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Effect of soil characteristics on sequential reduction and methane production in sixteen rice paddy soils from China, the Philippines, and Italy

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Abstract. The potentials for sequential reduction of inorganic electron acceptors and production of methane have been examined in sixteen rice soils obtained from China, the Philippines, and Italy. Methane, CO_2, Fe(II), NO_3^-, SO_4^{2-}, pH, E_h, H_2 and acetate were monitored during anaerobic incubation at 30 °C for 120 days. Based on the accumulation patterns of CO_2 and CH_4, the reduction process was divided into three distinct phases: (1) an initial reduction phase during which most of the inorganic electron acceptors were depleted and CO_2 production was at its maximum, (2) a methanogenic phase during which CH_4 production was initiated and reached its highest rate, and (3) a steady state phase with constant production rates of CH_4 and CO_2. The reduction phases lasted for 19 to 75 days with maximum CO_2 production of 2.3 to 10.9 μmol d^{-1} g^{-1} dry soil. Methane production started after 2 to 87 days and became constant after about 38–68 days (one soil >120 days). The maximum CH_4 production rates ranged between 0.01 and 3.08 μmol d^{-1} g^{-1}. During steady state the constant CH_4 and CO_2 production rates varied from 0.07 to 0.30 μmol d^{-1} g^{-1} and 0.02 and 0.28 μmol d^{-1} g^{-1}, respectively. Within the 120 d of anaerobic incubation only 6–17% of the total soil organic carbon was released into the gas phase. The gaseous carbon released consisted of 61–100% CO_2, <0.1–35% CH_4, and <5% nonmethane hydrocarbons. Associated with the reduction of available Fe(III) most of the CO_2 was produced during the reduction phase. The electron transfer was balanced between total CO_2 produced and both CH_4 formed and Fe(III), sulfate and nitrate reduced. Maximum CH_4 production rate (r = 0.891) and total CH_4 produced (r = 0.775) correlated best with the ratio of soil nitrogen to electron acceptors. Total nitrogen content was a better indicator for "available" organic substrates than the total organic carbon content. The redox potential was not a good predictor of potential CH_4 production. These observations indicate that the availability of degradable organic substrates mainly controls the CH_4 production in the absence of inorganic electron acceptors.

1. Introduction

Methane is one of the most important greenhouse gases and is an active component in atmospheric photochemistry. Understanding its source strength is subject of continuing experimental and theoretical interest and will remain important if its atmospheric mixing ratio continues to increase at the current rate (Rasmussen & Khalil 1981; Cicerone & Oremland 1988; Prinn 1994; Crutzen 1995). The biosphere with anaerobic soil ecosystems including rice paddies, natural wetlands, landfills, etc. accounts for 60–80% of the global annual release of CH_4 to the atmosphere. Thorough understanding of the anaerobic processes leading to CH_4 production in these ecosystems is essential to understand current and future emissions.

Chemical and microbiological soil properties control CH_4 production in wetland ecosystems (Neue & Roger 1993; Conrad 1989, 1993). Rice paddy soils are characterized by periodic waterlogging resulting in anoxic conditions. Generally, rice paddies become completely oxic during fallow periods, especially the dry season. Most anaerobic microorganisms, including methanogenic bacteria, are able to survive aeration and desiccation and recover after the field is flooded (Fetzer et al. 1993; Fukui & Takii 1990; Furusaka et al. 1991). Oxic soils become completely anoxic upon flooding (Ponamperuma 1981), except at the soil-floodwater interface and the zones around plant root (Conrad 1993). Chemical species which are reduced by microorganisms are: residual oxygen, nitrate, manganese(IV), iron(III), sulfate and carbon dioxide (Ponamperuma 1981; Patrick & Reddy 1978; Watanabe 1984; Achtnich et al. 1995; Peters & Conrad 1996). Organic electron acceptors like humic acids may also be involved (Lovley et al. 1996). Depletion of these electron acceptors paves the way to stable CH_4 production.

Thermodynamic theory predicts the sequential reduction of electron acceptors according to their redox potentials (Ponamperuma 1981; Zehnder & Stumm 1988). The reduction processes are closely related to soil chemical properties as well as to abundance and physiological potential of microorganisms. Many studies have already been carried out to isolate different physiological groups of bacteria from soils and sediments and to test their performances on individual electron acceptors (Garcia et al. 1974; Lovley 1991; Wolin & Miller 1987; Payne 1981). These studies are very helpful in understanding the interactions between electron acceptors and electron donors. However, it is difficult to apply these results to real soils with a much higher complexity than pure bacterial cultures.

Studies on the ability of microbial communities to reduce various electron acceptors are necessary to understand the CH_4 production process in rice fields. Focusing on the carbon flow from soil organic carbon to CO_2 and CH_4 should be helpful to elucidate the performance of various microbial

species and their roles during the degradation pathway. These pathways are not yet fully understood even for small organic compounds and H_2 coming from the fermentation of polymeric organic matter (Conrad 1993). Only few studies followed carbon consumption and balanced electron donors and electron acceptors (Takai 1961 (referenced in Watanabe 1984); Inubushi et al. 1984) and have compared the complete reduction process in different soils (Peters & Conrad 1996).

In this study we used long-term anaerobic incubations to simulate reduction processes that occur upon flooding of rice soil. The soils (16 rice soils from China, the Philippines, and Italy) represent a range of climatic conditions (temperate to subtropical zones) and rice cultivation. We investigated the effect of the soil chemical composition on the microbial reduction processes involved in organic matter degradation, and the carbon and electron balance at different reduction stages. The objective was to work out relationships between soil characteristics, soil reduction and CH_4 production.

2. Methods

Soil samples were taken during the fallow period. Samples were broken into lumps of 3–4 cm in diameter, air dried and stored in darkness at 4 °C until experiments were performed. The aerobic storage of dried soil has no significant effect on soil methane production capacity (Mayer & Conrad 1990). For experiments, soil samples were pulverized and passed through stainless steel sieves to obtain soil particles between 0.1 and 1 mm diameter. Ten grams of soil and 10 ml of distilled water were put into a 120-ml serum bottle. The distilled water was previously purged with N_2 for 30 min to remove dissolved O_2. The serum bottles were closed with butyl rubber stoppers and the headspace was flushed with N_2 at a rate of 300 ml min^{-1} for at least 20 min. All bottles were incubated upside down to minimize gas leakage through the rubber stopper. The incubation temperature was 30±0.3 °C. All measurements were carried out in triplicate. The time intervals between measurements varied between 0.5 and 30 days; they were short in the beginning and long at the end of the incubation.

One set of bottles ($n = 3$) was used to follow the partial pressures of CH_4, CO_2 and H_2 which were allowed to accumulate over the incubation. After vigorous manual shaking for about 30 s, gas samples were taken from the headspace of the bottles using a gas-tight pressure-lock syringe which had been flushed with N_2 before each sampling. Methane and CO_2 concentrations were analyzed with a Shimadzu GC 8A gas chromatograph equipped with a FID and a catalytic converter (Chrompack, nickat replacement reactor for methanizer). The separation column was a Propack Q 60–80 mesh column

of 80 cm length operated at a temperature of 50 °C. Hydrogen was analyzed on a Trace Analytical RGD2 HgO–Hg vapor conversion detector. Hydrogen was separated from other gases using a molecular sieve 5A column (80–100 mesh, 70 cm length) at 60 °C (Conrad et al. 1987). Total hydrocarbons were measured by FID connected to a capillary column (stainless steel), using CH_4 as the standard gas. Nonmethane hydrocarbons (NMHC) were determined by difference between total hydrocarbons and CH_4. Concentrations of CO_2 dissolved in the liquid phase were determined indirectly from the CO_2 partial pressure and the pH of the soil slurry using the equation given by Stumm and Morgan (1981). At the end of incubation, the total carbonate which was trapped in the slurry (both in dissolved form and carbonate precipitate) was measured by adding 1 ml of 1 M H_2SO_4 (containing 5% $FeSO_4.7H_2O$) to the soil slurry (0.5–1.5 g) and analyzing the released CO_2.

Another set of bottles (n = 3) was used for chemical analysis of soil slurries. Soil slurries were repeatedly sampled (1–2 ml) with a syringe after vigorous shaking of the bottles. The pH and E_h of the soil slurries were measured with a pH-meter and a Pt-electrode (Wissenschaftlich-Techische Werkstätten GmbH, PH-539). E_h readings were corrected by a reference electrode (210 mV). Iron was measured in sub-samples of 300 μl added to 5 ml of 0.5 N HCl and kept for more than 2 h at room temperature. For Fe(II) determination, 50–100 μl of the HCl suspension was added to 1 ml Ferrozin solution (0.1% w/w Ferrozin in 50 mM N-2-hydroxyl-ethylpiperazin-N′-2-ethane sulfonate, pH 7), mixed and centrifuged at 14,000 rpm for 5 min. The supernatant was measured in a photometer (Hitachi U-1100 photometer) at 562 nm. Total free iron (Fe(II) + Fe(III)) was determined by adding an aliquot (ca. 0.1 ml) of the HCl suspension to 2 ml 0.25 M hydroxylamine hydrochloride in 0.25 N HCl followed by incubation at 60 °C for 2 h and then analyzing Fe(II) as described above (modified according to Lovley & Phillips 1988). Dissolved sulfate was analyzed in another 300-μl subsample of the soil slurry after centrifugation and filtration through a 0.2-μm membrane filter (Sartorius, Minisart RC15). Part of the filtrate was stored frozen (−20 °C) for fatty acid analysis. Total sulfate (absorbed and dissolved sulfate) was determined after 12 h extracting 0.5–1.0 g slurry at room temperature with 5 ml phosphate mixture (15 mM $Ca(H_2PO_4)_2$ and 8 mM H_3PO_4) (Nelson 1982). Nitrate, nitrite and sulfate were determined on a Sykam ion chromatographic system containing a Sykam (LCA, A09) column and a conductivity detector serially connected to a UV detector at 218 nm (Bak et al. 1991). $Na_2CO_3/NaHCO_3$ (3 mM/1.5 mM) was used as the eluent. Dissolved acetate was measured using a Sykam HPLC system equipped with an Aminex HPX-87G ion exclusion column and a refraction index detector (Erma, CR Inc, ERC-7512) (Krumböck & Conrad 1991).

The following soil characteristics were determined in air-dry soil samples at the beginning of the incubation. Total free iron, dissolved sulfate, nitrate, pH and E_h were determined as described above. Total manganese (Mn(II) + Mn(IV)) was analyzed analogously to total iron after reduction with hydroxylamine. The resulting Mn(II) was then determined by atomic absorption spectrophotometry (Perkin Elmer AAS-1100). Soil organic carbon was measured using the potassium dichromate-sulfuric acid method (modified according to Nelson & Sommers 1982). In order to correct for the influence of reduced ions, samples without heating were used as blanks. For determination of total inorganic carbon content, soil samples (0.5–1.5 g) were accurately weighted into a serum bottle. Sulfuric acid solution (2 ml; 50% H_2SO_4 plus 7% $FeSO_4.7H_2O$) was injected into the serum bottle using a glass syringe. Inorganic carbon was determined by measuring the CO_2 concentration in the headspace of the serum bottle. Humic substances were measured using 5 g soil which was subsequently washed with 10 ml 0.05 N HCl and 10 ml distilled water to remove carbonates. The soil was then thinly spread on a glass plate and air-dried at room temperature. The air-dried soil was extracted with 50 ml of a pyrophosphate mixture (0.1 N NaOH, 0.1 M $Na_4P_2O_7$). The mixture was then bubbled with N_2 to remove dissolved air. The flask containing the mixture was then closed and shaken at room temperature for 24 h, followed by centrifugation at 5000 rpm for 10 min. The supernatant was measured in a photometer (Hitachi U-1100 photometer) at 380 nm, using commercially available humic acids (Fluka) dissolved in the sodium pyrophosphate mixture as standard. Labile organic carbon was obtained by mild oxidation of the soil organic carbon. Soil samples (5 g) were acidified with 10 ml of 2 M H_2SO_4 containing 2% $FeSO_4.7H_2O$ to remove all the carbonate. After 24 h, sub-samples (0.5–1 g) of the acidified slurries were put into 60-ml serum bottles, sealed with rubber stoppers, and then 2 ml of 0.4 N $K_2Cr_2O_7$ and 0.5 ml of 96% H_2SO_4 were injected into the serum bottles using a glass syringe. After incubation at 50 °C for 24 h, the oxidized organic carbon was measured by analyzing the CO_2 in the head space. Total soil nitrogen was analyzed using a CHN analyzer (Analytical Chemical Laboratory of the Philipps University, Marburg).

3. Results and discussion

3.1 *Initial soil characteristics*

Characteristics of air-dry soils before anaerobic incubation are given in Table 1. The 16 soils tested contained per gram dry weight soil: 8–26 mg organic carbon, 2.2–17.8 mg humic substances, 79–818 μmol labile organic carbon,

0.7–3 mg total nitrogen, 0–3.7 μmol nitrate, 83–421 μmol total free iron, 3.6–33.1 μmol total free manganese, and 0.2–5.1 μmol sulfate. The initial pH values of the soil slurries (1:1) were between 5.1 and 7.7. The initial redox potentials (E_h) of the soil slurries ranged between +340 and +510 mV.

3.2 *Methane and carbon dioxide production*

The temporal increase of CO_2 and CH_4 partial pressures was followed during the anaerobic incubation of the rice field soils. Typically, CO_2 was in all soils vigorously produced right from the beginning of the incubation, whereas CH_4 production started later (data not shown). The daily production rates of CO_2 and CH_4 were calculated from the first derivative of these accumulation curves using ORIGIN 5.0 (Microcal software Inc., Northampton, MA, U.S.A.). The CO_2 production rates were corrected for the CO_2 dissolved in the aqueous phase. The temporal pattern of the production rates of CO_2 and CH_4 during the incubation of the different rice soils is shown in Figure 1. The maximum CO_2 production rates (per gram dry weight soil) varied between 2.30 μmol d^{-1} g^{-1} (No. 13, Urdaneta) and 10.93 μmol d^{-1} g^{-1} (No. 11, Pila) (Table 2). After 19 (No. 8, Bugallon) to 75 days (No. 13, Urdaneta), the CO_2 production rate had decreased to about 15% of the maximum production rate and then remained relatively stable at between 0.02 μmol d^{-1} g^{-1} (No. 12, Gapan) and 0.28 μmol d^{-1} g^{-1} (No. 4, Beiyuan) during the remainder of the incubation (Figure 1, Tables 2, 3). The CO_2 produced during the first interval is probably the result of the microbial oxidation of organic carbon by various inorganic electron acceptors such as Fe(III), Mn(IV), sulfate, nitrate etc. In one exceptional case (No. 9, Luisiana) CO_2 partial pressures decreased after reaching a maximum on Day 19 resulting in net CO_2 uptake which is seen as small negative CO_2 production rate in Figure 1. Two processes have probably caused the decrease of CO_2, i.e., increased reduction of CO_2 to CH_4 and precipitation of $FeCO_3$. Using $H^{14}CO_3^-$ we found the proportion of CH_4 formed from H_2/CO_2 was 41% in this soil on Day 25 (detailed data are not shown) and thus higher than usually (29%) observed in methanogenic soils (Peters & Conrad 1996; Rothfuss & Conrad 1993). Furthermore, a large amount of carbonate precipitate had formed during the incubation. According to Neue and Bloom (1989) reduction processes usually produce Fe^{2+} and Mn^{2+} faster than carbonate precipitation can remove these ions from solution, especially in the initial stages of flooding. Generally, the ionic concentrations become and remain highly oversaturated with regard to the solubility equilibrium of the respective solid phases, like siderite, rhodochrosite, and calcite, because crystal growth sites are blocked by adsorption of soluble polymeric organic matter. In the Luisiana soil, however, the high free Fe

Table 1. Characteristics of the soils used.

No.	Origin	Country	Geographical site	Sampled date	pH	E_h mV	Total free iron μmol/g	Total free maganese μmol/g	Dissolved sulfate μmol/g	Nitrate μmol/g	Total nitrogen mg/g	Organic carbon mg/g	Humics mg/g	Labile org. C μmol/g
1	Zhenjiang	China	32.8° N, 119.5° E	10, 1995	7.7	460	130.2	12.1	0.89	0.16	0.74	10.4	4.7	79.5
2	Changchun	China	43.2° N, 125.4° E	11, 1995	6.0	510	142.4	13.8	0.39	<0.01	1.37	16.8	17.8	582.7
3	Guangzhou	China	23.1° N, 113.2° E	11, 1995	5.1	340	98.7	3.6	0.84	<0.01	1.27	18.5	6.0	713.9
4	Beiyuan	China	39.9° N, 116.3° E	12, 1995	7.4	360	92.0	8.2	5.15	1.41	0.90	13.4	3.2	376.5
5	Jurong	China	32.5° N, 118.5° E	11, 1995	6.3	460	177.7	13.6	0.75	<0.01	0.97	11.5	4.9	424.3
6	Shenyang	China	42.9° N, 123.1° E	11, 1995	6.7	450	140.4	10.2	0.90	0.01	0.70	13.5	5.6	477.3
7	Qinghe	China	40.1° N, 116.2° E	11, 1995	7.6	390	83.0	7.3	0.49	0.54	0.77	9.5	3.0	265.4
8	Bugallon	Philippine	16.2° N, 121.2° E	12, 1995	5.9	465	132.8	4.9	0.84	0.01	1.63	19.7	10.0	818.7
9	Luisiana	Philippine	14.2° N, 121.2° E	05, 1991	5.1	455	421.5	22.4	0.17	0.02	1.57	16.5	7.0	575.2
10	Maahas	Philippine	14.1° N, 120.1° E	05, 1995	6.2	460	273.4	22.3	1.38	<0.01	1.67	21.4	11.3	331.1
11	Pila	Philippine	14.2° N, 121.2° E	05, 1991	6.8	400	128.1	24.9	1.06	1.66	2.97	26.2	11.7	319.0
12	Gapan	Philippine	15.1° N, 120.5° E	12, 1995	6.0	505	261.2	13.0	0.29	0.01	1.17	15.1	7.3	200.4
13	Urdaneta	Philippine	17.5° N, 120.3° E	12, 1995	6.7	430	174.4	24.5	0.23	0.32	0.70	10.7	6.7	118.0
14	Maligaya	Philippine	15.4° N, 120.5° E	12, 1995	5.8	480	217.0	33.1	1.14	<0.01	1.07	13.9	10.1	272.3
15	Pavia	Italy	45.5° N, 171.1° E	03, 1993	6.1	440	88.5	4.3	0.39	0.45	0.70	8.1	4.1	405.9
16	Vercelli	Italy	45.9° N, 171.6° E	09, 1993	6.0	480	194.9	10.8	1.54	3.69	1.37	15.5	2.2	422.0

Data are averages of $n = 3$; CV was less than 10%. E_h and pH were from single measurements. Concentrations are given per gram dry weight soil.

Table 2. Duration and maximum CO_2 production during the reduction phase, conditions at the beginning of the methanogenic phase, and beginning of maximum CH_4 production.

Soil no.	Reduction phase and max. CO_2 production		Methanogenic phase					Maximum CH_4 production	
	duration (d)	Max. CO_2 μmol/g/d	Start (d)	H_2 (Pa)	Acetate (μM)	pH	E_h (mV)	Time (d)	Max. CH_4 μmol/g/d
1	26	5.91	32	1.1—1.5	110–450	6.7	30	36	0.23
2	21	4.11	24	2.5–4.1	30–130	6.9	45	47	0.30
3	24	4.31	3	1.1–23.0	2000–4000	6.0	160	9	1.42
4	25	4.95	17	1.1–2.8	70–120	7.2	−80	27	0.38
5	25	6.55	15	1.7–2.6	250–800	7.1	−60	19	0.42
6	29	3.87	18	2.2–3.2	60–100	7.1	−50	44	0.19
7	24	2.35	21	4.4–4.6	120–750	7.5	−55	27	0.17
8	19	8.25	3	2.7–16.0	100–1000	6.2	250	6	1.25
9	23	5.10	11	0.6–0.8	200–450	6.3	−5	16	0.40
10	29	7.21	22	0.3–0.4	300–450	6.9	−10	51	0.17
11	26	10.93	4	2.3–7.3	5000–8000	7.0	150	11	3.08
12	25	7.52	15	0.9–3.0	90–150	7.1	−25	27	0.47
13	75	2.30	87	0.9–1.5	>900	7.0	50	120	0.01
14	32	5.98	39	0.9–2.0	80–300	6.9	−35	78	0.18
15	21	3.68	2	2.3–15.0	900–1100	6.1	200	6	0.99
16	20	5.71	9	1.3–3.7	800–1200	7.0	10	18	0.51

Average of $n = 3$, CV <18%. Confidence limits for E_h and pH are ±35mV and ±0.23, respectively ($P = 0.05$). Acetate and H_2 are given as range of values observed between the beginning and the maximum of CH_4 production.

content provided sufficient Fe^{2+} upon flooding so that the precipitation of carbonate surpassed the CO_2 production after 19 days of reduction.

The CH_4 production profiles of all the different soils showed a similar pattern (Figure 1). The lag phase, before CH_4 production started, lasted between 2 (No. 15, Pavia) to 87 (No. 13, Urdaneta) days. After a short period of exponential increase, CH_4 accumulation slowed down and became almost linear during the later phase. The maximum CH_4 production rate ranged from 0.01 μmol d^{-1} g^{-1} (No. 13, Urdaneta) to 3.08 μmol d^{-1} g^{-1} (No. 11, Pila) (Table 2) while the final constant CH_4 production rates ranged from 0.07 μmol d^{-1} g^{-1} (No. 1, Zhenjiang) to 0.30 μmol d^{-1} g^{-1} (No. 5, Jurong) (Table 3). In most of the soils, the start of CH_4 production coincided with a decrease in the CO_2 production rate which then became relatively stable (Figure 1). In many soils (No. 2, 4, 5, 6, 7, 10, 12, 13, 14) there was a slight transient CH_4 production right at the beginning of the incubation (Figure 1). This phenomenon is described and discussed in detail elsewhere (Roy et al. 1997; Yao & Conrad 1999).

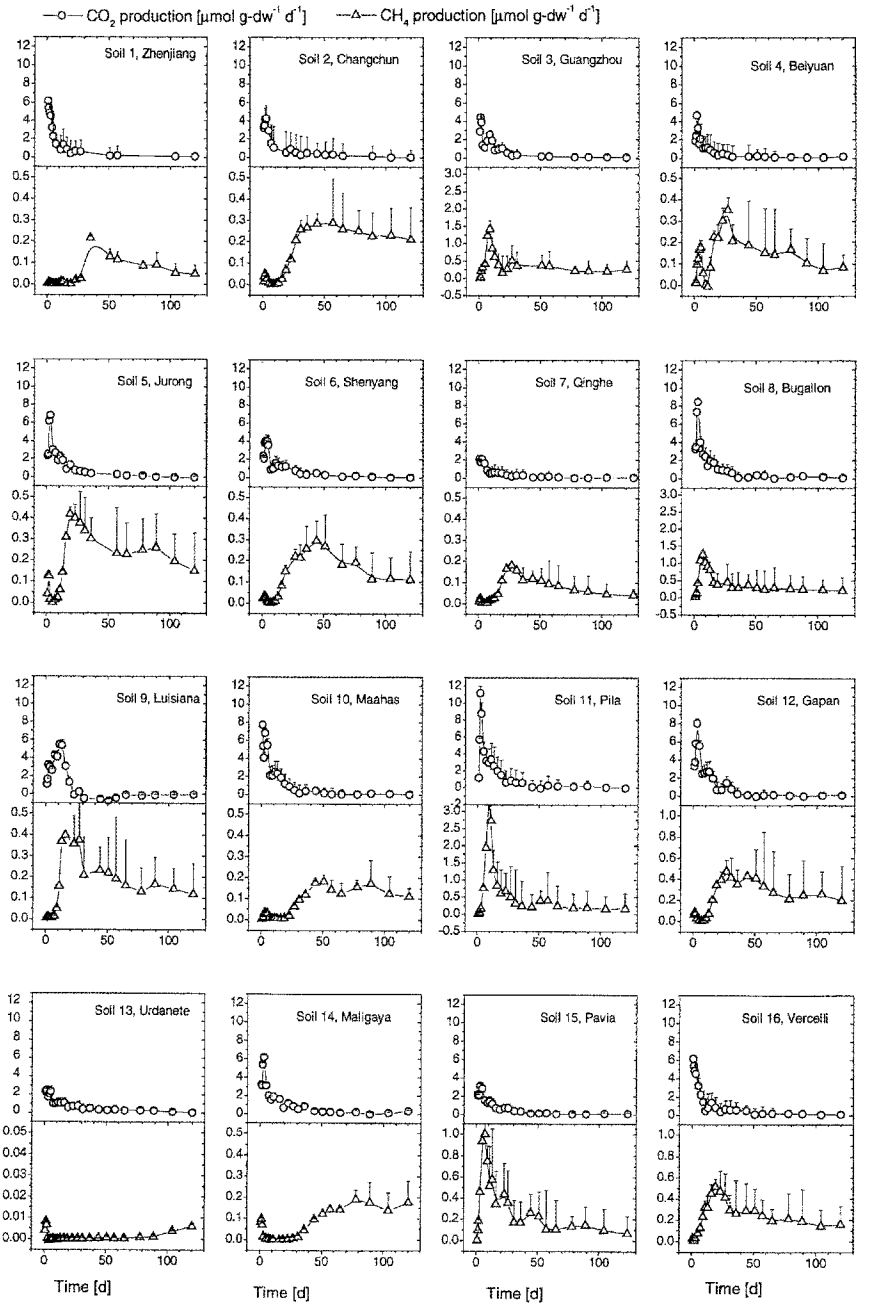

Figure 1. Production rates of CH_4 and CO_2 in anoxic slurries of 16 different soils. The CO_2 production was corrected for dissolved CO_2. Mean ± SD.

Table 3. Conditions and production rates of CH_4 and CO_2 during the steady state phase until end of incubation (120 d).

Soil no.	Start (d)	H_2 (Pa)	Acetate (μM)	Final pH	Final E_h (mV)	CH_4 prod. (μmol/g/d)	CO_2 prod. (μmol/g/d)
1	61	2.0–4.3	50–70	7.2	30	0.07	0.11
2	52	2.1–3.7	30–40	6.7	−15	0.28	0.14
3	43	1.8–3.7	30–70	6.8	−25	0.23	0.10
4	49	2.3–4.0	50–60	7.3	−135	0.14	0.28
5	38	2.2–3.5	30–50	7.0	−85	0.30	0.18
6	52	2.9–4.3	40–60	7.1	−70	0.16	0.23
7	47	2.6–5.0	40–80	7.4	−55	0.06	0.06
8	41	2.2–3.2	60–120	7.2	−25	0.25	0.16
9	42	0.9–2.2	80–100	7.2	−35	0.16	−0.29
10	49	2.0–3.8	100–120	7.1	−40	0.16	0.21
11	61	1.7–2.3	100–120	7.2	−40	0.12	0.13
12	52	2.3–3.0	90–130	6.6	−50	0.28	0.02
13	*	*	*	7.0	0	*	*
14	68	2.7–3.2	90–120	7.0	−30	0.18	0.05
15	38	3.4–6.4	50–70	6.9	−15	0.15	0.08
16	49	3.4–4.2	40–70	7.1	−35	0.21	0.26

Production rates of CH_4 and CO_2 were determined from a linear regression of the CH_4 and CO_2 partial pressures increasing with time during the steady state phase; mean of $n = 3$, CV <18%. Confidence limits for E_h and pH are ±35mV and ±0.23, respectively ($P = 0.05$). Acetate and H_2 are given as range of values observed during the steady state phase.

* Soil No. 13 (Urdaneta, Philippines) exhibited a very long lag phase before the onset of methane production. The start point of steady state is not defined. In this case, pH and E_h are the values measured at the end of incubation.

3.3 *Major electron acceptors*

The major electron acceptors monitored, i.e. nitrate, Fe(III) and sulfate, were always depleted successively during the incubation. Nitrate was reduced first. Reduction of Fe(III) and sulfate were completed later. The reduction of these two species partially occurred simultaneously. The duration of the reduction processes varied greatly between the soils. Figure 2 illustrates the reduction sequence of nitrate, Fe(III), and sulfate in soils No. 2 (Changchun), 8 (Bugallon) and 14 (Maligaya) as typical examples. The reduction sequences were the same as observed in previous studies (Klüber & Conrad 1998; Peters & Conrad 1996; Achtnich et al. 1995; Ponnamperuma 1981). Due to the reduction of the electron acceptors in the soil slurry, E_h values decreased

exponentially from levels above +340 (Table 1) to levels between −135 to + 30 mV at the end of incubation (Figure 2; Table 3).

In most soils the concentration of sulfate in the pore water of the slurry increased for a few days before sulfate reduction started (Figure 2). A similar pattern has been reported by Ponnamperuma (1981). The initial increase in porewater sulfate is due to the release of SO_4^{2-} adsorbed to ferric iron minerals such as goethite (Fleming & Alexander 1961; Parfitt & Smart 1978; Stanko-Golden et al. 1994) either by HCO_3^- or Fe-reduction. In acidic soils, clays and hydrous oxides of aluminum, such as basaluminite ($Al_4(OH)_{10}SO_4.5H_2O$) and jurbanite ($AlOHSO_4.5H_2O$), strongly sorb sulfate and release it when pH increases upon flooding. In acid sulfate soils (not included in the selected soils here) the dissolution of ferric oxide-sulfate complexes such as jarosite ($KFe_3(OH)_6(SO_4)_2$) release sulfate which is reduced at a lower E_h than Fe(III). The sulfate extracted by calcium phosphate-phosphoric acid mixture (Figure 2) supports this interpretation. It lacks the initial increase because above mentioned forms of sulfate are all extracted. This interpretation is in agreement with studies by Jäckel and Schnell (personal communication) in which the addition of increasing amounts of ferrihydrite to anoxic paddy soil resulted in a decrease of porewater sulfate.

The initial pH of the slurries of the 16 soils varied from 5.1 to 7.7. The pH gradually shifted toward a range of 6.0–7.5 as the incubation progressed (Figure 2; Table 2). The pH became stable (pH 6.6–7.4), especially when the CH_4 production rate became constant (Table 3). The variation of pH should be controlled by factors such as CO_2 and ammonia produced during incubation (Sparks 1995). The pH decrease to neutral in the calcareous soils and the final regulation of the pH rise to neutral in the acidic soils is regulated by carbonate solubility equilibria (Neue 1991). Above discussed oversaturations result in pH values higher than predicted by solubility equilibria.

3.4 *Major electron donors, redox potential and onset of methane production*

The chemical and physical conditions at the onset of CH_4 production were quite different among the soils (Table 2). However, during the transition from iron reduction and sulfate reduction to CH_4 production we always observed an increase of the H_2 partial pressure. For all soils, the H_2 partial pressures showed the following temporal pattern. The H_2 partial pressure exhibited a very sharp peak during the first 24 h of incubation (see soils No. 2, 8, 14 in Figure 2). The highest H_2 peaks reached 170 Pa (No. 5, Jurong). Subsequently, H_2 quickly decreased to about 0.4–0.8 Pa, and then again increased at the beginning of the CH_4 production phase. Afterwards H_2 stabilized at about 2–4 Pa, but with a slight decreasing trend after CH_4 production became constant (Figure 2).

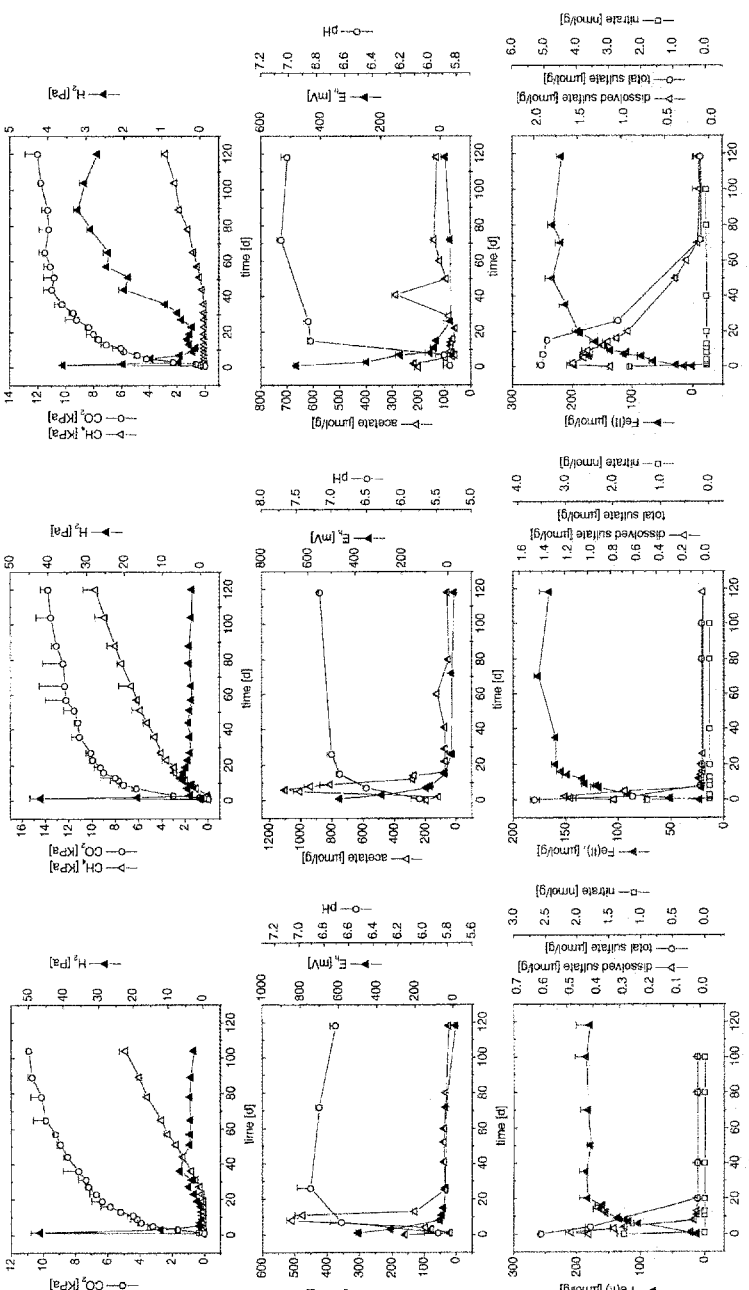

Figure 2. Production of CH_4 and CO_2 shown together with the temporal change of other soil variables in soils No. 2 (Changchun), 8 (Bugallon), and 14 (Maligaya). Mean $\pm$ SD.

Hydrogen is produced from the degradation of organic substances (Conrad 1996) and is rapidly consumed as electron donor in various redox reactions, so that the turnover time of H_2 is very short, typically on the order of minutes (Conrad et al. 1989). The sharp peak at the beginning of the incubation was probably caused by fermentation processes. The subsequent decrease of H_2 was likely due to consumption by bacteria using Mn(IV), Fe(III) and sulfate as electron acceptors. These bacteria are able to consume H_2 to relatively low concentrations because of the favorable energy yield of these redox reactions (Cord-Ruwisch et al. 1988; Lovley & Goodwin 1988). Finally, the increase of H_2 partial pressure at the end of these reduction processes allowed the beginning of hydrogenotrophic methanogenesis. The thermodynamic conditions for hydrogenotrophic methanogenesis are described in detail by Yao and Conrad (1999).

Because of the rapid H_2 turnover (Conrad et al. 1989) steady state conditions are rapidly reached. The H_2 steady state concentration is determined by the relative rates of H_2 production and H_2 consumption. The increase of H_2 at the onset of CH_4 production is probably characteristic for the transition from H_2 consumption by sulfate and/or iron oxidizers to H_2 consumption by methanogens (Cord-Ruwisch et al. 1988; Lovley & Goodwin 1988; Achtnich et al. 1995; Roy et al. 1997). Acetate which is, like H_2, produced from organic substances and consumed in various redox reactions does not give such a reliable signal, since its turnover time in paddy soil is much longer than that of H_2, typically in the range of hours or even days (Schütz et al. 1989; Sigren et al. 1997). Methane production was initiated at H_2 partial pressures between 1 and 23 Pa (Table 2). Acetate concentrations were at a larger range, i.e., 30–8000 μM, pH values were between 6.0 and 7.5, and E_h values were between −80 and +250 mV (Table 2). Eventually, acetate concentrations stabilized at values of 30–130 μM (Table 3) when CH_4 production became linear indicating that a steady state between production and consumption processes was reached.

Although the E_h of the soils generally decreased during the sequential reduction process, the E_h values did not allow the prediction when methanogenesis started. The E_h values at the onset of methanogenesis were relatively high when compared to the experimental results of Wang et al. (1993) and Masschelyn et al. (1993) who found that CH_4 production required E_h values of less than −150 mV. However, these authors adjusted the E_h of soil by titration with O_2. Treatment with O_2 may exert a toxic effect on the methanogenic bacteria besides increasing the E_h. In fact, experiments with cultures of methanogenic bacteria showed that O_2 had a much more adverse effect on methanogenic activity than high redox potentials and that methanogens were able to initiate CH_4 production in the absence of O_2 at E_h values up to + 420

mV (Fetzer & Conrad 1993). Other studies in which soils were not treated with O_2 often observed initiation of CH_4 production at E_h values around 0 to +100 mV (Garcia et al. 1974; Peters & Conrad 1996; Ratering & Conrad 1998). Based on our observations, we therefore believe that redox potential is not a good indicator for the onset of soil methanogenesis. In general it should only be used as an indicator when the soil and its CH_4 production behavior have been carefully characterized (e.g., Yagi et al. 1996; Sigren et al. 1997).

3.5 *Reduction patterns*

We found that the reduction patterns in all of the 16 different soils were generally similar. The major difference was that the reduction processes in some soils proceeded very fast while in others they proceeded only slowly. Similar observations were made in paddy soils from the Philippines (Neue et al. 1994; Wassmann et al. 1998). The patterns of the temporal change of production of CO_2 and CH_4 in all the soils suggested that the entire reduction process may be divided into three dynamic phases, i.e., phase 1 with predominant CO_2 production ("reduction phase"), phase 2 with predominant CH_4 production ("methanogenic phase"), and phase 3 as "steady state phase".

Figure 3 illustrates the patterns of the temporal accumulation of total CO_2 and CH_4 (in μmol per gram dry weight soil) as well as of the CO_2 and CH_4 production rates (first order derivative of the CH_4 and CO_2 accumulation) in a soil from Qinghe (No. 7). In this soil the CO_2 production rate increased to a maximum level during the first 1.4 days, then decreased rapidly within 24 d, and eventually stayed almost constant. On the other hand, CH_4 production started at Day 19 (Figure 3), i.e., when CO_2 production had almost decreased to its final low rate. By comparing the CH_4 and CO_2 production rates in all 16 soils, we found that CH_4 production usually was initiated at the time when the CO_2 production rates were greatly reduced and tended to stabilize at a constant rate. Therefore, we defined the interval between the start of incubation and the time when the CO_2 production rate had decreased to less than 15% of its peak rate as the reduction phase (Figure 3). This phase was followed by the initiation of vigorous CH_4 production (methanogenic phase), which eventually reached a constant rate (steady-state phase). We defined the beginning of the steady-state phase as the time when the CH_4 production rate was relatively constant, i.e., decreased less than 25% over 10 days (Figure 3). With these criteria, the duration of the reduction phase, the methanogenic phase and the steady-state phase was determined for all the soils tested (Tables 2, 3).

Please note, that in many soils CH_4 production began some time before the reduction phase was finished (Figure 3). In these cases, CH_4 production overlapped for some period with reduction of Fe(III) and sulfate. We never-

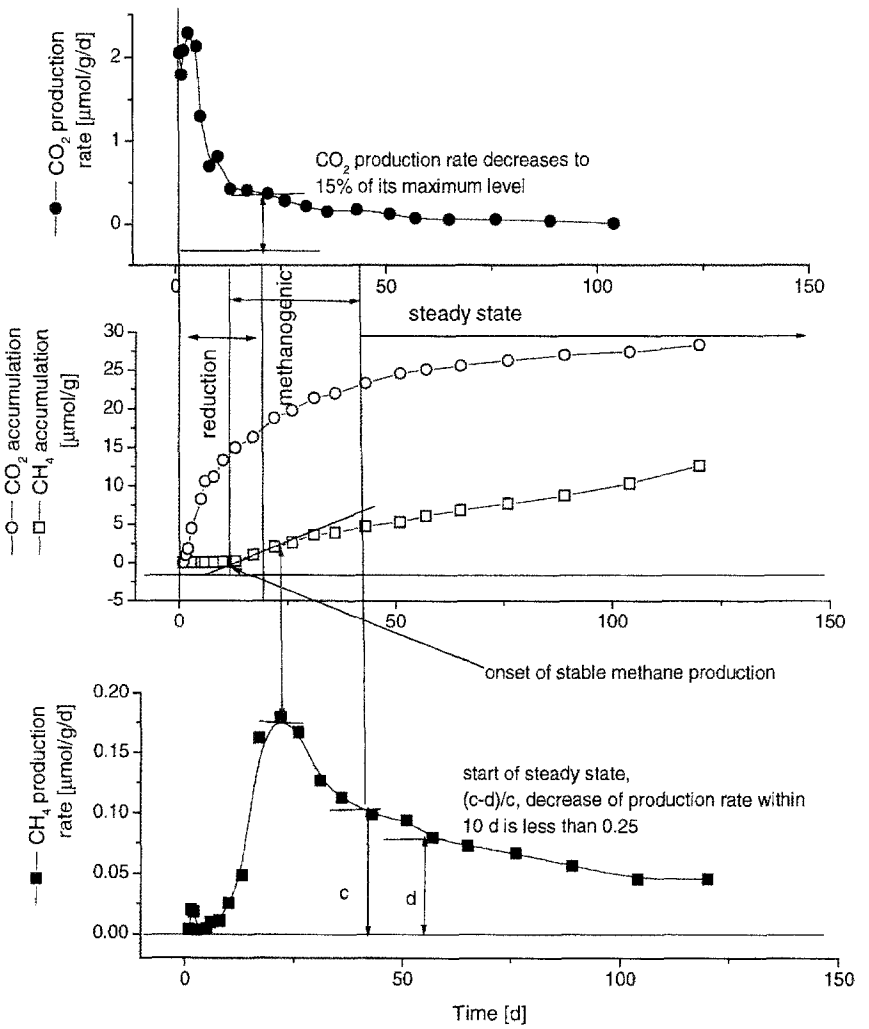

Figure 3. Scheme showing the different phases of production of CO_2 and CH_4 during the anoxic incubation of soil slurries. The data are taken from soil No. 7 (Qinghe).

theless, defined the shift from the reduction phase to the methanogenic phase as the time when the reduction of Fe(III) and sulfate was nearly finished, i.e., the time when CO_2 production had decreased to <15% of the peak rate.

During the reduction phase, the CO_2 production rates reached a maximum (Figure 3). The CH_4 production generally remained at a very low level within this phase. Nitrate and nitrite in the pore water became undetectable. Iron(II), which increased simultaneously with a change of soil color from brown to gray and a decrease in the redox potential, rose to more than 80% of its final value at the end of reduction phase. Sulfate in pore water became

almost undetectable by the end of the reduction phase. The rate of CO_2 production gradually became stable, indicating that most of the inorganic electron acceptors were depleted and the soil slurry entered another phase of reduction, i.e., the methanogenic phase (Figures 1, 3).

3.6 *Balance of electron donors and electron acceptors*

In a balanced redox reaction the amount of electrons transferred from the primary electron donors (e.g. polysaccharides etc.) to the different electron acceptors should be equal. An electron balance was calculated from the data at the end of the reduction phase and again at the end of the incubation assuming that during the reduction course, nitrate and nitrite were completely reduced to N_2, sulfate was reduced to sulfide, Mn(IV) to Mn(II), and Fe(III) to Fe(II), and further assuming that organic carbon at oxidation state zero was the only electron donor that was converted to either CO_2 or CH_4 (Figure 4). This is to our knowledge the first study which tested a stoichiometric balance between organic carbon oxidized, inorganic electron acceptors reduced and CH_4 produced. Our results demonstrate a fairly good balance at the end of the reduction phase and a good balance at the end of incubation (Figure 4). Most important for the overall electron balance was the reduction of Fe(III). During the reduction phase 58–79% of electrons transferred were due to iron reduction. Over longer periods CH_4 production became also important for the balance accounting to for 0.2–56% of the electrons transferred. In comparison to CH_4 production and iron reduction, the contribution of other electron acceptors to the total electron balance was quantitatively of minor importance (Figure 4).

Significant differences (t-test; $P < 0.01$) existed between the electrons donated and the electrons accepted in soils No. 4, 5, 7, 9, 10, 12, 14, 15, and 16 at the end of the reduction phase and in soils No. 1, 5, and 16 at the end of the incubation. At the end of the reduction phase there was a general deficit of CO_2 produced (Figure 4(A)). Deficits of CO_2 may have been caused by (i) the formation of carbonate precipitates (e.g., siderite formed from CO_2 and Fe^{2+}; Neue & Bloom 1989), (ii) by organic substances converting into a more oxidized form rather than into CO_2 (Saiz-Jimenez 1996), or (iii) by the presence of organic carbon with an overall oxidation state of other than zero (most likely less than zero). The first and/or the second explanation is the most likely, since finally, for most of the soils at the end of incubation (after 120 d), the CO_2 produced (this CO_2 includes eventually produced carbonate precipitates) was relatively well balanced with the total amount of electron acceptors reduced and CH_4 produced (Figure 4(B)). Linear regression of the amount of electrons donated (i.e., CO_2 production) in the different soils against those accepted (i.e., electron acceptors reduced) resulted in $y = 27$

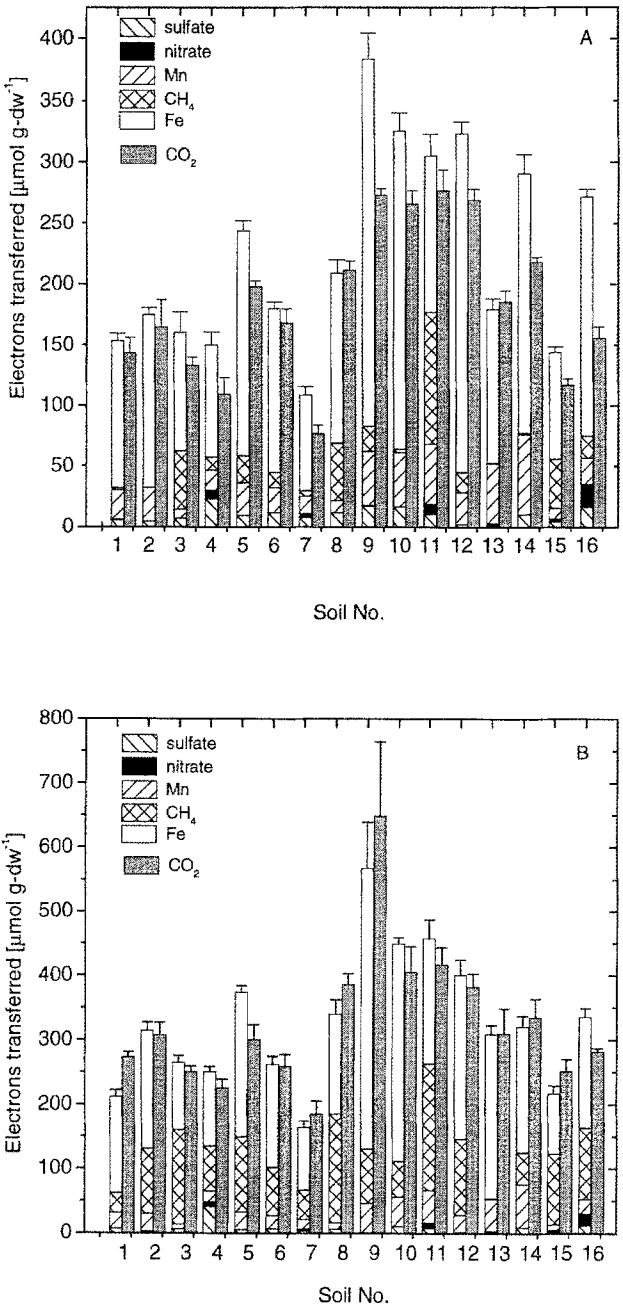

Figure 4. Electron balance between produced CO_2 and reduced electron acceptors at (A) the end of the reduction phase and (B) at the end of incubation. Total CO_2 in (A) was CO_2 in the gas phase plus dissolved CO_2. Total CO_2 in (B) was determined after acidification of the soil slurry.

+ 0.7x (r = 0.91) and y = 13 + 0.95x (r = 0.92) for the end of the reduction phase and the end of the incubation, respectively. The slopes of the regression analysis indicate a small deficit of CO_2 which was larger at the end of the reduction phase than at the end of the incubation.

3.7 *Balance of carbon*

At the end of incubation (120 d), the total CO_2 produced during the incubation was obtained by summing the CO_2 in gas phase and net increase of carbonate in soil slurry during the incubation. We also measured the soil organic carbon content after 120 days of incubation. For most of the soils the decrease in the organic carbon content was so small that it could not be detected significantly ($P < 0.05$). Based on the total production of CO_2 and CH_4, anaerobic incubation of soils for 120 days resulted in a loss of only 6.8–16.7% of the total organic carbon (Table 4). Similarly low losses (3–7%) have been observed before (Tsutsuki & Ponnamperuma 1987). The relative amounts of gaseous carbon lost (6.8–16.7%) were lower than the relative amounts of labile organic carbon (9–73%; calculated from Table 1) determined by mild oxidation of soil organic matter with dichromate. Thus, the labile organic carbon content was not representative for the release of gaseous carbon.

In our soils, 61–100% of the organic carbon consumed was converted to CO_2 (Table 4). This conversion was primarily coupled to the oxidation of inorganic soil oxidants, especially Fe(III) which accounted for 58–79% of organic carbon consumption (Figure 4). For soil No. 13 (Urdaneta), which had a long reduction phase and eventually only a low CH_4 production rate, almost all of the consumed carbon was used to reduce inorganic electron acceptors and to produce CO_2 (Table 2, Figure 4). Another possible loss of organic carbon is nonmethane hydrocarbons, but they accounted for just a small percentage (0–5%) of the total gaseous carbon released, expressed in equivalents of CH_4 (Table 3). They are therefore not significant compared to methane and CO_2, but provide some evidence that anaerobic soils may also be sources of atmospheric nonmethane hydrocarbons as suggested by Devai and DeLaune (1995).

3.8 *Classification of soils*

Although the general temporal pattern of the reduction processes, i.e. the distinction of reduction, methanogenic and steady state phase, was similar in the various soils, the CH_4 production rates and the total amounts of CH_4 produced during the 120-days incubation period were quite different. Neue et al. (1994) used the amount of CH_4 produced during 10 days to classify 20 different paddy soils from the Philippines into 4 different classes (<1,

Table 4. Balance of gaseous carbon produced during the incubation.

Soil no.	CH_4 μmol/g	CO_2[a] μmol/g	NMHC μmol/g	Initial org. C μmol/g	% org. C consumed[b]	% CO_2 of C consumed[c]
1	7.4 ± 0.6	68.2 ± 2.0	1.0 ± 0.1	850.0 ± 26.6	9.0 ± 0.4	89.0 ± 3.6
2	25.1 ± 2.4	77.0 ± 4.6	3.1 ± 0.5	1375.0 ± 45.4	7.7 ± 0.5	73.2 ± 5.7
3	36.6 ± 2.3	62.6 ± 2.0	3.8 ± 0.7	1525.0 ± 12.3	6.8 ± 0.2	60.8 ± 2.7
4	17.6 ± 0.6	56.3 ± 3.3	1.5 ± 0.2	1100.0 ± 10.9	6.9 ± 0.3	74.7 ± 5.5
5	29.1 ± 2.2	75.1 ± 5.8	3.5 ± 0.6	958.3 ± 46.1	11.3 ± 0.8	69.7 ± 6.7
6	18.4 ± 2.1	64.9 ± 4.5	3.3 ± 0.4	1116.7 ± 54.4	7.8 ± 0.6	74.9 ± 6.7
7	11.3 ± 1.9	46.3 ± 5.0	1.0 ± 0.3	725.0 ± 62.7	8.0 ± 1.0	79.0 ± 11.2
8	42.2 ± 4.7	96.6 ± 4.3	7.0 ± 0.7	1600.0 ± 35.9	9.4 ± 0.5	66.3 ± 4.1
9	21.1 ± 2.1	162.2 ± 29.1	2.9 ± 0.3	1358.3 ± 59.6	13.7 ± 2.2	87.1 ± 20.7
10	13.9 ± 0.6	101.4 ± 10.0	1.7 ± 0.2	1866.7 ± 63.6	6.3 ± 0.6	86.7 ± 11.3
11	49.0 ± 4.8	104.4 ± 6.6	7.2 ± 0.9	2158.3 ± 18.8	7.5 ± 0.4	65.0 ± 5.3
12	29.4 ± 5.1	95.7 ± 5.1	3.4 ± 0.5	1225.0 ± 44.7	10.5 ± 0.7	74.5 ± 5.7
13	0.1 ± 0.0	77.6 ± 9.6	0.0 ± 0.0	891.7 ± 40.7	8.7 ± 1.2	99.9 ± 17.5
14	12.5 ± 1.3	84.0 ± 7.1	2.2 ± 0.2	1200.0 ± 31.3	8.2 ± 0.6	85.1 ± 9.5
15	27.4 ± 1.8	63.0 ± 4.6	2.6 ± 0.3	558.3 ± 17.7	16.7 ± 1.0	67.7 ± 6.2
16	27.8 ± 1.9	70.5 ± 1.3	4.5 ± 0.4	1225.0 ± 63.4	8.4 ± 0.5	68.6 ± 2.0

Mean ± SD; $n = 3$

[a] CO_2 = gaseous CO_2 + dissolved CO_2 + precipitated carbonate

[b] 100 (CH_4 + CO_2 + NMHC)/initial org. C

[c] 100 CO_2/(CH_4 + CO_2 + NMHC)

1–10, 10–100, >100 μg CH_4 g^{-1} soil). Wassmann et al. (1998) classified 11 different paddy soils from the Philippines into 3 different classes, those with almost spontaneous CH_4 production, those with a delay for 2 weeks, and those with a long-term suppression for >8 weeks. The behavior of the soils used in the present investigation was consistent with these types of classification with soils No. 3, 8, 9, 11, 15, and 16 belonging to class I, soils No. 1, 2, 4, 5, 6, 7, 10, 12, and 14 to class II and soil No.13 to class III (Figure 1, Table 2). However, to predict the influence of soil type on production of CH_4 during submerged conditions it is important to find out which basic soil characteristics determine CH_4 production. Ideally, prediction would be based on process theory and the measurement of simple soil analytical data. A plausible theoretical assumption is that CH_4 is produced from organic matter as soon as inorganic electron acceptors have been depleted. This assumption is in agreement with the good electron balance shown in Figure 4. This assumption is also in agreement with the experiments of Takai (1961) (referenced by Watanabe (1984)) that the ratio of CO_2/CH_4 produced increases with the relative oxidizing capacity of a soil that is measured by the ratio of NH_4^+ formed to inorganic electron acceptors reduced. The amount

Table 5. Pearson correlation matrix of CH_4 production parameters against basic soil variables.

	Maximum CH_4 production rate	Total CH_4 produced	Steady state CH_4 production rate
Max. CO_2 production rate	0.640**	0.647***	0.331
Organic carbon	0.653**	0.487	0.009
Humus	0.215	0.235	0.288
Labile carbon	0.284	0.613*	0.579*
Total nitrogen	0.780***	0.681**	0.164
Length of lag phase	−0.492	−0.738***	−0.520*
Total free iron	−0.259	−0.132	0.144
Total electron acceptors	−0.247	−0.150	0.111
Ratio org.N/total e^--acceptors	0.891***	0.775***	0.120
E_h at onset of methanogenesis	0.658**	0.587*	0.056
E_h at start of steady state	0.064	0.094	0.135
E_h at end of incubation	0.040	−0.130	−0.248

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

of NH_4^+ formed was taken as a proxy for the amount of organic carbon oxidized. However, it is impractical to make long incubation experiments in order to assess the CH_4 production potential of a soil. Therefore, we tested whether the CH_4 production potential may be predicted from simple soil characteristics that can be measured without long-term incubation. For this purpose we calculated Pearson correlation coefficients between parameters of CH_4 production and various soil variables, such as pH, E_h, concentrations of electron donors (H_2, acetate, organic carbon etc.) and electron acceptors (nitrate, sulfate, iron) or combinations of them (Table 5).

When correlating either the maximum CH_4 production rates or the total CH_4 produced, a highly significant correlation was obtained with the maximum CO_2 production rate ($r > 0.64$) indicating that degradable organic carbon is an important driving force. The correlation of the maximum CH_4 production rates with the total organic carbon content was similar ($r < 0.653$), but the correlation with the total nitrogen content was stronger ($r > 0.78$). On the other hand, there was no significant correlation with the content of humus (Table 5). Labile organic carbon (measured by mild oxidation of organic carbon) also showed only a relatively weak correlation with the total CH_4 produced and with the rate of CH_4 production during the steady state phase, and no correlation at all with the maximum CH_4 production rate. We assume that only part of the total organic matter is degradable and thus can

serve as electron donor for the reduction processes including CH_4 production. Unfortunately, there is no standard procedure for the determination of this fraction of the organic carbon pool. Our results suggest, however, that the initial rate of CO_2 production (which is more or less equivalent to the maximum CO_2 production rate; see Figure 1) or the total nitrogen content (representing mainly organic nitrogen) are relatively good proxies. The suitability of organic nitrogen is consistent with the hypothesis of Takai (1961) that the amount of NH_4^+ formed is equivalent to the amount of organic matter degraded.

As theoretically expected, CH_4 production parameters were negatively correlated to the amount of reducible inorganic electron acceptors. However, the correlation was only weak and statistically not significant (Table 5). The reason for the weak correlation may be that the amount of inorganic electron acceptors which was extractable did not always represent the amount which was actually available for the reducing bacteria. In addition it is likely that the CH_4 production parameters are a function of both available organic carbon serving as electron donor and inorganic compounds serving as electron acceptors. Indeed, a very significant correlation was obtained between the total amount of CH_4 produced or the maximum CH_4 production rate and the ratio of organic nitrogen (as proxy for available electron donors) to total electron acceptors (Table 5; Figure 5). Hence, the oxidizing capacity of the soil, as represented by the total inorganic electron acceptors, did have an effect on the CH_4 production parameters, confirming the earlier suggestion by Takai (1961).

Correlation of CH_4 production parameters to other soil variables were not significant. In particular, we did not observe a negative correlation with the E_h values (Table 5), indicating that the redox potentials that were measured with the platinum electrode were not a suitable proxy for the oxidizing capacity of the soil. On the contrary, we even found a positive correlation of maximum CH_4 production rates ($r = 0.658$) and total CH_4 produced ($r = 0.587$) with the E_h values at the beginning of methanogenesis (Table 2). The reason for this positive correlation was that soils with high CH_4 production often exhibited such a short lag phase that the reduction phase and the methanogenic phase overlapped. In these soils CH_4 production started before the inorganic electron acceptors were fully depleted, i.e., at a relatively high E_h (e.g. soil No. 8; Figure 2). Our results are in contrast to an earlier study by Garcia et al. (1974) of Senegalese rice field soils which has been evaluated using correlation analysis by Neue and Roger (1993). This study reports a significant negative correlation ($r = -0.646$; $P < 0.01$) between the E_h at 7 days after flooding and the logarithm of the potential CH_4 production rate that was measured during anaerobic incubation at 37 °C 8–12 days after flooding. The reason

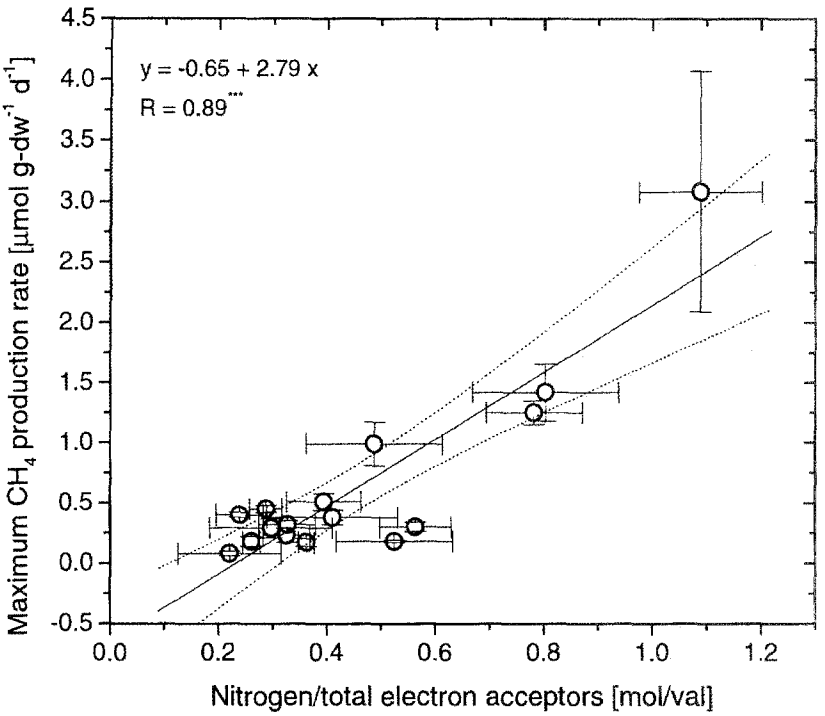

Figure 5. Regression of the maximum CH_4 production rates observed in the different soils against the ratio of total soil nitrogen divided by total inorganic electron acceptors (mainly iron). Error bars indicate the overall standard deviation, the dotted line the 95% confidence limit.

for the discrepancy between our and the earlier results is presently unclear. We speculate that methanogenesis is limited by available inorganic electron acceptors (e.g. Fe^{3+}) which compete for limited electron donors in form of labile organic substrates. Since the Fe^{3+}/Fe^{2+} redox couple at a given solubility equilibrium determines the E_h of the soil solution, the methanogenic activity would indirectly depend on the E_h value and increase with decreasing E_h, even if methanogenesis is not directly affected by E_h (see Discussion 3.4). Indeed, such a negative correlation between CH_4 production and E_h was evident for each individual soil (e.g. Figure 2). However, it was only valid within one and the same soil, but obviously not among different soils with different mineral compositions and solubility equilibria (Neue 1991). Therefore, we conclude that a negative correlation between CH_4 production and E_h may only become apparent over a limited range of related soils. The analysis of total electron acceptors, which is possible even before the soil is flooded, seems to us a more direct approach to quantify the oxidizing capacity of soil

than the measurement of the signal of a platinum electrode at a particular time point after the flooding of the soil.

4. Conclusions

We found that maximum CH_4 production rates as well as total CH_4 production are related to both the oxidizing and reducing capacities of soil, which are best represented by the amount of total inorganic electron acceptors and the amount of available organic carbon, respectively. About 60% of the variability of total CH_4 produced could be explained by these two soil variables which can relatively easily be determined in soil samples. The predictability of CH_4 production could be further improved when only the amount of organic carbon that is available to microorganisms could be quantified. Although organic nitrogen or the initial rate of CO_2 production seem to be good proxies, there may be better ways to estimate this variable. A possible approach may be the use of the enriched fractions of organic carbon or nitrogen, i.e., the differential between topsoil and subsoil concentrations of the respective compounds (Gaunt et al. 1977; Wassmann et al. 1998). Another approach may be to distinguish between clay-protected and non-clay-protected soil organic matter fractions (Gaunt et al. 1997) and to explain the seasonal CH_4 emission increases with the percentage of sand (Sass et al. 1994; Huang et al. 1997). The availability of organic carbon seems to be under the control of soil minerals, with a positive relationship between noncrystalline minerals and recalcitrant organic carbon (Torn et al. 1997). The characterization of the mineralogy would also be helpful with respect to the availability inorganic electron acceptors for soil microorganisms. This is especially important for the various iron and manganese minerals which obviously are not all easily available to microbes (Lovley 1991).

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