

# Long-term growth at elevated carbon dioxide stimulates methane emission in tropical paddy rice

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## Abstract

Recent anthropogenic emissions of key atmospheric trace gases (e.g. CO<sub>2</sub> and CH<sub>4</sub>) which absorb infra-red radiation may lead to an increase in mean surface temperatures and potential changes in climate. Although sources of each gas have been evaluated independently, little attention has focused on potential interactions between gases which could influence emission rates. In the current experiment, the effect of enhanced CO<sub>2</sub> (300 µL L<sup>-1</sup> above ambient) and/or air temperature (4 °C above ambient) on methane generation and emission were determined for the irrigated tropical paddy rice system over 3 consecutive field seasons (1995 wet and dry seasons 1996 dry season). For all three seasons, elevated CO<sub>2</sub> concentration resulted in a significant increase in dissolved soil methane relative to the ambient control. Consistent with the observed increases in soil methane, measurements of methane flux per unit surface area during the 1995 wet and 1996 dry seasons also showed a significant increase at elevated carbon dioxide concentration relative to the ambient CO<sub>2</sub> condition (+49 and 60% for each season, respectively). Growth of rice at both increasing CO<sub>2</sub> concentration and air temperature did not result in additional stimulation of either dissolved or emitted methane compared to growth at elevated CO<sub>2</sub> alone. The observed increase in methane emissions were associated with a large, consistent, CO<sub>2</sub>-induced stimulation of root growth. Results from this experiment suggest that as atmospheric CO<sub>2</sub> concentration increases, methane emissions from tropical paddy rice could increase above current projections.

*Keywords:* carbon dioxide, climate change, methane, rice

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## Introduction

Considerable evidence is now available which suggests that human activities have altered the concentration of key trace gases in the earth's atmosphere. Because these gases (carbon dioxide, CO<sub>2</sub>; methane, CH<sub>4</sub>; nitrous oxides, N<sub>x</sub>O; chlorofluorocarbons, CFCs) are transparent to incoming radiation but trap part of the terrestrial long-wave radiation (heat) re-radiated back into space, increases in global surface temperature and subsequent climate change are possible (Watson *et al.* 1990; Pachauri *et al.* 1992). Although climate change and its consequences

are uncertain, its potential importance is such that much effort has been given to determining the sources and radiative effect of these gases.

Assessments of climate change induced by these trace gases have focused primarily on the observed increases in atmospheric CO<sub>2</sub> and CH<sub>4</sub> since these gases are considered to be the most important with respect to radiative forcing, and because their effects can be reasonably well quantified with present knowledge (Duxbury 1994).

In assessing the current concentration of these gases, it is important to remember that each gas has a biological component. Carbon dioxide, for example, is the sole

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source of carbon for photosynthesis and plant growth. It is clear from numerous experiments that increasing CO<sub>2</sub> can have a significant effect on biological activity in agricultural and unmanaged eco-systems (cf. Kimball 1983; Drake & Leadley 1991). In turn,  $\approx 80\%$  of all methane emissions are biotic in nature (e.g. flooded rice, ruminant populations). Given that biology plays a key role in determining the concentration of each gas, is there a biological system whereby the rise of one global warming gas (CO<sub>2</sub>) can result in further stimulation of another global warming gas (CH<sub>4</sub>)?

Agriculture, especially flooded rice production, is a significant source of atmospheric methane. Although estimates of the contribution of flooded rice to global methane production have ranged widely (18–280 Tg y<sup>-1</sup>, see Khalil & Rasmussen 1990 for a review), recent estimates suggest a flux rate of 60–80 Tg y<sup>-1</sup>, or about 16–21% of the total annual methane budget (Bachelet & Neue 1993; Duxbury 1994). Although the percentage of plant mediated CH<sub>4</sub> transport may vary depending on the quantity of organic inputs, a significant portion of the CH<sub>4</sub> that is released from paddy soils can be transferred to the atmosphere through the rice plant (Wassmann *et al.* 1996). In addition to its role in transport, rice enhances the production of methane directly by providing carbon substrates in the form of root exudate or root autolysis products (e.g. carbohydrates) (Neue *et al.* 1996).

Given that the flux of methane in flooded rice is responsive to the supply of carbon from roots, as well as dependent on roots and tillers for transport from soil to atmosphere, stimulation of photosynthesis and growth parameters with increasing carbon dioxide concentration could lead to an additional or synergistic increase in the current rate of methane emission in rice paddies. Because paddy rice is a significant contributor to the concentration of global methane, the additional release of methane with increasing carbon dioxide could be significant. Preliminary data from controlled environmental growth chambers suggest a potential interaction in rice between CO<sub>2</sub> concentration and increased methane flux, especially at high air temperature (i.e. > 35 °C) (Allen *et al.* 1994). To date, however, no multiseason field data on the effects of increasing CO<sub>2</sub> concentration on methane emission rates in tropical paddy rice are available.

To evaluate the potential effect of increasing carbon dioxide on methane emission in rice, we determined if growth at ambient and ambient + 300  $\mu\text{L L}^{-1}$  concentrations of carbon dioxide altered dissolved soil methane and methane flux in a paddy rice community over a two year period. Because increasing CO<sub>2</sub> concentration may also result in elevated air temperatures, we also examined if simultaneous increases in CO<sub>2</sub> and air temperature (4 °C above ambient) would further alter methane production and release.

## Materials and methods

### Field control system

All experiments were conducted using 12 open top chambers (OTCs) located in an irrigated (paddy) field at the International Rice Research Institute (IRRI), Los Baños, Philippines (121°15'E, 14°13'N). The OTC system was designed, constructed and operated to control both CO<sub>2</sub> and temperature to within  $\pm 10\%$  of a desired setpoint 24 h a day for the duration of a given experiment. Each chamber was 2.0 m tall with an octagonal base area of  $\approx 3.5 \text{ m}^2$  and a total volume of 7.2 m<sup>3</sup> including that of the extended frustrum. An extended frustrum with a 0.35 m<sup>2</sup> opening was attached to the top of each chamber to prevent wind intrusion and to minimize temperature fluctuations and CO<sub>2</sub> loss. All walls and the extended frustrum were covered with 0.13 mm thick clear film ('Mylar', Dupont Corp., Wilmington, DE) that transmitted 89% of the incoming photosynthetically active radiation (PAR). Additional details concerning the operation of the system and microclimate within the chambers can be found in Collins *et al.* (1995) and Moya *et al.* (1997), respectively.

### Experimental details

OTCs were assigned a CO<sub>2</sub> concentration of either ambient ( $\approx 360 \mu\text{L L}^{-1}$ ) or ambient + 300  $\mu\text{L L}^{-1}$  (660  $\mu\text{L L}^{-1}$ ) and one of two different air temperatures (ambient and ambient + 4 °C). Fixed levels of elevated CO<sub>2</sub> were not used since night-time ambient CO<sub>2</sub> can be as high as 500  $\mu\text{L L}^{-1}$  at this field site. All treatments (CO<sub>2</sub> and temperature) were maintained over a 24 h time period for each growing season from germination until maturity.

Experiments involving measurement of methane production and emission were determined for IR72, a standard semidwarf indica cultivar, over 3 field seasons (1995 dry season, 1995 wet season and 1996 dry season). Dry seasons (DS) in Los Baños generally run from January through April while wet seasons (WS) are from early July through November. For each growing season rice seeds were germinated in flats within each chamber for a given growth CO<sub>2</sub>/temperature treatment. Fourteen days after sowing, seedlings were taken out of the flats and planted into the paddy surrounded by the OTC. Hills were placed at 20 × 20 cm intervals, 3 plants per hill (i.e. 75 plants per m<sup>2</sup>, the standard plant density for commercial production in this environment). The area surrounding the chambers was also transplanted at this time to the same density. The overall statistical design was a 2 × 2 factorial (i.e. 2 CO<sub>2</sub> treatments at 2 air

temperatures) with 3 replications in a randomized block design.

Crop care and management practices were standard for all seasons. Basal applications of 40 and 60 kg N ha<sup>-1</sup> (as ammonium sulphate) were carried out during each wet and dry season, respectively. After transplanting, timing of supplemental N was determined by chlorophyll meter (SPAD, model 502, Minolta Corp., Tokyo, Japan) measurements. The actual amount of N applied was determined using the relationship between uptake and cumulative degree days as given by Cassman *et al.* (1994). Total amounts of N added were 110 kg N ha<sup>-1</sup> in the wet season and 220 kg N ha<sup>-1</sup> in the dry season which came from either ammonium sulphate or urea. All treatments received the same amount of fertilizer at the same time.

#### *Methane measurements*

Dissolved methane was determined using porous ceramic cups which could be placed at different depths and allowed to come to equilibrium with the surrounding soil. Soil water from a given depth was collected at selected intervals by attaching to evacuated stoppered test tubes. Air was injected into the test tube and then the tube was vortexed and the air in the head space of the tube was sampled using a syringe. Air from this syringe was then injected into a gas chromatograph (Shimadzu Corp., Kyoto, Japan) with flame ionization detector. Dissolved methane was calculated based on the weight/volume of the soil solution in the tube as described by Wassmann *et al.* (1996). Measurements of dissolved methane were made for each treatment OTC at a depth of 10 cm for the 1995 dry season from 5 to 104 days after transplanting (DAT). Because no interaction between increased temperature and carbon dioxide was observed (relative to elevated carbon dioxide alone), measurements of dissolved soil methane focused on the ambient and elevated CO<sub>2</sub> treatments for the 1995 wet and 1996 dry seasons (0–83 DAT and 3–103 DAT for each season, respectively), but at different depths [0 cm (soil water interface), 5, 10, and 15 (cm)].

Although the OTCs used in this experiment provided stable concentrations of carbon dioxide and temperature, the flux of methane is too small to be determined given their rapid air changes. Based on the initial observations involving dissolved methane for the 1995 dry season, an attempt was made to measure methane flux in the 1995 wet season using smaller separate chambers (40 × 40 × 80 cm high) which could be placed and sealed within the larger OTC. The smaller chamber enclosed four hills. Initial measurements of methane flux were made for the ambient and ambient + 300 µL L<sup>-1</sup> CO<sub>2</sub> treatments at ambient temperature (i.e. six chambers in total, one for each rep and treatment) over a three week

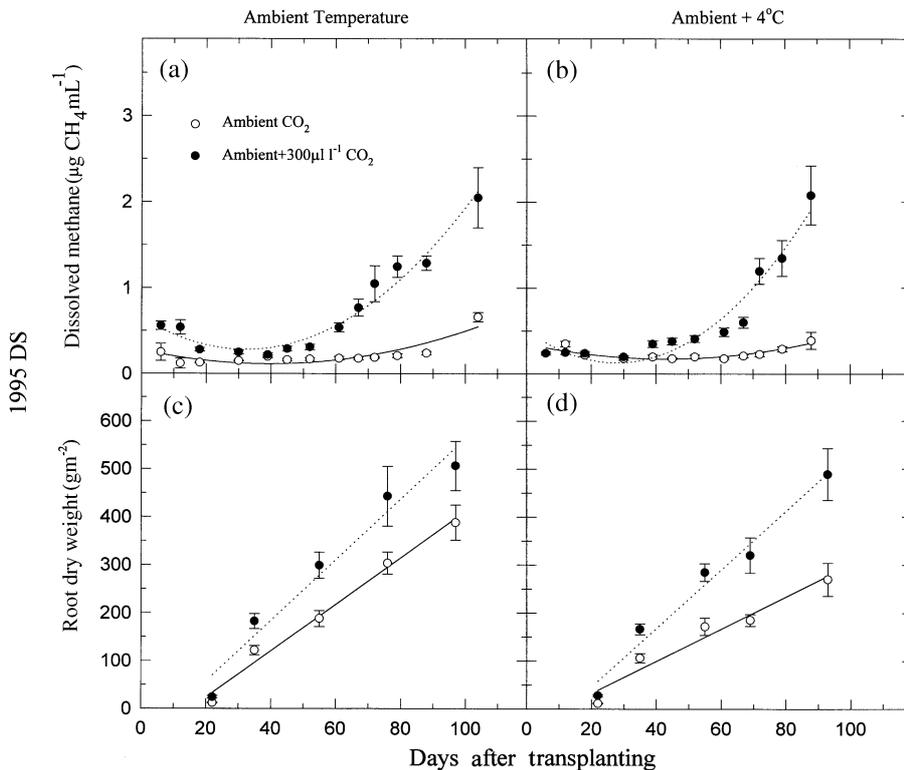
period beginning 62 DAT during the 1995 wet season (4 measurements). More extensive measurements of methane flux were made during the 1996 dry season beginning at 22 DAT and continuing at approximately weekly intervals until 80 DAT (eight measurements) for ambient and ambient + 300 µL L<sup>-1</sup> CO<sub>2</sub> treatments at both ambient and ambient + 4 °C air temperatures.

To determine methane emissions from the rice canopy, air samples were taken through a rubber diaphragm on the smaller sealed boxes at 5 minute intervals for time spans of 20 min at different times of day (dawn for the 1995 wet season, 07.00–08.00; dawn, noon and evening for the 1996 dry season, 07.00–08.00, 12.00–13.00, 16.00–17.00 hours). Methane emission rates were derived from the temporal increase of methane concentration inside the chamber which was roughly linear. Emission rates obtained at different daytimes were averaged for one sampling date while the values of all sampling dates were averaged for seasonal emission rates. The CH<sub>4</sub> concentration was determined in each sample in replicate runs by a gas chromatograph equipped with a flame ionization detector. CO<sub>2</sub> concentration would be expected to decrease due to photosynthesis during the time of methane measurement (i.e. 20 min), but subsequent changes in stomatal conductance would not be expected to influence methane release which is primarily through micropores (see Nouchi *et al.* 1990).

#### *Growth measurements*

For the 1995 dry season, extensive measurements were made of root growth. Root biomass was determined from plants grown in 20 cm diameter, 30 cm deep PVC cylinders that were filled with soil and buried in each OTC prior to the start of the experiment. These cylinders were perforated along the sides to prevent root binding. Estimates of root biomass were made at 22, 35, and 55 DAT as well as at flowering (76 and 69 DAT for the ambient and elevated temperature treatments, respectively) and maturity or final harvest (97 and 93 DAT for the ambient and elevated temperature treatments, respectively). At sampling times the cylinders were lifted from the ground and roots outside the cylinders were discarded. Soil was screened and roots gently washed with water. Roots were then oven dried and weighed. Based on the amount of dry biomass within the root core, root weight was then determined on a square meter basis. Extensive root sampling however, was both time-consuming and destructive. Since in the 1995 wet and 1996 dry seasons space was limited in the OTCs (due to additional cultivars being grown), root sampling was limited to flowering and maturity for the 1995 wet and 1996 dry seasons for the IR72 cultivar.

Since methane flux from the rice paddy can be influ-



**Fig. 1** Changes in the amount of dissolved methane (as  $\mu\text{g}$  of methane per mL of soil water at a depth of 10 cm) and root dry weight ( $\text{g m}^{-2}$ ) for rice (IR72) grown at two  $\text{CO}_2$  concentrations (ambient and ambient +  $300 \mu\text{L L}^{-1} \text{CO}_2$ ) and two air temperatures (ambient and ambient +  $4^\circ\text{C}$ ) for the 1995 dry season. A second degree quadratic was used for the 'best-fit' function in drawing regressions in (a) and (b); while a linear function was used to describe root growth in C and D. Bars are  $\pm$  SE,  $n = 3$ .

enced in part by above ground components, vegetative growth was determined from a subsample of 4 hills per OTC per treatment at flowering and maturity in the 1995 wet and 1996 dry season. At these times, 4 hills per OTC were sampled for plant height, number of tillers, then cut at ground level and separated into stems (culms) and leaf blades. All plant material was oven dried at  $70^\circ\text{C}$  for 72 h or until dry weights were constant. Additional details concerning vegetative and reproductive parameters at increasing  $\text{CO}_2$ /temperature can be found in Ziska *et al.* (1997).

#### Data analysis

Data for methane flux for the 1995 wet season were compared using a standard *t*-test (elevated vs. ambient  $\text{CO}_2$ ) assuming unequal variances. Methane flux for the 1996 dry season and rice growth data for the 1995 and 1996 seasons were analysed using a two-way ANOVA (SuperANOVA, Abacus Concepts, Berkeley, CA, USA) comparing the effects of air temperature and  $\text{CO}_2$  in combination. Treatment effects were separated using least square means. Unless otherwise stated all differences were compared to the ambient condition of  $\text{CO}_2$  concentration and air temperature at the 0.05 level of significance.

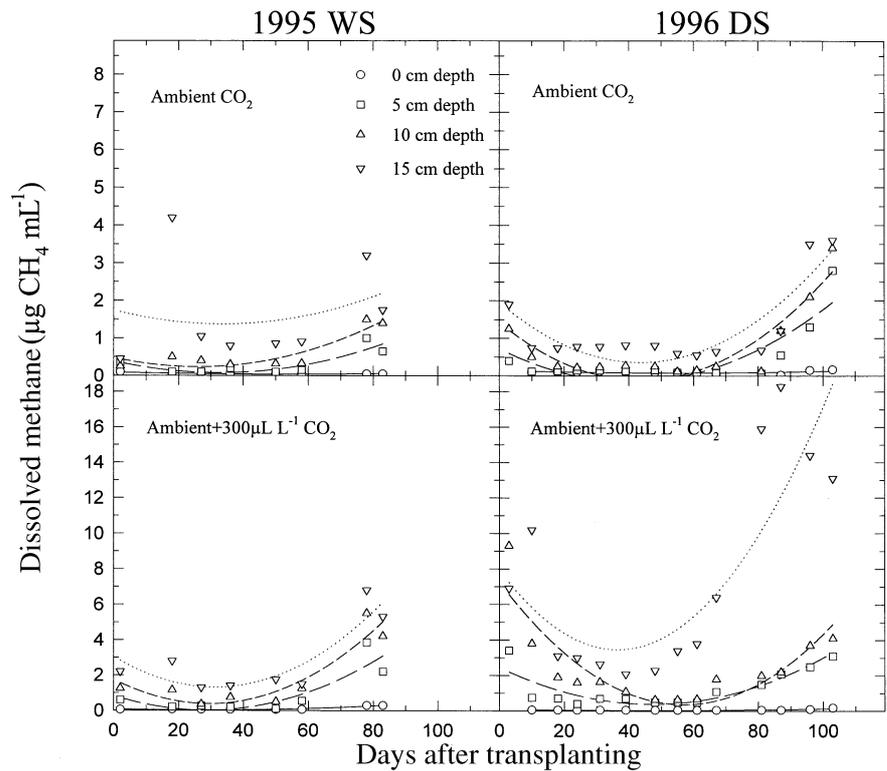
#### Results

Dissolved soil methane at a depth of 10 cm increased markedly at the elevated  $\text{CO}_2$  concentration, irrespective

of air temperature from 40 DAT until maturity in the 1995 DS (Fig. 1a,b). The rise in dissolved methane was positively correlated with an increase in root dry weight at either  $\text{CO}_2$  concentration over this same measurement period. However, the correlation was significant for the elevated  $\text{CO}_2$  relative to the ambient  $\text{CO}_2$  treatment ( $R^2 = 0.83$  vs.  $0.53$  for the elevated and ambient  $\text{CO}_2$  treatments, respectively). No additive effect between increasing air temperature (i.e.  $4^\circ\text{C}$  above ambient) and elevated  $\text{CO}_2$  concentration was observed for either dissolved methane or increasing root weight (Fig. 1b,d). Average (i.e. 24 h) air temperature was approximately the same between the 1995 wet and 1996 dry seasons ( $25.8$  and  $26.3^\circ\text{C}$ , respectively) during the experimental period.

Additional measurements taken during the 1995 wet and 1996 dry seasons showed increasing amounts of dissolved methane with depth for both seasons (Fig. 2). This is consistent with the anaerobic conditions required for methanogenesis. However, as with the 1995 dry season, increasing  $\text{CO}_2$  concentration resulted in a greater stimulation of dissolved methane compared to the ambient  $\text{CO}_2$  control at greater soil depths (i.e. 10 and 15 cm) with no additive effect between increasing  $\text{CO}_2$  and air temperature (data not shown). Overall, greater amounts of dissolved methane were observed at the end of the season for the 1996 dry season compared to the 1995 wet season.

Preliminary measurements of average methane flux from the rice canopy during the 1995 wet season



**Fig. 2** Changes in the amount of dissolved methane (as  $\mu\text{g}$  of methane per mL of soil water) at four different depths, 0 cm (soil-water interface), 5, 10 and 15 cm for rice (IR72) grown at two CO<sub>2</sub> concentrations (ambient and ambient + 300  $\mu\text{L L}^{-1}$ ) at ambient air temperature for the 1995 wet and 1996 dry season. (Note the difference in scale for the  $y$ -axis for the ambient + 300  $\mu\text{L L}^{-1}$  CO<sub>2</sub> treatment.) A second degree quadratic was used for the 'best-fit' function in determining regression of dissolved methane over time.

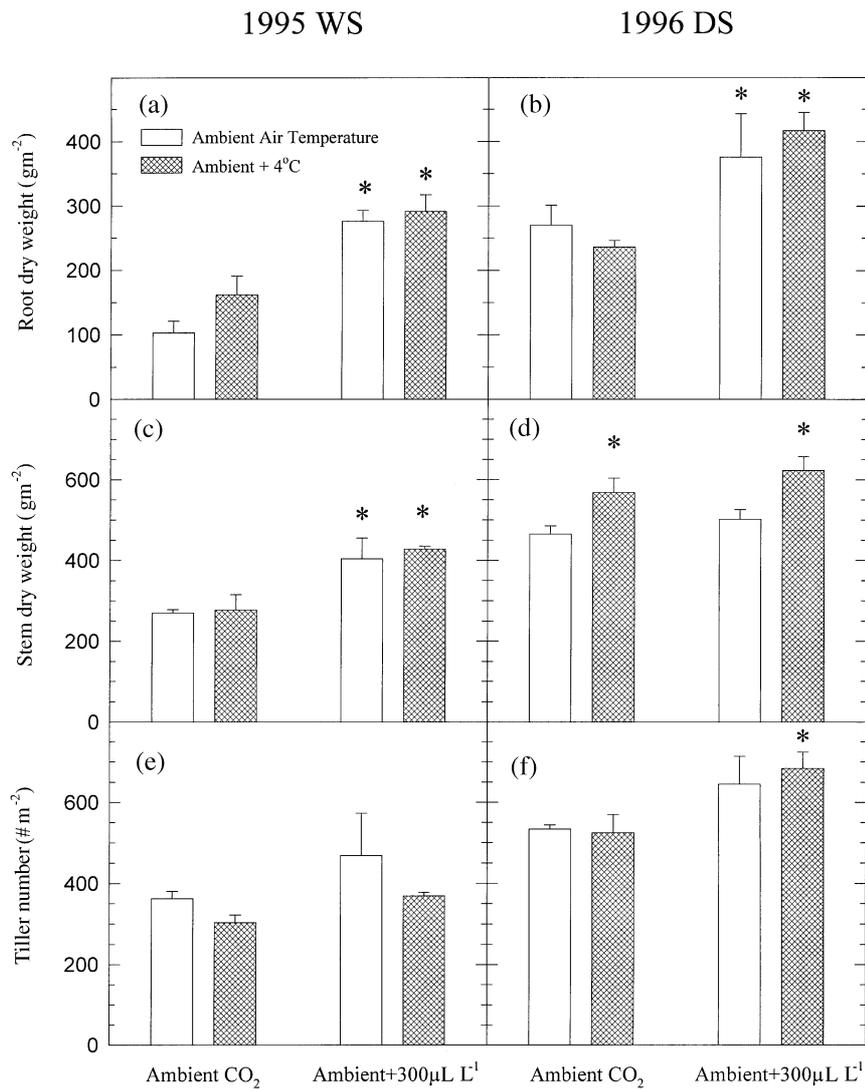
**Table 1** Daily methane flux rates and total biomass (leaves, stems, panicles and roots) at maturity for paddy rice (cv IR72) grown at ambient and ambient + 300  $\mu\text{L L}^{-1}$  (CO<sub>2</sub>) at two different air temperatures (ambient and ambient + 4 °C). Data presented are the average data for each treatment over the sampling period in a given year. Additional details are given in the Methods. \* indicates a significant increase in methane flux or total biomass ( $P < 0.05$ ) relative to the control [ $t$ -test assuming unequal variances for the 1995 WS data, and a two-way ANOVA (temperature and CO<sub>2</sub>) for the 1996 DS data].

Season	CO <sub>2</sub>	Temp.	Methane flux ( $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ )	Total biomass ( $\text{g m}^{-2}$ )
1995WS	Amb.	Amb.	142.2 $\pm$ 19.9	681 $\pm$ 58.6
	+ 300	Amb.	227.5 $\pm$ 28.8*	937 $\pm$ 155*
1996DS	Amb	Amb.	260.2 $\pm$ 28.6	1892 $\pm$ 105
	Amb.	+ 4 °C	119.7 $\pm$ 10.9	1880 $\pm$ 15.2
	+ 300	Amb.	385.8 $\pm$ 51.6*	2387 $\pm$ 124*
	+ 300	+ 4 °C	376.5 $\pm$ 37.7*	2059 $\pm$ 78.2

(63–82 DAT) show a significant increase in daily estimates of methane flux with the elevated CO<sub>2</sub> treatment (Table 1). More extensive measurements of daily methane flux during the 1996 dry season demonstrate a similar increase in average methane flux with elevated CO<sub>2</sub>, ( $P = 0.001$ ). Over both CO<sub>2</sub> levels there was also a significant decrease in methane emissions with elevated air temperature ( $P = 0.038$ ). However, no observed synergy between increased CO<sub>2</sub> concentration and air temperature on methane flux (relative to elevated CO<sub>2</sub> per se) was observed. The reduction in methane flux observed at the higher air temperature is primarily because of the large reduction in methane emission observed at ambient CO<sub>2</sub> and high (+ 4 °C) air temperature relative to ambient CO<sub>2</sub>, air

temperature conditions (i.e. at + 300  $\mu\text{L L}^{-1}$ , no significant reduction in methane flux was observed between air temperatures). Overall, rates of methane flux were higher (at either treatment CO<sub>2</sub> level) during the 1996 dry compared to the 1995 wet season.

Flux of methane, in contrast to dissolved methane from rice paddies, may depend not only on roots, but on vegetative growth as well. Measurements of rice growth taken during maturity for the 1995 wet and 1996 dry season show a significant increase in root dry weight with increasing CO<sub>2</sub> for both seasons, with no influence of increasing air temperature at either CO<sub>2</sub> concentration (Fig. 3a,b). Stem weight (i.e. the dry weight of the culm plus leaf sheath) was also increased with the elevated



**Fig. 3** Changes in selected growth parameters: root weight, stem weight and tiller number at maturity for rice (IR72) grown at two CO<sub>2</sub> concentrations (ambient and ambient + 300 µL L<sup>-1</sup> CO<sub>2</sub>) and two air temperatures, ambient (open bars) and ambient + 4 °C (hatched bars) for the 1995 wet and 1996 dry season. \* indicates a significant difference relative to the ambient CO<sub>2</sub>, ambient temperature control for a given growth parameter for that season. Bars are ± SE. See methods for additional details.

CO<sub>2</sub> concentration for the 1995 wet season. Increases in stem weight are also noted in the 1996 dry season, but only at the higher growth temperature (Fig. 3c,d). There is a slight, but nonsignificant ( $P = 0.11$ ) effect of increasing CO<sub>2</sub> concentration on tiller number when averaged for both seasons (Fig. 3e,f). Overall, all aspects of biomass (irrespective of treatment) were greater in the 1996 dry relative to the 1995 wet season (Table 1). However, the relative effect of increasing CO<sub>2</sub> on stem and root parameters was greater in the 1995 wet compared to the 1996 dry season. (e.g. a 112 and 56% increase in root growth for the 1995 wet and 1996 dry season, respectively).

## Discussion

Although the response of rice to elevated CO<sub>2</sub> concentration is quite variable, a number of studies have shown significant increases in growth and yield (cf Imai &

Murata 1978; Imai *et al.* 1985; Baker *et al.* 1990; Kim *et al.* 1993; Morokuma *et al.* 1996). Specific increases have been observed for panicle weight, tiller number and root growth up to CO<sub>2</sub> concentrations of ≈ 600 µL L<sup>-1</sup> (Baker *et al.* 1990; Baker *et al.* 1992). Previous work with IR72 at this experimental site for the 1994 wet and 1995 dry season had demonstrated that a significant stimulation in relative growth rate, biomass and yield was evident with increasing CO<sub>2</sub> concentration (see Ziska *et al.* 1997). The additional information reported here on vegetative parameters for later growing seasons was part of an ongoing study to determine the long-term effects of increasing CO<sub>2</sub> and temperature on rice productivity.

Clearly, absolute values of growth (e.g. roots, stems) will differ between wet and dry seasons in part because of large differences in solar radiation in the tropics (See Matthews *et al.* 1995). However, low solar radiation should not, by itself, eliminate a positive growth response to increasing CO<sub>2</sub> concentration. Because increasing

atmospheric CO<sub>2</sub> can reduce the loss of CO<sub>2</sub> via photorespiration, light compensation points should be lowered (see Long 1991 for a discussion). Consequently, the relative effect of CO<sub>2</sub> concentration can be greater under low solar irradiance.

Because increasing CO<sub>2</sub> levels can alter rice growth and development in either season, it can, potentially, influence the release of methane from paddy soils. This is due, in part, to the transfer of CH<sub>4</sub> from paddy soils to the atmosphere through the rice plant (Schutz *et al.* 1989a). Water-logged conditions create soil O<sub>2</sub> deficiency of roots, and rice has developed mechanisms of gas exchange alternative to that of diffusion. Hydrophytic adaptations include the development of large air spaces (lacunae) in the cortex of the roots and tillers which allow a continued supply of O<sub>2</sub> to the roots (Armstrong 1978; Nouchi *et al.* 1990). However, the lacunar system also provides a route by which methane formed in the anoxic areas of flooded soils can be released into the atmosphere (Cicerone & Shetter 1981; Holzappel-Pschorn & Seiler 1986). This release of CH<sub>4</sub> does not occur through stomata, but through micropores, small openings in the culm of the rice plant (Nouchi *et al.* 1990). Unvegetated fields, when compared to rice paddies, demonstrate a substantial reduction in CH<sub>4</sub> emission (Seiler *et al.* 1984). Measurements in flooded rice indicate that gas exchange across the air-water interface contributes less than 25% of the overall flux when the field is fertilized by urea whereas the contribution may exceed 50% under organic fertilization (Wassmann *et al.* 1996). Other aquatic species such as *Echinochloa crus-galli*, can facilitate gas exchange between the submerged soil and atmosphere, but do not necessarily stimulate methanogenesis (Holzappel-Pschorn *et al.* 1986). Unlike rice, weedy plants induce a relatively high redox potential in the submerged soil so that 95% of the produced CH<sub>4</sub> becomes oxidized before it can reach the atmosphere (Holzappel-Pschorn *et al.* 1986).

In addition to its role in methane transport, rice may also enhance the production of CH<sub>4</sub> directly by providing carbon substrates in the form of root exudates or root autolysis products (e.g. carbohydrates) which are easily decomposed by fermentive bacteria to CO<sub>2</sub>, H<sub>2</sub> and acetate (Schutz *et al.* 1989a; Schutz *et al.* 1989b) and then utilized by methanogenic bacteria. Root exudates and root litter may therefore contribute between 10 and 50% of the carbon substrate needed for methanogenesis (Seiler *et al.* 1984).

Given what is known concerning the role of rice in methane generation and transfer, elevated CO<sub>2</sub> concentrations could alter methane flux by increasing the potential difference in methane concentration between soil and air, and/or altering the resistance of methane transfer through the rice plant. Among all growth parameters which could influence the flux of methane, it is clear that

root growth is particularly sensitive to increasing CO<sub>2</sub> in the current experiment. Other reported responses of root growth to enriched CO<sub>2</sub> levels have been dramatic (Rogers & Runion 1994; Prior *et al.* 1994); and restriction of root growth by small pots or insufficient nutrients has been suggested as an underlying cause of photosynthetic acclimation to high CO<sub>2</sub> concentrations (Thomas & Strain 1991; Arp 1991).

Although limited space prevented extensive below ground sampling during the 1995 wet and 1996 dry season, there was a good correlation between increasing root growth and dissolved methane at the elevated carbon dioxide concentration for the 1995 dry season. Similarly, the increase in root biomass seen at maturity for both the 1995 wet and 1996 dry season was consistent with the increase in both dissolved and emitted methane for each season. Interestingly, when averaged across all CO<sub>2</sub> and temperature treatments for both field seasons there is a significant correlation between root biomass at maturity and average seasonal methane flux ( $R^2 = 0.71$ ). Given that root products are a substantial source of carbon for methanogenesis it seems likely that CO<sub>2</sub> induced increases in root growth led to increases in dissolved methane and subsequent methane emissions. Although not measured for the 1995 dry season, noted increases in root growth at elevated CO<sub>2</sub> imply a continuous enhancement of methane emission at the higher CO<sub>2</sub> concentration during the course of the experiment. No attempt was made however, to separate pre-existing soil organic matter and recently formed root organic matter as sources of carbon for the observed changes in methane flux. Presumably, if the <sup>13</sup>C ratio of the CO<sub>2</sub> source was different enough from ambient CO<sub>2</sub> concentration, this type of analysis could be done in the future.

If increased CO<sub>2</sub> concentration results in increased root growth and greater methane production, does it also influence the transfer pathway of methane from soil to air? As stated previously, CO<sub>2</sub>-induced increases in leaf area would not increase methane flux from the canopy since CH<sub>4</sub> does not pass primarily through the stomata. However, CO<sub>2</sub> induced increases in tiller number/stem weight at maturity (i.e. increased micropores) would increase the conductivity of methane flux from soil to air. Although stem dry weight was stimulated with elevated CO<sub>2</sub> concentration in the 1995 wet season, tiller number/stem weight per se does not seem to correspond directly to methane flux (e.g. no increase in stem weight at elevated CO<sub>2</sub> for the 1996 dry season). Because tiller number or stem weight is a limiting factor for the methane pathway, increasing root production at elevated CO<sub>2</sub> concentration appears to stimulate methane emissions primarily by increasing the supply of carbon either through root exudates or root autolysis products. However, this assumes that the elevated CO<sub>2</sub> treatment did

not alter aerenchyma size, intercellular space systems or micropore development – anatomical changes which could influence the transfer pathway and require further investigation.

It is well known that methanogenesis is temperature dependent (Neue 1991). In our experimental set up, however, only air temperature was elevated by 4 °C. Measurements of water temperature showed a minimal (i.e. < 0.5 °C) increase and no significant effect was observed on soil temperature in the respective OTC. In contrast, preliminary experiments in controlled environment chambers showed an interaction/synergism between CO<sub>2</sub> concentration and air temperature for increased methane emission in rice (Allen *et al.* 1994). However, the reported daytime air temperatures in this experiment (i.e. up to 38 °C) may have been sufficiently high enough to also increase soil temperature (Allen *et al.* 1994). In any case, it is important to emphasize the temporal and spatial nature of the current field experiment and to stress that with climate change, soil temperature on average may increase.

Increases in the biological uptake of CO<sub>2</sub> by rice plants could moderate the rise of atmospheric CO<sub>2</sub> concentration; however, the CO<sub>2</sub> induced changes in the emission of methane would exacerbate any potential effect on global warming through increased CH<sub>4</sub> emission since CH<sub>4</sub> has a larger degree of radiative forcing. Field and growth-chamber studies have also shown a positive correlation between increasing CO<sub>2</sub> concentration and methane emission of individual marsh species such as *Scirpus olneyi* and *Orontium aquaticum* (Darcy *et al.* 1994, Megonigal & Schlesinger 1997), but it is unclear if wetlands, as a whole, will demonstrate increased methane emissions as atmospheric CO<sub>2</sub> increases. In contrast, irrigated flooded rice, of and by itself, makes a significant contribution to the global CH<sub>4</sub> budget. If the findings of the current study are typical, increases of 50–60% over the current contribution of irrigated, paddy rice to global methane (i.e. 60–80 Tg y<sup>-1</sup>, Bachelet & Neue 1993) are possible as atmospheric CO<sub>2</sub> concentration increases.

Such effects could be exacerbated as atmospheric CO<sub>2</sub> continues to rise and as the need for additional rice production increases with future populations. Increases in methane flux could be mediated by CO<sub>2</sub>-induced stimulation of vegetation (especially below-ground) since vegetation supplies both the organic matter for the biosphere and the emission of other atmospheric trace gases such as N<sub>2</sub>O (Dacey *et al.* 1994). Consequently, direct effects of carbon dioxide on biological processes could lead to greater emission rates of key atmospheric trace gases than predicted based on individual sources and flux rates. Potential feedback mechanisms between biological systems should therefore be taken into consideration with

respect to current IPCC predictions (e.g. Houghton *et al.* 1996) of future methane emissions.

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