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# Oxidation of methane in the rhizosphere of rice plants

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**Abstract** Oxidation of $CH_4$ in the rhizosphere of rice plants was quantified using (1) methyl fluoride, a specific inhibitor of $CH_4$ oxidation, and (2) measuring changes in plant-mediated $CH_4$ emission after incubation under air, $N_2$, or 40% $O_2$. No significant rhizospheric $CH_4$ oxidation was observed from rice plants in the ripening stage. $CH_4$ emission from rice plants 1 week before panicle initiation increased by 40% if $CH_4$ oxidation in the rhizosphere was blocked. The growth stage of the rice plant is an important factor determining the rhizospheric $CH_4$ oxidation. Fluctuation of rhizospheric $CH_4$ oxidation during the growing season may help to explain the observed seasonal $CH_4$ emission patterns in field studies. Measurements from four rice varieties showed that one variety, Pokkali, had higher rhizospheric $CH_4$ oxidation. This was probably because Pokkali was in an earlier growth stage than the other three varieties. Both in the early and in the late growth stages, incubation under $N_2$ caused a much stronger $CH_4$ flux than inhibition of $CH_4$ oxidation alone. Apparently, $N_2$ incubation not only blocked $CH_4$ oxidation but also stimulated methanogenesis in the rhizosphere. Incubation under a higher $O_2$ atmosphere (40% $O_2$) than ambient air decreased the $CH_4$ flux, suggesting that increasing the oxidation of the rice rhizosphere may help in reducing $CH_4$ fluxes from rice agriculture. The $O_2$ pressure in the rhizosphere is an important factor that reduces the plant-mediated $CH_4$ flux. However, inhibition of methanogenesis in the rhizosphere may contribute more to $CH_4$ flux reduction than rhizospheric $CH_4$ oxidation.

**Key words** Methane emission · Methane oxidation · Methyl fluoride · Plant-mediated gas transport · Rice

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## Introduction

$CH_4$ is an important greenhouse gas and a key factor in tropospheric and stratospheric chemistry (Wang et al. 1976; IPCC 1992). Wetland rice fields are an important source of $CH_4$, accounting for approximately 20% of the global anthropogenic methane emission (IPCC 1992). $CH_4$ is produced by strictly anaerobic bacteria that are common in anoxic soils such as wetland rice fields (Cicerone and Oremland 1988). $CH_4$ emission from a rice field is the net effect of $CH_4$ production (methanogenesis) and $CH_4$ oxidation (methanotrophy). Methanotrophs are obligate aerobes because the enzyme monooxygenase, responsible for the oxidation of $CH_4$ to $CH_3OH$, requires molecular $O_2$ (Bedard and Knowles 1989; King 1992; Knowles 1993). Therefore, methanotrophs occur and are active close to oxic-anoxic interfaces where the concentration gradients of $CH_4$ and $O_2$ overlap.

In rice fields oxic-anoxic interfaces are found at the floodwater-soil interface and in the rice rhizosphere. The rice plant relies on aerobic respiration for growth and transports atmospheric $O_2$ to its roots to survive in the anaerobic environment (Armstrong 1978). Oxidation of the rice rhizosphere is caused partly by enzymatic oxidation but mostly by radial $O_2$ loss through the root wall (Ando et al. 1983). Hereby a very thin oxidized layer forms around the rice roots, creating a habitat for aerobic microorganisms, like methanotrophs, in an otherwise anoxic soil environment. Methanotrophy can be an important sink for $CH_4$ produced in anaerobic soils (King 1992). Unfortunately, investigations of methanotrophy in ecosystems have been hampered by the lack of a specific inhibitor that inhibits $CH_4$ oxidation only and has no inhibitory effect on other processes such as methanogenesis (Bedard and Knowles 1989). Therefore, $CH_4$ oxidation was often estimated as $CH_4$ production minus $CH_4$ emission (Schütz et al. 1989; Sass et al. 1990; Conrad and Rothfuss 1991; Denier van der Gon and Neue 1995b).

A large number of $CH_4$-oxidizing bacteria is present in the rhizosphere, indicating a high potential for $CH_4$ oxidation (de Bont et al. 1978). But $C_2H_2$ (an inhibitor of $CH_4$

oxidation) did not increase plant-mediated emission of $CH_4$, suggesting little or no $CH_4$ oxidation in the rhizosphere (de Bont et al. 1978). However, $C_2H_2$ inhibits not only oxidation (de Bont and Mulder 1976), but also the production of $CH_4$, at least at higher concentrations (Oremland and Taylor 1975; Sprott et al. 1982). Recently, Oremland and Culbertson (1992a,b) reported that $CH_3F$ and dimethyl ether are specific inhibitors of methanotrophs, although dimethyl ether was a less effective inhibitor than $CH_3F$. The use of $CH_3F$ as a specific inhibitor allows a direct quantification of methanotrophy in ecological systems and the ambiguities in indirect assessments of methane oxidation can be avoided. Using $CH_3F$, Oremland and Culbertson (1992a) showed that methanotrophs at the soil-water interface can consume more than 90% of the potentially available $CH_4$. This confirmed estimates for $CH_4$ oxidation, obtained by indirect assessments, at the soil-water interface of wetlands (King 1990; King et al. 1990) and rice fields (Conrad and Rothfuss 1991; Denier van der Gon and Neue 1995b).

Preliminary experiments with rice plants, using $CH_3F$, showed that between 10 and 47% of the potential $CH_4$ flux was oxidized in the rhizosphere (Epp and Chanton 1993). Increasing $CH_4$ oxidation in the rhizosphere could be a potential mitigation option that may reduce the $CH_4$ source strength of paddy fields without affecting rice yields. The objective of the present work was to investigate the significance of $CH_4$-oxidizing activity in the rice rhizosphere by using $CH_3F$ as a specific inhibitor of $CH_4$ oxidation. To investigate whether the $CH_4$-oxidizing activity in the rhizosphere is limited by $O_2$ availability, $CH_4$ emission was measured from plants incubated under variable $O_2$ concentrations.

## Materials and methods

Maahas clay soil from the International Rice Research Institute (IRRI) research farm (Block C) in the Philippines was air-dried and sieved. Plastic 5-liter pots were filled with 2.5 kg air-dried, ground soil mixed thoroughly with 0.27 g urea, equivalent to 100 kg N $ha^{-1}$. The pots were flooded and puddled by hand. The next day, two 2-week-old seedlings, rice variety IR 72 unless otherwise stated, were transplanted into each pot. IR 72 was also used in several studies that monitored $CH_4$ emission from rice fields (Denier van der Gon and Neue 1994; Wassmann et al. 1994; Denier van der Gon and Neue 1995a,b). The pots were placed in the greenhouse and a floodwater layer of about 5 cm was maintained.

$CH_4$ emission rates from the planted plots were measured with closed chambers as described by Denier van der Gon and van Breemen (1993). Before a $CH_4$ flux measurement, the stems of the rice plants were cut 5 cm above the floodwater. Earlier experiments revealed no significant differences in the $CH_4$ emitted by cut and intact plants, both a high and low levels of emission (Denier van der Gon and van Breemen 1993). The $CH_4$ flux into a headspace with normal air was measured. Next, the pots were separated into different batches, each batch with a different headspace composition. The headspace compositions studied comprised air, air with 1.5% $CH_3F$, air with 3% $CH_3F$, 100% $N_2$, and air with 40% $O_2$. Throughout each experiment, the batch with air in the headspace served as the control treatment. At the end of each experiment the tiller number, root length, dry weight of panicles, aboveground biomass, and belowground biomass were measured for each pot.

### $CH_4$ flux measurements

After an incubation, five headspace samples were collected with 5–10 min intervals. The gas samples were injected immediately into a 2-ml sample loop connected to a gas chromatograph equipped with a flame ionization detector, $N_2$ as carrier gas,and a column oven temperature of 45°C. The $CH_4$ flux was calculated by a linear regression from the increase in the $CH_4$ concentration in the headspace over time. The $r^2$ of the linear regressions is typically >0.95. Each flux measurement was repeated at least once. Modification of the system used in this study, compared to the set-up described by Denier van der Gon and van Breemen (1993), was that the floodwater layer was not separated from the headspace. The plant-mediated $CH_4$ flux was differentiated from the flux through the floodwater layer by additional measurements with a 140-ml glass beaker placed upside down over the stems of the rice-plant hill with the rim of the beaker submerged in the floodwater. Thus emission via the stems of the rice plant into the headspace was blocked and only $CH_4$ emission via the floodwater was measured.

### Methyl fluoride treatments

Epp and Chanton (1993) reported that for measurements on rhizospheric $CH_4$ oxidation, one 16 to 18-h incubation period with a 1.5% $CH_3F$ chamber headspace concentration was sufficient to inhibit $CH_4$ oxidation, without significantly affecting methanogenesis. In the present work we adopted their methodology. After measuring the $CH_4$ flux under air, the headspace of the closed chambers was spiked with 80 or 160 ml pure (99+%) $CH_3F$ (Scott Specialty Gases) to obtain about 1.5 or 3% (vol/vol) $CH_3F$ in the headspace, respectively. After incubation the $CH_3F$ concentration in the headspace was measured to check whether it remained constant. $CH_3F$ was measured on the same gas chromatograph with a flame ionization detector as $CH_4$. Standards of $CH_3F$ in air were made by injecting pure $CH_3F$ into glass bottles with a known volume to obtain 0, 1, and 5% (vol/vol) $CH_3F$. After 15 h of incubation, the average $CH_3F$ concentration in the headspace above the planted pots was 1.4% (±0.1) and 2.9% (±0.2) $CH_3F$ for the 1.5 or 3% $CH_3F$ treatment, respectively. This indicates that the closed chambers were gastight and that the $CH_3F$ concentration remained high enough for inhibition of methanotrophy throughout the incubation period. After an incubation of 16 h the closed chambers were removed and the pots were allowed to equilibrate for 1–3 h. Next, the $CH_4$ flux was measured as described above. We did not observe a significant difference in $CH_4$ flux after incubation with 1.5 or 3% $CH_3F$ (data not shown), in line with Oremland and Culbertson (1992b) and Epp and Chanton (1993). Therefore, all other experiments with $CH_3F$ treatments reported in this paper were performed with 1.5% (vol/vol) $CH_3F$.

### $N_2$ and 40% $O_2$ treatments

The headspace of the closed chambers was flushed for about 1.5 h with either $N_2$ or 40% $O_2$ (balance 60% $N_2$) at a high rate. Earlier experiments had shown that flusing for 1.5 h is sufficient to change the headspace composition for >99% (Denier van der Gon and van Breemen 1993). The atmosphere inside the cover was kept at a small over-pressure throughout the incubation period, causing a slow continuous escape of bubbles from the water seal separating inner and outer atmosphere, to ensure a fixed headspace composition. After 15 h the flushing rate was increased for 1 h to remove any accumulated $CH_4$. Next, the flushing was stopped and the $CH_4$ flux was measured as described above. One hour later the headspace was flushed again with 100% $N_2$ or 40% $O_2$ to remove trapped $CH_4$ and refresh the headspace gas composition, and a second $CH_4$ flux was measured.

A number of experiments were performed using the techniques described above.

Experiment 1: $CH_4$ oxidation in the rice rhizosphere at ripening stage

Seventeen pots prepared as described above were planted with IR 72. Because a limited number of closed chambers was available the seventeen pots were divided into a group of eleven pots and a group of six pots. The plants from the eleven pots were cut 65 days after transplanting (ripening stage). The $CH_4$ fluxes from each pot were measured, 0, 18, 24, and 24 h after cutting, under an air headspace. Next, three of the pots were incubated under $N_2$, four pots under 40% $O_2$, and four pots under air. The $CH_4$ fluxes were measured after 19 and 26 h of incubation. In between, after 23 h of incubation, $CH_4$ emission via the floodwater only was measured. The plants of the remaining six pots were cut 5 days later and $CH_4$ fluxes were measured 0, 4, and 6 h after cutting the plants. Next, half the pots were incubated under air, the other half under 1.5% $CH_3F$. $CH_4$ fluxes were measured after incubation as described above.

Experiment 2: Measurements in the field

$CH_4$ emissions from rice fields have a distinct diurnal pattern (Schütz et al. 1989; Denier van der Gon and Neue 1994, 1995a) which complicates any interpretation of the impact of headspace incubations on $CH_4$ emissions. To reduce the influence of diurnal variation in $CH_4$ emission, fluxes measured at a particular time of day are compared only with fluxes measured at exactly the same time the next day. Six hills of IR 72 were cut at harvest stage, about 100 days after transplanting. A closed chamber as used to measure the $CH_4$ flux from planted pots (Denier van der Gon and van Breemen 1993) was placed over each hill. $CH_4$ fluxes were measured at 11.30 and 15.00 h. Next, three hills were incubated under air with 1.5% $CH_3F$, while the remaining three were incubated under air. The incubation was stopped after 16 h and the chambers were removed. After 3 h of equilibration, the $CH_4$ fluxes were measured at 11.30 and 15.00 h.

Experiment 3: Varietal differences

Three pots each, prepared as described above, were planted with rice variety IR 72, IR 65597, Pokkali, or Dular. The stems of the rice plants were cut 65 days after transplanting. The $CH_4$ flux from the pots was measured 0, 3, and 5 h after cutting. Next, the pots were incubated under air with 1.5% $CH_3F$, and the $CH_4$ fluxes were measured the next day (see above). After two flux measurements, the pots were incubated under $N_2$ (as described under $N_2$ treatments) for 20 h and the $CH_4$ flux was measured twice.

Experiment 4: $CH_4$ oxidation in the rice rhizosphere before panicle initiation

About 30 days after transplanting (1–2 weeks before panicle initiation) 12 rice plants (IR 72) were collected in the field by using a 5-liter pot with a cut-out bottom and a sharpened edge as a corer. The soil core with the rice plant was immediately transferred to a pot and placed in the greenhouse. After 1 week the plants were cut, and the $CH_4$ fluxes were measured 0 and 4 h after cutting under an air atmosphere. In between, 2 h after cutting, $CH_4$ emitted through the floodwater only was measured by excluding plant-mediated $CH_4$ emission. Next, four pots each were incubated under 40% $O_2$, 1.5% $CH_3F$, and air for 16–18 h. After equilibration for 2 h, two $CH_4$ fluxes (each based on five headspace samples over time) were measured. Next, the pots that had been incubated under $CH_3F$ were incubated for 20 h under 100% $N_2$ while the remaining eight pots were incubated under air, and two $CH_4$ fluxes (each based on five headspace samples over time) were measured after the incubation.

## Results

### Plant-mediated $CH_4$ emission

$CH_4$ transport directly through the floodwater layer, either diffusive or by ebullition, contributed less than 10% to the total $CH_4$ flux, irrespective of the incubation gas or age of the rice plants (data not shown). So, plant-mediated transport consistently contributed over 90% (average 95%) to the total $CH_4$ emission from the pots. Therefore, changes in $CH_4$ emission upon incubation with different gases can be attributed to changes in the plant-mediated $CH_4$ emission. Keeping a control set of plants under air headspace throughout the experiment proved a useful approach which facilitated interpretation.

### Experiment 1: $CH_4$ oxidation in the rhizosphere of IR 72 in ripening stage

Although all pots were prepared in the same manner, the $CH_4$ emissions from the pots differed by a factor of 3–4 (Table 1). No correlation between the number of tillers or plant biomass and the rate of $CH_4$ emission was found. The variation shows how variable $CH_4$ emission may be, even under apparently similar conditions. With this large variation in $CH_4$ fluxes it was not possible to compare the mean $CH_4$ emissions per treatment and attribute different flux rates to different treatments. However, the $CH_4$ emission level per pot varied little with time, as can be seen from the standard deviation in Table 1. For comparison, $CH_4$ fluxes of each pot are scaled by setting the first measured $CH_4$ flux under air for each pot at 100%. $CH_4$ emissions of seven pots from Table 1 are plotted relative to the first measurement under air for each pot on the right half of Fig. 1. After incubation under $N_2$ for 19 h, the $CH_4$ emission was strongly stimulated, to about 210% of the first flux measurement (Fig. 1A). The relative effect of $N_2$ was the same despite the large differences in absolute emission levels, indicating that the scaling procedure is useful. The $CH_4$ emission from the control pots under air

**Table 1** Experiment 1: $CH_4$ flux into air from planted pots measured 0, 18, 25, and 47 h after cutting and some plant parameters. $CH_4$ flux is expressed as means (SD), and biomass as dry weight

| Pot | $CH_4$ flux ($\mu g\ CH_4\ pot^{-1}\ h^{-1}$) | Tillers | Aboveground biomass (g) | Belowground biomass (g) | Root length (cm) |
|---|---|---|---|---|---|
| 1 | 72.2 (6.1) | 29 | 90.5 | 20.3 | 42.0 |
| 2 | 169.7 (19.5) | 28 | 88.1 | 23.7 | 51.5 |
| 3 | 151.0 (9.4) | 27 | 88.6 | 17.5 | 42.3 |
| 4 | 234.2 (27.2) | 25 | 85.4 | 14.4 | 34.1 |
| 5 | 199.9 (47.8) | 27 | 90.7 | 16.2 | 46.5 |
| 6 | 78.8 (3.6) | 27 | 90.4 | 16.0 | 35.4 |
| 7 | 209.5 (51.6) | 28 | 85.5 | 21.1 | 33.1 |
| 8 | 310.3 (34.3) | 26 | 88.5 | 11.6 | 37.3 |
| 9 | 252.5 (27.3) | 30 | 89.6 | 19.3 | 44.2 |
| 10 | 82.6 (5.2) | 29 | 101.2 | 19.2 | 44.4 |
| 11 | 148.6 (22.0) | 26 | 95.5 | 14.5 | 38.6 |

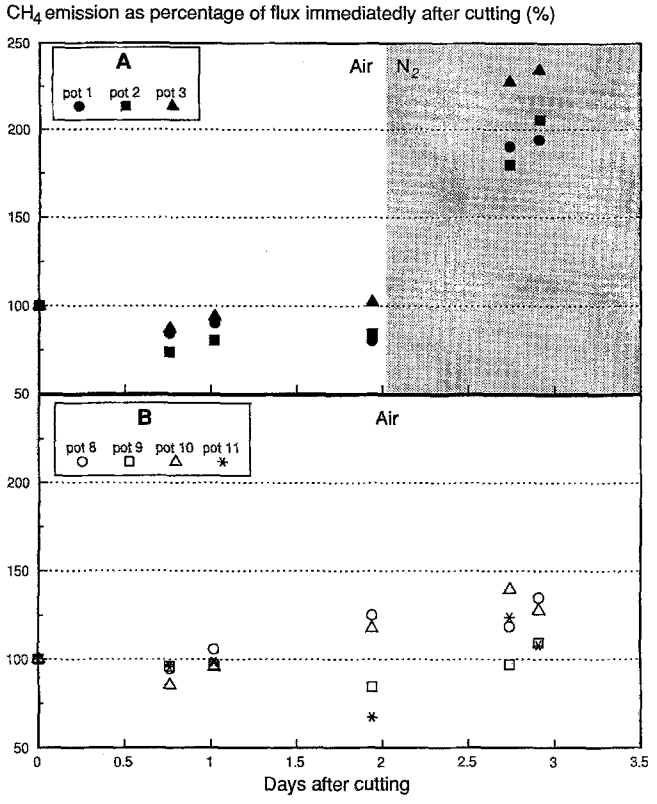


**Fig. 1A,B** Experiment 1: $CH_4$ flux from pots with rice plants (ripening stage) in the first 3 days after cutting the stems, expressed as a percentage of the initial $CH_4$ flux under air. Pot numbers refer to those given in Table 1. Pots 1–3 were under air and $N_2$ (**A**) and pots 8–11 under air as the control (**B**)

increased over time to about 125% (Fig. 1B). The flux increase after $N_2$ incubation had to be corrected for this "baseline drift" to give the impact of $N_2$ incubation only. Figure 2 shows the impact of incubation under various gases scaled to the $CH_4$ flux measurement under air and corrected for a drift in the $CH_4$ flux over time as derived from the control measurement under air. The standard deviation for each treatment, represented by the error bars, indicates the uniformity of the relative fluxes of different pots with the same headspace composition. Adding 1.5% $CH_3F$ did not alter the $CH_4$ flux. Incubation under $N_2$ or 40% $O_2$ resulted in a $CH_4$ flux of about 190 or 80% of the $CH_4$ flux under air, respectively.

## Experiment 2: Measurements in the field

For comparison, the $CH_4$ fluxes were scaled in the same way as in Fig. 2. The $CH_4$ emission from rice plants at harvest stage in the field did not significantly increase upon incubation under 1.5% $CH_3F$ (Fig. 3). This suggests little or no $CH_4$ oxidation in the rhizosphere, confirming the result of the pot experiment (experiment 1).

## Experiment 3: Varietal differences

$CH_4$ emission 65 days after transplanting was much higher from IR 72 and IR 65597 than from Pokkali or Dular (Table 2). Other differences included the high tiller number of IR 72, the high biomass of Pokkali, and the long roots of Dular plants. Furthermore, Pokkali had not yet flowered whereas the other three varieties had already panicles. The results cannot be scaled as in Figs. 2 and 3, because the number of closed chambers available did not allow control measurements under air headspace. The $CH_4$ flux from Pokkali increased by 37% under 1.5% $CH_3F$, whereas fluxes from the other varieties did not change significantly (Table 3). The results for IR 72 under 1.5% $CH_3F$ were in line with the observations in the previous experiments, where a control measurement under air was available. Therefore, we assume that the 37% flux increase from Pokkali plants is representative for the amount of $CH_4$ consumed by $CH_4$ oxidation in the rhizosphere of Pokkali. Under $N_2$, the flux from Pokkali increased by

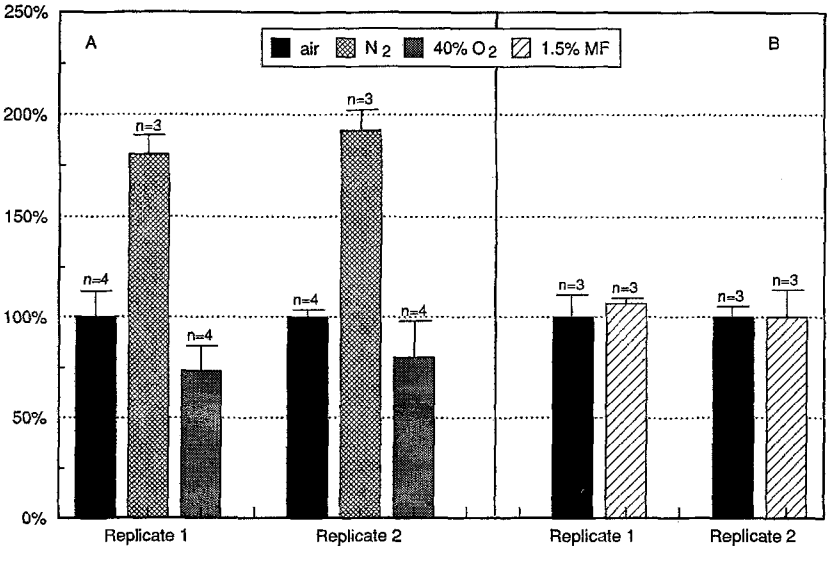


**Fig. 2** Experiment 1: $CH_4$ flux from pots with rice plants (ripening stage) under air, $N_2$, 40% $O_2$, or 1.5% $CH_3F$ (*MF*) relative to the previous $CH_4$ flux under air, corrected for changes in emissions from the control measurements. *Error bars* indicate standard deviation

**Table 2** Experiment 3: Average $CH_4$ emission, tiller number, panicle weight, biomass, and root length per pot of pots planted with two seedlings each, about 70 days after transplanting. Values are expressed as means (SD), $n=3$. Panicle weight and biomass represent dry weights. Aboveground biomass includes panicle weight. No panicles had developed for the Pokkali variety and those for the two IR varieties were not fully developed

| Rice variety | $CH_4$ emission ($\mu g\ pot^{-1}\ h^{-1}$) | Tiller (no.) | Panicle weight ($g\ pot^{-1}$) | Aboveground biomass ($g\ pot^{-1}$) | Belowground biomass ($g\ pot^{-1}$) | Root length (cm) |
|---|---|---|---|---|---|---|
| IR 72 | 483 (45) | 33 (2) | 17 (2) | 89 (2) | 19 (2) | 37 (1) |
| IR 65597 | 375 (35) | 19 (2) | 8 (0.2) | 61 (2) | 20 (4) | 36 (1) |
| Pokkali | 84 (45) | 19 (1) | – | 107 (9) | 31 (1) | 37 (3) |
| Dular | 44 (17) | 20 (3) | 21 (2) | 97 (4) | 19 (3) | 46 (1) |

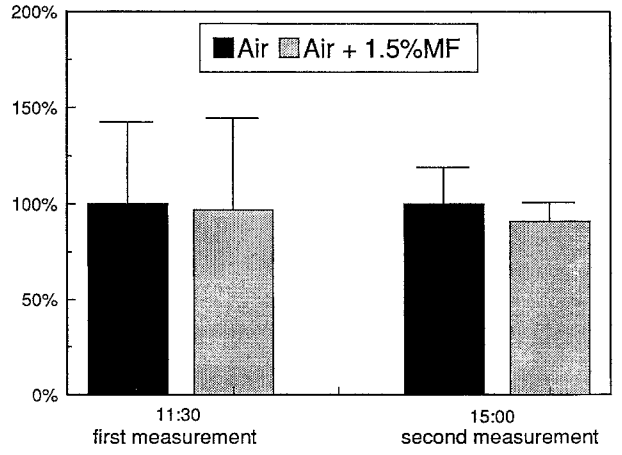


**Fig. 3** Experiment 2: $CH_4$ flux from plants in the field under air or 1.5% $CH_3F$ (*MF*) relative to the previous $CH_4$ flux under air, corrected for changes in emissions from the control measurements under air. *Error bars* indicate standard deviation ($n=3$)

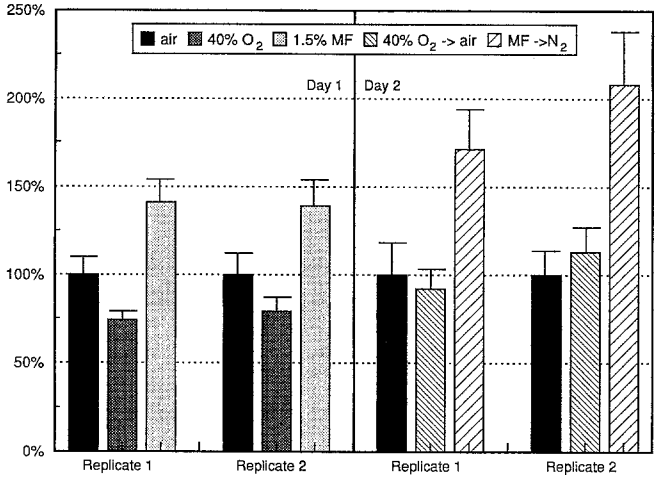


**Fig. 4** Experiment 4: $CH_4$ flux from pots with rice plants about 1 week before panicle initiation, under air, 40% $O_2$, or 1.5% $CH_3F$ (*MF*) (*day 1*) and under air or $N_2$ (*day 2*) relative to the previous $CH_4$ flux under air, corrected for changes in emissions from the control measurements under air. *Error bars* indicate standard deviation ($n=4$)

50% and the flux from IR 72, IR 65597, and Dular increased by about 10%. This 10% flux increase after incubation under $N_2$ was small compared to the twofold flux increase observed in the previous experiment. However, since control flux measurements under air were not available we cannot exclude the possibility that other factors influenced the $CH_4$ emission as well.

**Table 3** Experiment 3: Mean relative change (SD) in $CH_4$ flux for four rice varieties upon incubation under 1.5% $CH_3F$ or $N_2$ compared to previous measurements under air

| Variety | Relative change in $CH_4$ flux (%) | | $CH_4$ flux relative to IR 72 (%) | |
|---|---|---|---|---|
| | 1.5% $CH_3F$ | $N_2$ | 1.5% $CH_3F$ | $N_2$ |
| IR 72 | –1 (1) | +9 (3) | 100 | 100 |
| IR 65597 | –2 (2) | +9 (3) | 99 | 100 |
| Pokkali | +37 (5) | +50 (8) | 134 | 134 |
| Dular | +4 (1) | +11 (7) | 101 | 99 |

## Experiment 4: $CH_4$ oxidation in the rice rhizosphere before panicle initiation

The variation in $CH_4$ emission levels of IR 72 about 1 week before panicle initiation was similar to that of plants in the ripening stage. Again, $CH_4$ emission from various pots differed by a factor of about 4, but the emission level of each individual pot varied little with time (data not shown). The observed $CH_4$ fluxes from the planted pots after different incubations were scaled in the same way as those shown in Figs. 2 and 3. $CH_4$ emission from rice plants under 1.5% $CH_3F$ increased to 140% of the $CH_4$ flux under air (Fig. 4, left). Next, the plants previously treated with 1.5% $CH_3F$ were incubated under $N_2$. The $CH_4$ flux increased from 140 to about 190% of the flux under air (Fig. 4, right). So, on top of the 40% flux increase due to blocking of $CH_4$ oxidation, the $CH_4$ flux increased by another 50% due to stimulation of methanogenesis. The flux reduction under 40% $O_2$ (Fig. 4, 100%→77%) was similar to that observed for plants in the ripening stage (Fig. 2). On the second day, when the plants previously incubated under 40% $O_2$ were incubated again under air, the $CH_4$ flux fully recovered to about 100%.

## Discussion

Emission of $CH_4$ is not influenced by cutting the stems above the floodwater because plant-mediated gas transport between soil and atmosphere is independent of the transpiration rate or stomatal opening (Lee et al. 1981; Seiler et al. 1984) and takes place mostly via micropores in the leaf sheaths (Nouchi et al. 1990). Cutting the plants has several advantages: (1) The covers can be smaller, making the experimental set-up simpler and the $CH_4$ detection limit lower because of volume reduction; (2) photosynthesis is strongly reduced or stopped, and does not influence the gas composition of the headspace; and (3) the total distance from the atmosphere via the leaves, stems, and roots to the rhizosphere is reduced, creating a quicker response to headspace composition changes. A more detailed discussion on measurements with cut plants compared to intact plants has been presented previously (Denier van der Gon and van Breemen 1993). $CH_4$ transport from the soil to the atmosphere through rice plants is driven by diffusion (Denier van der Gon and van Breemen 1993). The headspace compositions used in our experiments do not alter the rate of $CH_4$ diffusion. Therefore, changes in plant-mediated $CH_4$ emission rates can only be invoked by changes in the concentration gradient between rhizosphere and atmosphere. If the $CH_4$ concentration in the rhizosphere increases because $CH_4$ oxidation is inhibited or methanogenesis is stimulated, the $CH_4$ flux from rhizosphere to atmosphere will increase.

The doubling of the $CH_4$ flux under $N_2$ (Fig. 2; experiment 1) cannot be attributed to decreased or blocked methanotrophy because the 1.5% $CH_3F$ incubation indicated that no significant $CH_4$ oxidation occurred. So, the increased $CH_4$ flux from plants in the ripening phase under $N_2$ must be due to increased methanogenesis. Indeed, Kimura et al. (1991) showed that $CH_4$ was produced from substances released by rice roots. This suggests that methanogenesis close to the oxic-anoxic rhizosphere interface is an important component of total plant-mediated $CH_4$ emission and that if $CH_4$ production is measured by incubating rhizosphere soil samples under $N_2$ this could lead to an overestimate because the inhibition of methanogenesis in the oxidized parts of the rhizosphere is no longer present.

Rhizospheric $CH_4$ oxidation in rice plants appears to be less effective than in some other wetland plants such as *S. lancifolia* and *P. cordata* (Epp and Chanton 1993). In IR 72 rhizospheric $CH_4$ oxidation was of minor importance at the end of the growing season (experiments 1 and 2). This is in line with de Bont et al. (1978) who found that $C_2H_2$ had no effect on the rate of $CH_4$ emission from rice plants in the ripening stage. Epp and Chanton (1993) reported that the $CH_4$ oxidation in the rhizosphere of 3-month-old rice plants was 14–52% of the potential $CH_4$ flux, or (recalculated) 116–208% of the original flux under air. Although Epp and Chanton's (1993) results are not fully comparable with our results and those by de Bont et al. (1978) because they did not have a control set of plants under air their results indicate that at the end of the growing season rhizospheric $CH_4$ oxidation is not always negligible. Blocking rhizospheric $CH_4$ oxidation increased the plant-mediated $CH_4$ flux by 40% in IR 72 just before panicle initiation (experiment 4) and in Pokkali before flowering (experiment 3). Rhizospheric $CH_4$ oxidation apparently varies with the growth stage of the rice plant. This may be related to the observation that oxidizing activity of rice roots varies with the growth stage (Armstrong 1969). Growth stage dependence of rhizospheric $CH_4$ oxidation may halp explain why at tillering and especially at ripening larger fractions of the produced $CH_4$ were emitted from rice fields than a panicle initiation (Denier van der Gon and Neue 1995a). Clearly, the relationship between the growth stage of rice and the $CH_4$ oxidation in its rhizosphere should be the subject of further study.

Schütz et al. (1989), using $CH_4$ production minus $CH_4$ emission to estimate $CH_4$ oxidation, reported that at the end of the growing season only 6% of the total produced $CH_4$ was emitted and over 90% of the total produced $CH_4$ was oxidized (implying that the $CH_4$ flux should increase $\sim$17-fold after incubation with $CH_3F$). Similar high oxidation rates were reported in other studies that assessed $CH_4$ oxidation indirectly. (Holzapfel-Pschorn et al. 1986; Frenzel et al. 1992). Rhizospheric $CH_4$ oxidation quantified with the "$CH_4$ production minus $CH_4$ emission" approach may be overestimated because in-situ methanogenesis is probably overestimated by the anaerobic incubation of soil samples in the laboratory. This can be illustrated by re-evaluating the results of Holzapfel-Pschorn et al. (1986). These authors measured the $CH_4$ flux from a microcosm with rice plants incubated under air, air with 5% $C_2H_2$, and $N_2$. The $CH_4$ flux under 5% $C_2H_2$ and $N_2$ was 136 and 342% of the flux under air, respectively. The flux increase upon $N_2$ incubation compared reasonably well with their indirect assessment of $CH_4$ oxidation ($CH_4$ production minus $CH_4$ emission) in the field but the 5% $C_2H_2$ incubation indicated a much lower $CH_4$ oxidation rate. The large difference between $N_2$ and 5% $C_2H_2$ incubation was not discussed by Holzapfel-Pschorn et al. (1986). However, the much larger flux increase under $N_2$ than under 1.5% $CH_3F$ in our experiments shows that most of the increase under an $N_2$ atmosphere is due to increased methanogenesis, not to blocked $CH_4$ oxidation. The $CH_4$ flux increase observed after incubation with 5% $C_2H_2$ by Holzapfel-Pschorn et al. (1986) is similar to the $CH_4$ flux increases under 1.5% $CH_3F$ observed in our experiments and by Epp and Chanton (1993). This suggests that in the experiments of Holzapfel-Pschorn et al. (1986) the $CH_4$ flux increase under 5% $C_2H_2$ was a better estimate of rhizospheric $CH_4$ oxidation than the incubation under $N_2$. The high $CH_4$ oxidation efficiency reported for intact soil columns from rice fields (Conrad and Rothfuss 1991; Denier van der Gon and Neue 1995b) are representative for the soil-water interface but not for the rhizosphere soil.

The four varieties screened in experiment 3 represent different plant types. IR 72 is representative of the successful, high-tillering, high-yielding varieties. IR 65597, the so-called new plant type, is a more recent development in plant breeding, with fewer tillers but more filled pani-

cles. Pokkali is a tall, salt-tolerant, long-duration variety from India, and both Dular and Pokkali are traditional varieties. Dular and Pokkali emitted very little $CH_4$ compared to both IR varieties. However, a thorough comparison of the amount of $CH_4$ emitted by different varieties was not possible because (1) the measurements did not cover the full growing season and (2) during the experiment the varieties were in different growth stages. Remarkably, first results with the same varieties in a field experiment, where $CH_4$ emission was continuously monitored, indicate exactly the opposite trend, with higher emissions from Dular and Pokkali (H.-U. Neue, unpublished data, 1995). Possibly the larger root biomass of Pokkali and the deeper rooting of Dular (Table 4) result in higher $CH_4$ fluxes under field conditions because the roots are not confined to the limited amount of soil in a pot. However, no good explanation is available at present and more research on variety-specific $CH_4$ emission is required.

To compare the varieties, the measurements were scaled with the response of IR 72 upon different incubations as 100% (Table 3). IR 72, IR 65597, or Dular responded in the same way to changing headspace compositions. Pokkali behaved differently, with a 34% increasing $CH_4$ flux under 1.5% $CH_3F$ or $N_2$. Clearly, $CH_4$ oxidation was a more important $CH_4$ sink in the rhizosphere of Pokkali plants than in the rhizosphere of IR 72, IR 65597, and Dular plants. When the impact of blocking $CH_4$ oxidation with $CH_3F$ was subtracted from the flux increase for Pokkali under $N_2$, the relative $CH_4$ flux increase for Pokkali was equal to that of IR 72, IR 65597, and Dular. So, the stimulation of methanogenesis by $N_2$ was equal for all four varieties. Although the higher $CH_4$ oxidation in the rhizosphere of Pokkali may be a varietal trait, it is more likely related to the growth stage. All varieties were planted on the same day, but at the time of the experiments Pokkali was behind in physiological development and had not flowered (Table 4). $CH_4$ oxidation efficiency in young IR 72 plants (experiment 4, Fig. 4) was similar to that in the Pokkali plants.

The $CH_4$ flux decrease after incubation under 40% $O_2$ indicates that increased oxidation of the rhizosphere may be a promising mitigation option. The recovery of the $CH_4$ flux to its original value when the plants were incubated under air again shows that the $CH_4$ flux decrease under 40% $O_2$ is reversible. The relatively fast recovery suggests that changes in microbial activity rather than death and/or new growth of microbes causes the changes in $CH_4$ emission. Since the variability in root-oxidizing power among rice cultivars is high (Armstrong 1969; Ando et al. 1983) it may be possible to breed high-yielding varieties with high root-oxidizing power. The most important mechanism behind $CH_4$ emission reduction due to a more highly oxidized rhizosphere could well be increased inhibition of methanogenesis in the rhizosphere instead of increased $CH_4$ oxidation in the rhizosphere.

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