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Effect of rice plants on methane production and rhizospheric metabolism in paddy soil

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Abstract. In order to elucidate the effects of rice plants on CH_4 production, we conducted experiments with soil slurries and planted rice microcosms. Methane production in anoxic paddy soil slurries was stimulated by the addition of rice straw, of unsterile or autoclaved rice roots, and of the culture fluid in which rice plants had axenically been cultivated. The addition of these compounds also increased the concentrations of acetate and H_2, precursors of CH_4 production, in the soil. Planted compared to unplanted paddy soil microcosms exhibited lower porewater CH_4 concentrations but higher CH_4 emission rates. They also exhibited higher sulfate concentrations but similar nitrate concentrations. Concentrations of acetate, lactate and H_2 were not much different between planted and unplanted microcosms. Pulse labeling of rice plants with $^{14}CO_2$ resulted during the next 5 days in transient accumulation of radioactive lactate, propionate and acetate, and after the second day of incubation in the emission of $^{14}CH_4$. Most of the radioactivity (40–70%) was incorporated into the aboveground biomass of rice plants. However, during a total incubation of 16 days about 3–6% of the applied radioactivity was emitted as $^{14}CH_4$, demonstrating that plant-derived carbon was metabolized and significantly contributed to CH_4 production. The sequence of the appearance of radioactive products and their specific radioactivities indicate that CH_4 was produced from root exudates by a microbial community consisting of fermenting and methanogenic bacteria.

Introduction

Flooded rice fields are an important source of atmospheric methane with an annual emission of about 60 Tg (range of 20–150 Tg) (Prinn 1994). The large range of the source strength is partially due to the tremendous seasonal variation in emission. In some sites in China, India and the Philippines for example, CH_4 emissions change from season to season, but without a clear pattern (Wassmann et al. 1993, 1994; Parashar et al. 1996). These variations may be caused by many different factors, especially organic amendments, water management and fertilization (Yagi and Minami 1990; Watanabe et al.

1995b; Sass et al. 1991a, 1992; Wassmann et al. 1994; Neue et al. 1996; Yagi et al. 1996). However, rice fields in Italy (Holzapfel-Pschorn et al. 1986; Schütz et al. 1989), Japan (Yagi and Minami 1990; Minoda et al. 1996), Texas-USA (Sass et al. 1990, 1991a,b), China (Yao & Chen 1994), Indonesia (Nugroho et al. 1996) and India (Debnath et al. 1996) show a seasonal pattern with two or three pronounced maxima. It has been hypothesized (Holzapfel-Pschorn et al. 1986; Schütz et al. 1989) that the first maximum is associated with decomposition of straw from the previous season, the second maximum with root exudation during the reproductive growth of the plants, and the last maximum with decaying roots at the ripening stage in the end of the season.

However, few studies have addressed the effect of rice plants on CH_4 production. Recent studies in which rice plants were pulse-labeled with $^{13}CO_2$ showed a significant emission of $^{13}CH_4$ indicating that plant photosynthates are a major source of CH_4 (Minoda & Kimura 1994; Minoda et al. 1996). One may hypothesize that photosynthates are excreted from the roots or that roots are decaying, and that the recently assimilated carbon is thus made available to soil microorganisms that eventually degrade it to CH_4. Therefore, we investigated the effect of rice roots and root exudates on CH_4 production in soil, measured the concentrations of CH_4, methanogenic precursors and oxidants in planted and unplanted rice microcosms, and studied the fate of radioactive carbon after its fixation by photosynthesizing plants.

Materials and methods

Soil samples were obtained from Italian rice fields at the Rice Research Institute in Vercelli in the valley of the river Po. The general soil characteristics and the procedures used for sampling and storage have been described (Holzapfel-Pschorn & Seiler 1986; Conrad et al. 1987). Soil was sampled from dry fields in early spring 1992 and 1993. The soil contained 2.0% organic carbon in 1992 and 1.7% in 1993.

Soil slurries were 10 g dw sieved (2 mm mesh) soil and 10 ml distilled water, prepared in 120-ml serum bottles. The bottles were closed with black rubber stoppers, 3 times evacuated and sparged with N_2, and then incubated with slight shaking (120 rpm) under a N_2 overpressure of 0.2 bar at 25 °C in darkness. Gas samples were taken every 2–3 days and analyzed for CH_4 and CO_2 by gas chromatography (Conrad et al. 1989). The initial partial pressures were <1 Pa CH_4 and <100 Pa CO_2. Some of the soil samples had been amended with either 0.5 g dw rice straw (equivalent to 200 mg C), 1 g fresh rice roots (equivalent to 0.1 g dw or 30 mg C) or 1 ml exudate solution (equivalent to <0.4 mg C). Rice straw was obtained from harvested rice plants which were air-dried and cut into about 1-cm pieces. Rice roots were

also cut into about 1-cm pieces and were applied either fresh or autoclaved (10 min). The dry weight of the roots was determined after drying at 105 °C for 24 h. Carbon contents were determined in a CHN analyzer (Analytical Chemical Laboratory of the Philipps University, Marburg).

The exudate solution was obtained by growing rice plants (*Oryza sativa*, type japonica, variety Roma) in an axenic growth medium (Hurek et al. 1994). The seeds were surface sterilized for 40 min with 30% sodium hypochlorite solution, washed with sterile water, then germinated on sterile agar plates. The plants were transferred into sterile 20-ml tubes containing glass beads (1 mm mesh) and 5 ml growth medium. After 5 weeks the plants were transferred into larger culture tubes that contained 50 ml medium. The medium was exchanged again after 4 weeks and further incubated for another 4 weeks. This medium was then used as exudate solution. The constituents of this solution were analyzed by high pressure liquid chromatography (HPLC) (Krumböck & Conrad 1991). Monosaccharides were detected by thin-layer chromatography using commercial thinlayer plates (Merck, Darmstadt, Germany; HPTLC silica gel 60), a mixture of 2-propanol: aqueous 0.75% boric acid: glacial acetic acid (40:5:1) for development (80 min) and a reagent solution of 0.5 g 4-aminohippuric acid in 100 ml ethanol. The reagent was sprayed onto the plates, followed by 10 min heating at 140 °C. The monosaccharides were detected as blue-fluorescent spots under UV light (365 nm).

Rice plants also were grown in two types of soil microcosms. A large microcosm consisted of a plastic tube (height = 15 cm; diameter = 6 cm) closed at the bottom with a stopper and filled with flooded soil (about 360 g dw) up to 1 cm below the upper rim (Wind & Conrad 1995). The smaller microcosms consisted of specially constructed plastic tubes (height = 6 cm; diameter = 6 cm) filled with flooded soil (about 180 g dw) and with drainage tubes for sampling of porewater. These microcosms were gas-tight when closed with a plastic cover over the rice phyllosphere (volume = 2090 ml) that contained a ventilator. A detailed description will be given elsewhere (Dannenberg & Conrad, in prep.). After pre-incubation at 25 °C for 1 week, both types of microcosms were planted with 4 rice seedlings each. The rice seeds had been germinated on water-soaked filter paper until they had reached a length of about 6 cm. The walls of the soil microcosms were covered with alufoil to allow illumination of the soil surface only. Weeds were regularly removed from both planted and unplanted microcosms.

The larger microcosms were incubated at 25 °C, 70% relative humidity in a climatic room under fluorescent light (Osram type L 36W\72) at about 156 μE m^{-2} s^{-1} (34 W m^{-2}) photosynthetically active radiation (PAR) and a light/dark cycle of 12/12 h. Planted and unplanted microcosms were prepared (N = 17) and one was sacrificed at each sampling date. Dissolved H_2

was determined within the intact microcosms using a gas diffusion probe (Krämer & Conrad 1993). The upper 10 cm of the soil core was pushed upwards out of the tube and cut into 1-cm sections. Half of each section was transferred into Eppendorf cups, centrifuged at 14,000 rpm, and the supernatant filtered through 0.2-μM membrane filters (regenerated cellulose; Sartorius, Göttingen, Germany) and stored frozen (–20 °C) until analysis by HPLC (Krumböck & Conrad 1991). The other half was transferred into a N_2-flushed 150-ml serum bottle, diluted with the same amount of sterile demineralized and N_2-saturated water, stoppered, and flushed with N_2 again (Bosse & Frenzel 1998). The dissolved CH_4 and gas bubbles were extracted into the headspace by vigorous hand-shaking for 2 min and analyzed by gas chromatography (Conrad et al. 1987). The fresh and dry weight of the soil were then also determined. The data that were obtained from the soil sections were averaged between 3 to 7 cm depth.

The smaller microcosms were prepared in triplicate and incubated for 11 weeks under the same conditions. Porewater was obtained from perforated medical drainage tubes (diameter = 4.5 mm, type Ch14; Sterimed, Püttlingen, Germany) that were inserted into the soil at 2.5 cm depth through openings in the wall of the microcosm (Dannenberg and Conrad, in preparation). The drained porewater was collected into a plastic syringe (5 ml) which was connected to the drainage tube. The syringe was flushed with N_2 to prevent oxidation of the porewater constituents. The porewater was then membrane-filtered and stored frozen until analysis by HPLC (Krumböck & Conrad 1991). Dissolved CH_4 (Rothfuss & Conrad 1994; Rothfuss et al. 1994) and H_2 (Krämer & Conrad 1993) were determined within the intact microcosms using gas diffusion probes. Emission rates of CH_4 were determined by closing the microcosms with a cover and measuring the time-linear increase of the CH_4 mixing ratio within the enclosed headspace.

We carried out pulse-labeling experiments with $^{14}CO_2$ using the small microcosms. In the first experiment (August 1994) duplicate microcosms with 9 week-old plants, incubated under 12 h light/12 h dark cycles at a light intensity of 70 μE $m^{-2}s^{-1}$ PAR, were transferred to a hood at room temperature, and illuminated with 2 halogen flashlights (Lobi-Lux, Kirchain, Germany) at 120 μE $m^{-2}s^{-1}$ PAR and 12 h light and 12 h dark. The headspace of the microcosms was closed and about 10 MBq $^{14}CO_2$ were injected. Uptake of $^{14}CO_2$ into the soil was avoided by covering the surface of the flooding water with plastic. The $^{14}CO_2$ was prepared by acidifying a $NaH^{14}CO_3$ solution (0.5 ml; 37 MBq; Amersham-Buchler, Braunschweig, Germany) with 1 ml 1 M H_2SO_4 plus 0.5 ml H_2O in a stoppered 12-ml glass bottle. Gas samples were taken from the headspace and analyzed for radioactive and total CH_4 and CO_2 (Conrad et al. 1989), in order to calculate

Table 1. Effect of root exudates, fresh and autoclaved roots, and rice straw on the production of CH_4 and CO_2 in slurries of anoxic paddy soil. Shown are final partial pressures over soil slurries (10 g dw soil containing 170 mg organic C plus 10 ml water) which were incubated in 120-ml serum bottles under a N_2atmosphere for 23 days at 25 °C; mean ± SE of n = 4.

Additions	organic C added (mg)	CO_2 (Pa)	CH_4 (Pa)	CH_4 (Pa) per mg C added
Unamended control	0	4420 ± 190	15 ± 2	0
Exudates of rice roots	<0.4	5600 ± 400	30 ± 6	>38
Fresh rice roots	30	4710 ± 310	1200 ± 210	40
Autoclaved roots	30	5680 ± 270	3070 ± 780	102
Dry rice straw	200	21900 ± 600	42800 ± 1150	214

the daily emission rate of CH_4. Porewater was sampled with drainage tubes as described above and analyzed by HPLC connected to a radioactive detector (Krumböck & Conrad 1991). The measurements were done every day before the light was switched on, then the gas phase was exchanged with fresh pressurized air (10 min flushing). This procedure was repeated daily until Day 6, then it was repeated every second or third day. At the end of the experiments, total radioactivity was quantified in the gas phase, the porewater, the dry soil (including microbial biomass) and the plants. The plant carbon was converted into CO_2 by oxidation with chromsulfuric acid (Schlichting & Blume 1966).

In the second experiment (November 1996), the planted microcosms were permanently kept in a greenhouse at ambient light conditions at a temperature of 25 °C. In addition the microcosms were illuminated from above with mercury lamps (Philips IP55) providing a light intensity of 133 μE $m^{-2}s^{-1}$ PAR. The light was switched on at 06.00 h and switched off at midnight giving a light phase of 18 h. The pulse labeling was done with 13 MBq $^{14}CO_2$, and the daily flushing of the gas headspace was done in the morning at 09.00 h.

Results

1. *Stimulation of CH_4 production in soil slurries*

The unamended soil slurry produced little CH_4 during the 23-d incubation. The other treatments produced more CH_4 after a lag phase of about 10–15 d. At the end of incubation the final CH_4 partial pressures were higher in the treatment with rice straw > autoclaved roots > fresh roots > root exudates (Table 1). Production of CO_2 was only stimulated by the rice straw, but this

Table 2. Porewater concentrations of different compounds and CH_4 emission rates in planted and unplanted rice soil microcosms after 11 weeks of incubation. The microcosms (height = 6 cm; diameter = 6 cm) were filled with soil sampled in 1993 from unflooded Italian rice fields, and were kept submerged at 25 °C under 12 h light-12 h-dark cycling. Dissolved CH_4 and H_2 were measured in-situ with gas diffusion probes, the other compounds with HPLC after extraction.

Compound	unplanted	planted
CH_4 (μM)	518.0 ± 58*	128.0 ± 22*
Sulfate (μM)	3.4 ± 0.6	7.5 ± 2.0
Thiosulfate (μM)	ND	ND
Nitrate (μM)	0.9 ± 0.3	2.8 ± 0.9
H_2 (nM)	6.6 ± 0.9	18.6 ± 5.8
Acetate (μM)	72.0 ± 18	65.0 ± 14
Lactate (μM)	5.0 ± 3	15.0 ± 3
CH_4 emission (mmol m^{-2} d^{-1})	<0.4	7.3 ± 0.5*

Mean ± SE of n = 3; ND = not detectable (<1 μM); *Students t-test indicates significant difference at $\alpha < 0.05$.

effect was mainly due to the larger amount of dry matter and carbon contained in the straw compared to the roots. The relatively small stimulation of CH_4 production by root exudates was also caused by the relatively low amount of organic matter added (Table 1). A qualitative analysis of the root exudates showed in particular xylose, lactate and acetate as organic constituents. Lactose and fructose (or ribose) were only observed during the initial 4 weeks of plant growth. Fructose and ribose could not be separated by chromatography.

2. *Analysis of porewater concentrations in planted and unplanted rice soil microcosms*

After 80 days the plants had reached their maximum height of 70–75 cm. Flowering started after about 90 days. The presence of plants resulted in a significant decrease of dissolved and bubble CH_4 during the middle of the vegetation period (Figure 1A; Bosse & Frenzel 1998). However, measurement of only the dissolved portion of CH_4 (using gas diffusion probes) in a different set of 77-days old small microcosms also showed significantly lower CH_4concentrations in planted compared to unplanted soil (Table 2).

The presence of rice plants also resulted in a significant increase of sulfate concentrations in the soil porewater during the middle of the vegetation period (Figure 1B). Thiosulfate concentrations reached 13 μM in planted microcosms versus generally <1 μM (detection limit) in unplanted soil (data not shown). On the other hand, presence of plants had no significant effect

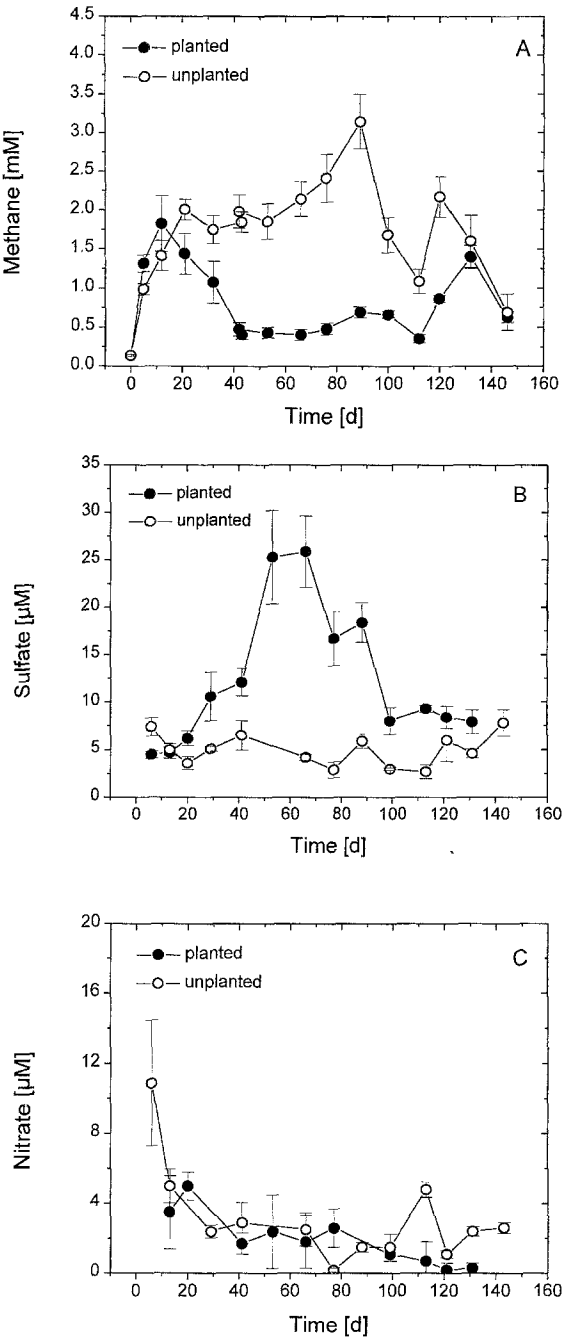

Figure 1. Temporal change of the concentrations of (A) CH_4, (B) sulfate, (C) nitrate, (D) H_2, (E) acetate, and (F) lactate in planted (closed symbols) and unplanted (open symbols) rice microcosms (the larger type cores) incubated under flooded conditions at 25 °C and 12/12 h light/dark cycle. The cores were sectioned into 1-cm slices. The data give the mean ± SE of the sections from 3–7 cm depth (n = 5). Graph (A) is adapted from Bosse and Frenzel (1998).

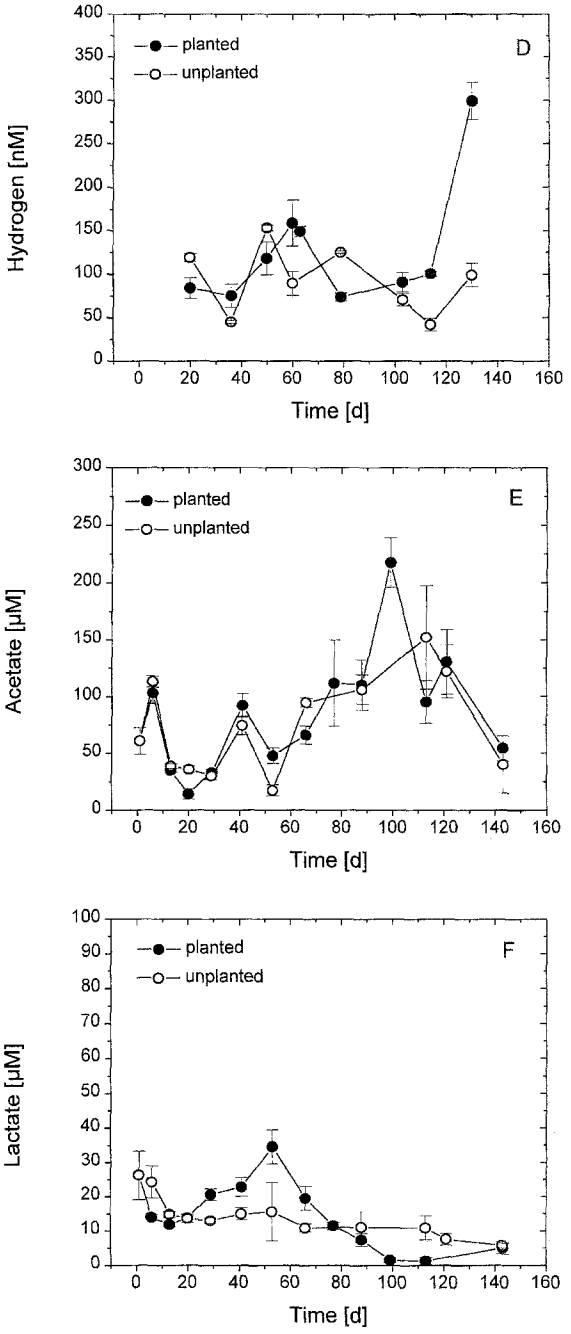

Figure 1. Continued.

on the porewater concentration of nitrate which showed in both planted and unplanted microcosms a rapid decrease and stabilized at around 4 μM (Figure 1C).

The presence of rice plants did not have a significant effect on the concentrations of the two immediate methanogenic precursors acetate (Figure 1D) and H_2 (Figure 1E), as well as on lactate (Figure 1F), a common product of fermenting bacteria and regular intermediate in the methanogenic degradation of organic matter in paddy soil (Chin & Conrad 1995). Formate and propionate occurred occasionally. The experiments with the smaller microcosms (height = 6 cm), evaluated after 77 days of incubation (Table 2), also showed lower CH_4 concentrations in the planted than in the unplanted microcosms. Emission rates of CH_4 were significantly higher in the planted than in the unplanted microcosms (Table 2) corroborating earlier observations (Holzapfel-Pschorn et al. 1986). Unplanted microcosms emitted little CH_4 unless a sudden ebullition event occurred (Holzapfel-Pschorn et al. 1986). The emission rates of planted microcosms were at the lower end of the range of rates observed in Italian rice fields (Schütz et al. 1989). The concentrations of compounds other than CH_4 were not significantly different between planted and unplanted microcosms. It should be noted that for the smaller microcosms another batch of paddy soil (sampled in 1993) was used than for the larger ones (soil sampled in 1992).

3. *Pulse labeling of rice plants with $^{14}CO_2$*

Two experiments were conducted with duplicate 9-weeks old planted microcosms. The results of these experiments are shown in Figures 2 and 3, respectively. The initial specific radioactivity of $^{14}CO_2$ in the headspace was about 350 Bq nmol^{-1}. After 2 h incubation in the light, >80% of the injected $^{14}CO_2$ had been taken up by the plants. After 5.5 h, less than 1.5% of the initial $^{14}CO_2$ remained in the headspace. The CO_2 mixing ratio decreased from ambient (approximately 350 ppmv) to values of 10–30 ppmv and stayed in this range thereafter. After 3 h, radioactivity occurred in the soil porewater. During the next 4–5 days radioactive acetate, lactate, and propionate occurred in the porewater. In addition some minor unidentified peaks appeared in the HPLC chromatogram. After 24 h (12 h light + 12 h dark) $^{14}CO_2$ and $^{14}CH_4$ was found in the headspace. Radioactive CH_4 showed a maximum between the 3rd and 4th day after the pulse labeling and was still detectable at the end of the incubation, i.e. 16 days. The daily rates of CH_4 emission stayed relatively constant at 35–55 mmol m^{-2}d^{-1} for the first 8 days of incubation (Figure 4), which are at the upper end of the range of rates observed in Italian rice fields (Schütz et al. 1989). After the 8th day of incubation the CH_4 emission rates decreased, probably due to deterioration of plant health

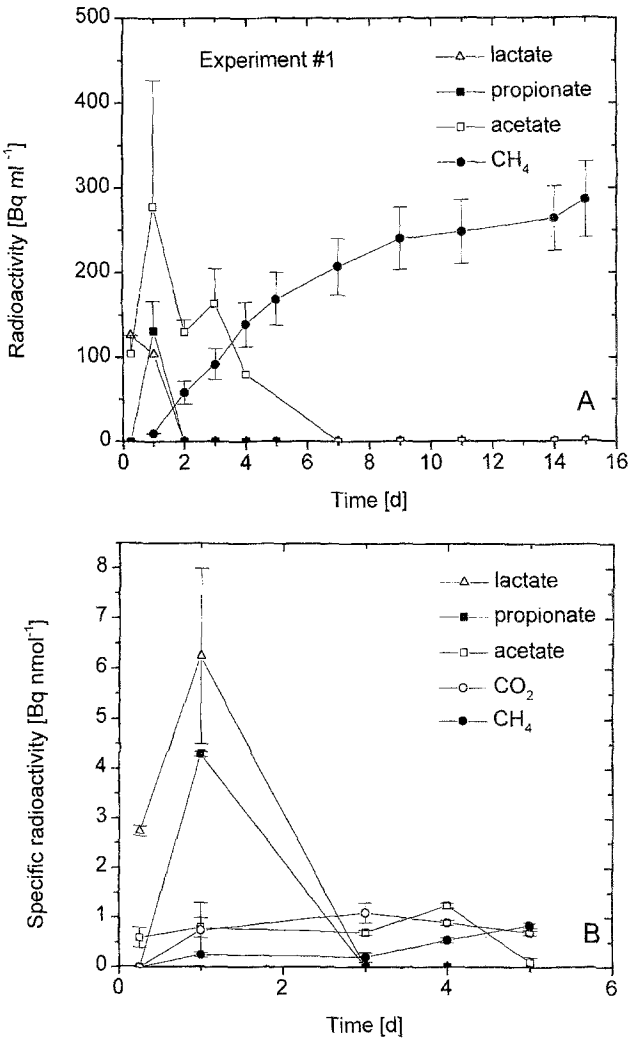

Figure 2. First experiment: temporal change of (A) the radioactivity in the pore water constituents and the cumulative accumulation of $^{14}CH_4$ in the gas phase and (B) the specific radioactivities of pore water constituents and emitted CH_4 and CO_2 after pulse-labeling (at Day zero) of the rice plants with $^{14}CO_2$. Mean ± range of duplicate microcosms.

(Figure 4). At this time, the radioactive acetate that had been excreted into the porewater was depleted and the cumulative release of $^{14}CH_4$ had slowed down (Figures 2 and 3).

At the end of incubation most of the radioactivity (43–69%) occurred in the above-ground biomass of the plants, followed by the soil (7–13%), the roots (6–9%) and the CH_4 (3–6%) that was emitted during the incubation (Table 3). We also observed emission of $^{14}CO_2$ (13% of the assimilated ^{14}C)

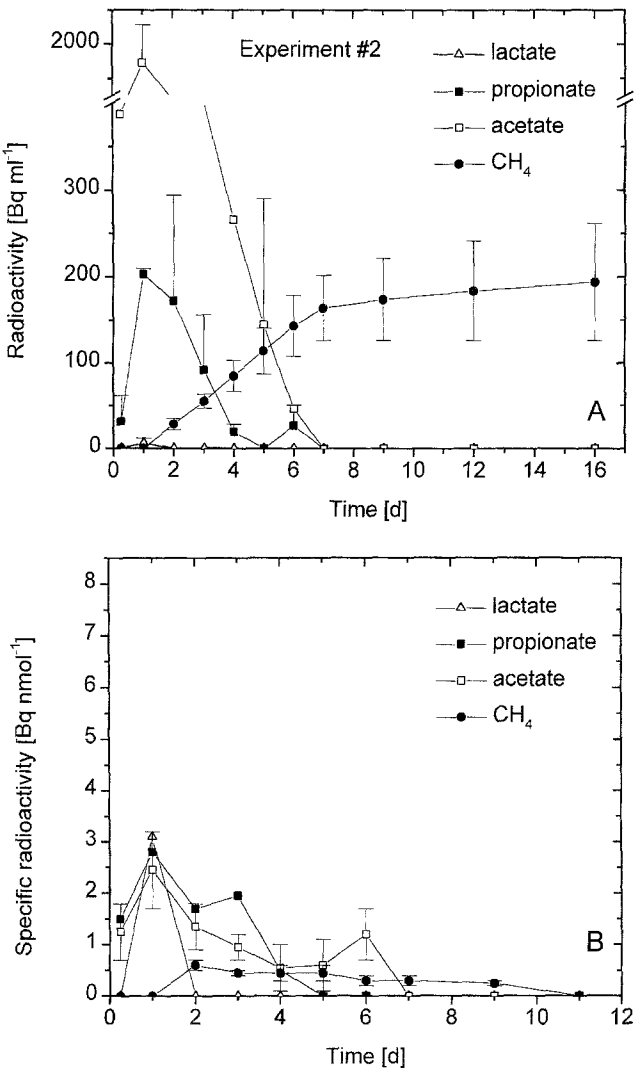

Figure 3. Second experiment: temporal change of (A) the radioactivity in the pore water constituents and the cumulative accumulation of $^{14}CH_4$ in the gas phase and (B) the specific radioactivities of pore water constituents and emitted CH_4 after pulse-labeling (at Day zero) of the rice plants with $^{14}CO_2$. Mean ± range of duplicate microcosms.

during the first experiment but not during the second one. This discrepancy was probably due to the timing of the daily flushing of the headspace. In the first experiment (climatic room), the headspace was flushed after the dark phase before the lights were switched on again, in the second experiment (greenhouse) this was done 3 h after the light was switched on (and after

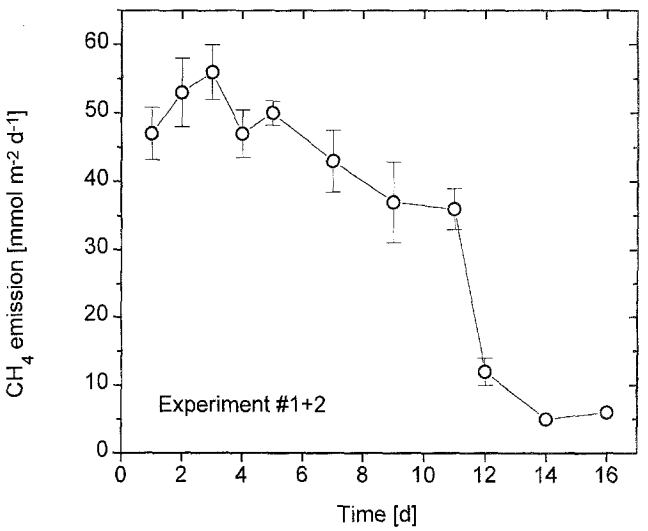

Figure 4. Temporal change of the CH_4 emission rates during two pulse labeling experiments with $^{14}CO_2$. Mean ± SE of 4 microcosms (two in each of the two experiments).

Table 3. Percentage recovery of radioactivity at the end of $^{14}CO_2$ pulse labeling experiments (16–17 days incubation) in different compartments of the rice microcosms (mean ± range; n = 2).

Compartment	1. Experiment	2. Experiment
Plant above ground	42.6 ± 3.6	69.1 ± 17.4
Roots	9.3 ± 0.4	6.0 ± 0.1
Soil	7.3 ± 0.5	12.5 ± 7.7
Pore water	2.1 ± 0.1	<0.5
Gas phase – CO_2	12.7 ± 1.2	<0.5
Gas phase – CH_4	5.8 ± 0.6	2.9 ± 0.5
Total	79.8 ± 3.9	90.5 ± 19.0

sunrise), so that the $^{14}CO_2$ that was possibly released during the night had probably been assimilated by photosynthesis.

During the 1st experiment, lactate, propionate and acetate concentrations were in a concentration range of 5–75, 5–50, and 5–560 μM, respectively. During the second experiment the concentration ranges were 5–70, 5–110, and 5–1100 μM, respectively. The specific radioactivities of lactate, propionate, acetate and CH_4 are shown in Figures 2 and 3. The specific radioactivities in the organic acid pools were generally less than 8 and 4 Bq

$nmol^{-1}$ in the first and second experiment, respectively. The $^{14}CH_4$ emitted until the 8th day of incubation had still lower specific radioactivities (0.1–0.9 Bq $nmol^{-1}$). The CO_2 emitted during the 1st experiment exhibited specific radioactivities of 0.2–1.3 Bq $nmol^{-1}$.

Discussion

Our pulse labeling experiment demonstrates that rice plant photosynthates were indeed precursors of CH_4 production and contributed to CH_4 emission. This observation confirms the hypothesis of Holzapfel-Pschorn et al. (1986) and is consistent with experiments using ^{13}C-enriched CO_2 (Minoda & Kimura 1994; Minoda et al. 1996). It furthermore indicates that CH_4 production is stimulated by the release of freshly photo-assimilated carbon in the form of compounds (e.g. acetate) that serve as methanogenic precursors.

The specific radioactivities of emitted CO_2 and CH_4 were lower than those of the radioactive organic compounds in the soil porewater and again much lower than the specific radioactivity of the CO_2 that was primarily assimilated by plant photosynthesis. These data are thus consistent with a conceptual carbon flow in which atmospheric CO_2 is assimilated into plant photosynthates, followed by excretion of photosynthates from the roots into the soil where the excreted organic compounds are then converted by microorganisms to CO_2 and CH_4. Emission of $^{14}CO_2$ was only observed after dark phases but not when the plants had the chance to photo-assimilate CO_2. The specific radioactivity of the emitted CO_2 was similar to that of acetate suggesting that it also served as precursor for methanogenesis (see below). Some of the emitted $^{14}CO_2$ may have been the product of plant respiration and of microbial oxidation of $^{14}CH_4$ taking place in the oxic rhizosphere (Holzapfel-Pschorn et al. 1985; Denier van der Gon & Neue 1996; Bosse & Frenzel 1997). The specific radioactivities in the organic soil solutes showed a decreasing tendency from lactate to propionate to acetate to CH_4. This tendency is consistent with a microbial community in which lactate is fermentatively converted via propionate and acetate to CH_4. Such a pathway is not unexpected, since fermentation of lactate to propionate and acetate by propionic acid bacteria is well known (Stams 1994). Lactate can furthermore be degraded by syntrophic bacteria to acetate, bicarbonate and H_2 (Stams 1994). Propionate can also be degraded by syntrophic bacteria to acetate, bicarbonate and CO_2 (Stams 1994). Acetate, bicarbonate and H_2 are then direct precursors of acetotrophic and hydrogenotrophic methanogenic bacteria, respectively (Stams 1994). The observation that radioactive lactate and propionate in the porewater appeared earlier than acetate and CH_4 is also consistent with such a carbon flow. However, our results do not exclude that some CH_4 may in addition be produced

by methanogenic bacteria that live on the root surface (Kimura et al. 1991; Frenzel & Bosse 1996; Liesack et al. 1997) and metabolize root exudates (e.g. acetate) directly before they can equilibrate with the bulk soil porewater. Our results do also not exclude that some of the $^{14}CH_4$ may have originated from the methanogenic reduction of $^{14}CO_2$ that may either have diffused from the headspace through the aerenchyma of the plants and the roots into the soil or may have been released by root respiration. CO_2 may be reduced to CH_4 by methanogens utilizing fermentatively produced H_2 as substrate. Earlier experiments showed that CO_2 reduction contributes 24 $\pm$ 7% to total CH_4 production in Italian rice field soil (Rothfuss & Conrad 1993).

About 3–6% of the primarily assimilated pulse of $^{14}CO_2$ was subsequently released as $^{14}CH_4$. Our estimate is probably conservative, since for safety reasons the planted microcosms had to be kept in a closed vessel after application of radioactivity. This treatment was not optimal to obtain a realistic contribution of photosynthates to total CH_4 production, since CO_2 partial pressures were only about 5–10% of those under ambient conditions. Although CH_4 emission rates were in a range similar as observed under field conditions (Schütz et al. 1989) for most of the incubation time, the plants were limited by CO_2 that was only replenished once a day, and eventually deteriorated so much, that CH_4 emission rates dropped after the 8th day. The plants were additionally limited by light during the first pulse labeling experiment, but not so much during the second one when the plants were incubated in the greenhouse at ambient light conditions. Using $^{13}CO_2$ Minoda et al. (1996) found that the contribution of photosynthates to CH_4 emission was 3.8–52% under field conditions. The importance of plant photosynthates for CH_4 production is consistent with the observation that CH_4 emission from wetlands is positively correlated with net ecosystem productivity (Whiting & Chanton 1993) and is enhanced by increased CO_2 (Dacey et al. 1994; Hutchin et al. 1995; Megonigal & Schlesinger 1997).

The stimulation of CH_4 production by photosynthesized carbon may be due to root exudation or decomposition of root material. Our experiments with anoxic soil slurries showed that CH_4 production can be stimulated by addition of root exudates, fresh or autoclaved roots or dry rice straw. Relative to the organic carbon contents dry rice straw and autoclaved roots showed a larger effect than fresh roots and root exudates. However, these data should not be over-interpreted, since the different treatments exhibited different lag phases of CH_4 production so that the magnitude of the final CH_4 partial pressure was not equivalent to the potential CH_4 production rate.

Root exudates contain hexoses, pentoses, and various organic acids, but vary in composition depending on the conditions under which they were

obtained (MacRae & Castro 1966; Boureau 1977; Hale & Moore 1979; Chaboud & Rougier 1984; Lin & You 1989; Waschütza et al. 1992). The qualitative analysis of our root exudates showed the presence of xylose, lactose, fructose (or ribose), lactate and acetate. In any case, root exudates are readily available substrates for microbial degradation to CH_4. On the other hand, more complex organic matter such as rice straw can also be rapidly degraded, at least to some extent (Neue & Scharpenseel 1987), resulting in the formation of dissolved organic compounds and CH_4 (Araragi & Tangcham 1979; Yagi & Minami 1990; Sass et al. 1991a; Wang et al. 1992; Kimura et al. 1992, 1993). Labeling studies showed that straw is besides plant photosynthates apparently the main source for CH_4 production (Chidthaisong & Watanabe 1997). Fresh organic matter such as roots or green manure are likewise decomposed and result in stimulated CH_4 production (Murakami et al. 1990; Lauren et al. 1994; Denier van der Gon & Neue 1995). The increased availability of easily available organic compounds that are derived from decomposed complex organic matter also accelerates the reduction of oxidants such as ferric iron so that the time needed for sequential reduction of oxidants after flooding of soil is shortened and CH_4 production is initiated relatively early (Inubushi et al. 1984). Our experiments did not allow to determine whether the organic acids observed in soil pore water were due to root exudation or root decay.

The concentrations of organic acids and H_2 in soil porewater were within the range of earlier observations (Rothfuss & Conrad 1993; Krämer & Conrad 1993). However, the presence of plants had no clear stimulatory effect on these concentrations. Perhaps root exudation was low because the plants were grown at relatively low light intensities or the effect of plant roots on dissolved organic compounds was too localized to be detectable in the bulk soil by significant concentration changes. Recently, Sigren et al. (1997) observed in rice fields that the type of rice cultivar influences the soil porewater concentration of acetate. Similarly, the emission of CH_4 from rice fields was shown to be dependent on the type of rice cultivar (Watanabe et al. 1995a; Wang et al. 1997; Butterbach-Bahl et al. 1997; Singh et al. 1997; Shao & Li 1997; Sigren et al. 1997).

Rice plants had a clear effect on the concentrations of sulfate and CH_4. Concentrations of sulfate (and thiosulfate) were increased in the presence of plants during the middle of the vegetation period. The same effect has been observed in vertical profiles (Wind & Conrad 1995, 1997) and is most probably due to the oxidation of reduced sulfur to sulfate, as O_2 and other oxidants become available in the rhizosphere due to the leakage of O_2 from the rice roots (Frenzel et al. 1992; Kirk et al. 1993; Flessa & Fischer 1992). Likewise, CH_4 is oxidized in the rhizosphere and in addition is ventilated through the

rice plants into the atmosphere (Holzapfel-Pschorn et al. 1985; Nouchi & Mariko 1993; Gilbert & Frenzel 1995; Denier van der Gon & Neue 1996; Bosse & Frenzel 1998). Because of these two processes CH_4 concentrations were lower in planted than in unplanted microcosms.

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