

## **Vertical profiles of CH<sub>4</sub> concentrations, dissolved substrates and processes involved in CH<sub>4</sub> production in a flooded Italian rice field**

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**Abstract.** Vertical profiles were measured in soil cores taken from flooded rice fields in the Po valley during July and August 1990. Methane concentrations generally increased with depth and reached maximum values of 150–500  $\mu\text{M}$  in 5–13 cm depth. However, the shape of the profiles was very different when studying different soil cores. The CH<sub>4</sub> content of gas bubbles showed a similar variability which apparently was due to spatial rather than temporal inhomogeneities. Similar inhomogeneities were observed in the vertical profiles of acetate, propionate, lactate, and formate which showed maximum values of 1500, 66, 135, and 153  $\mu\text{M}$ , respectively. However, maxima and minima of the vertical profiles of the different substrates usually coincided in one particular soil core. Large inhomogeneities in the vertical profiles were also observed for the rates of total CH<sub>4</sub> production, however, the percentage contribution of H<sub>2</sub>/CO<sub>2</sub> to CH<sub>4</sub> production was relatively homogeneous at  $24 \pm 7\%$  (SD). Similarly, the H<sub>2</sub> content of gas bubbles was relatively constant at  $93.3 \pm 9.6$  ppmv when randomly sampled in the rice field at different times of the day. A small contribution (6%) of H<sub>2</sub>/CO<sub>2</sub> to acetate production was also observed. Vertical profiles of the respiratory index (RI) for [2-<sup>14</sup>C] acetate showed that acetate was predominantly degraded by methanogenesis in 5–11 cm depth, but by respiration in the surface soil (3 cm depth) and in soil layers below 13–16 cm depth which coincided with a transition of the colour (grey to reddish) and the physical characteristics (porosity, density) of the soil. The observations indicate that the microbial community which degrades organic matter to CH<sub>4</sub> is in itself relatively homogenous, but operates at highly variable rates within the soil structure.

## **Introduction**

Flooded rice fields are an important source in the budget of atmospheric methane (Aselmann & Crutzen 1989). CH<sub>4</sub> fluxes from rice fields in different regions have intensively been studied during the last years (Cicerone & Shetter 1981; Seiler et al. 1984, Holzapfel-Pschorn & Seiler

1986; Schütz et al. 1989a; Sass et al. 1990; Yagi & Minami 1991; Khalil et al. 1991). The  $\text{CH}_4$  fluxes cover a broad range of values depending on daytime, season, region and field management. The  $\text{CH}_4$  fluxes are largely controlled by microbial production and oxidation of  $\text{CH}_4$  and by the processes involved in transport of  $\text{CH}_4$  from the soil into the atmosphere (Conrad 1989; Schütz et al. 1989b; Conrad 1993a).

Methane in aquatic environments originates from the anaerobic degradation of organic matter by a complex microbial community. This community may consist of many different hydrolytic, fermenting, homoacetogenic, and syntrophic bacteria which degrade cellulose and other plant polymers mainly via alcohols and fatty acids to  $\text{H}_2$  and acetate. Only these two compounds serve as substrates for the methanogenic bacteria (Zehnder 1978; Dolfig 1988). In rice fields, most of the studies of microbial processes have concentrated on laboratory measurements in slurries of anoxic paddy soil or in rice microcosms (Yamane & Sato 1964; Conrad et al. 1989b; Krumböck & Conrad 1991; Thebrath & Conrad 1992). Among others, these studies showed that glucose is degraded mainly via acetate to  $\text{CH}_4$ , but that a small part of the acetate is also produced from  $\text{CO}_2$  by homoacetogenic bacteria. Acetate seems to be the predominant substrate for methanogenesis, but  $\text{H}_2$  also contributes to  $\text{CH}_4$  production mainly by interspecies- $\text{H}_2$ -transfer within microbial methanogenic associations. So far, however, field studies on these processes are lacking. Because of the large inhomogeneities which have to be expected in vegetated soil it would be interesting to know their effects on the production of  $\text{CH}_4$  and on the processes involved in  $\text{CH}_4$  production.

Here, we report on measurements of vertical profiles of different compounds and microbial activities involved in  $\text{CH}_4$  production during the main vegetation period of a rice field in the Po valley (Italy). Our observations show that the contribution of  $\text{H}_2$  to methanogenesis was relatively constant, although concentrations and  $\text{CH}_4$  production rates changed dramatically with soil depth and in-between the different sampling sites.

## Materials and methods

The soil samples were taken in a flooded rice field in Vercelli, Italy (Holzapfel-Pschorn & Seiler 1986) during July and August 1990. The field was planted with rice (*Oryza sativa*, variety *roma*, type *japonica*). For one experiment, a soil core was taken from a field treated with  $\text{K}_2\text{SO}_4$  (1860 kg/ha) in spring. All other samples were taken from plots which

were unfertilized. Temperatures were measured by temperature probes fixed in different depths before planting of rice.

Soil cores were taken with stainless steel corers (50 mm diameter, 350 mm length) with predrilled side holes every 2 cm over the whole length (Schütz et al. 1989b). The end of the corer was sharpened to cut through the root mat of the rice plants. Before taking the core the side holes were closed by tape.

Porosity and density of the soil cores were measured in the following way. The soil core was pushed up by a piston into a plexiglass-tube and cut into disks of 2 cm. The weight and volume of each disk was measured in fresh state and the weight in dry state after drying at 105 °C. These data were used to calculate the porosity (P), the density (D) of the fresh soil and density of the solid particles (DSP) of the soil.

Samples for measurement of dissolved substrates were taken in the following way: Sediment disks (see above) were transferred into centrifuge tubes and weighted (fw). Then, they were suspended in 5 ml distilled water and were centrifuged (1400 g, 5 min) immediately. The substrate concentrations ( $C_m$ ) in the supernatant were analysed by HPLC, the pellet was dried at 105 °C and weighed again (dw). The concentration of the substrates in porewater (C) was calculated from the concentration ( $C_m$ ) in the supernatant by

$$C = C_m [(fw - dw) + 5] / [fw - dw]$$

Methane dissolved in soil pore water was measured as described by Frenzel et al. (1991). The diffusive flux of  $CH_4$  was calculated from the vertical profiles of dissolved  $CH_4$  using Fick's law as described by Conrad & Rothfuss (1991).

Gas bubbles which were forced out of soil were sampled with inverted funnels as described by Holzapfel-Pschorn et al. (1985). Gas samples from the funnel were stored as gas bubbles in 10 ml glass reaction vessels filled with saturated  $Na_2SO_4$  solution and closed with a black rubber stopper. The vessels were stored for up to 11 days in inversed position so that the gas bubble was only in contact with the glass and the saturated  $Na_2SO_4$  solution. Then the gas bubbles were analyzed for  $CH_4$  and  $H_2$ . A random sample of 10 vessels which contained  $95.3 \pm 10.9$  (SD) ppmv  $H_2$  was stored for 10 months and then, showed  $H_2$  mixing ratios of  $91 \pm 37$  ppmv.

For radioactive measurements soil samples (5 ml) were taken through the predrilled side holes with a cut 5 ml syringe and transferred into pressure tubes (26 ml). The tubes were closed with black rubber stoppers,

evacuated and gassed 5 times with  $N_2$  and pressurized to 1.5 bar. For the measurement of the respiratory index (RI), 0.5 ml solution of carrier-free  $[2-^{14}C]$  acetate (10000 Bq, 1.94 GBq/mmol, Amersham-Buchler, Braunschweig, FRG) was injected into the tubes. The tubes were shaken vigorously for 1 min and incubated at ambient temperature for 6 to 9 days. Within that time they were transported from Vercelli to Konstanz. Then, the microbial activity was stopped by adding 1 ml 1 N  $H_2SO_4$  to each tube followed by vigorously shaking. After this treatment the gas phase was analysed for  $^{14}CH_4$  and  $^{14}CO_2$ .

For the determination of the conversion of  $^{14}C$ -bicarbonate into different substrates two soil cores were taken side by side and transported to Konstanz within 11 h. Subsamples from one core (core 7) were filled into pressure tubes as described above. Carrier-free  $NaH^{14}CO_3$  solution (0.5 ml; 37000 Bq; 2.07 GBq/mmol; Amersham-Buchler, Braunschweig, FRG) was injected into each tube, and the tubes were shaken vigorously. The tubes were incubated at room temperature (22–23 °C) for 3 days. The gas phase was analysed daily after shaking vigorously (without acidification) for 1 min using the GC system described below. Methane production rates were calculated from the temporal increase of  $CH_4$  mixing ratios in the tubes. The specific radioactivities (SR) of  $^{14}CH_4$  and  $^{14}CO_2$  were determined from the radioactivity (Bq/ml) and the concentration (nmol/ml) of the  $CH_4$  and the  $CO_2$ , respectively. In the end, the samples were centrifuged (1400 xg) and the supernatant was analysed for the concentrations and radioactivities in the substrates. The second core (core 8) was stored for 2 days at 4 °C and was then handled in the same way as the first core.

The fraction ( $f_{CO_2-CH_4}$ ) of methane formed from  $H_2/CO_2$  was calculated by

$$f_{CO_2-CH_4} = SR_{CH_4}/SR_{CO_2}$$

The fraction ( $f_{CO_2-Ac}$ ) of acetate formed from bicarbonate was calculated by

$$f_{CO_2-Ac} = SR_{Ac}/(2 SR_{CO_2})$$

The fraction ( $f_{CO_2-Prop}$ ) of propionate formed from bicarbonate was calculated by

$$f_{CO_2-Prop} = SR_{Prop}/SR_{CO_2}$$

Unlabeled and radioactive  $CH_4$  and  $CO_2$  were analyzed in a gas chro-

matograph equipped with a methanizer, a flame ionization detector and a gas proportional counter (Conrad et al. 1987, 1989b). Hydrogen was measured by gas chromatography as described by Conrad et al. (1987). For calculation of  $H_2$  and  $CH_4$  concentrations from their gas partial pressure we used a Henry constant at 25 °C of 0.70 and 1.32 mM bar<sup>-1</sup>, respectively (Crozier & Yamamoto 1974; Médard et al. 1976). Acetate, lactate, propionate, formate and other compounds were measured in a HPLC system which contained a column for organic acid analysis (Biorad), a refraction index detector and a scintillation monitor for radioactivity as described by Krumböck & Conrad (1991).

## Results

Soil cores taken from the flooded rice field showed a typical layering. The depths of the individual layers varied from core to core. Some typical features are described in Fig. 1. The cores mostly showed a transition from greyish to reddish brown in about 13–16 cm depth. The upper greyish soil layers consisted of a fine clay-rich material. The lower reddish soil layers consisted of a hard material which could not be penetrated by the stainless steel corer for more than 2–4 cm. The porosity of the soil layers decreased from 0 to 2 cm depth and then stayed constant down to

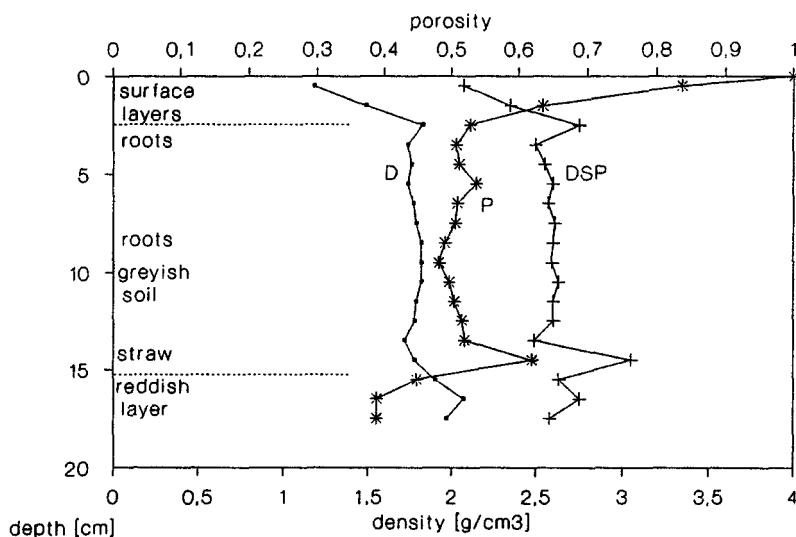


Fig. 1. Vertical profiles of porosity (P), density of fresh soil (D), and density of solid particles (DSP) in the rice field.

about 14 cm depth (Fig. 1). The specific weight of the soil layers and the density of solid particles (DSP) increased from 0 to 2 cm depth and also stayed constant down to about 14 cm depth. The average values in 2–14 cm depth were about 0.5, 1.75 g cm<sup>-3</sup>, and 2.59 g cm<sup>-3</sup> for porosity, density, and DSP, respectively. The roots of the rice plants were mainly

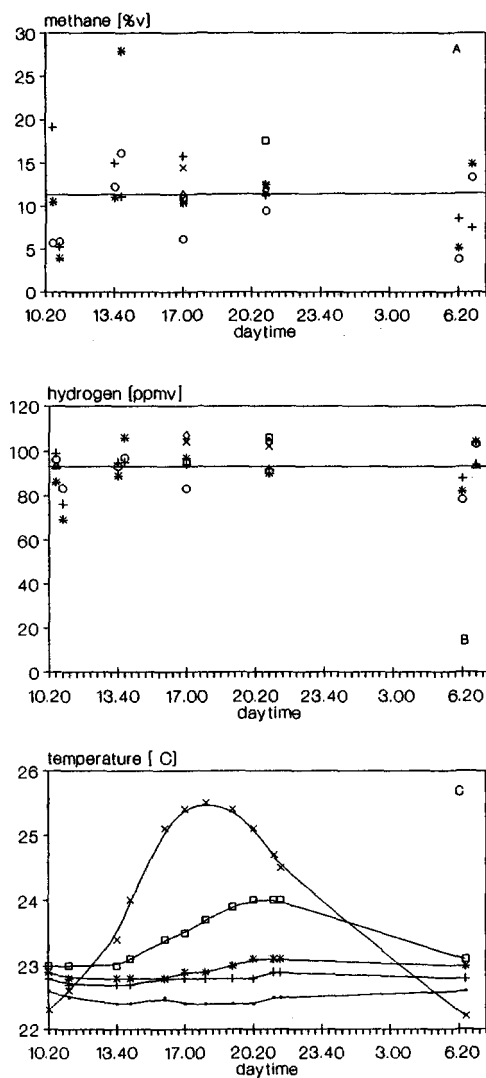


Fig. 2. Diurnal change of the mixing ratios of CH<sub>4</sub> (A) and H<sub>2</sub> (B) and of temperatures (C) in gas bubbles from the rice field. The temperature was continuously recorded in the flooding water (×) and in the soil in depth of 5 cm (□), 10 cm (\*), 15 cm (+), and 20 cm (●). The gas bubbles were stirred out of the soil at different times on 3–4 Aug 1990. The dotted lines in (A) and (B) give the arithmetic mean of all data points ( $n = 30$ ).

found in these soil layers with highest density in about 2–8 cm depth. Decomposing rice straw from the last year was mainly found in 8–14 cm depth. The hard reddish layer below 14 cm depth showed a decreased porosity and slightly increased specific weight and DSP.

The content of  $\text{CH}_4$  and  $\text{H}_2$  of gas bubbles was measured during the course of a day (Fig. 2). The temperatures in the flooding water and in the soil (5 cm depth) showed a typical diurnal rhythm (Fig. 2C). The mixing ratios of  $\text{CH}_4$  and  $\text{H}_2$  in the gas bubbles, on the other hand, showed no significant diurnal trend. However, the  $\text{CH}_4$  mixing ratios varied within a broad range from 5 to 20%v with an average value ( $\pm$  SD) of  $n=30$  data of  $11.3 \pm 5.1\%v$  ( $\pm 45\%$ ) (Fig. 2A). Interestingly, the mixing ratios of  $\text{H}_2$  in the same gas bubbles were relatively constant at an average value of  $93.3 \pm 9.6 \text{ ppmv}$  ( $\pm 10\%$ ) (Fig. 2B).

Profiles of  $\text{CH}_4$  concentrations were measured in 3 different cores taken from different sites of the rice field and at different times of the day (Fig. 3). The resulting profiles were very different indicating a high spatial and/or temporal variability. Methane concentrations in the soil cores ranged between 45 and 525  $\mu\text{M}$  equivalent to partial pressures between about 30 and 380 mbar. These figures are consistent with the  $\text{CH}_4$  mixing ratios measured in the gas bubbles. Each vertical  $\text{CH}_4$  profile showed a distinct maximum of the  $\text{CH}_4$  concentrations which was reached in 5, 9, or

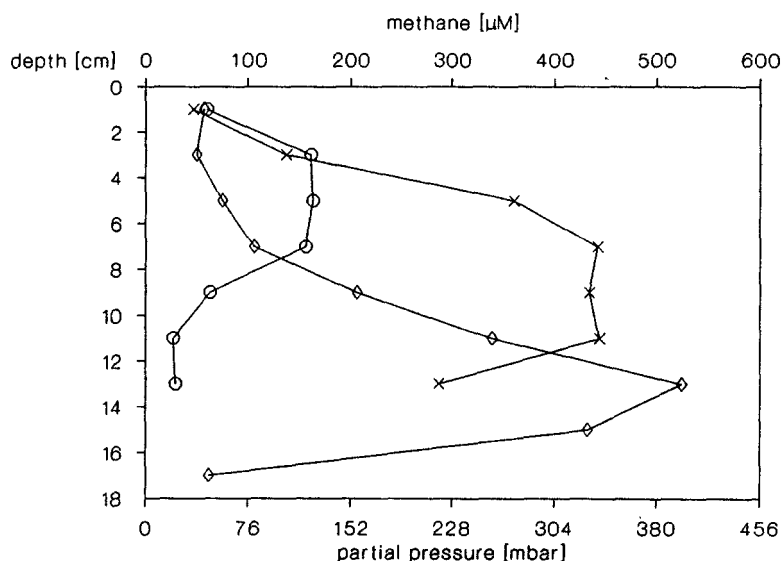


Fig. 3. Vertical profiles of methane concentrations in the porewater of 3 different cores taken from the rice field. Core 1 (□) was taken on 25 Jul, 13.30; core 2 (×) was taken on 30 Jul, 9.00; and core 3 (◇) was taken on 30 Jul, 18.30.

13 cm depth. The diffusive  $\text{CH}_4$  flux calculated from the steepest gradient of dissolved  $\text{CH}_4$  in Fig. 3 was  $0.25 \text{ mmol d}^{-1} \text{ m}^{-2}$ .

Concentration profiles of substrates dissolved in porewater were measured in soil cores taken within 24 h from a small area ( $< 1 \text{ m}^2$ ) in the rice field (Fig. 4). Concentrations were generally low in the upper 2–3 cm depth. In deeper soil layers (about 4–14 cm depth), the measured concentrations showed a very inhomogeneous pattern, sometimes with several maxima and differences between the individual cores. However, within one particular core the concentration maxima and minima were found in the same depth for each substance, i.e. acetate, propionate, lactate, and formate. Acetate concentrations were between 16 and  $97 \mu\text{M}$  with maxima reaching up to  $1500 \mu\text{M}$  (Fig. 4A). Propionate concentrations ranged from the detection limit of our analytical system ( $5 \mu\text{M}$ ) to  $20 \mu\text{M}$  with maxima reaching up to  $66 \mu\text{M}$  (Fig. 4B). Lactate concentrations were between 5 and  $31 \mu\text{M}$  with maxima reaching up to  $135 \mu\text{M}$  (Fig. 4C). Formate concentrations ranged between the detection limit ( $5 \mu\text{M}$ ) and  $60 \mu\text{M}$  with maxima reaching up to  $153 \mu\text{M}$  (Fig. 4D). Caproate

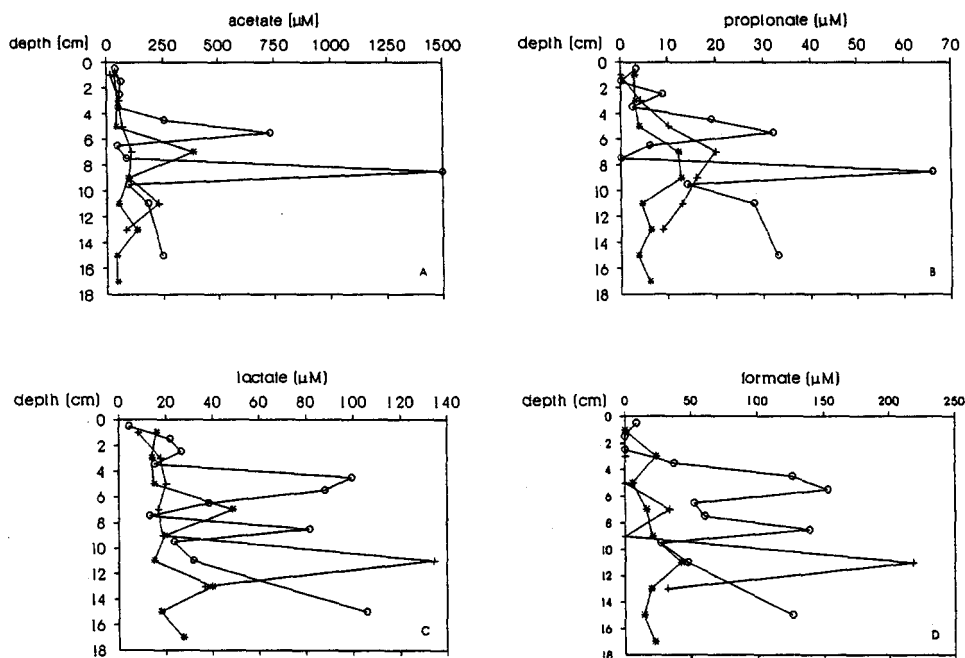


Fig. 4. Vertical profiles of the concentrations of acetate (A), propionate (B), lactate (C), and formate (D) in 3 different soil cores from the rice field. Core 4 (○) was taken on 27 Jul, 11.30, core 5 (+) was taken on 27 Jul, 17.30, and core 6 (\*) was taken on 28 Jul, 6.00. The cores were taken within an area of  $1 \text{ m}^2$ .



could be detected in some layers between 2 and 14 cm depth showing low concentrations  $< 20 \mu\text{M}$ . 1-Propanol, 2-propanol, 2-methylbutyrate, 3-methylbutyrate, valerate, 1-butanol and 2-butanol could not be detected (i.e. concentrations  $< 5 \mu\text{M}$ ). Succinate, methanol, butyrate and ethanol were also not detected (i.e. concentrations  $< 20 \mu\text{M}$ ).

The vertical profile of  $\text{CH}_4$  production rates were measured in two different cores taken almost simultaneously adjacent to each other (Fig. 5). The profiles in the two cores were very different, but showed relatively low activities in the top 2 cm soil layer and in depths below about 18 cm depth. Integration of the  $\text{CH}_4$  production rates with depth resulted in a total  $\text{CH}_4$  production of 79 and 128 mmol  $\text{CH}_4$  per  $\text{m}^2$  and day. The average ( $\pm \text{SD}$ ) of all  $\text{CH}_4$  production rates ( $n = 20$ ) was  $464 \pm 282 \text{ nmol ml}^{-1}\text{d}^{-1}$  ( $\pm 61\%$ ).

Vertical profiles of  $\text{CH}_4$  production from  $\text{H}_2/\text{CO}_2$  were determined in the same cores used for measuring the total  $\text{CH}_4$  production rates (Fig. 6). The  $\text{H}_2$ -dependent  $\text{CH}_4$  production was determined from the conversion of  $^{14}\text{CO}_2$  to  $^{14}\text{CH}_4$ . Beside  $\text{H}_2$ -utilizing methanogens the conversion of  $^{14}\text{CO}_2$  to  $^{14}\text{CH}_4$  may also be due to methanogens utilizing formate, 2-propanol or ethanol as electron donors (Schauer & Ferry 1980; Widdel 1986). We presently do not know the role of these electron donors relatively to that of  $\text{H}_2$  and thus, tentatively subsume all these under  $\text{H}_2/\text{CO}_2$ -dependent methanogenesis. The percentual contribution of  $\text{H}_2/\text{CO}_2$  to

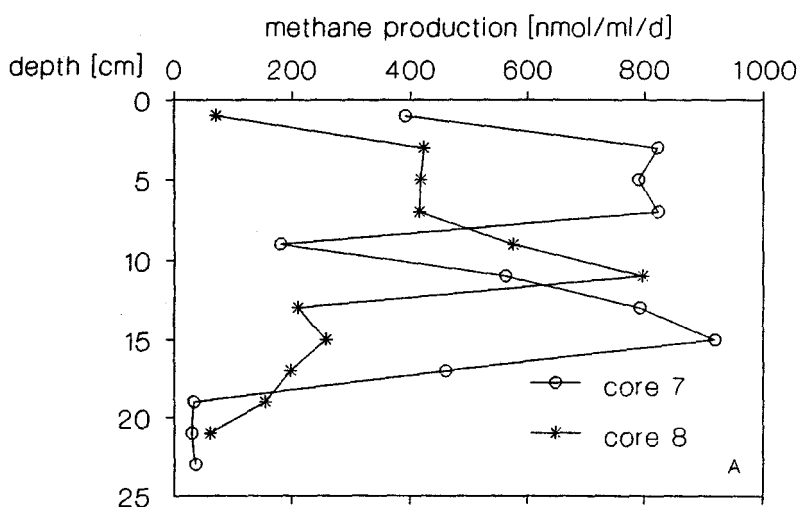


Fig. 5. Vertical profiles of rates of methane production measured in two different soil cores adjacent to each other. Both, core 7 (O) and core 8 (\*) were taken on 4 Aug 1990. Core 7 was analyzed 11 h after sampling, core 8 was analyzed on 6 Aug 1990.

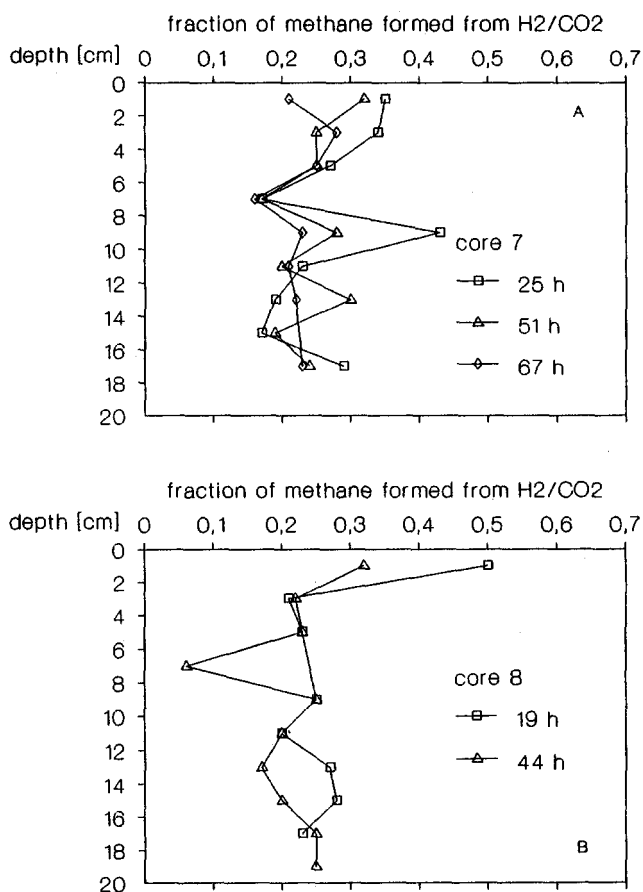


Fig. 6. Vertical profiles of the fraction of CH<sub>4</sub> (f<sub>H<sub>2</sub>-CH<sub>4</sub></sub>) produced from H<sub>2</sub>/CO<sub>2</sub> in soil core 7 (A) and core 8 (B) (compare Fig. 5). The cores were treated with NaH<sup>14</sup>CO<sub>3</sub> and incubated for different times.

total methanogenesis did not show a temporal trend during the incubation period of 19–67 h. The vertical profiles showed a relatively even distribution of the percentual contribution of H<sub>2</sub>/CO<sub>2</sub> to total methanogenesis and furthermore, showed a good agreement between the two different soil cores (Fig. 6A, B) with average values ( $\pm$ SD) of  $25 \pm 6\%$  (Fig. 6A) and  $24 \pm 8\%$  (Fig. 6B), respectively. The average deviation from the mean value of 24% was about 29%.

We also detected incorporation of <sup>14</sup>CO<sub>2</sub> into acetate and propionate. The comparison of the specific ratioactivities of these compounds with that of <sup>14</sup>CO<sub>2</sub> gives the relative amount of carbon atoms derived from CO<sub>2</sub> (Table 1). The results indicate that significant amounts of <sup>14</sup>CO<sub>2</sub> were

incorporated into the acetate and propionate which was recovered from the rice soil.

Acetate is an important methanogenic substrate in rice fields, but may also be consumed by oxidation with electron acceptors such as  $O_2$ , nitrate, sulfate, iron—III. The respiratory index (RI) of  $[2-^{14}C]$  acetate ranged between 0.19 and 0.29 in the soil layers of 5–11 cm depth demonstrating that acetate was used predominantly by methanogenesis. Only in surface soil and in the deep reddish soil layers, the RI reached values of 0.89 demonstrating oxidative degradation of acetate. In soil cores taken from a field plot which had been treated with potassium sulfate the RI values measured in the methanogenic soil layers (3–13 cm) were higher than in those of the untreated field plots (Table 2).

*Table 1.* Fractions of acetate ( $f_{CO_2-Ac}$ ) and propionate ( $f_{CO_2-Prop}$ ) formed from radioactive bicarbonate in different soil depths of the rice field. The measurements were done with soil core 8 taken on 4 Aug 1990.

Depth [cm]	Specific radioactivity (SR) [Bq nmol <sup>-1</sup> ] of			$f_{CO_2-Ac}$	$f_{CO_2-Prop}$
	CO <sub>2</sub>	acetate	propionate		
1	0.807	0.030	0.134	0.019	0.17
3	1.715	0.130	0.291	0.038	0.17
5	3.426	0.417		0.061	
7	2.269	0.148		0.033	

*Table 2.* Vertical profile of the respiratory index (RI) for the utilization of  $[2-^{14}C]$  acetate in soil cores from an untreated rice field (duplicates) and a field which was treated with  $K_2SO_4$  in spring 1990 (cores 9–11).

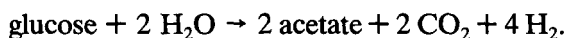
Field	untreated		$K_2SO_4$
Sampling time	31 Jul; 14.00	2 Aug; 10.30	1 Aug; 13.30
Incubation [d]	8	6	7
Depth [cm]			
1	0.85	0.18	0.94
3	0.64	0.86	0.47
5	0.22	0.29	0.42
7	0.25	0.21	0.42
9	0.22	0.19	0.19
11	0.19	0.19	0.30
13	0.89		0.32
15			0.89

## Discussion

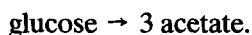
Our observations show that the percentage contribution of  $H_2/CO_2$  to  $CH_4$  production was relatively homogeneous with depth and between different soil cores demonstrating that about  $24 \pm 7\%$  of the  $CH_4$  production was due to  $H_2$ -dependent methanogenesis. This homogeneity was surprising, since rice fields are a very inhomogeneous environment. In fact, we observed large spatial and vertical inhomogeneities with respect to  $CH_4$  concentrations and total rates of  $CH_4$  production which were probably due to gas ebullition (Chanton & Dacey 1991), plant effects (Sass et al. 1990) and/or uneven distribution of old plant residues (straw) in the soil (Sass et al. 1991). Obviously, there is a discrepancy between the heterogeneous distribution of total activity of  $CH_4$  production and the homogeneous distribution of  $H_2$ -dependent activity of  $CH_4$  production. This discrepancy is also seen in the composition of gas bubbles with a high variation of the  $CH_4$  content versus a low variation of the  $H_2$  content.

This discrepancy may be explained by the existence of microbial associations of a relatively homogeneous composition. These microbial associations would consist of hydrolytic, fermenting, syntrophic, homo-acetogenic, and methanogenic bacteria which together accomplish the complete degradation of organic matter to  $CH_4$  and  $CO_2$ . The existence of  $H_2$ -syntrophic methanogenic bacterial associations which account for  $>90\%$  of the  $H_2$ -dependent methanogenesis by interspecies- $H_2$ -transfer has been demonstrated in anoxic paddy soil (Conrad et al. 1989b; Conrad 1989). Laboratory experiments have shown that the percentage contribution of  $H_2$  to methanogenesis increases with time from about 2–6% in the beginning to about 25–30% after one month of submergence (Conrad et al. 1989b) indicating that at this time a stable methanogenic bacterial community has established. This percentage we have now also observed in the rice field under in-situ conditions.

A fermenting microbial community would degrade carbohydrates by the following stoichiometry:



Conversion of the produced acetate and  $H_2$  into  $CH_4$  would result in 66% of the methane being produced from acetate and 33% from  $H_2/CO_2$ . If the microbial community exists exclusively of homoacetogenic bacteria the stoichiometry would be



In this case, 100% of the methane would be produced from acetate and 0% from  $\text{H}_2/\text{CO}_2$ . It has been shown that homoacetogenic bacteria play a significant role in  $\text{H}_2$  turnover (Conrad et al. 1989a), glucose degradation (Krumböck & Conrad 1991) and conversion of  $\text{CO}_2$  to acetate (Thebrath & Conrad 1992) in anoxic paddy soil. Our observation of 24% contribution of  $\text{H}_2$  to methanogenesis indicate that some of the acetate might be produced by homoacetogens. In fact, some of the acetate (<6%) may even be produced from  $\text{H}_2/\text{CO}_2$  by chemolithotrophic homoacetogens.

Hydrogen is an intermediate in the degradation process and is produced and consumed in the methanogenic microbial associations resulting in the establishment of a steady state  $\text{H}_2$  concentration. This  $\text{H}_2$  concentration is under thermodynamic control and thus is kept at a relatively constant value (Zehnder 1978; Schink 1992; Conrad 1993b). In anoxic paddy soil slurries, steady state  $\text{H}_2$  partial pressures established in a range of about 10–120 ppmv mainly depending on temperature (Conrad et al. 1987) and dilution (Conrad & Babbel 1989). The gas bubbles in the rice field showed over one day a relatively constant value of about 93 ppmv  $\text{H}_2$ , which is well within the range observed in the laboratory. Earlier studies in the Italian paddy fields showed  $\text{H}_2$  partial pressures in gas bubbles between 10 and 41 ppmv  $\text{H}_2$  (Schütz et al. 1988) which is also within the range observed by laboratory studies.

Acetate is also an intermediate in the degradation process, but the thermodynamic control of its concentration is less strict. In contrast to  $\text{H}_2$ , anaerobic degradation processes usually are not inhibited by high acetate concentrations (Schink 1992). Our observations show that the turnover of acetate was highly dynamic in the rice field soil resulting in a very heterogenous distribution of acetate concentrations ranging over 2 orders of magnitude. This observation is in agreement with laboratory studies (Thebrath et al. 1992).

Similar as for acetate, concentrations of other fermentation products such as lactate, propionate, and formate also showed a very heterogenous distribution in the rice field soil. However, they usually increased and decreased in parallel to each other indicating that the factors causing their heterogeneities were identical. We assume that one reason was the existence of local spots with high fermentative activity due to the presence of decaying plant material. These factors probably also resulted in the large differences observed for the rates of total  $\text{CH}_4$  production and for the  $\text{CH}_4$  concentrations.

Lactate and propionate were measured in concentrations of <135 and <66  $\mu\text{M}$ , respectively. Lactate is fermented by propionate-producing bacteria either via the succinate or the acrylate pathway (Gottschalk 1986). In both pathways bicarbonate is incorporated into propionate with

a theoretical molar ratio of 0.5 and 1.0 in the succinate and the acrylate pathway, respectively. In the rice field soil, we observed a ratio of 0.17 indicating that either propionate was formed by other fermentation pathways, e.g. as a product of amino acid degradation or of degradation of odd-numbered fatty acids by  $\beta$ -oxidation, or that the bicarbonate in the soil did not completely exchange with the intracellular bicarbonate pool of the propionate-producing bacteria.

The vertical profiles of RI values usually showed values around 0.2 which are typical for the methanogenic degradation of acetate (Conrad & Schütz 1988). However, we also observed higher RI values, especially in surface soil and in the field which had been treated with  $K_2SO_4$ . The high RI values indicate that oxidative processes such as sulfate reduction or reduction of iron—III contributed or even dominated the degradation of acetate in these soil samples. We also cannot exclude that substrates other than acetate were degraded oxidatively in those soil layers where acetate was exclusively degraded by methanogens. This situation may arise if sulfate reducers and methanogens would exhibit preferences for particular electron donors as indicated by observations in littoral sediments of Lake Constance (Bak & Pfenning 1991; Thebrath et al. 1993). All these factors may cause a tremendous heterogeneity of activities and substrate conversion rates which would be consistent with our field observations. Interestingly,  $H_2$  turnover seems to be an exception.

The total  $CH_4$  production rates in the soil cores were between 79 and 128  $mmol\ d^{-1}m^{-2}$ . These rates were within the range of those (75–490  $mmol\ d^{-1}m^{-2}$ ) measured in previous experiments in the Italian rice field during July/August (Schütz et al. 1989b) and were also similar to those (55–94  $mmol\ d^{-1}m^{-2}$ ) measured in a flooded rice microcosm (Frenzel et al. 1992). Because of  $CH_4$  oxidation in the oxic rhizosphere of the rice microcosms, the emission of  $CH_4$  showed lower rates (8–12  $mmol\ d^{-1}m^{-2}$ ) than the total production of  $CH_4$  (Frenzel et al. 1992).  $CH_4$  emission rates (22–45  $mmol\ d^{-1}m^{-2}$ ) reported for Italian rice fields during July/August (Schütz et al. 1989a) were also lower than the total  $CH_4$  production rates measured in the soil cores. Most of the  $CH_4$  emission is due to ebullition and to vascular transport through the rice plants, whereas the diffusive  $CH_4$  flux is marginal (Hozapfel-Pschorn et al. 1985; Schütz et al. 1989b). A very low diffusive  $CH_4$  flux of about 0.25  $mmol\ d^{-1}m^{-2}$  was also indicated from the gradients observed in the vertical profiles of  $CH_4$  concentrations in the soil cores (Fig. 3).

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