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Processes involved in formation and emission of methane in rice paddies

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Abstract. The seasonal change of the rates of production and emission of methane were determined under in-situ conditions in an Italian rice paddy in 1985 and 1986. The contribution to total emission of CH_4 of plant-mediated transport, ebullition, and diffusion through the flooding water was quantified by cutting the plants and by trapping emerging gas bubbles with funnels. Both production and emission of CH_4 increased during the season and reached a maximum in August. However, the numbers of methanogenic bacteria did not change. As the rice plants grew and the contribution of plant-mediated CH_4 emission increased, the percentage of the produced CH_4 which was reoxidized and thus, was not emitted, also increased. At its maximum, about 300 ml CH_4 were produced per m^2 per hour. However, only about 6% were emitted and this was by about 96% via plant-mediated transport. Radiotracer experiments showed that CH_4 was produced from H_2/CO_2 (30 – 50%) and from acetate. The pool concentration of acetate was in the range of 6 – 10 mM. The turnover time of acetate was 12 – 16 h. Part of the acetate pool appeared to be not available for production of CH_4 or CO_2.

Introduction

Paddy fields apparently are one of the most important sources in the budget of atmospheric methane (Ehhalt & Schmidt 1978; Khalil & Rasmussen 1983; Seiler 1984; Blake 1984; Crutzen 1987; Bolle et al. 1987). However, the present estimates of CH_4 emission from rice paddies are relatively uncertain and vary between 95 and 190 Tg CH_4 per year. This uncertainty is partially due to the fact that the CH_4 emission rates from paddy fields show a high variability with respect to daytime, season, location and management (Cicerone & Shetter 1981; Cicerone et al. 1983; Seiler et al. 1984; Holzapfel-Pschorn & Seiler 1986; Schütz et al., in prep.). Hence, it is difficult to extrapolate the available field data to global conditions.

The emission of CH_4 is obviously the result of different processes which each may change with the conditions in the field during the cultivation period. The most important process for CH_4 emission is the production of

CH_4 by methanogenic bacteria which starts only after anoxic, reduced soil conditions have established in the flooded paddies. The methanogenic bacteria can only utilize a limited number of substrates for production of CH_4 and growth, e.g., H_2/CO_2, formate, methanol, methylamines, acetate (Zeikus 1977; Balch et al. 1979; Mah & Smith 1981; Jones et al. 1987). Acetate and H_2CO_2 seem to be the two most important methanogenic substrates in anoxic paddy soil (Takai 1970). These substrates are provided by a complex food chain of various anaerobic bacteria depolymerizing and fermenting organic matter (Zehnder 1978; Zeikus 1983; Schink 1988). The nature and origin of the primary organic substrates is not quite clear. They either may originate from soil humus or plant residues (e.g., rice straw) incorporated into the soil, or may be autolysation products or exudates of the rice plants roots. Observations in the laboratory and in the field indicate that both types of organic matter are involved in production and emission of CH_4 (Holzapfel-Pschorn et al. 1986; Holzapfel-Pschorn & Seiler 1986).

Another important process affecting the quantity of CH_4 ultimately emitted is the reoxidation of the produced CH_4. Methane is relatively inert in anoxic environments, but is oxidized by methanotrophic bacteria as soon as oxygen becomes available (Hanson 1980; Rudd & Taylor 1980). Bacteria able to anaerobically oxidize CH_4 have so far not been isolated, although the possibility of their existence in marine sediments is seriously discussed because of geochemical evidence (see Alperin & Reeburgh 1984). Aerobic methanotrophic bacteria are present in the oxic surface layer of the submerged paddy soil and in the rhizosphere where oxygen is available in a shallow layer around the rice roots (DeBont et al. 1978). It has recently been shown that CH_4 oxidation is taking place within these zones of the paddy soil so that part of the produced CH_4 does not reach the atmosphere (Holzapfel-Pschorn et al. 1985).

Finally, the fate and the emission pattern of CH_4 is greatly influenced by its transport pathway into the atmosphere. Methane may escape from the submerged soil by diffusion through the flooding water, by ebullition, or by plant-mediated transport. In fact, the flow of CH_4 through the intercellular gas space system of the rice appears to be the most important transport pathway (DeBont et al. 1978; Seiler et al. 1984; Holzapfel-Pschorn et al. 1986). Since size and morphology of the rice plants is changing during the growing season, the transport pathway of CH_4 may also be affected.

Here, we report on measurements in an Italian rice paddy which were conducted to study methanogenic processes in the submerged soil. Furthermore, we quantified rates of CH_4 emission in relation to CH_4 production, as well as the transport pathway of CH_4 during the growing season.

Methods

Field site

The studies were conducted in 1985 and 1986 in rice paddies of the Italian Rice Research Institute in Vercelli, located in the valley of River Po (45° 20′N; 8° 25′W). The soils consist of a sandy loam having a pH of 6. The fields were dry fallow during the winter, ploughed in the beginning of April, flooded and planted with pregerminated rice seeds (variety Roma, type japonica) in May, and drained and harvested in September. The water level of the paddy field was ca. 5–10 cm deep during the cultivation period and was exchanged by a slow flow of flooding water at a rate of 5000 $l\ ha^{-1}\ h^{-1}$. The paddy field was parceled out in 16 experimental plots of ca. 9 m^2 each which were used for different studies. The individual plots could be reached via footbridges constructed prior to flooding. The studies described here were done in two plots which were not fertilized or otherwise treated during the cultivation period. Temperature was routinely measured once per hour in the paddy water, and in the soil at depths of 1, 5, 10, and 15 cm by using Pt 100 thermocouples.

Methane production

Soil cores of the submerged paddy soil were taken with a stainless steel corer (length = 26 cm; i.d. = 7 cm) shown in Fig. 1. Holes were drilled in the corer body in intervals of 2 cm, and were closed by tape for sampling. The corer was forced into the soil until the top submerged in the flooding water, the top was then closed with the screw cap, the corer was retrieved, and closed at the other end with a rubber bung. The corer had a sharpened lower end so that it was possible to cut through the rice roots. The tape was removed and subcores (length = 5–7 cm) were taken through the predrilled holes by means of plastic syringes (10 ml) with the needle lock cut-off. The subcores were taken immediately after sampling at the field site at ambient temperature by using an Atmosbag (Aldrich, Steinheim) filled with N_2. The subcores were transferred into serum bottles (120 ml) or pressure tubes (25 ml) which had been flushed with nitrogen and were closed with butyl rubber stoppers. The bottles were evacuated six times and refilled with N_2 to a total pressure of 150 kPa and incubated at the temperature recorded at a soil depth of 5 cm. This procedure guaranteed the removal of dissolved CH_4 and did not significantly affect the partial pressur of CO_2 (about 2 kPa) which established immediately after closure of the bottles. The production of CH_4 was measured by repeated analysis of the CH_4 mixing ratio in gas

samples (1 ml) taken from the gas headspace by using a gas chromatograph equipped with flame ionization detector (Holzapfel-Pschorn et al. 1985). The analysis was routinely repeated over a period of 3 to 6 h, but sometimes up to 24 h. During this incubation time CH_4 always increased linearly, without a lag. The dry weight of the subcore was determined gravimetrically at the end of the incubation. Methane production rates in the individual subcores were calculated on a dry weight basis and integrated over the depth of the whole core. In 1985 and 1986, the measurements and the integration was done over a depth of 14 and 24 cm, respectively. Since the measurements down to a depth of 14 cm apparently did not cover the active methanogenic zone completely, the data in 1985 were corrected by multiplication with a factor of two. This correction assumes that an equal amount of CH_4 was produced in soil depths of 14 to 24 cm which was missed by our sampling in 1985. The CH_4 production rate per unit area of submerged soil was calculated from the integrated production rate and the density of the soil core.

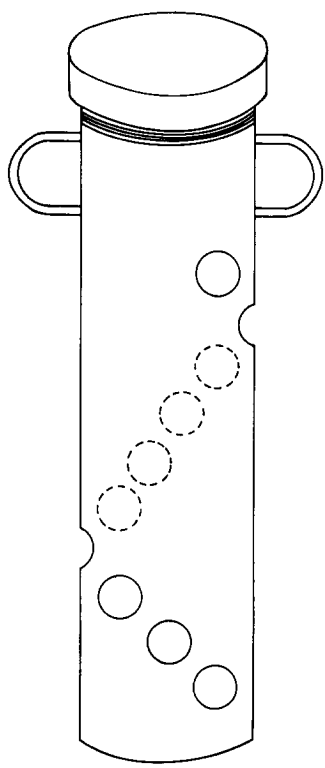

Fig. 1. Design of the stainless steel corer used for sampling anoxic soil in the paddy field.

Methane emission

Methane emission rates were measured by the static box technique. The rectangular collector boxes, consisting of plexiglas, had a width and breadth of 65 cm and a height of 90 cm. The details of the boxes, the installation, and the operation have been described in detail by Holzapfel-Pschorn & Seiler (1986). During our field studies, the boxes were equipped with a top lever which could be opened and closed automatically, and the whole CH_4 sampling and analyzing system was operated automatically and continuously by computer. The automatization allowed the determination of CH_4 emission rates at 16 individual field plots once every 3 hours; the system will be described elsewhere (Schütz et al., in prep.). To determine the influence of plant-mediated CH_4 release into the atmosphere, emission rates were measured at a field plot immediately before and after the rice plants had been cut below the water surface. After cutting, the plexiglas box was usually replaced by a shallow glass box (H = 18 cm). To determine the diffusional CH_4 flux, the plant-free soil surface was covered with a nylon screen (100 μm mesh), which prevented the escape of gas bubbles but allowed the diffusional exchange of gas during the course of the measurements. The ebullition of CH_4 was quantified by trapping emerging gas bubbles with glass funnels over a period of 2–3 hours. The funnels, which were shaped like longitudinally-cut tubes, had an effective trapping area of 375 cm^2 and were installed ca. 1 cm above the soil-water interface between the rows of the rice plants. Gas samples were analyzed for CH_4 by using a gas chromatograph equipped with a flame ionization detector and for CO_2 by using an infrared analyzer (Holzapfel-Pschorn et al. 1985).

Acetate concentration

Soil cores were taken with the stainless steel corer, and transferred into a N_2-filled Atmosbag (Aldrich). Soil from 5 to 15 cm depth was pooled, and aliquots of 5–10 g fresh weight soil were transfered into pressure tubes (25 ml). The tubes were closed inside the Atmosbag with butyl rubber stoppers and once again flushed with N_2. To minimize changes due to metabolic activities, the tubes were stored on ice until the experiments were initiated (within 24 hours). Aliquots of soil were poisoned by adding NaOH to a final concentration of 0.1 N, and stored on ice. After transport to the laboratory (within 24 h) the soil was centrifuged and the alkaline pore water was stored frozen. Acetate was analyzed by gas chromatography as described by Schink (1985).

Rate measurements

Radiotracer experiments were done after the gas phase of the pressure tubes was exchanged by evacuating and refilling with N_2. The experiments were initiated by adding 1 ml (ca.35–70 kBq) of a solution of carrier-free $NaH^{14}CO_3$, or (2-^{14}C) acetate (Amersham) having a specific radioactivity of 2.15 and 2.07 TBq mole^{-1} (129 and 124 dpm μmole^{-1}), respectively. The tubes were incubated in several replicates at 24 °C. The reaction was stopped after different time periods by adding 1 ml of 5N NaOH. After gaseous CO_2 had been absorbed by the alkali (within 3 h), an aliquot (1 ml) of the gaseous headspace was analyzed for CH_4 concentration, and another aliquot (1 ml) was analyzed for radioactive CH_4 using the method described by Zehnder et al (1979). The gas was injected into a scintillation vial fitted with a septum, equilibrated with 20 ml of toluene-based Quickszint 501 (Zinsser, Frankfurt), and counted in a liquid scintillation counter. The efficiency of dissolution of CH_4 in the scintillation cocktail was 72%, as determined by injecting known amounts of CH_4 and analyzing the remaining portion after dissolution. SRMeth, the specific radioactivity of CH_4 (dpm μmol^{-1} = 16.67 Bqnmol^{-1}), was calculated from the radioactivity and the concentration of CH_4 in the gas phase. The tubes were then opened and flushed to remove the $^{14}CH_4$. After closing them again, 1 ml of 7 N H_2SO_4 was added to acidify the anoxic soil sample and liberate total CO_2. The total carbonate pool contained dissolved CO_2 and bicarbonate, but no significant amounts of solid carbonate. Aliquots (1 ml) of the gaseous headspace were analyzed for CO_2 concentration and radioactive CO_2. $^{14}CO_2$ was counted by injection into a scintillation vial fitted with a septum, absorption in 8 ml of ethanolamine ehtylene glycol monomethylether (1:7), and addition of 12 ml of Quickszint 501 (Zinsser, Frankfurt). SR_{Carb}, the specific radioactivity of total CO_2 (dpm μmole^{-1}), was calculated from the radioactivity and the concentration of CO_2 in the gas phase. After opening and flushing the tubes to remove the $^{14}CO_2$, the radioactivity in the anoxic soil was determined by using Instagel (Packard, Frankfurt). The counting efficiency of ^{14}C was > 90% and was routinely determined by external standardization. The recovery of total radioactivity was 70–100%

The fraction of CH_4 produced from H_2 + CO_2 (fH_{ydr}) was calculated from the specific radioactivities of CH_4 (SR_{Meth}), and total CO_2 (SR_{Carb}) by

$$fH_{ydr} = SR_{Meth} / SR_{Carb} \quad (1)$$

The acetate turnover time (Tt) was calculated from the logarithmic transformation with time of (2-^{14}C) acetate into $^{14}CH_4$ plus $^{14}CO_2$ (Phelps &

Zeikus, 1984). We assumed that the maximum value achieved for total $^{14}CH_4$ and $^{14}CO_2$ production was 100% of the radioactivity available to the reaction, and thus

$$Tt = t/\ln\left(1 - \frac{\text{dpm of } ^{14}CO_2 + ^{14}CH_4}{\text{maximum dpm of } ^{14}CO_2 + ^{14}CH_4}\right) \quad (2)$$

The acetate turnover rate ($_{VAc}$) was calculated using the acetate pool size ($_{CAc}$) by equation (3)

$$_{VAc} = (_{CAc}/T_t)\,\frac{\text{maximum dpm of } ^{14}CO_2 + ^{14}CH_4}{\text{total dpm of } ^{14}C \text{ added}} \quad (3)$$

The respiratory index (RI) was calculated from the amounts of total $^{14}CO_2$ and $^{14}CH_4$ produced at each time point by

$$RI = {}^{14}CO_2/({}^{14}CO_2 + {}^{14}CH_4) \quad (4)$$

The rate of the acetate-dependent CH_4 production rate ($_{Pac}$) was calculated from the acetate turnover rate by,

$$p_{Ac} = v_{ac}\,(1 - RI) \quad (5)$$

or was calculated from the total CH_4 production rate ($_{Ptot}$) by assuming that CH_4 was only produced from acetate or from H_2/CO_2:

$$p_{Ac} = p_{tot}\,(1 - f_{Hydr}) \quad (6)$$

Bacterial numbers

The population density of methanogenic bacteria was determined in three-tube most-probable-number (MPN) analyses using a carbonate-buffered medium containing vitamins and 1 mM acetate as carbon source (Widdel 1986). Hydrogen-utilizing methanogens were counted by using a headspace of H_2/CO_2 (8:2), acetate-utilizing methanogens by using a headspace of N_2/CO_2 (8:2) and supplementing the medium with 10 mM acetate. The tubes were incubated at 30 °C for up to 12 weeks and analyzed at least two times for production of CH_4 by gas chromatography. Tubes were counted as positive when the headspace contained $CH_4 > 500$ ppmv.

Results

Production and emission of methane

The flooded paddy fields showed an oxic soil surface layer of 1–2 cm depth which had a light brown colour. Below, the colour of the soil changed to a greyish brown and the soil conditions were anoxic and reduced. Methane production was only detected in the soil below the oxic surface layer of ca. 1–2 cm depth.

Incubation of individual soil samples under anaerobic conditions resulted in a linear increase of CH_4 without lag phase (Fig. 2). This increase was due to microbial production (methanogenesis) and not to degassing of CH_4 dissolved in the pore water (Kiene & Capone 1985), as the increase of CH_4 stopped immediately after addition of formaldehyde (Fig. 2A), ethanol (not shown), or heating to 100 °C (not shown). Degassing of pore water was negligible, since the water content in the bulk soil was low (15–30 g water per 100 g fresh weight), and the soil samples were evacuated and flushed with N_2 prior to incubation.

The anoxic soil samples were handled with special care to avoid contamination with oxygen. However, contamination with O_2 apparently was not

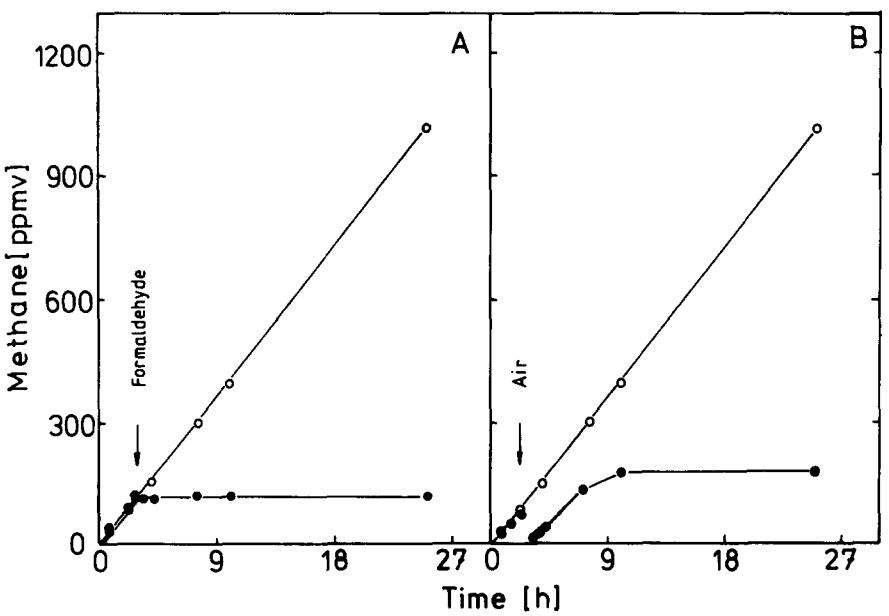

Fig. 2. Production of CH_4 in anoxic soil samples without (○) or with (●) addition of 5% formaldehyde (A) or air (B).

critical for the measurement of methanogenesis, since the initial rate of CH_4 increase was the same for aerobic as for anaerobic incubation and only ceased after 6 h under aerobic conditions (Fig. 2B). Methane production was completely inhibited within 1 h, when 10 μM chloroform was added to inhibit the methyl CoM reductase activity of methanogenic bacteria (not shown).

The vertical distribution of methanogenesis in the submerged paddy soil is shown in Figs. 3, 4 for different times of the growing seasons 1985 and 1986, respectively. In 1985, we used a soil corer of 15 cm length only. Maximum activities were reached at the deepest point of sampling. Obviously, the length of the core did not cover the entire zone of methanogenic activity. Coring was therefore extented to a depth of 25 cm in 1986. This depth completely covered the zone of rice roots and the zones of maximum methanogenic activity, although CH_4 was still produced in 25 cm depth.

Sampling of soil and measuring CH_4 production was routinely done in the morning (8.30 to 10.00 a.m.). Methanogenic activities were similar when two soil cores were sampled simultaneously in the morning (Fig. 4C). They were also similar at two occasions, when sampled in the morning and again in the afternoon (Fig. 3B, 4A), but at two other occasions (Fig. 4B,D) they were higher when sampled in the afternoon. The reason for this result is not yet clear. Since the soil and incubation temperatures were similar, diel temperature changes can be ruled out. It is more likely that the observations

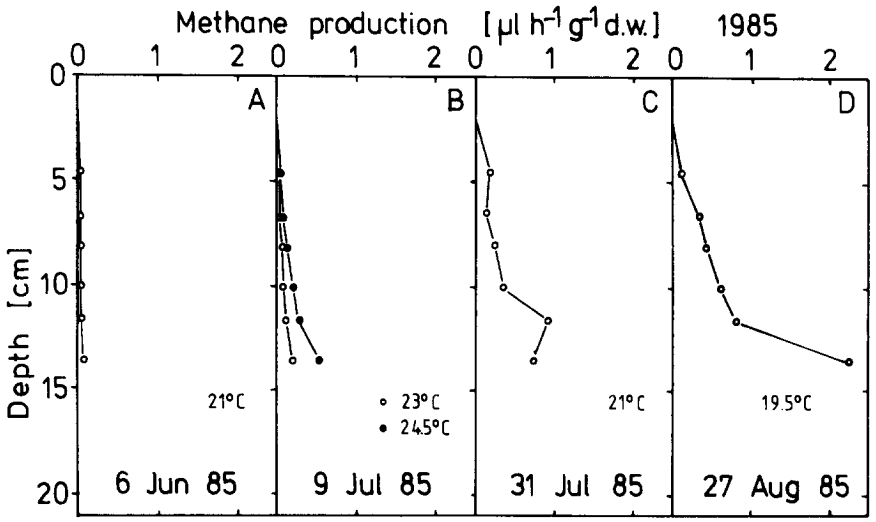

Fig. 3. Vertical profiles of CH_4 production in a rice paddy in Vercelli, Italy, during the cultivation period of 1985. ○ measured in the morning (8.30–10.00 am); ● measured in the afternoon (2.00–5.00 pm).

indicate spatial inhomogeneities or sporadic events stimulating methanogenesis; e.g., increased concentration of CH_4 precursors due to root exudation. Since CH_4 was produced without lag and at a constant rate for up to 24 h of incubation, the pieces of roots which might have been cut by the steel corer should not have influenced CH_4 production because of the time lag between root decomposition and production of CH_4 precursors. However, we do not exclude that some CH_4 precursors (e.g., acetate) may have been released upon cutting the roots. This would imply, however, that roots contain a larger amount of potential CH_4 precursors in the afternoon than in the morning.

The CH_4 production rates calculated per unit area of the paddy soil are shown in Table 1 and 2. The rates were calculated by integrating the data of the vertical profiles shown in Fig. 3 and 4. When two vertical profiles were measured, in the morning and in the afternoon, the tables show the average values and the range of the integrated CH_4 production rates. In order to account for the too short soil cores taken in 1985, the values given in Table 1 have been corrected by assuming that an equal amount of methanogenic activity had been missed. The values shown in the tables still give the lower limits of CH_4 production rates per unit area, since it is possible that significant methanogenic zones still existed below 25 cm depth and were missed by our sampling. Despite the uncertainties due to spatial and diurnal variabili-

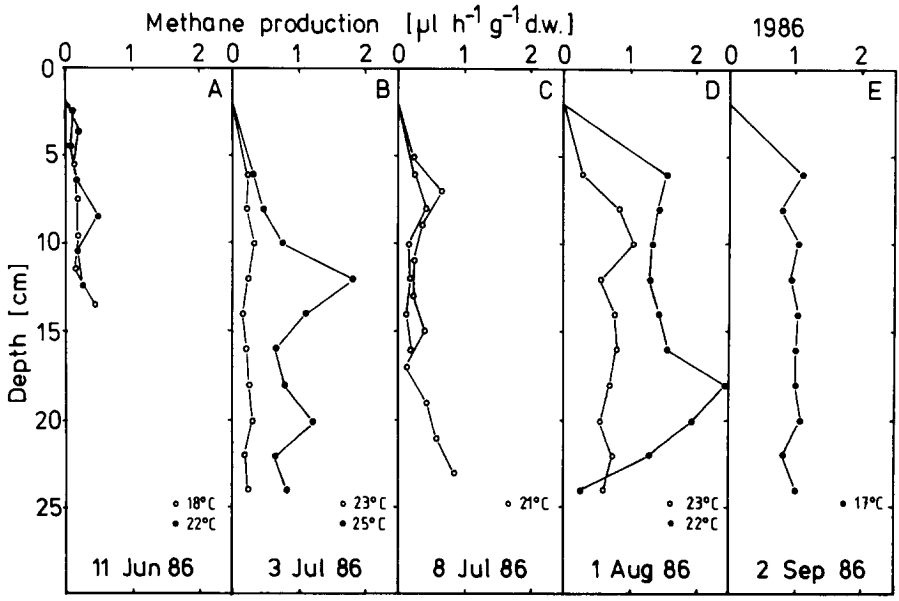

Fig. 4. See Fig.3, cultivation period of 1986.

Table 1. Seasonal change of CH_4 production and emission in a paddy field in Vercelli, Italy, 1985.

Date	Plant height (cm)	CH_4[1] Production ($ml\,m^{-2}\,h^{-1}$	CH_4 Emission ($ml\,m^{-2}\,h^{-1}$)	% emitted	CH_4 Ebullition ($ml\,m^{-2}\,h^{-1}$)	CH_4 Emission (plants cut) ($ml\,m^{-2}\,h^{-1}$)	Diffusion ($ml\,m^{-2}\,h^{-1}$)	% emitted via plants	Methanogenic population (bacteria g^{-1} d.w.) growing on	
									H_2/CO_2	Acetate
6 Jun	5–10	20	11	56	12.3	12.7	1.0	0		10^4
9 Jul	50–60	67	24	36	11.6	13.2	0.4	48	10^6	10^4
31 Jul	60–80	192	40	21	3.8	4.1	0.5	90	10^6	10^4
27 Aug	60–80	373	33	9	0.6	1.4	0.6	97	10^6	10^4

[1] Methane production rates per unit area were obtained by integrating the vertical profiles shown in Fig.3 and multiplying them with a correction factor of two to account for CH_4 production in soil depths of 14–25 cm which were not reached by sampling.

Table 2. Seasonal change of CH_4 production and emission in a paddy field in Vercelli, Italy, 1986.

Date	Plant height (cm)	CH_4[1] production ($ml\,m^{-2}\,h^{-1}$)	CH_4[2] Emission ($ml\,m^2\,h^1$)	(%) emitted	CH_4[2] Ebullition ($ml\,m^{-2}\,h^{-1}$)	CH_4 Emission[2] (plants cut) ($ml\,m^{-2}\,h^{-1}$)	Diffusion[2] ($ml\,m^{-2}\,h^{-1}$)	(%) emitted via plants	CH_4[3] produced from H_2/CO_2 (%)	Acetate[3] concentration	
										(mM)	($\mu mol\,g^{-1}$ d.w.)
11 Jun	18–22	58.5 ± 1.3	24.0 ± 0.1	41	4.4	6.4	0.7	77			
3 Jul	45–55	161 ± 85	38.3 ± 4.7	24	5.0 ± 1.6	5.3 ± 1.1	0.5 ± 0.2	87	34	7.1	4.2
1 Aug	60–80	363 ± 132	20.2 ± 4.9	6	1.5	3.4	0.8	90	51	6.4	2.9
2 Sep	60–80	297	8.3 ± 1.3	3		0.3		96	28	9.9	3.8

[1]Values were obtained by integrating the vertical profiles shown in Fig.4 and are average values ± range of duplicate profiles taken in the morning and in the afternoon. [2]Fluxes were measured simultaneously with sampling of the vertical soil profiles for determination of CH_4 production rates. [3]Samples were taken and processed one week after the date indicated.

ties (see above) and to the correction of the data of 1985, integrated CH_4 production rates were comparable in 1985 and 1986 and showed a significant trend to increase during the season.

Since CH_4 emission rates show strong diurnal variations (Holzapfel-Pschorn & Seiler 1986; Schütz et al., in prep.), they were determined within 3 h of sampling the soil cores for determination of CH_4 production rates. The data are compiled in Table 1 and 2. They include the rates of CH_4 ebullition and the rates of CH_4 emission that were measured immediately after cutting the rice plants below the water surface. They both resulted in fairly similar values and essentially represent the CH_4 flux which is not due to plant-mediated transport. The tables also include the rates of CH_4 diffusion through the flooding water. Since the magnitude of the measured fluxes was strongly influenced by daytime, all determinations had to be done within a short time period of sampling the soil cores. Therefore, no replicate measurements were made in 1985. In 1986, some of the measurements were repeated in the afternoon. Table 2 shows the average values and the range of these duplicate determinations. In general, the range of variability of duplicate determinations of integrated CH_4 production rates and of CH_4 fluxes was less than 10–50%.

The percentage of CH_4 emitted was calculated from the average CH_4 production in the soil core (Table 1 and 2). Generally, only part of the produced CH_4 was emitted, the rest was apparently oxidized. This was also true, if not the averages but the lower limits of CH_4 production rates were used for calculation. e.g., on 3 July 1986 (Fig. 4B) 54% and 17% of the CH_4 were emitted using the lower or the higher data set of CH_4 production rates, respectively. The percentage of emitted CH_4 decreased during the season and was paralleled by a decrease of CH_4 ebullition and an increase of plant-mediated CH_4 emission. Incubation of anoxic soil samples with radioactive bicarbonate resulted in production of radioactive CH_4. The specific radioactivity of total CO_2 (CO_2 plus bicarbonate) decreased during the incubations indicating that unlabeled CO_2 was produced from organic matter degradation. The specific radioactivity of CH_4 decreased correspondingly resulting in a constant ratio (f_{Hydr}) between both specific radioactivities (Fig. 5). This result indicates that during the incubations CH_4 was produced by CO_2/H_2-dependent methanogenesis at a constant percentage. The percentage contribution (Table 2) seemed to be higher during August, when CH_4 production had reached its maximum, than during July or September. Acetate concentrations changed in an antiparallel manner. More data are required to clearly document a seasonal variation. The numbers of H_2-dependent methanogens were by two orders of magnitude higher than those of acetate-dependent methanogens. However, the bacterial numbers did not change during the cultivation period (Table 1).

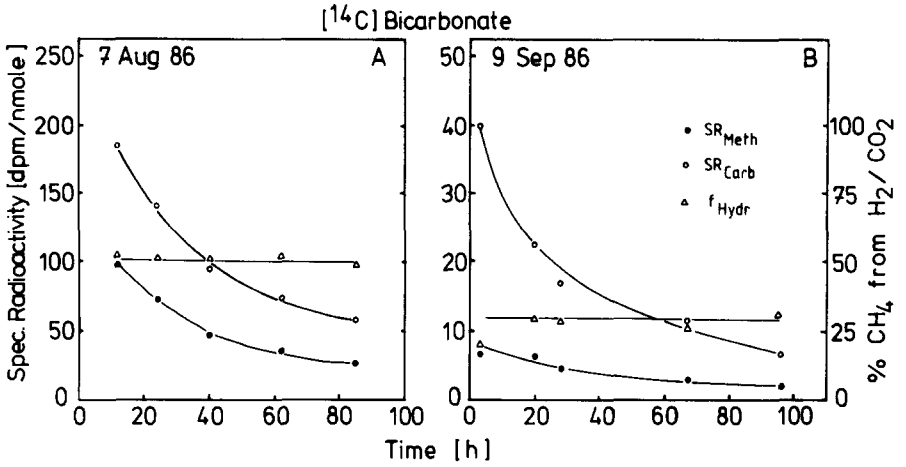

Fig. 5. Conversion of (^{14}C) bicarbonate to CH_4. Anoxic soil samples were incubated at 25 °C in presence of 3.7 × 10^6dpm (*A*) or 1.1 × 10^6 dpm (*B*) of carrier-free $NaH^{14}CO_3$, and the specific radioactivities of CH_4 (SR_{Meth}) and of CO_2 (SR_{Carb}) were measured. The percentage of CH_4 produced from H_2/CO_2 (f_{Hydr}) was calculated from the ratio of SR_{Meth} to SR_{Carb}. Each time point represents the average value determined from triplicate incubation tubes.

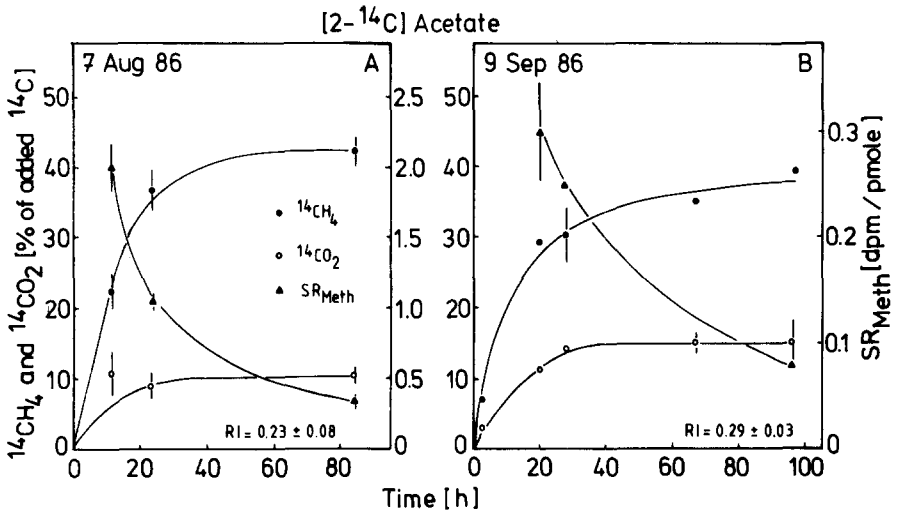

Fig. 6. Conversion of (2-^{14}C) acetate to CH_4 and CO_2. Anoxic soil samples were incubated at 24 °C in the presence of 4.4 × 10^6dpm (*A*) or 2.3 × 10^6dpm (*B*) of carrier-free sodium (2-^{14}C) acetate, and the production of $^{14}CH_4$ and total (CO_2 + HCO_3^-) $^{14}CO_2$ as well as the specific radioactivity of CH_4 (SR_{Meth}) were measured. Each time point represents the average determined from triplicate incubation tubes.

The methyl group of acetate was rapidly converted to CH_4 and CO_2 (Fig. 6) with CH_4 being the predominant (> 70%) product. However, only 45–50% of the radioactive acetate added were actually transformed to CH_4 and CO_2; the rest was apparently assimilated, irreversibly absorbed, or transformed to products not available for methanogens or fermenting bacteria. Acetate was transformed to CH_4 and CO_2 with a turnover time (Tt) of 12–16 h (Table 3). Extraction of anoxic soil samples resulted in an acetate pool size of 3–4 μmoles per gram dry weight, or 6–10 mM in the pore water. Based on the relatively high in-situ acetate concentrations the turnover rates of acetate and consequently, the acetate-dependent rates of methanogenesis were by a factor of 2–4 higher than the rates of methanogenesis estimated from rates of in-situ CH_4 production and the fraction of CH_4 which was not derived from H_2/CO_2 (Table 3). This difference seems to be significant even if the two acetate turnover rates were measured one week later than the in-situ CH_4 production rates. This difference seems to be also significant if we assume that sampling of the soil cores had induced a release of acetate by cutting the rice roots, since the released acetate should simultaneously have stimulated the CH_4 production rates measured in the soil samples. More experiments are necessary to show definitely, whether acetate turnover and methane production are affected differently by the sampling procedure, or whether the extracted acetate pool may be higher than the acetate pool which is available for formation of CH_4 and CO_2.

Discussion

Methane production rates increased during the season. Due to the weather situation (cold and wet spring), plant growth and CH_4 production rates increased less rapidly in 1985 than in 1986. In 1985, the maximum was reached in September, in 1986, it was reached in August. Methane emission rates showed a similar delay. In general, rates increased from May to July and decreased again from August to September. A similar pattern has been observed in 1983 (Holzapfel-Pschorn & Seiler 1986) and in 1984 (Holzapfel-Pschorn et al 1986).

Most of the CH_4 was emitted by plant-mediated transport. The rest was emitted by bubbles. The ebullition rate measured by collecting the emerging bubbles resulted in fairly similar values to those measured by the static box technique after cutting the rice plants below the water surface. Methane emission by diffusion through the flooding water was very low and usually amounted to less than 1% of total emission. The percentage of CH_4 which was emitted by ebullition decreased during the season from 100% on June

Table 3. Acetate turnover and methanogenesis in anoxic soil samples from an Italian paddy field.

Parameter	7 Aug 1986	9 Sep 1986
T_1, Acetate turnover time (h)	11.9 ± 0.3	15.9 ± 0.3
Maximum fraction of acetate converted to CH_4 and CO_2	0.52	0.46
RI value for ($2^{-14}C$) acetate	0.23 ± 0.02	0.29 ± 0.03
C_{Ac}, Acetate pool (μmol g^{-1} d.w.)	2.9 ± 0.6	3.8 ± 0.8
V_{Ac},Acetate turnover rate	127 ± 29	110 ± 25
P_{tot}, Total CH_4 production [1] (nmole $h^{-1}g^{-1}$d.w.)	50 ± 25	45
f_{Hydr}, Fraction of CH_4 produced from H_2/CO_2	0.51 ± 0.01	0.28 ± 0.02
P_{Ac}, CH_4 production not from H_2 [2] (nmol $h^{-1}g^{-1}$ d.w.)	25 ± 13	32
P_{Ac}, CH_4 production from turnover [3] of acetate (nmole $h^{-1}g^{-1}$ d.w.)	98 ± 15	78 ± 15

[1] Average rate estimated from data given in Fig. 4 D, E
[2] Calculated from eq. (6).
[3] Calculated from eq. (5).

6, 1985, when rice plants had not yet developed, to 4–9% in September, briefly before the harvest of the rice. The relative contribution of plant-mediated CH_4 emission increased correspondingly. In 1986, the seasonal change was similar, however, ebullition was relatively low from the beginning which was most probably due to the better plant growth.

Only part of the produced CH_4 was emitted into the atmosphere, the rest was apparently reoxidized. This observation confirms earlier measurements (Holzapfel-Pschorn et al 1985). The percentage of CH_4 emitted relative to the CH_4 produced decreased in parallel to the decrease of ebullition and the increase of plant-mediated CH_4 emission. Reoxidation of CH_4 obviously was higher when methane was emitted by plant-mediated transport rather than by ebullition. The oxidized zones around the rice roots which constitute a habitat for methanotrophic bacteria (DeBont et al 1978) are probably the reason for this behaviour. The methanotrophs apparently constitute an efficient sink for the CH_4 before it can enter the roots and be transported by the plant's aeration system (Armstrong 1979).

The pathway of CH_4 from the paddy soil into the atmosphere is schematically shown in Fig.7. A similar scheme may generally apply for methanogenic ecosystems which are covered by aquatic plants that have the capacity for gas exchange between leaves and roots and by that way, may influence CH_4 flux into the atmosphere (Dacey 1980; Sebacher et al. 1985). A similar scheme may likewise apply for other reduced compounds, as the

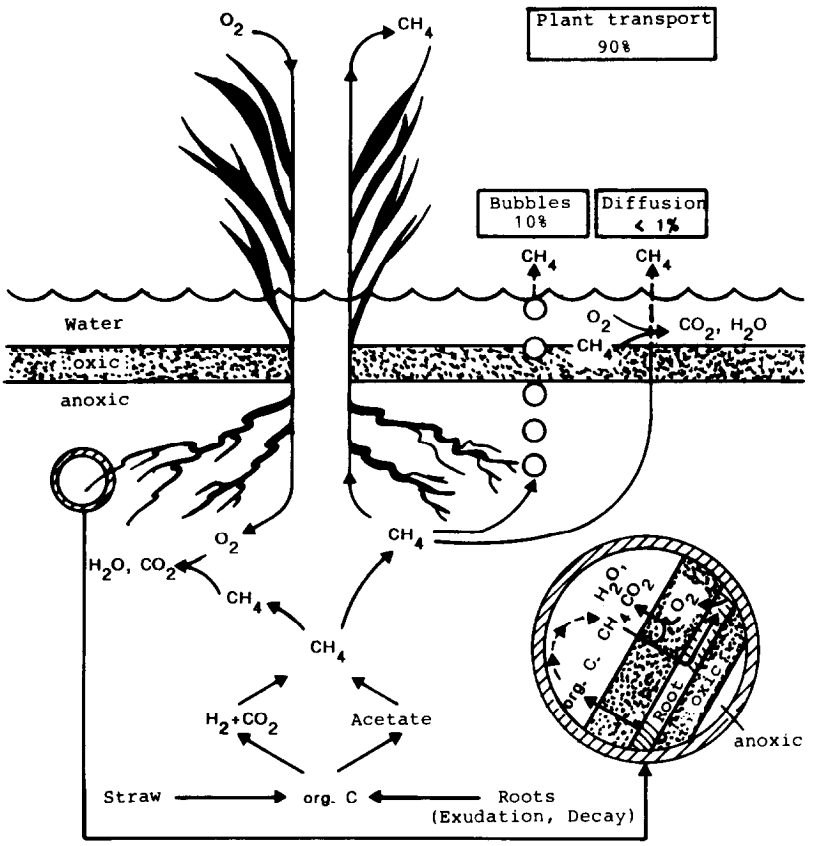

Fig. 7. Scheme of production, reoxidation, and emission of CH_4 in a paddy field.

paddy rhizosphere is not only important for the oxidation of CH_4, but also for oxidation of ammonia (Reddy & Patrick 1984; Garcia & Tiedje 1982) and of reduced sulfur (Joshi & Hollis 1976; Freney et al. 1982). However, it seems to be less important for the oxidation of hydrogen, most probably because H_2 concentrations in this environment are too low for aerobic hydrogen bacteria (Conrad 1984; Schütz et al. 1987).

The bacterial numbers were in a similar range as reported for a profundal eutrophic lake sediment (Phelps & Zeikus 1985) with H_2-oxidizing methanogens being more numerous than acetate-utilizing methanogens. Garcia et al. (1974) studied various paddy soils of Senegal and reported numbers of methanogenic bacteria being in some cases significantly lower than our estimates.

Despite the seasonal increase of CH_4 production rates, the numbers of methanogenic bacteria did not change significantly during the season. This

discrepancy is certainly due to their different cultivation techniques which applied soil extract media instead of substrate-supplemented mineral media. They observed, however, that the bacterial counts were highly dependent on the pH, the chloride content, and the redox potential of the soil studied.

Hydrogen was an important methane precursor and accounted for 30–50% of total CH_4 production. Takai (1970) observed a smaller contribution ($< 20\%$) of H_2 to CH_4 production, however, he diluted the anoxic soil by 50% with water. When using diluted soil suspensions we also observed lower values (Conrad & Babbel 1988). Our results do neither prove nor disprove a seasonal change of the relative contribution of H_2 to CH_4 production, however, such a change is not unlikely. Although the methanogenic population did not increase during the season, the relative contribution of H_2 may have changed due to temperature changes and due to availability of substrates that could serve as H_2 precursors. We recently have shown that temperature limits H_2 production and thus H_2-dependent methanogenesis (Conrad et al 1987). We are presently studying the influence of the availability of fermentable organic substrates on H_2-dependent methanogenesis.

Acetate was an equally important precursor of CH_4. Turnover times were relatively high (12–15 h) compared to other methanogenic environments (0.3–3 h) (Lovley & Klug 1982; Schink 1985; Jones & Simon 1985). The respiratory index (RI) of the methyl group of acetate was in a low range (< 0.3) indicating that sulfate reduction or other metabolic processes producing CO_2 instead of CH_4 were not important. Similar results have been obtained in other methanogenic environments where sulfate is limiting (Ward & Winfrey 1985).

Compared to other methanogenic environments, where acetate concentrations usually are $< 100\,\mu M$, the paddy soil exhibited high concentrations of extractable acetate (about $3\,\mu mole\,g^{-1}$ d.w. or 6.5 mM). Similar values ($2\text{-}10\,\mu mole\,g^{-1}$ d.w.) were reported by Takai (1970) for different Japanese paddy soils and by Hollis & Rodriguez-Kabana (1967) for Louisiana paddy fields. It is yet unclear why acetate concentrations in paddy soils are so high. Possible explanations are root exudations or artefacts created by cutting the rice roots during sampling. An alternative explanation may be the relatively high activity of homoacetogenic bacteria which is observed in anoxic paddy soil and which is presently studied in our laboratory.

The acetate concentrations observed in paddy fields are slightly higher than the K_m value for acetate (3 mM) of Methanosarcina barkeri (Schönheit et al 1982) or of about 5–10 times the K_m (0.5 mM) of Methanothrix soehngenii (Zehnder et al. 1980). Hence, these methanogens would be able to operate close to V_{max}. Slight changes in acetate concentration would

therefore not result in a change of methanogenic activity or would result in a response being less than proportional to the change in acetate concentration.

Acetate concentrations may be highly variable not only on a temporal, but also on a spatial basis. Moreover, it is very likely that soil is compartmentalized with respect to acetate availability. For example, the acetate turnover due to methanogenesis which was calculated from the in-situ concentration and the turnover time of acetate was 2–4 times higher than the rates of CH_4 production measured. Artefactual release of acetate from cut rice roots should also have resulted in stimulated methane production rates. Since such a stimulation was not observed, it is more likely that only part of the acetate pool was available for methanogenesis. Acetate pools with different availability have also been reported for marine sediments (Christensen & Blackburn 1982; Parkes et al. 1984; Shaw et al. 1984) where ca. 50% of the acetate pool was recalcitrant, and have even been demonstrated in culture fluids of anaerobic model ecosystems (Thompson & Nedwell 1985).

Our studies have shown that the emission of CH_4 from paddy fields is the result of very dynamic processes which include CH_4 formation from H_2 and acetate, reoxidation of CH_4, and transport into the atmosphere. All these processes obviously can be influenced by the presence and the developmental state of rice plants, by temperature changes during the day and the season, and by the management of the paddy field. More research is needed to understand these dynamic processes in order to better evaluate the importance of rice paddies for the atmospheric CH_4 budget.

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