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Effects of elevated carbon dioxide concentration on biological nitrogen fixation, nitrogen mineralization and carbon decomposition in submerged rice soil

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Abstract Controlled-environment chambers were used to study the effects of elevated CO_2 concentrations on biological N fixation, N mineralization and C decomposition in rice soil. In three chambers, CO_2 concentration was maintained at 353±15/396±23 μmol mol^{-1} (day/night; ambient CO_2), while in another three, CO_2 was maintained at 667±36/700±41 μmol mol^{-1} (day/night; elevated CO_2) throughout the growing season. Rice (var. Nipponbare*)* seedlings were grown under either ambient or elevated CO_2 concentrations, and then transplanted into the soils in the corresponding chambers. At different growth stages, soil samples were taken from surface (0–1cm) and sub-surface (1–10cm) layers at the centre of four hills, then sieved (<1 mm) to remove root residues. Fresh soil was used to measure N fixation activity (using the acetylene reduction assay), NH_4^+ content and organic C. Separate sets of soil samples were transferred to serum bottles and anaerobically incubated at 30°C for 30 days to measure potential rates of N mineralization and C decomposition. Under an elevated atmospheric CO_2 concentration, acetylene reduction activity significantly increased in the surface soil layer during the early cultivation stages and in the sub-surface soil layer during the latter part of cultivation. There was no difference in the amount of NH_4^+ in fresh soils between elevated and ambient CO_2 chambers, while the rate of N mineralization was increased by elevated CO_2 during the latter part of cultivation. Soils from the elevated CO_2 chambers had obviously higher rate of C decomposition than that from the ambient CO_2 chambers. CH_4 production gradually increased with the growth of rice plants. These results suggest that elevated CO_2 affected biological N fixation, N mineralization and C decomposition in submerged rice soil during the different growth stages of rice.

Keywords Carbon decomposition · Carbon dioxide enrichment · Dinitrogen fixation · Nitrogen mineralization · Paddy soil

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Introduction

The global atmospheric CO_2 concentration is currently increasing, and this trend is projected to continue and result in an approximate doubling of the current CO_2 concentration by the end of the twenty-first century (Bolin 1986; IPCC 1995). An increase in the atmospheric level of CO_2 has the potential to affect terrestrial ecosystems by influencing plant photosynthesis and productivity. Most of the recent studies on this subject have focused on the effects of elevated CO_2 concentrations on aboveground plant processes such as photosynthesis, transpiration and biomass accumulation (Lekkerkerk et al. 1990; Norby 1994). Few studies have documented the effects of an elevated atmospheric CO_2 concentration on the dynamics of soil C and N in different ecosystems (Zak et al. 1993; Soussana and Hartwig 1996; Hungate et al. 1997), and most of these data were obtained from aerobic soils.

Rice soils account for a large fraction of wetland ecosystems and provide a staple food to a large portion of the world's population, especially in Asia. The dynamics of C and N in submerged rice soil is different from that of aerobic soil because submerged rice soils are maintained at lower redox potentials. N fixation is an important process in flooded soils planted to rice. The potential for associative biological N fixation is higher in submerged rice soil than in aerobic soil (Yoshida and

Ancajas 1973). It is believed that submerged rice soil can maintain its soil fertility for a long time due to the fixation of atmospheric N by microorganisms in the soil (Ladha et al. 1997). On the other hand, in submerged rice soils, decomposed C is not only mineralized to CO_2, but also fermented to CH_4, which has a potential for thermal absorption which is about 30 times higher than that of CO_2 (Bouwman 1990). Due to the lower redox potential of submerged rice soils, NH_4^+ cannot be transformed to NO_3^- by nitrification, and so NH_4^+ remains as the dominant form of mineral N in submerged rice soils.

According to our previous study using microcosm experiments without rice plants, the amounts of microbial biomass C and chlorophyll-type compounds in surface soil were increased under an elevated atmospheric CO_2 concentration (Inubushi et al. 1999). Also, the CH_4 emission rate was decreased due to the increase in the CH_4 oxidation rate in the surface soil layer under an elevated atmospheric CO_2 concentration (Cheng et al. 2000b). It is possible that N fixation activities may be increased in surface soil layers by elevated CO_2 concentrations due to increased algal growth (Cheng et al. 2000a). Algal photosynthesis can increase the concentration of O_2 in floodwater (Thind and Rowell 1999), whereas algal growth can prevent N losses and increase fertilizer-N use efficiency (Thind and Rowell 2000). A number of studies on the response of the rice plants to an elevated CO_2 concentration have shown significant increases, not only in growth and yield but also in root biomass and tiller number (Imai et al. 1985; Baker et al. 1993; Kim et al. 1996). An elevated CO_2 concentration increased root dry weight by 13% at early heading, 20% at mid-maturity, and 27% at harvest (Kobayashi et al. 1999). Increased root weight and subsequent root exudation will indirectly influence C and N cycling in soil.

The objectives of this research were to evaluate the effect of an elevated atmospheric CO_2 concentration on biological N fixation, N mineralization and C decomposition in submerged rice soil.

Materials and methods

Controlled environment chambers and experimental design

This research was conducted in a set of growth chambers (Climatron) of the National Institute of Agro-Environmental Sciences, Tsukuba, Japan (NIAES). The system consists of six controlled environment chambers whose dimensions are 4×3×2 m (length× width×height; L×W×D), each providing 4×2×2 m for plant growth. Each chamber housed two stainless steel containers (150× 150×30 cm, L×W×D) filled with paddy soil. The frames, rear (north) walls, and floor of the chamber were made of stainless steel. The frames were glazed with 5-mm-thick tempered glass whose transmittance of visible light is >80%. Air temperature and relative humidity in each chamber were controlled by electrical resistance heaters (with a bubbling system for humidification) and cold-water heat exchangers using proportional integral derivative controllers (DB1000; CHINO, Tokyo). Air temperature and relative humidity in each chamber were measured with temperature-humidity sensors (HN-Q500–1; CHINO) shielded against direct solar radiation and mounted above a rice canopy. In this experiment, air temperature was controlled to track ambient air temperature with the seasonal mean temperature being 23.4°C. Relative humidity was kept at 80±1.9%. In three chambers, the CO_2 concentration was maintained at 353±15/396±23 µmol mol^{-1} (day/night; ambient CO_2), while in another three, CO_2 was maintained at 667±36/700±41 µmol mol^{-1} (day/night; elevated CO_2) throughout the growing season. In the daytime, CO_2 was maintained by a computer-controlled pure CO_2 injection system, which compensated for CO_2 uptake by the rice canopy. During the night, CO_2 increased due to plant respiration, but was kept at a level which did not exceed the daytime one by >100 µmol mol^{-1} by a computer-controlled air ventilation system, which introduced ambient air to reduce the level of CO_2. The ambient air temperature was measured with a platinum resistance thermometer, which was shielded, aspirated and placed outside the chambers. Environmental data in each chamber and ambient air temperature were monitored every 10 s, and 5-min means were recorded.

Experimental soil, rice cultivar and cultural practices

Bulk soil was collected from the plough layer (top 20 cm) of a rice field in Yawara, Ibaraki Prefecture, Japan. The soil was classified as alluvial soil with a clay content of about 35%. As the soil had been piled up for 2 years outside before being used, visible plant resides were absent. The rice cultivar used was Nipponbare, a popular variety used by Japanese farmers. Rice seedlings were grown under ambient or elevated CO_2 concentrations separately at 23°C for 25 days in incubators in the laboratory of Micrometeorology of NIAES, and then transplanted to the soil inside the containers at the same two targeted CO_2 levels. One day before transplanting, basal fertilizers ($N:P_2O_5:K_2O$, 50:150:150 kg ha^{-1}) and rice straw chopped to about 3 cm in length (1,500 kg ha^{-1}) were added to produce a homogeneous mixture with the soil. Seedlings were transplanted by hand with three seedlings per hill at 20×20 cm spacing. Top-dressing with NH_4Cl was undertaken between maximum tillering and panicle initiation stages at the rate of 30 kg N ha^{-1}. The flooded water was maintained at about 3–5 cm depth throughout the cultivation period.

Sampling and analysis

On 21, 41, 63, 89 and 112 days after transplanting (DAT), representing early growth, maximum-tillering, panicle initiation, flowering and grain-filling stages of rice growth, respectively, soil samples were collected from the surface layer (0–1cm) and subsurface layer (1–10 cm) at the centre of four hills in each chamber by using a spatula and core sampler, respectively. Sampled soils were pushed through 1-mm sieve in the laboratory to remove root residues. Sieved soil was used to measure N fixation activity, N mineralization, and C decomposition. The detailed procedures for measuring these parameters were as follows.

Biological N fixation activity

The acetylene reduction method (Yoshida and Ancajas 1971; Reddy and Patrick 1979) was used to measure N fixation activity. With this technique, soil samples (3 g on an oven-dried basis) were weighed into a 30-ml Erlenmeyer flask which were sealed with a W-shaped butyl-rubber stopper. Ten percent (volume) of the flask headspace air was replaced with pure acetylene gas using a syringe. After the flasks were incubated at 30°C for 48 h, ethylene production was measured by a gas chromatograph (Shimadzu GC-7A) with a FID detector (Yoshida and Ancajas 1971).

Amounts of NH_4^+ and soluble organic C in fresh soils

Fresh soil samples equivalent to 20 g on an oven-dry basis were weighed into 100-ml plastic bottles with screw caps. Soils were immediately extracted with 50 ml of 0.5 M K_2SO_4 solution by shaking for 30 min on a reciprocal shaker at 70 r.p.m. and filtered

through Advantec no. 6 filter paper (Toyo). Soil extracts were stored in a freezer at –18°C prior to analysis.

The NH_4^+ concentration was measured by the nitroprusside method (Keeney and Nelson 1982) utilising the modifications described by Cheng et al. (2000a). Soluble organic C was measured by a TOC analyser (Shibahara and Inubushi 1995).

Anaerobic incubation experiment for measuring N mineralization and C decomposition of soil

Fresh surface or sub-surface soils (equivalent to 10 g dry soil) were sampled from chambers, and transferred to 100-ml serum bottles. Then 20 ml of O_2-free water was added to make the soil submerged. Since we attempted to see the indirect effect of elevated CO_2 on C and N decomposition processes in soil via plant and/or other habitats in the system, the headspace gas in the bottles was replaced with N_2 (not CO_2) gas before sealing tightly with a butyl stopper. The bottles were then incubated at 30°C for 30 days, the CO_2 and CH_4 concentration in the headspace of the bottles determined by a gas chromatograph (Shimadzu GC-7A) with TCD and FID detectors, respectively. After the gases were measured, the incubated soil was immediately extracted with 20 ml of 1 M K_2SO_4 solution by shaking for 30 min on a reciprocal shaker. The amounts of NH_4^+ and soluble organic C in the soil extracts were measured as described above. The mineralized N was calculated as the amount of NH_4^+ in soil after anaerobic incubation minus the amount of NH_4^+ in fresh soil. The decomposed C was considered as the sum of produced CO_2 and CH_4 plus net solubilized C. The net solubilized C was calculated as the amount of soluble C in soil after anaerobic incubation minus the amount of soluble C in fresh soil.

Data analysis

Significant difference between the two treatments of elevated and ambient CO_2 in relation to biological N fixation, N mineralization and produced soluble C was determined by a paired *t*-test. Data on CO_2 and CH_4 levels were statistically analysed by ANOVA, *F*-test and least significant difference at the 95% confidence level.

Results

Biological N fixation activity

The acetylene reduction assay is a feasible and rapid way to measure N fixation activity, because it is much more sensitive than the Kjeldahl or the ^{15}N method (Yoshida and Ancajas 1971). Changes in acetylene reduction activity (ARA) from surface and sub-surface soils over the duration of the experiment are shown in Fig. 1. Under both ambient and elevated CO_2 conditions, ARA rapidly increased in the surface layer soils to its maximum between panicle initiation (63 DAT) and heading (89 DAT) stages, and then decreased gradually until the end of the experiment. From early to mid-rice growth (up to about panicle initiation, 63 DAT), ARA in the surface soils was significantly higher under elevated CO_2 than that under ambient CO_2. However, after this period ARA in the surface soils was similar under both ambient and elevated CO_2 conditions.

The ARA of sub-surface soil layers under both ambient and elevated CO_2 conditions increased gradually from the early stage of rice growth through the grain-filling stage (112 DAT). The ARA in this case showed a clearly different pattern from that of the surface soil layers. In contrast to the ARA trend of the surface soil layers, elevated CO_2 promoted significantly higher ARA than ambient CO_2 in sub-surface soil layers only after the panicle initiation (63 DAT) through grain-filling (112 DAT) stages of rice growth. During the early period of plant growth (up to about maximum tillering, 41 DAT), there was no significant difference in the ARA between ambient and elevated CO_2 conditions.

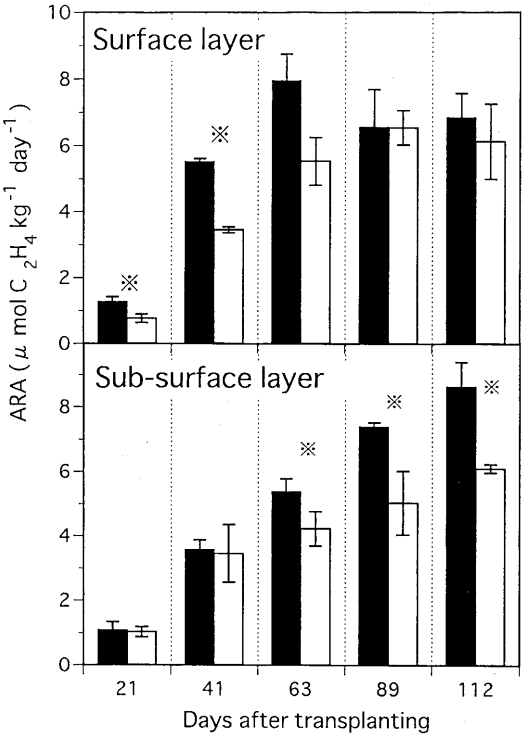

Fig. 1 Acetylene reduction activity (*ARA*) of the surface (0–1 cm) or sub-surface soil layers (1–10 cm) at 2 days of incubation at 30°C. *Stars* indicate significant difference ($P<0.05$) between elevated (■) and ambient (□) CO_2 treatments. *Bars* indicate SDs

Amounts of NH_4^+ detected in fresh soils

The seasonal variations in NH_4^+ concentrations in the surface and sub-surface soil layers are presented in Fig. 2. At 21 DAT, a large amount of NH_4^+ was extracted from the surface and sub-surface soil layers under both the ambient and elevated CO_2 conditions. NH_4^+ content was higher in surface soil (55–60 mg N kg^{-1}) than in the sub-surface soil layers (25–30 mg N kg^{-1}). The large NH_4^+ content at the first sampling may reflect the effect of basal N fertilizer, which was applied 1 day before transplanting. There was a sharp decrease to about 10 mg N kg^{-1} in the soil NH_4^+ level at maximum tillering (41 DAT), and this small amount of NH_4^+ was maintained throughout the rest of the growth period. There was no significant difference in the soil NH_4^+ level between the ambient and elevated CO_2 treatments.

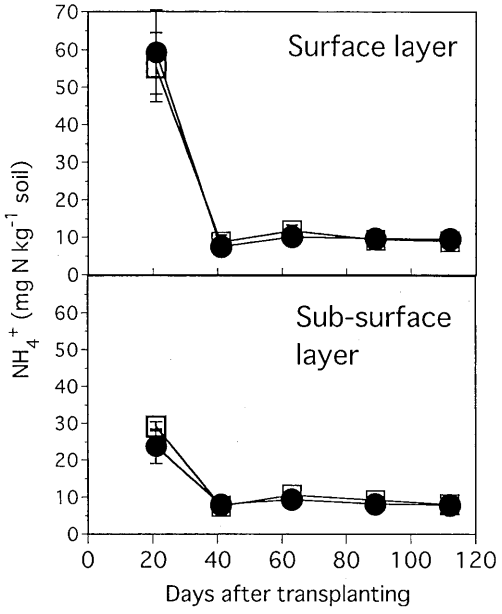

Fig. 2 The amounts of NH_4^+ in the surface (0–1 cm) or sub-surface soil layers (1–10 cm) in elevated (□) and ambient (●) CO_2 treatments. *Bars* indicate SDs

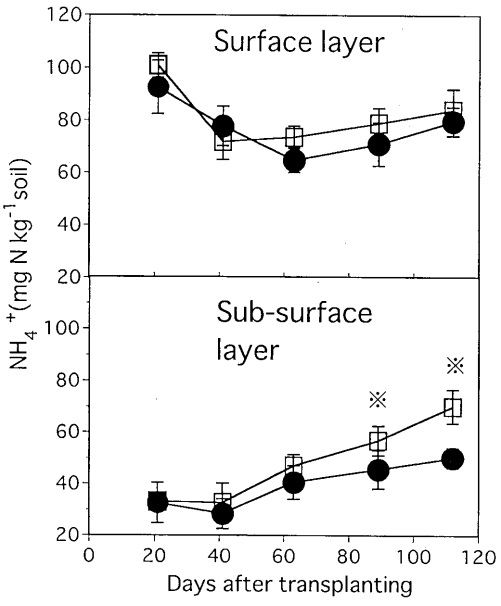

Fig. 3 The mineralized NH_4^+ produced in the soil taken from surface (0–1 cm) or sub-surface soil layers (1–10 cm) under elevated (□) and ambient (●) CO_2 treatments at 30 days of incubation at 30°C. *Stars* indicate significant difference (P<0.05). *Bars* indicate SDs

N mineralization

The N mineralized from organic matter in surface and sub-surface soil layers under the elevated and ambient CO_2 conditions after 30 days of anaerobic incubation at 30°C are shown in Fig. 3. For surface soil layers, there was no difference in the NH_4^+ produced under anaerobic incubation between the elevated and ambient CO_2 treatments throughout the growth of the rice plants. However, in the sub-surface soil layers, the amount of NH_4^+ produced was significantly larger in soils sampled under the elevated CO_2 conditions than in those under ambient CO_2 conditions during the flowering and grain-filling stages, though no difference was found during the early growth, maximum tillering and panicle initiation stages.

The amount of NH_4^+ produced during the anaerobic incubation of surface soil was larger than that produced by sub-surface soil layers, especially during early stages of rice growth (Fig. 3).

C decomposition

The decomposition of organic C in rice soil can be divided into two steps. Organic C is initially decomposed into soluble C, which is then converted into gases, such as CO_2 and CH_4, which are entrapped by the soil or lost to the atmosphere. There were no significant differences in the amount of soluble C in fresh soils taken from surface or sub-surface soil layers between the elevated and ambient CO_2 treatments throughout rice growth (Fig. 4). However, after anaerobic incubation of the fresh soils for 30 days at 30°C, net solubilized C tended to be significantly higher in soils under elevated CO_2 conditions than

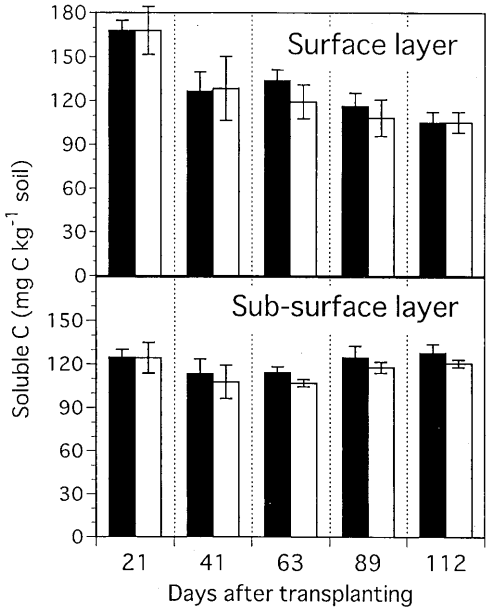

Fig. 4 The amounts of soluble C in the surface (0–1 cm) or sub-surface soil layers (1–10 cm) under elevated (■) and ambient (□) CO_2 treatments. *Bars* indicate SDs

ambient CO_2, especially during flowering and grain-filling stages (Fig. 5). Both CO_2 and CH_4 produced under anaerobic incubation are shown in Tables 1 and 2. Generally, the amount of CO_2 produced was much larger than that of CH_4 on the basis of C molecules during early growth stages. During the middle and late growth stages,

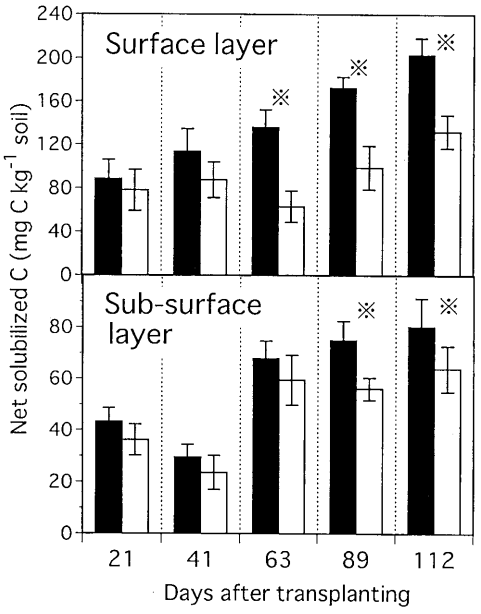

Fig. 5 Net solubilized C produced in the soils taken from surface (0–1 cm) or sub-surface soil layers (1–10 cm) under elevated (■) and ambient (□) CO_2 treatments at 30 days of incubation at 30°C. *Stars* indicate significant difference (P<0.05). *Bars* indicate SDs

Table 1 CO_2 production from two soil layers taken at elevated or ambient CO_2 after 30 days of incubation at 30°C (mg C kg^{-1} soil). Values within each row followed by *same letter* do not differ significantly (P<0.05)

Days after transplanting	Surface layer		Sub-surface layer	
	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2
21	243.3 a	203.7 b	197.8 b	165.1 b
41	286.3 a	221.4 b	210.2 b	169.5 b
63	309.6 a	243.2 b	260.9 b	191.5 c
89	299.8 a	256.7 b	250.3 b	212.3 c
112	254.1 a	215.6 b	229.7 b	160.4 c

Table 2 CH_4 production from two soil layers taken at elevated or ambient CO_2 after 30 days of incubation at 30°C (mg C kg^{-1} soil). Values within each row followed by *same letter* do not differ significantly (P<0.05)

Days after transplanting	Surface layer		Sub-surface layer	
	Elevated CO_2	Ambient CO_2	Elevated CO_2	Ambient CO_2
21	4.60 a	1.0 b	1.1 b	0.6 b
41	22.6 a	15.5 b	13.6 b	10.9 b
63	83.3 a	69.2 b	57.6 b	40.0 c
89	89.4 a	72.8 ab	67.7 b	60.4 b
112	104.4 a	88.9 b	80.8 b	77.5 c

the production of CH_4 gradually increased. There were significant differences in C decomposition rates between the two soil layers (surface and sub-surface), and between ambient and elevated CO_2 conditions. C decomposition was highest in the surface layer soil under elevated CO_2 conditions during flowering and grain-filling stages of rice.

Discussion

Generally, biological N fixation is enhanced by organic matter application in rice soil due to the supply of available C source for microorganisms (Adachi et al. 1989; Yoo et al. 1991). Results from our previous study showed that microbial biomass C and chlorophyll-type compounds in the surface soil were increased by an elevated atmospheric CO_2 concentration (Inubushi et al. 1999). It is therefore possible that N-fixation activities may be increased in surface soil layers by elevated CO_2 conditions due to enhanced algal growth (Cheng et al. 2000a). The results from this research are consistent with this hypothesis for the early period of rice cultivation (Fig. 1). However, during the later stages of growth, the rice plants shaded the surface soil layers, so no difference was found between ambient and elevated CO_2. On the other hand, there was a significant difference in sub-surface layer soils between ambient and elevated CO_2 conditions during later periods of cultivation (Fig. 1). Elevated CO_2 presumably led to an increase in the release of root exudates and sloughed-off roots to the soils.

Many studies have dealt with N mineralization in submerged and upland soils. A large number of factors can influence N mineralization in soils, including pre-treatments, temperature, soil moisture, properties of soils, and stress (Shioiri and Aomine 1937; Stanford et al. 1973; Inubushi and Wada 1987; Manguiat et al. 1996; Aulakh et al. 2000). Under elevated atmospheric CO_2 conditions, a short-term laboratory assay of N mineralization indicated that N availability was significantly higher in the aerobic bulk soil than under ambient CO_2 (Zak et al. 1993). From a previous laboratory incubation experiment in the absence of rice plants, it was shown that an elevated CO_2 concentration and temperature accelerated N mineralization in submerged soil even when mineralization was examined under N_2 conditions (Cheng et al. 2000a). From the current study, the amount of NH_4^+ in the fresh soils showed no difference between elevated and ambient CO_2 conditions (Fig. 2) in spite of the fact that the potential rate of N mineralization was increased in response to elevated CO_2 during the latter period of cultivation (Fig. 3). This indicates that rice plants probably took up mineralized N as quickly as it was formed for their growth, because the NH_4^+ concentration was low (about 10 mg N kg^{-1} soil) in the soils after the maximum-tillering stage. This result was similar to the effect of elevated CO_2 on the decomposition of organic C to soluble C (Fig. 4. and Fig. 5). These results imply that there may be differences in the decomposability of soil organic matter (C and N) under ambient and elevated CO_2 conditions during the latter period of rice cultivation. A similar result was found in an upland wheat crop where elevated CO_2 was supposed to influ-

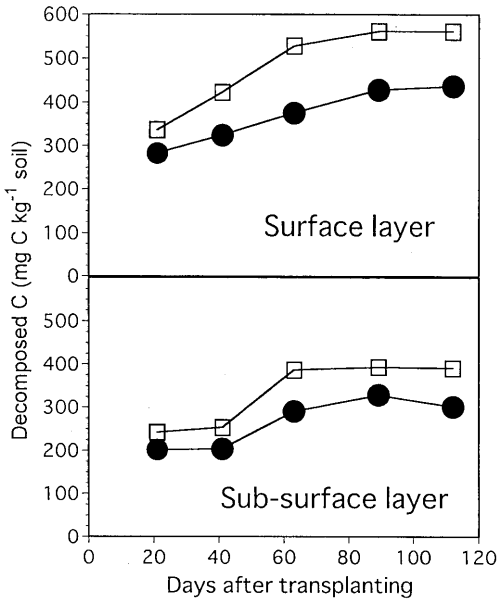

Fig. 6 The amounts of decomposed C as soluble C plus CO_2 plus CH_4 of the soils taken from the two treatments and two layers at 30 days of incubation at 30°C

ence the soil organic matter through the production of higher plant biomass with a resultant high root biomass and root exudates (Cheng and Johnson 1998).

In submerged rice soils, the major pathways for the losses of C are those of the production and emission of CO_2 and CH_4 during aerobic respiration and anaerobic fermentation (Takai 1970). CH_4 emission from submerged rice soils to the atmosphere has been identified as an important source of atmospheric greenhouse gas that contributes to global warming (IPCC 1995). According to Table 2, the produced CH_4 (or CH_4 production potential) gradually increased with the rice growth stages. This result is consistent with the CH_4 emission inside the chambers (data not shown). This suggests that elevated CO_2 accelerates soil organic C turnover to CH_4 in the rice plant-soil agro-ecosystem. From the sum of the produced soluble C, CO_2 and CH_4 (Fig. 6), it can be seen that elevated CO_2 increased the rate of C decomposition in both surface and sub-surface layers. However, elevated CO_2 did not lead to an increase in soluble C in fresh soils (Fig. 4); a possible explanation for this is that elevated CO_2 levels accelerate the turnover rate of soil organic C in the rice plant-soil agro-ecosystem.

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