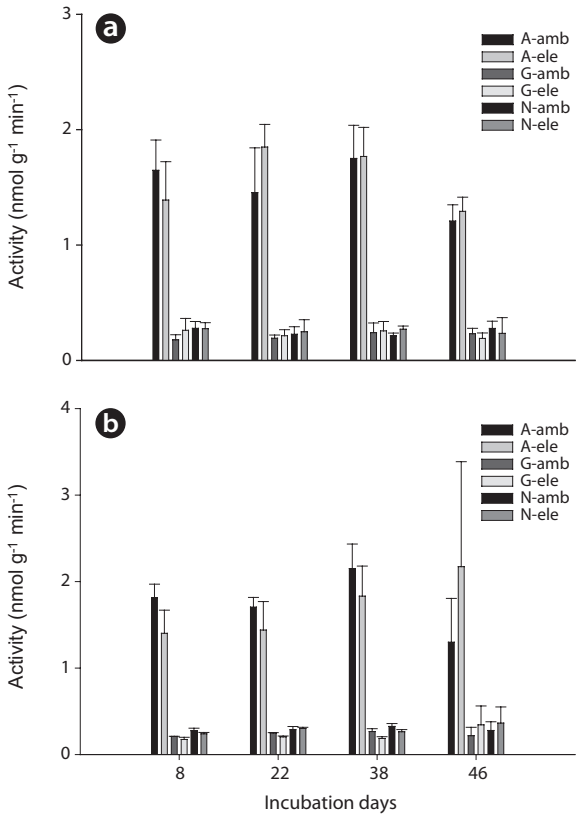

Fig. 1. Change in extracellular enzyme activities in surface (a) and rhizome soil (b). Error bar indicates standard deviation ($N = 2$ or 3). A, aminopeptidase; G, β-glucosidase; N, N-acetylglucosaminidase; amb, 380 ppm CO_2; ele, 760 ppm CO_2.

The ratio of enzyme activities

The ratio of aminopeptidase activity to β-glucosidase activity was reported to be 7.17 and 7.24 on average at 380 ppm CO_2 and 760 ppm CO_2, respectively. In rhizome soil, the ratios were (on average) 7.14 at 380 ppm CO_2 and 7.74 at 760 ppm CO_2 (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_2 and 8.33 at 760 ppm CO_2. In the rhizome soil, the ratios were (average) 5.87 at 380 ppm CO_2 and 5.86 at 760 ppm CO_2 (Table 4). The ratio of β-glucosidase activity to N-acetylglucosaminidase activity was 0.92 and 1.09 (on average) at 380 ppm CO_2 and 760 ppm CO_2, respectively. In rhizome soil, the ratios were 0.82 and 0.77 at 380 ppm CO_2 and 760 ppm CO_2, respectively (Table 4).

No differences in the ratios of extracellular enzyme activities were noted between the surface and rhizome soils at 380 ppm CO_2. At 760 ppm CO_2, however, only the ratio of β-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 38th day of incubation. Additionally, we noted the effects of elevated CO_2 on the ratio of β-glucosidase activity to N-acetylglucosaminidase activity in rhizome soils, which were significantly lower at 760 ppm CO_2 than at 380 CO_2 ppm (Mann-Whitney U test, $N = 7$, $P < 0.01$) (Table 3).

Community structure of sulfate-reducing bacteria

Amplification of the *dsr*AB gene was noted in 38 of the 41 total samples. According to the results of T-RFLP analysis of the amplified *dsr*AB 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_2 than at 380 ppm CO_2 (Fig. 2). The diversity index in rhizome soil at 760 ppm CO_2 increased at an early stage of incubation as well; however, it was sustained at a certain level. At CO_2 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_2 concentration on the community structure of sulfate-reducing

Table 4. Descriptive statistics of enzyme ratios

Soil part	CO_2 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, β-glucosidase activity; N, N-acetylglucosaminidase activity.

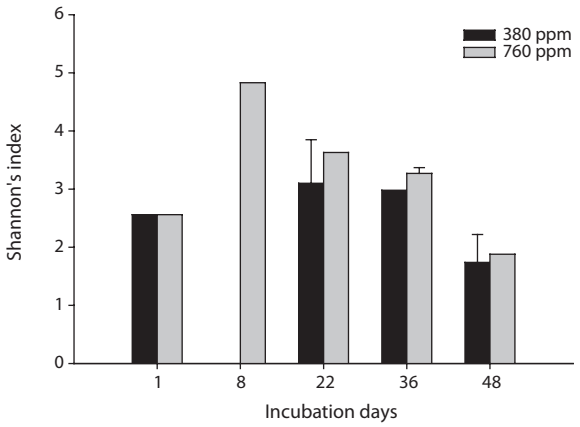

Fig. 2. Shanon-Wiener diversity index of community structure of sulfate-reducing bacteria in surface soil.

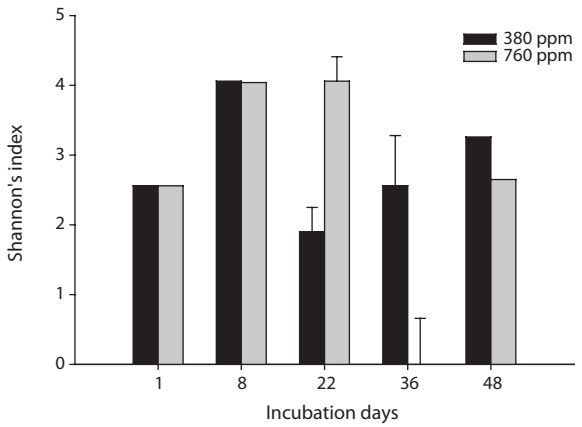

Fig. 3. Shanon-Wiener diversity index of community structure of sulfate-reducing bacteria in rhizome soil.

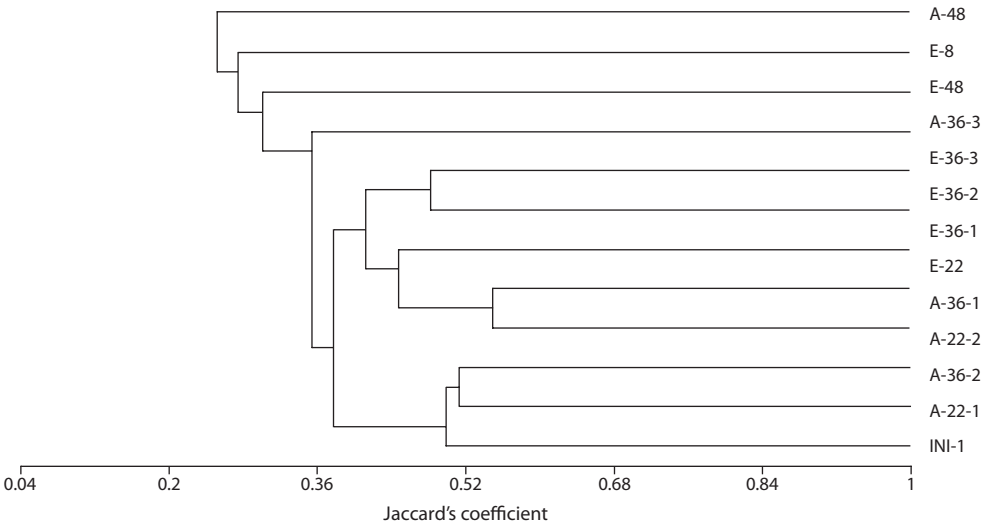

Fig. 4. Similarity of community structure of sulfate-reducing bacteria in surface soil. INI, initial soil; A, ambient (380 ppm CO_2); E, elevated (760 ppm CO_2). Each label consists of CO_2 condition-sampling date-replicates.

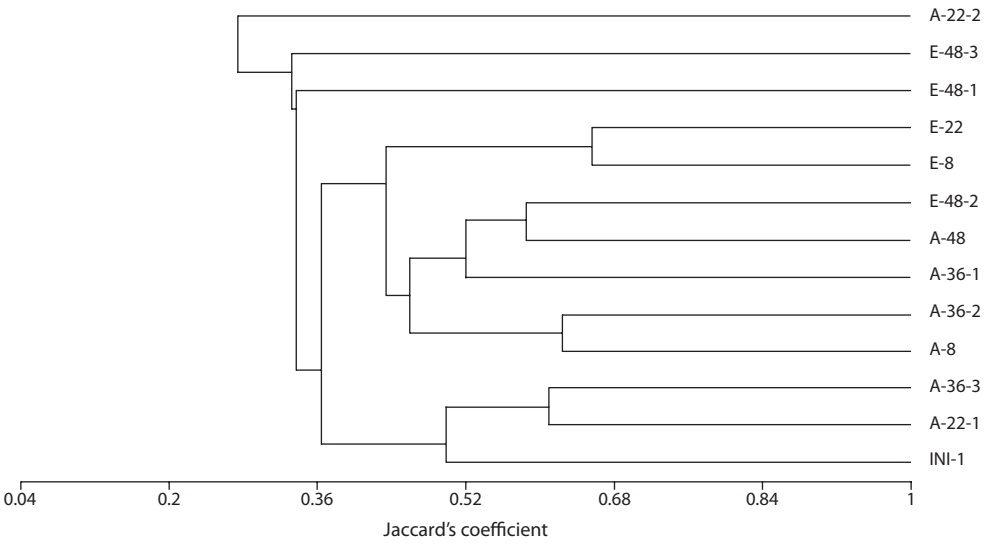

Fig. 5. Similarity of community structure of sulfate-reducing bacteria in rhizome soil. INI, initial soil; A, ambient (380 ppm CO_2); E, elevated (760 ppm CO_2). Each label consists of CO_2 condition-sampling date-replicates.

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_2 and at 760 ppm CO_2. 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_2 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_2 increased labile carbon in the rhizosphere, through which products of stimulated photosynthesis are transported under elevated CO_2 levels (Cheng and Johnson 1998), even though this differed according to plant type (C_3 or C_4) (Paterson et al. 1996). C_3 plant growth and root biomass were enhanced under elevated CO_2 concentration conditions, whereas C_4 plant growth evidenced declining patterns or no effects (Curtis et al. 1990, Arp et al. 1993, Gray and Mogg 2001). Elevated CO_2 also affects the quantity of labile carbon in pore water in wetland soils. Elevated atmospheric CO_2 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_3-sedge predominated was reported to increase under-elevated CO_2.

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_2 treatment were detected in surface soil or in rhizome soil. The responses of soil microbial enzyme activities to elevated atmospheric CO_2 were generally enhancements (Dhillion 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_2 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_2 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_2 treatments, as nitrogen is a key factor in regulating the responses of the soil carbon pool to elevated atmospheric CO_2 (Hungate et al. 2009). Considering that the pronounced effects of enhanced atmospheric CO_2 on soil carbon pool were mainly associated with C_3 plants (Long et al. 2004), *S. japonica* may not truly be a C_3 plant; this would support the findings of this study regarding the quantity and quality of DOC.

Elevated CO_2 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_2 ppm than at 380 CO_2 ppm, demonstrating that the contribution of fungi to soil organic matter decomposition under elevated CO_2 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_2 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_2 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_2 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 *dsr*AB 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_2 concentrations may affect the community structures of sulfate-reducing bacteria. Diversity indices were altered by elevated CO_2 concentration in both surface and rhizome soils. Additionally, unlike extracellular enzyme activities, the community structure of sulfate-reducing bacteria was separated according to CO_2 treatment groups. Previously, shifts in bacterial community structures by CO_2 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_2 concentration, the separation of sulfate-reducing bacteria by elevated CO_2 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_2 treatment group (data not shown). Therefore, elevated atmospheric CO_2 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_2 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 detected 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_2. Keller et al. (2009) demonstrated that sulfate reduction rates in brackish marshes were enhanced by elevated CO_2. However, in this study, factors that enhanced sulfate reduction rates, such as DOC, were unaffected by CO_2 treatments. Thus, elevated CO_2 was not considered likely to enhance sulfate reduction rates in this study.

According to the results observed herein, elevated CO_2 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_2 treatments. However, alterations in microbial communities as the result of CO_2 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_2 conditions than under ambient CO_2 conditions; however, the activity of N-acetylglucosaminidase which is produced principally by fungi, did not increase significantly under elevated CO_2 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_2. In this study, factors that regulate sulfate reduction, such as DOC, were unaffected by elevated CO_2; however, the community structures of sulfate-reducing bacteria were unaltered by elevated CO_2 concentrations. This finding indicates that elevated atmospheric CO_2 may affect microbial diversity rather than microbial activity, or that the response of microbial diversity to elevated CO_2 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_2. However, the relative contributions of fungi and bacteria to organic matter decomposition were appar-

ently altered under elevated CO_2 conditions. Additionally, the community structure of sulfate-reducing bacteria was also altered by elevated CO_2. Therefore, elevated atmospheric CO_2 may affect microbial diversity rather than microbial activity; alternatively, the response of microbial diversity to elevated CO_2 may be more rapid than that of microbial activity.

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