Biogeochemistry **41:** 199–214, 1998.

Methane emissions from rice microcosms: The balance of production, accumulation and oxidation

ULRIKE BOSSE* & PETER FRENZEL**
Max-Planck-Institut für terrestrische Mikrobiologie, D-35043 Marburg, Germany (Present address: Department of Natural Resource Sciences, McGill University, Macdonald Campus, 21,111 Lakeshore Road, Ste. Anne de Bellevue, QC H9X 3V9, Canada; ** Corresponding author: Tel.: +49-6421-178821, Fax: +49-6421-178809, e-mail: frenzel@mailer.uni-marburg.de)*

Accepted 1 October 1997

Key words: methane balance, methane emission, methane oxidation, methane production, paddy soil

Abstract. In rice microcosms (*Oryza sativa*, var. Roma, type japonica), CH_4 emission, CH_4 production, CH_4 oxidation and CH_4 accumulation were measured over an entire vegetation period. Diffusive CH_4 emission was measured in closed chambers, CH_4 production was measured in soil samples, CH_4 oxidation was determined from the difference between oxic and anoxic emissions, and CH_4 accumulation was measured by analysis of porewater and gas bubbles. The sum of diffusive CH_4 emission, CH_4 oxidation, and CH_4 accumulation was only 60% of the cumulative CH_4 production. The two values diverged during the first 50 days (vegetative phase) and then again during the last 50 days (late reproductive phase and senescence) of the 150 day vegetation period. During the period of day 50–100 (early reproductive phase/flowering), the processes were balanced. Most likely, gas bubbles and diffusion limitation are responsible for the divergence in the early and late phases. The effect of rice on CH_4 production rates and CH_4 concentrations was studied by measuring these processes also in unplanted microcosms. Presence of rice plants lowered the CH_4 concentrations, but had no net effect on the CH_4 production rates.

Introduction

From preindustrial values of 0.7 ppmv, the atmospheric CH_4 mixing ratio has increased to currently about 1.7 ppmv (Raynaud et al. 1993; Kahlil & Rasmussen 1994). CH_4 is a very effective greenhouse gas (Lelieveld et al. 1993), and so its increase is cause for concern. Wetlands and irrigated rice fields are among the most important sources of atmospheric CH_4 (e.g. Cicerone & Oremland 1988; Wassmann et al. 1993). CH_4 emission from these sources is controlled by biological processes – microbial production and consumption – and by physical processes – diffusion, ebullition and ventilation. CH_4 production is a strictly anaerobic process. In contrast, CH_4 oxidation is almost exclusively an aerobic process. Only in marine sediments

and in some saline inland waters there is evidence for anaerobic CH_4 oxidation (Iversen & Joergensen 1985; Iversen et al. 1987).

The availability of O_2 is limited in wetland soils and in sediments, restricting aerobic CH_4 oxidation. The top soil of a few millimeters thickness is oxygenated, and a large percentage (about 90%) of the CH_4 diffusing through this layer is oxidized (Kuivila et al. 1988; Frenzel et al. 1990; King 1990; Conrad & Rothfuss 1991). Ebullition is often rapid and then CH_4 oxidation probably plays no role. Wetland plants constitute another effective pathway between the sediment and the atmosphere as their aerenchyma serves as a conduit for gases such as O_2 or CH_4 (Dacey & Klug 1979; Sebacher et al. 1985). While CH_4 will be transported into the atmosphere in this way, O_2 will be transported from the atmosphere downwards. O_2 may also leak from the plant roots into the surrounding sediment (Armstrong 1971) resulting in oxidized conditions in the rhizosphere (Crowder et al. 1987; Trolldenier 1988). Hence, favorable conditions for CH_4-oxidizing bacteria may be expected around roots of wetland plants, and indeed there is growing evidence that CH_4 oxidation in the rhizosphere is important in controlling the amount of CH_4 which is emitted from wetlands and rice paddies (King 1992; Bosse & Frenzel 1997). Measuring CH_4 oxidation in these systems is difficult, but it is a necessary step for understanding the mechanistic basis of CH_4 emissions, and CH_4 turnover in rice fields can be explained and modeled only if quantitative data are available. We measured these processes in rice microcosms over an entire vegetation period. To our knowledge this is the first attempt to compile such a process-based CH_4 balance in wetland rice.

Material and methods

Microcosms

Soil was taken from a rice field of the Istituto Sperimentale per la Cerealicoltura in Vercelli (Italy) in the spring of 1992 before flooding, and stored air dried. Both methanogenes and CH_4 oxidizing bacteria survive drying for prolonged periods (Le Mer et al. 1996; Mayer & Conrad 1990; Whittenbury et al. 1970). Field management and soil type at this site have been described (Holzapfel-Pschorn et al. 1986). The soil contained stubbles and rice roots from the last season. Before use the soil together with this organic matter was crushed and sieved to a diameter of <2 mm. Microcosms were constructed from Plexiglas®-tubes (inner diameter 6 cm), which were filled with water-logged soil to a depth of 14 cm and incubated at 25 °C under 2 cm of floodwater as described (Bosse & Frenzel 1997). They were planted with 4 rice seedlings each (*Oryza sativa*, var. Roma, type japonica) or left unplanted

as a control. Bulk soil had a density of 1.65 gfw ml^{-1} and a water content of 0.28 g H_2O (gfw soil)$^{-1}$.

Gas analyses

CH_4 and CH_3F were analyzed by gas chromatography with a flame ionization detector, O_2 was analyzed by gas chromatography with a thermal conductivity detector (Bosse & Frenzel 1997).

CH_4 concentration

To measure CH_4 concentration, one subcore was taken from the center of the microcosms, including one to two riceplants. Subcores were taken with a stainless steel corer (inner diameter 2.6 cm). The soil was pushed out with a piston and cut into 1–2 cm slices. The slices were immediately transferred into N_2-flushed 150 ml flasks and diluted with the same volume of N_2-bubbled demineralized water. The flasks were stoppered with rubber septa and flushed again with N_2. CH_4 was extracted into the headspace by shaking for 2 min, and freshweight and dryweight of the soil were determined. By this method both porewater- and bubble-CH_4 was measured. This was shown using CH_4 probes (Rothfuss & Conrad 1994; Rothfuss et al. 1994) in the same microcosms, because with these probes it is possible to distinguish between porewater and gas bubbles. But some gas bubbles could also escape during the preparation. To measure total CH_4, microcosms (soil volume ca. 0.4 l) were completely transferred into 2 l Erlenmeyer flasks containing 0.5 l degassed water. A Tedlar®-bag was connected to the flask to avoid pressure changes while the soil was transferred into the flask. The CH_4 was extracted into the headspace and freshweight and dryweight were determined.

CH_4 production

CH_4 production was measured in two ways: as anoxic fluxes (see next paragraph) and in soil slurries. For the slurries, slices and flasks with water were prepared as described. After transfer into the flasks, soil and water were mixed and root fragments picked out with forceps while the headspace was flushed with N_2. The flasks were stoppered with rubber septa, flushed again with N_2 and incubated at 25 °C in the dark. Before sampling the headspace for CH_4, the flasks were shaken to equilibrate porewater- and headspace-CH_4. CH_4 production in the soil was calculated from the increase of CH_4 in the headspace with time and from the dryweight of the soil slices, and expressed in [nmol (gdw)$^{-1}$ h^{-1}]. CH_4 production per area was calculated by summing up rates from [nmol (soil slice)$^{-1}$ h^{-1}] to give [nmol subcore^{-1} h^{-1}], and by

converting this to [mmol m^{-2} d^{-1}] for the given production depth (14 cm) using the surface area of a subcore.

CH_4 emission and CH_4 oxidation

For measure emission, a chamber (volume 0.6–2.4 l) with a built-in fan was put over the microcosms into the water. Samples were taken with a gas-tight syringe through a butyl septum and the sampled volume was replaced with air. Fluxes were usually measured within 1 h (n = 5–8). Emission was calculated from the increase of CH_4 with time.

CH_4 oxidation was measured by two different methods. The first was to compare CH_4 emissions under oxic and anoxic conditions (also termed oxic and anoxic fluxes) and to use the difference between them as an estimate of CH_4 oxidation. For measurements of anoxic fluxes, the chamber was placed in the dark to prevent photosynthetic O_2 production, flushed with N_2 until O_2 was no longer detected and preincubated for $\geq$1.5 h before the flux was measured. The other method was the application of CH_3F. CH_3F inhibits methane monooxygenase, the key enzyme of CH_4 oxidation in methanotrophic bacteria. CH_4 oxidation is then determined from the difference between the fluxes with and without CH_3F. CH_3F was injected into the headspace to give a final mixing ratio of 1.6–2.4% and the chamber was preincubated for 3.5 h before the flux was measured. Anoxic fluxes and fluxes under CH_3F were usually measured within 2.5 hours (n = 3–8)

Dataset

CH_4 fluxes under an oxic atmosphere were measured from 22 planted microcosms. Eight of these were then treated with N_2 to measure anoxic fluxes and two with CH_3F to inhibit CH_4 oxidation. The 12 untreated microcosms and in addition 26 planted and 30 unplanted microcosms were used in the destructive experiments: Porewater CH_4 concentration was measured in 16 unplanted and in 15 planted microcosms, CH_4 production in 11 unplanted and in 10 planted microcosms, and CH_4 accumulation in 3 unplanted and 3 planted microcosms.

Results

The vegetation period of the rice plants in the microcosms was 150 days. The density of plants was high as compared with the field, but in contrast to field plants, tillers were only formed at the end of the vegetation period. Roots developed mostly between 30 and 60 days after planting. The flowering stage

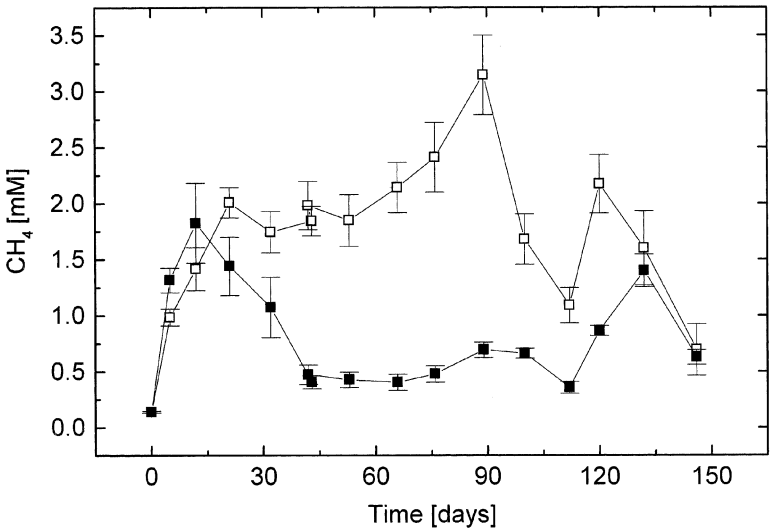

Figure 1. CH_4 concentration in planted (■) and unplanted (□) microcosms. CH_4 concentration was determined in each microcosm in different depths (n = 7–11) and then averaged. Concentrations could be higher than in CH_4-saturated water because both porewater and bubble-CH_4 are included. Each datapoint represents mean and standard error from one microcosm; standard errors are added as an empirical measure for the variation between the different soil layers.

was reached after 90 days. At this time, about 70% of the root biomass was located in the upper 3 cm of the soil, creating a root layer similar to that found in the field. The remaining 30% of the roots grew mostly along the walls and the bottom of the microcosms. A reddish tinge of the soil around the roots and on the roots themselves indicated Fe(III) plaques and thus sites where O_2 may have been released. Entrapped gas bubbles were observed frequently in the soil, especially in 1–2 cm depth in unplanted microcosms.

CH_4 concentration

Measurements on soil subcores showed that CH_4 concentrations were 2–4 times higher in unplanted than in planted microcosms during most of the vegetation period (Figure 1). The total CH_4 content, i.e. porewater- and bubble-CH_4, was also higher in unplanted microcosms (Table 1). A microcosm contained about 180 ml of porewater (calculated from freshweight, soil density and water content of the soil). At 25 °C, the maximum solubility of CH_4 is 1300 μm, which is equivalent to a total of 240 μmol CH_4 dissolved in the porewater of a microcosm. Comparing this value with the total CH_4 content (Table 1) shows that in unplanted and planted microcosms, at least 85% and 50% of the CH_4 was in the form of gas bubbles, respectively.

Table 1. CH_4 content in porewater and in gas bubbles of microcosms; one microcosm per measurements.

Age of microcosms [days]	CH_4 content [μmol microcosm^{-1}]	
	Unplanted	Planted
0		658
20	n.d.[1]	1635
80	1631	546
130	1764	661

[1] not determined

CH_4 production

CH_4 production measured as anoxic fluxes are described in the next section. In slurry measurements of CH_4 production rates, the r^2 of the linear regression was $\geq$0.98 (n = 4–7) in 95% of all cases. Usually, rates were measured within 24 h. However, rates were linear for a wide variety of sampling frequencies. Sampling intervals between 1 and 24 h were tested by measuring CH_4 production as described, but with varying sampling frequencies. For larger sampling intervals, the procedure was modified. After measuring the initial CH_4 concentration at time 0 in a total of 40 flasks, at every time point after that, the CH_4 concentration was determined in 2 flasks which were discarded afterwards. Thus each flask was only agitated once after the initial measurement. CH_4 production was constant for 42 days. This showed that it was independent of the sampling frequency.

The average CH_4 production was very similar in planted and unplanted microcosms (Figure 2). CH_4 production rates from day 30–150 were 52.1 mmol m^{-2} d^{-1} ($\pm$4.2, 95%CL, n = 16, n_i = accumulated production of one profile). CH_4 production rates averaged over time for each depth were also very similar in planted and unplanted microcosms (data not shown). Finally, in both planted and unplanted microcosms CH_4 production rates at 0–3 cm depth were somewhat lower (10.5$\pm$2.1 nmol gdw^{-1} h^{-1}, 95%CL, n = 50) than in the soil layers below 3 cm (14.0$\pm$1.1 nmol gdw^{-1} h^{-1}, 95%CL, n = 103).

CH_4 emission and CH_4 oxidation

For all CH_4 emission rates, the r^2 of the linear regression was $>$0.99. Diffusive CH_4 emission rates under oxic conditions (oxic fluxes) are shown in Figure 3. The mean value was 26.3 mmol m^{-2} d^{-1} ($\pm$5.7 95% CL, n = 22, range 1.8–54 mmol m^{-2} d^{-1}). Diffusive CH_4 emission rates under anoxic conditions (anoxic fluxes) were higher than oxic fluxes, indicating CH_4 oxidation rates

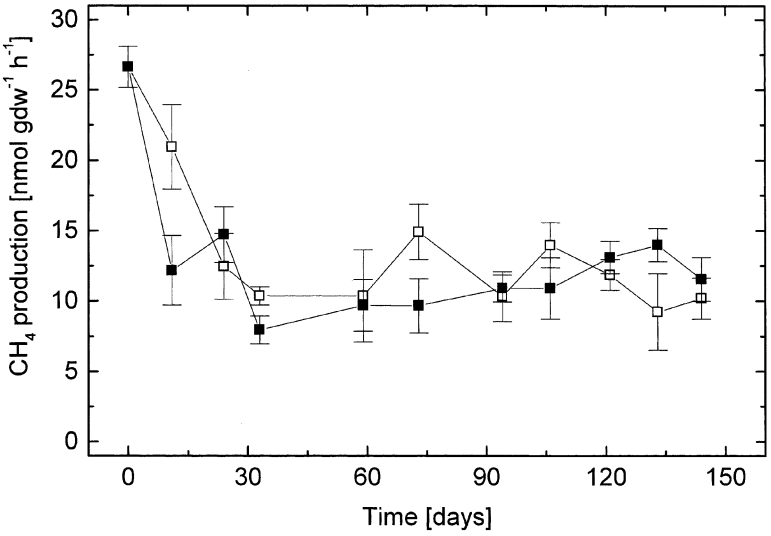

Figure 2. CH_4 production in planted (■) and unplanted (□) microcosms. CH_4 production was determined in each microcosm in different depths (n = 4–10) and then averaged. Each datapoint represents mean and standard error from one microcosm; standard errors are added as an empirical measure for the variation between the different soil layers.

between 1 and 19 mmol m^{-2} d^{-1} (Figure 4). The average percentage oxidation rate was 33% (±9 SD, n = 8; range 17–43%) of the CH_4 production. If the inhibition experiments with CH_3F were included, the average oxidation rate was still 33% (±9 SD, n = 10) (Figure 4). The percentage oxidation rates were constant with time (r = 0.60, $p > 0.05$, n = 10).

CH_4 balance

The seasonal CH_4 balance for a microcosm is

production = accumulation + oxic emission + oxidation.

For

anoxic emission = oxic emission + oxidation,

this converts to

production = accumulation + anoxic emission.

We calculated the CH_4 production from the integrated production measured in slurry (Figure 2) and measured the accumulated CH_4 as total CH_4 content

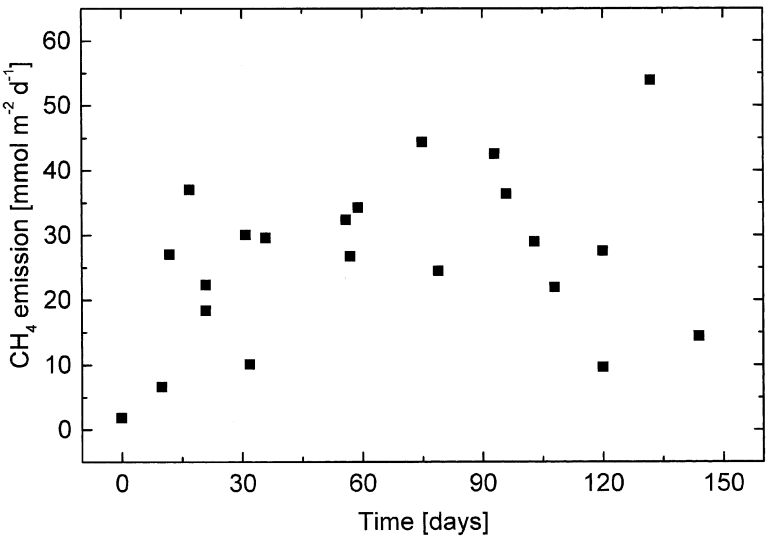

Figure 3. Diffusive CH_4 emission from planted microcosms under oxic conditions.

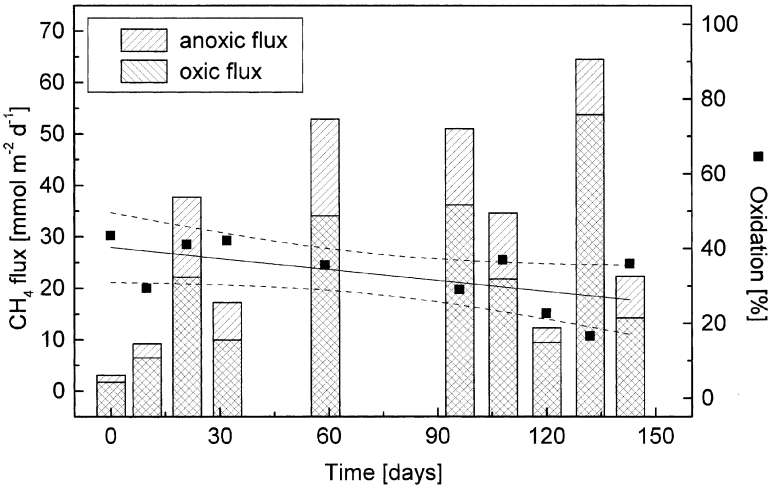

Figure 4. Diffusive CH_4 emission from planted microcosms under oxic and anoxic conditions. On each sampling date, the CH_4 emission from one microcosm was measured first under oxic (\\\\\) and then under anoxic (////) conditions. All the fluxes under oxic conditions shown here are also shown in Figure 3. The difference between the two fluxes (where bars do not appear cross-hatched) was considered to be CH_4 oxidation. It was expressed as percentage of the anoxic flux (■, linear regression and 95% CL).

(Table 1). Diffusive anoxic emissions were measured directly (Figure 4). A seasonal balance was estimated from the measured values and is shown in Figure 5. First, values between measured data were interpolated linearly. This

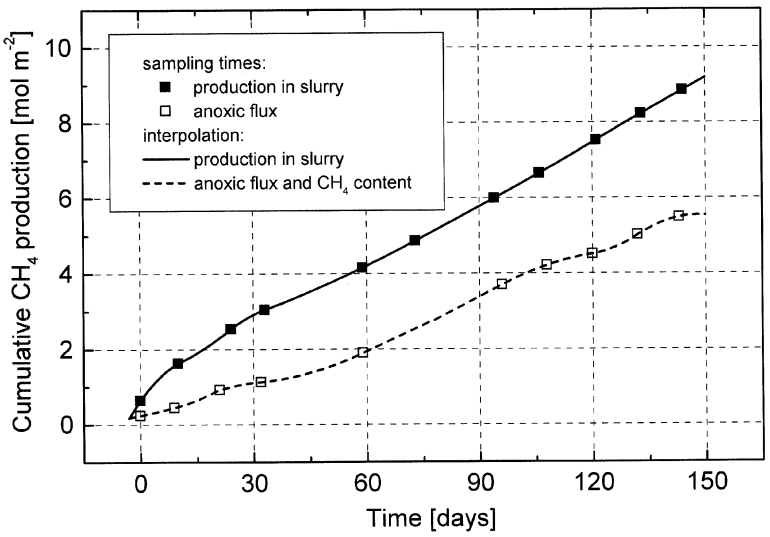

Figure 5. Cumulative CH_4 production in planted microcosms interpolated from slurry measurements (—■—) and from the diffusive anoxic flux plus the CH_4 content in the microcosms (—□—). For details of interpolation, see text.

was done with the CH_4 production rates, the CH_4 emission rates and the CH_4 content. The interpolated daily production rates were then summed up to give the cumulative production values shown as a solid line. The dashed line is composed of the interpolated daily anoxic emission rates summed up in the same way, and the CH_4 content. The daily CH_4 content was added directly, not as a cumulative value, as it was not a rate. Figure 5 shows that the sum of accumulation and diffusive anoxic emission represents only 60% of the production. The two lines diverge during the first 50 days and again after 100 days, but run more or less parallel in between.

Discussion

Processes

The results of the process-measurements mostly confirmed earlier studies. Planted microcosms contained less CH_4 than unplanted (Figure 1; Nouchi et al. 1994; Gilbert & Frenzel 1995). The CH_4 production rates were not affected by rice plants (Figure 2). In other studies, plant effects ranged from stimulation to inhibition of CH_4 production (Schütz et al. 1989; Sass et al. 1990; Butterbach-Bahl 1993; Frenzel et al. submitted). Many parameters, for example soil type, field treatment and rice variety probably influence CH_4

production. CH_4 emission rates (Figure 3) did not show a clear pattern. In field studies often more distinct peaks were observed, although the patterns vary considerably. It has been suggested that these peaks are the result of the sequential availability of straw, exudates and finally root biomass itself (Conrad 1993). Minoda et al. (1996), using $\delta^{13}C$ analyses, could show that the percentage of CH_4 derived from photosynthates had its maximum in July/August. Temperature can also influence CH_4 emissions (Butterbach-Bahl 1993). Our incubation conditions (constant light, constant temperature and no addition of straw) may thus have favored a simpler emission pattern. CH_4 oxidation was determined separately for the period of complete CH_4 balance (day 50–100) to be about 13 mmol m^{-2} d^{-1} or 25% of the CH_4 production. Our results indicate a constant percentage oxidation rate over the vegetation period. This was also found in other rice microcosms by Gilbert and Frenzel (1995), but is in contrast to field measurements in *Typha* (Lombardi et al. 1997). Percentage oxidation over different other studies varies a lot (Reeburgh et al. 1993). However, recent studies tend to give lower values (Denier van der Gon & Neue 1996) than older ones (Holzapfel-Pschorrn et al. 1986). The absolute values in our experiment are within the range estimated in a rice field (5–15 mmol m^{-2} d^{-1}, Holzapfel-Pschorn et al. 1985) and in other microcosms (mean of 5 mmol m^{-2} d^{-1}, Gilbert & Frenzel 1995).

Balance

CH_4 turnover could be balanced for a 50-day period, but a complete balance was not possible (Figure 5). Two methods were used to determine the CH_4 production: measuring in slurry and measuring anoxic fluxes. For a considerable part of the vegetation period, CH_4 production was higher if measured in slurry. Schipper and Reddy (1996) reported similar problems with point measurements comparing slurry measurements with CH_3F fluxes for *Sagittaria lancifolia*.

For a balance, the reliability of the experimental procedures is very important. Production measurements in slurries may be biased in different ways, resulting in both over- or underestimation. Roots which were cut during the preparation could provide additional C-sources. Roots were therefore excluded from the slurries to prevent an overestimation of CH_4 production. However, exclusion of roots may instead result in underestimation of CH_4 production as roots give off organic compounds *in situ* which may stimulate CH_4 production. Rooted soil may contain oxidants such as O_2, NO_3^-, SO_4^{2-}, Fe(III) and Mn(IV) which can serve as alternative electron acceptors (Bacha & Hossner 1977; Trolldenier 1988; Frenzel et al. 1992; Wind & Conrad 1995; Arth et al. in press). Anoxic measurements will lead to a depletion of these electron acceptors, resulting in an overestimation of CH_4 production. How-

ever, from the depth profiles of CH_4 production, the CH_4 production of the main root zone (top 3 cm) was calculated. On average, this zone contributed only 15% to the overall CH_4 production. In contrast, the total difference between the production measured in slurry and the production measured as anoxic flux was larger (40%, including periods of correspondence). Finally, slurrying (i.e. homogenizing and suspending the soil) might have improved the substrate availability and thereby increased CH_4 production rates. However, we tested different sampling frequencies and found no effect on CH_4 production. We conclude that slurry measurements were unlikely to critically overestimate CH_4 production. Instead, CH_4 flux measurements under anoxic conditions may have underestimated CH_4 production.

If plants are incubated in a closed chamber, changes in temperature, humidity, light and CO_2 will occur and they will affect transpiration. However, in rice no correlation was seen between transpiration and CH_4 emissions (Nouchi et al. 1990; Butterbach-Bahl 1993), and in our own experiments, we measured linear increases of headspace-CH_4 without any indication of a chamber effect.

But flux measurements might be biased in other ways. During the first 50 days, anoxic diffusive fluxes probably underestimated CH_4 production, because the plants were still small and some CH_4 might have left the system as gas bubbles. Ebullition was not measured separately because the plant density was too high to install a sampling device. We saw one gas bubble flux in the very beginning (distinguished from the diffusive flux by its high CH_4content), but we did not see any others. However, gas bubbles may be rare events and detection will be limited by the incubation time. The cumulative CH_4 production was 3700 mmol m^{-2} after the first 50 days, contrasting with a cumulative anoxic CH_4 emission (equivalent to oxic emission + oxidation) of 1100 and a total CH_4 content of 400 mmol m^{-2}. The difference of 2200 mmol m^{-2} is 24% of the seasonal cumulative CH_4 production of 9200 mmol m^{-2}. We assume that this CH_4 emitted as gas bubbles. In a field study, Butterbach-Bahl (1993) found that about 10% of the seasonal CH_4 emission emitted as gas bubbles during the first weeks.

CH_4 production from flux measurements would also be underestimated if the root system was not completely anoxic during the anoxic fluxes. Webb and Armstrong (1983) showed that when the green parts of 40 day-old rice plants were incubated under N_2, roots stopped releasing O_2 after 15 min. A preincubation under N_2 of $\geq$1.5 h should therefore be enough to ensure anoxic conditions even in the root system of the larger plants. Gas bubbles might have been a problem, because even a small amount of O_2 in a gas bubble represents a large reservoir compared to the amount dissolved in the surrounding porewater. This effect is difficult to assess. However, anoxic fluxes did agree with slurry measurements at a stage when the root system

was well developed and O_2 was probably present in the rooted layer (Frenzel et al. 1992). This indicates that O_2 in gas bubbles was not a problem and that CH_4 production was not underestimated in this way.

On the contrary, one argument against the N_2 technique is that an anoxic headspace favors CH_4 production in the soil. The anaerobic metabolism of the roots itself may stimulate root-associated CH_4 production, especially by releasing ethanol (Drew et al. 1994; Perata & Alpi 1994). Additional CH_4 production induced by the anoxic incubation would mean that the real divergence between the different estimates was even higher. An inhibitor like CH_3F would avoid this, and in our experiments, the average percentage oxidation rate measured under N_2 didn't change if the fluxes measured with CH_3F were included. But it was not used extensively because it may inhibit CH_4 production, too (Frenzel & Bosse 1996).

Finally, CH_4 production measured as CH_4 emission under anoxic conditions would be underestimated if anaerobic CH_4 oxidation took place. Anaerobic CH_4 oxidation has been reported mostly for marine and saline environments. In a Japanese rice soil it was suggested to occur in deeper soil layers coinciding with reduction of ferric iron (Kimura et al. 1992; Miura et al. 1992). However, anaerobic CH_4 oxidation would affect both slurry- and flux-measurements and so wouldn't help explain the difference between them.

Therefore, we think that the escape of CH_4 in gas bubbles offers the most plausible explanation for the observed discrepancy between CH_4 production measured in slurry and measured as CH_4 emission under anoxic conditions.

Between approximately day 50 and day 100, results from the slurry- and the flux measurements agreed well. The root system had reached its maximum biomass, providing a large aerenchyma for CH_4 to pass through. At the same time, escape of gas bubbles is probably mechanically impeded by the dense root mat. The potential for CH_4 oxidation in rhizospheric soil and roots was easily sufficient for the observed oxidation rate of 13 mmol m^{-2} d^{-1}(Bosse & Frenzel 1997).

After this period of good correspondence between flux measurements and slurry measurements, results diverged again after day 100. Gas transport through rice plants is mainly diffusive (Nouchi et al. 1990; Mariko et al. 1991; Denier van der Gon & van Breemen 1993). The capacity for transport may be limited, for example by the size of the root surface and especially at the transition zone between root and shoot (Armstrong 1979; Butterbach-Bahl 1993; Denier van der Gon & van Breemen 1993). A change in transport capacity due to morphological changes could affect gas transport under both oxic and anoxic conditions, but we found no information about this aspect of morphology during the late growth stage in the literature. However, we

observed more blackened, soft roots during this stage, especially after day 120, suggesting that plant-mediated transport might have become limited again.

Conclusion

Our results show that for a CH_4 balance in rice microcosms, CH_4 production, CH_4 emission and CH_4 oxidation were most important, while CH_4 accumulation was negligible. CH_4 production rates measured (a) in slurry and (b) as anoxic flux corresponded well for about one third of the vegetation period (day 50–100). Measuring CH_4 production with either of these methods, oxidation rates could then be calculated from the difference between the production and the oxic emission. The results confirmed that CH_4 oxidation is an important factor controlling CH_4 emissions, as 25% of the CH_4 production was oxidized.

However, a large amount of CH_4 was "lost" from the system during the vegetative phase (day 0–50) and some was lost during the late reproductive phase and senescence (day 100–150), most likely as episodic gas bubbles (ebullition). The total "loss" was 40% of the CH_4 produced. Under these conditions, CH_4 oxidation would be overestimated if it is calculated from the difference between oxic flux and production measured in slurry. We conclude that in principle it is possible to approach a balance, but that during the early vegetative phase and during senescence, CH_4 pathways seem to be quite variable and that more studies are needed to elucidate the role of gas bubbles and diffusion limitations in this system.

This study was done with microcosms, and because of the large heterogeneity in a field, a similar study would be difficult to do under field conditions. However, CH_4 emission rates were within the range observed in the field for this rice variety and this soil (Holzapfel-Pschorn & Seiler 1986; Butterbach-Bahl 1993). Thus it seems possible that the principle conclusions discussed here may also apply to the field situation.

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (FR 1054/1-1, 1-2) and the EC (Contract No EV5V-CT94-0499). We thank Dr. R. Conrad for helpful discussions, and Dr. S. Russo from the Istituto Sperimentale per la Cerealicoltura in Vercelli, Italy, for the paddy soil samples.

References

Armstrong W (1971) Radial oxygen losses from intact rice roots as affected by distance from the apex, respiration and waterlogging. Physiol. Plant 25: 192–197

Armstrong W (1979) Aeration in higher plants. Adv. Bot. Res. 7: 225–332

Arth I, Frenzel P & Conrad R (in press) Denitrification coupled to nitrification in the rhizosphere of rice. Soil Biol. Biochem.

Bacha RE & Hossner LR (1977) Characteristics of coatings formed on rice roots as affected by iron and manganese additions. Soil Sci. Soc. Am. J. 41: 931–935

Bosse U & Frenzel P (1997) Activity and distribution of CH_4-oxidizing bacteria in flooded rice microcosms and in rice plants (*Oryza sativa*). Appl. Environ. Microbiol. 63: 1199–1207

Butterbach-Bahl K (1993) Mechanismen der Produktion und Emission von Methan in Reisfeldern. IFU-Schriftenreihe 14–93

Cicerone RJ & Oremland RS (1988) Biogeochemical aspects of atmospheric methane. Global Biogeochem. Cycles 2(4): 299–327

Conrad R (1993) Mechanisms controlling methane emission from wetland rice fields. In: Oremland RS (Ed) Biogeochemistry of Global Change (pp 317–335). Chapman & Hall, New York

Conrad R & Rothfuss F (1991) Methane oxidation in the soil surface layer of a flooded ride field and the effect of ammonium. Biol. Fert. Soils 12: 28–32

Crowder AA, Macfie S, Conlin T, St. Cyr L & Greipsson S (1987) Iron hydroxide plaques on roots of wetland plants. Heavy Metals in the Environment 1: 404–406

Dacey JWH & Klug MJ (1979) Methane efflux from lake sediments through water lilies. Science 203: 1253–1255

Denier van der Gon HAC & van Breemen N (1993) Diffusion-controlled transport of methane from soil to atmosphere as mediated by rice plants. Biogeochemistry 21(3): 177–190

Denier van der Gon HAC & Neue HU (1996) Oxidation of methane in the rhizosphere of rice plants. Biol. Fertil. Soils 22: 359–366

Drew MC, Cobb BG, Johnson JR, Andrews D, Morgan PW, Jordan W & He CJ (1994) Metabolic acclimation of root tips to oxygen deficiency. Ann. Bot. 74(3): 281–286

Frenzel P & Bosse U (1996) Methyl fluoride, an inhibitor of methane oxidation and methane production. FEMS Microbiol. Ecol. 21: 25–36

Frenzel P, Thebrath B & Conrad R (1990) Oxidation of methane in the oxic surface layer of a deep lake sediment (Lake Constance). FEMS Microbiol. Ecol. 73: 149–158

Frenzel P, Rothfuss F & Conrad R (1992) Oxygen profiles and methane turnover in a flooded rice microcosm. Biol. Fert. Soil 14: 84–89

Frenzel P, Bosse U & Janssen PH (submitted). Rice roots and methanogenesis in a paddy soil: Ferric iron as an alternative electron acceptor in the rooted soil.

Gilbert B & Frenzel P (1995) Methanotrophic bacteria in the rhizosphere of rice microcosms and their effect on porewater methane concentration and methane emission. Biol. Fert. Soils 20: 93–100

Holzapfel-Pschorn A & Seiler W (1986) Methane emission during a cultivation period from an Italian rice paddy. J. Geophys. Res. 91D: 11803–11814

Holzapfel-Pschorn A, Conrad R & Seiler W (1985) Production, oxidation and emission of methane in rice paddies. FEMS Microbiol. Ecol. 31: 343–351

Holzapfel-Pschorn A, Conrad R & Seiler W (1986) Effects of vegetation on the emission of methane from submerged paddy soil. Plant Soil 92: 223–233

Iversen N & Joergensen BB (1985) Anaerobic methane oxidation rates at the sulfate-methane transition in marine sediments from Kattegat and Skagerrak (Denmark). Limnol. Oceanogr. 30(5): 944–955

Iversen N, Oremland RS & Klug MS (1987) Big Soda Lake (Nevada). 3. Pelagic methanogenesis and anaerobic methane oxidation. Limnol. Oceanogr. 32: 804–814

Khalil MAK & Rasmussen RA (1994) Global emissions of methane during the last several centuries. Chemosphere 29(5): 833–842

Kimura M, Miura Y, Watanabe A, Murase J & Kuwatsuka S (1992) Methane production and its fate in paddy fields. 1. Effects of rice straw application and percolation rate on the leaching of methane and other soil components into the subsoil. Soil Sci. Plant Nutr. 38(4): 665–672

King GM (1990) Dynamics and controls of methane oxidation in a Danish wetland sediment. FEMS Microbiol. Ecol. 74: 309–324

King GM (1992) Ecological aspects of methane oxidation, a key determinant of global methane dynamics. In: Marshall KC (Ed) Advances in Microbial Ecology, Vol. 12 (pp 431–468). Plenum Press, New York

Kuivila KM, Murray JW, Devol AH, Lidstrom ME & Reimers CE (1988) Methane cycling in the sediments of Lake Washington. Limnol. Oceanogr. 33: 571–581

Lelieveld J, Crutzen PJ & Brühl C (1993) Climate effects of atmospheric methane. Chemosphere 26(1–4): 739–768

Lombardi JE, Epp MA & Chanton JP (1997) Investigation of the methyl fluoride technique for determining rhizospheric methane oxidation. Biogeochem. 36: 153–172

Mariko S, Harazono Y, Owa N & Nouchi I (1991) Methane in flooded soil water and the emission through rice plants to the atmosphere. Environ. Exp. Bot. 31: 343–350

Mayer HP & Conrad R (1990) Factors influencing the population of methanogenic bacteria and the initiation of methane production upon flooding of paddy soil. FEMS Microbiol. Ecol. 73: 103–112

Minoda T, Kimura M & Wada E (1996) Photosynthates as dominant source of CH_4 and CO_2 in soil water and CH_4 emitted to the atmosphere from paddy fields. J. Geophys. Res. 101: 21091–21097

Miura Y, Watanabe A, Murase J & Kimura M (1992) Methane production and its fate in paddy fields. 2. Oxidation of methane and its coupled ferric oxide reduction in subsoil. Soil Sci. Plant Nutr. 38(4): 673–679

Nouchi I, Mariko S & Aoki K (1990) Mechanism of methane transport from the rhizosphere to the atmosphere through rice plants. Plant Physiol. 94: 59–66

Nouchi I, Hosono T, Aoki K & Minami K (1994) Seasonal variation in methane flux from rice paddies associated with methane concentration in soil water, rice biomass and temperature, and its modeling. Plant Soil 161(2): 195–208

Perata P & Alpi A (1993) Plant responses to anaerobiosis. Plant Sci. 93(1–2): 1–17

Raynaud D, Jouzel J, Barnola JM, Chappellaz J, Delmas RJ & Lorius C (1993) The ice record of greenhouse gases. Science 259: 865–1092

Reeburgh WS, Whalen SC & Alperin MJ (1993) The role of methylotrophy in the global methane budget. In: Murrell JC & Kelly DP(Ed) Microbial Growth on C1 Compounds (pp 1–14). Intercept, Andover

Rothfuss F & Conrad R (1994) Development of a gas diffusion probe for the determination of methane concentrations and diffusion characteristics in flooded paddy soil. FEMS Microbiol. Ecol. 14: 307–318

Rothfuss F, Frenzel P, Conrad R (1994) Gas diffusion probe for measurement of CH_4 gradients. In: Stal LJ & Caumette P (Eds) Microbial Mats, Vol. G35 (pp 167–172). NATO ASI Series

Sass RL, Fisher FM, Harcombe PA & Turner FT (1990) Methane production and emission in a Texas rice field. Global Biogeochem. Cycles 4: 47–68

Schipper LA & Reddy KR (1996) Determination of methane oxidation in the rhizosphere of *Sagittaria lancifolia* using methyl fluoride. Soil Sci. Soc. Am. J. 60(2): 611–616

Schütz H, Seiler W & Conrad R (1989) Processes involved in formation and emission of methane in rice paddies. Biogeochemistry 7: 33–53

Sebacher DI, Harriss RC & Bartlett KB (1985) Methane emission to the atmosphere through aquatic plants. J. Environ. Qual. 14(1): 40–46

Trolldenier G (1988) Visualization of oxidizing power of rice roots and of possible participation of bacteria in iron deposition. Z. Pflanzenern. Bodenk. 151: 117–122

Wassmann R, Papen H & Rennenberg H (1993) Methane emission from rice paddies and possible mitigation strategies. Chemosphere 26(1–4): 201–217

Webb T & Armstrong W (1983) The effects of anoxia and carbohydrates on the growth and viability of rice, pea, and pumpkin roots. J. Exp. Bot. 34: 579–603

Whittenbury R, Davies SL & Davey JF (1970) Exospores and cysts formed by methane-utilizing bacteria. J. Gen. Microbiol. 61: 219–226

Wind T & Conrad R (1995) Sulfur compounds, potential turnover of sulfate and thiosulfate, and numbers of sulfate-reducing bacteria in planted and unplanted paddy soil. FEMS Microbiol. Ecol. 18: 257–266