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Contribution of methanol to the production of methane and its ^{13}C-isotopic signature in anoxic rice field soil

RALF CONRAD* and PETER CLAUS
*Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Str., 35043 Marburg, Germany; *Author for correspondence (e-mail: Conrad@staff.uni-marburg.de; phone: +49-6421-178-801; fax: +49-6421-178-809)*

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Abstract. Conversion of methanol to CH_4 has a large isotope effect so that a small contribution of methanol-dependent CH_4 production may decrease the $\delta^{13}CH_4$ of total CH_4 production. Therefore, we investigated the role of methanol for CH_4 production. Methanol was not detectable above 10 μM in anoxic methanogenic rice field soil. Nevertheless, addition of ^{13}C-labeled methanol (99% enriched) resulted in immediate accumulation of $^{13}CH_4$. Addition of 0.1 μM ^{13}C-methanol resulted in increase of the $\delta^{13}CH_4$ from -47 to $-6‰$ within 2 h, followed by a slow decrease. Addition of 1 μM ^{13}C-methanol increased $\delta^{13}CH_4$ to $+500$ ‰ within 4 h, whereas 10 μM increased $\delta^{13}CH_4$ to $+2500‰$ and continued to increase. These results indicate that the methanol concentrations *in situ*, which diluted the ^{13}C-methanol added, were ≤ 0.1 μM and that the turnover of methanol contributed only about 2% to total CH_4 production at 0.1 μM. However, contribution increased up to 5 and 17% when 1 and 10 μM methanol were added, respectively. Anoxic rice soil that was incubated at different temperatures between 10 and 37 °C exhibited maximally 2–6% methanol-dependent methanogenesis about 1–2 h after addition of 1 μM ^{13}C-methanol. Only at 50 °C, contribution of methanol to CH_4 production reached a maximum of 10%. After longer (7–10 h) incubation, however, contribution generally was only 2–4%. Methanol accumulated in the soil when CH_4 production was inhibited by chloroform. However, the accumulated methanol accounted for only up to 0.7 and 1.2% of total CH_4 production at 37 and 50 °C, respectively. Collectively, our results show that methanol-dependent methanogenesis was operating in anoxic rice field soil but contributed only marginally to total CH_4 production and the isotope effect observed at both low and high temperature.

Introduction

In anoxic sediments and soil, methanol is produced during the anaerobic decomposition of pectin (Schink and Zeikus 1982). Pectin, which is a common constituent of plant and algal cells, is a polymer of galacturonic acid that is methoxylated at the carboxy groups to a varying extent. This methoxy group is released as methanol during anaerobic microbial degradation (Schink and Zeikus 1980). Therefore, methanol can be expected to be a common metabolite in anoxic soils and sediments. There are only few investigations of methanogenic methanol turnover in anoxic environments (Winfrey et al. 1977; Oremland et al. 1982; King et al. 1983; Lovley and Klug 1983; Nüsslein and Conrad

2000). All these studies indicate only a marginal importance of methanol as methanogenic substrate, generally contributing less than 5–10% to total methanogenesis. In marine sediments, methanol is a non-competitive substrate of methanogenesis, i.e. it supports CH_4 production despite the simultaneously occurring sulfate reduction that successfully competes for the other methanogenic substrates (i.e. H_2 and acetate) (Oremland et al. 1982; Oremland 1988). Even in this environment, however, methanol was found to be a far less important methanogenic substrate than trimethylamine, which is another non-competitive substrate of methanogenesis (Oremland et al. 1982).

Several genera of the methanogenic archaea are able to utilize methanol as substrate, i.e. *Methanosphaera* within the *Methanobacteriaceae*, and *Methanosarcina*, *Methanolobus* and other genera within the *Methanosarcinaceae* (Boone et al. 1993). *Methanosarcinaceae* and *Methanobacteriaceae* are abundant constituents of the active methanogenic microflora in anoxic rice field soils (Lueders and Friedrich 2002). However, it is unknown whether they are able to utilize methanol and how important this metabolism is.

Even a moderate methanol-dependent methanogenesis might have a dramatic effect on the stable isotopic signature of the produced CH_4, since conversion of methanol to CH_4 exhibits a large isotope effect of about $\varepsilon = -74‰$ for ^{13}C (Krzycki et al. 1987). Stable isotopic signatures are frequently used to constrain CH_4 production pathways in anoxic soil and sediments (Sugimoto and Wada 1993; Tyler et al. 1997; Avery et al. 1999). Recently, we observed a rather large isotope effect ($\varepsilon \approx -70‰$) for the conversion of CO_2 to CH_4 in rice field soil (Conrad et al. 2002; Fey et al. 2003). Studies on other rice field soils mostly reported smaller isotope effects ($\varepsilon = -45$ to $-60‰$) for the conversion of CO_2 to CH_4 (Sugimoto and Wada 1993; Tyler et al. 1997; Chidthaisong et al. 2002). The larger isotope effect is relatively robust, since it has been constrained by radiotracer measurements, in particular at incubation temperatures of 50 °C, where CH_4 is exclusively produced from CO_2 reduction (Fey et al. 2001, 2003). However, our previous studies did not consider that methanol-dependent CH_4 production might have contributed to the strong isotopic fractionation observed.

Therefore, we determined the contribution of methanol to total CH_4 production in anoxic rice field soil, by measuring the conversion of $^{13}CH_3OH$ to $^{13}CH_4$ and the accumulation of methanol upon inhibition of methanogenesis.

Materials and methods

The soil samples and incubation procedures were the same as described by Fey and Conrad (2000). The soil was collected in March 1998 (after plowing) from the not yet flooded rice fields of the Italian Rice Research Institute in Vercelli, Italy. The soil was air-dried and stored in dry lumps at room temperature. These storage conditions were found to be optimal for studies of CH_4

production (Mayer and Conrad 1990). The dry lumps were broken and passed through a stainless steel sieve (2 mm). Soil slurries were prepared by mixing 200 g dry soil with 200 ml anoxic, sterile distilled water giving 0.75 g dry soil per ml slurry. The soil slurry was then kept in stoppered 500-ml glass bottles under N_2 atmosphere at 37 °C for about 2 months. In some experiments it was also kept at other temperatures.

Incubation of soil with ^{13}C-labeled methanol ($^{13}CH_3OH$; 99% enriched; Cambridge Isotope Laboratories, MA, USA) was done in triplicate by filling 10-ml aliquots of the preincubated anoxic slurry into 35-ml serum bottles, which were closed with black rubber stoppers and gassed with N_2. Appropriate amounts of a solution of 0.1 M $^{13}CH_3OH$ were injected to give a final concentration of 0.1, 1.0 or 10 μM (relative to the volume of the slurry). Gas samples were repeatedly taken during the further incubation and analyzed for CH_4 and $\delta^{13}CH_4$. The recovery of $^{13}CH_3OH$ was measured at the end of two experiments (1 and 10 μM initial $^{13}CH_3OH$) by mass balance with the total $^{13}CO_2$ and $^{13}CH_4$ formed, resulting in 102 $\pm$ 33%.

Aliquots of soil slurry were analyzed for CH_3OH concentration. Accumulation of CH_3OH was measured upon inhibition of CH_4 production in anoxic soil slurries incubated at 37 and 50 °C. Glass bottles (150 ml) were filled with 40 g-dw soil and 40 ml anoxic, sterile distilled water, stoppered and gassed with N_2 and incubated in 8 replicates at each temperature until CH_4 production rates become constant. During this time, the ^{13}C-isotopic composition of CH_4 and CO_2 was analyzed. Then, the gas headspace was replaced by fresh N_2 and chloroform was injected into 4 replicates to give a final concentration of 200 μM. Incubation was continued and CH_4 concentrations were repeatedly measured. At two time points, 2 ml slurry was removed, centrifuged, the supernatant membrane-filtered (PTFE; 0.2 μm; Schleicher & Schüll, Dassel, Germany) and immediately analyzed for methanol.

CH_4 and CO_2 were analyzed by gas chromatography. CO_2 was detected by FID upon conversion to CH_4 with a methanizer (Ni-catalyst at 350 °C, Chrompack, Middelburg, The Netherlands). Methanol was analyzed by gas chromatography using a flame ionization detector (Carlo Erba, Milano, Italy). For methanol analysis, the liquid samples were amended with amylalcohol as internal standard. Methanol (1 μl sample) was separated on a capillary column (BP 20, 0.32 mm ID, 25 m length, 0.5 μm film thickness, SGE, Victoria, Australia) using a split/splitless injector, N_2 as carrier gas at 2.4 ml min^{-1} and a temperature program of 50 °C (5 min), 10 °C min^{-1}, 200 °C (2 min). The detection limit was 10 μM CH_3OH.

Stable isotope analysis of $^{13}C/^{12}C$ in gas samples was performed using a gas chromatograph combustion isotope ratio mass spectrometer (GCC-IRMS) system that was purchased from Thermoquest (Bremen, Germany).The principle operation was described (Sugimoto et al. 1991; Brand 1996). The isotope ratios were detected with a Finnigan MAT delta plus IRMS. The CH_4 and CO_2 in the gas samples (10–400 μl) were first separated in a Hewlett Packard 6890 gas chromatograph operating with a Pora Plot Q column (27.5 m length;

0.32 mm i.d.; 10 μm film thickness; Chrompack, Frankfurt, Germany) at 25 °C and He (99.996% purity; 2.6 ml min^{-1}) as carrier gas. After conversion of CH_4 to CO_2 in the Finnigan Standard GC Combustion Interface III the gases were transferred into the IRMS. The working standards were CO_2 (99.998% purity; Messer-Griesheim, Düsseldorf, Germany) and methylstearate (Merck). The latter was intercalibrated at the Max-Planck-Institut für Biogeochemie, Jena, Germany (courtesy of Dr W. Brand) against NBS22 and expressed in the delta notation versus PDB carbonate:

$$\delta^{13}C = 10^3(R_{sa}/R_{st} - 1) \quad (1)$$

with $R = {}^{13}C/{}^{12}C$ of sample (sa) and standard (st), respectively.The precision of repeated analysis was $\pm 0.2‰$ when 1.3 nmol CH_4 was injected.

Fractionation factors for a reaction A $\rightarrow$ B are defined according to Hayes (Hayes 1993) by

$$\alpha_{A/B} = (\delta_A + 1000)/(\delta_B + 1000), \quad (2)$$

$$\varepsilon_{A/B} = (1 - \alpha_{A/B})1000 \approx \delta_B - \delta_A \quad (3)$$

The contribution of methanogenic methanol consumption to the overall isotopic signature of the produced CH_4 ($\delta^{13}CH_4$) was calculated by the following mass balance

$$\delta^{13}CH_4 = f_{CH_3OH}\delta_m + (1 - f_{CH_3OH})\delta_x \quad (4)$$

where f_{CH_3OH}, fraction of CH_4 produced from methanol; δ_m, $\delta^{13}CH_4$ derived from methanol; δ_x, $\delta^{13}CH_4$ derived from other methane precursors, i.e. acetate and CO_2. The $\delta^{13}CH_4$ of newly formed CH_4 was calculated from mass balance of the CH_4 detected at two subsequent time points. We do not know the isotopic composition of methanol in rice field soil. Therefore, we assumed that it was the same as that of soil organic C and thus, $\delta_m = \delta^{13}C_{org} + \varepsilon_{CH_3OH/CH_4}$. With $\delta^{13}C_{org} = -26‰$ (Fey et al. 2003) and $\varepsilon_{CH_3OH/CH_4} = -74‰$ (Krzycki et al. 1987) we arrive at $\delta_m = -100‰$.

The contribution of $^{13}CH_3OH$ to total CH_4 production was calculated in the following way: the $\delta^{13}C$ values were converted back into R_{sa} (at.%) using Eq. (1) (done automatically by the software of the GC-C-IRMS). The $R_{^{13}CH_4}$ of the newly formed CH_4 is calculated from the temporal change of the mixing ratio (m; ppmv) and the isotopic signature ($\delta^{13}C$ converted to R) of the CH_4 accumulated in the headspace of the bottles:

$$R_{^{13}CH_4} = (R_m - R_0m_0)/(m - m_0) \quad (5)$$

with R_0 and m_0 being the values at the beginning of incubation. The fraction (f_{CH_3OH}) of CH_4 formed from $^{13}CH_3OH$ is then given by

$$f_{CH_3OH} = R_{^{13}CH_4}/R_{^{13}CH_3OH}. \quad (6)$$

The values of $R_{^{13}CH_4}$ were derived from measured values using Eq. (5). Those of CH_3OH can be calculated from the concentration and R = 99 at.% of the $^{13}CH_3OH$ added, and from the concentration and R = 1.1 at.% (natural abundance) of the CH_3OH originally present in the soil. The concentration of the original CH_3OH is unknown, but must be <10 μM (i.e. detection limit of our analysis). Hence, $R_{^{13}CH_3OH}$ must be >50, >91 and >99% for application of 0.1, 1.0 and 10 μM $^{13}CH_3OH$, respectively. For application of 1 and 10 μM $^{13}CH_3OH$, f_{CH_3OH} is thus virtually identical with $R_{^{13}CH_4}$.

Results

Production of CH_4 was measured in slurries of anoxic rice field soil incubated at 37 and 50 °C. Production rates were in the order of 9–11 nmol h^{-1} g-dw^{-1}. The produced CH_4 exhibited a much lower $\delta^{13}CH_4$ at 50 °C than at 37 °C. The newly formed CH_4 had on the average a $\delta^{13}CH_4$ of -80‰ at 50 °C and -46‰ at 37 °C. When $\delta^{13}CO_2$ was plotted against $\delta^{13}CH_4$, a larger apparent fractionation factor was obtained at 50 °C ($\alpha_{CO_2CH_4}$ = 1.07–1.08) than at 37 °C ($\alpha_{CO_2CH_4}$ = 1.01–1.04) (Figure 1). These results corroborate our earlier experiments (Fey et al. 2003), where we ascribed the relatively large $\alpha_{CO_2CH_4}$ at 50 °C to the fact that CH_4 was exclusively produced from H_2/CO_2, while at 37 °C acetate was the major precursor for CH_4. We neglected that methanol-

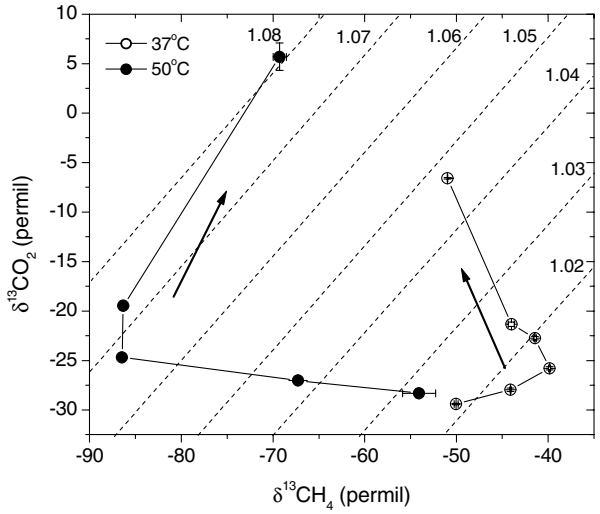

Figure 1. Values of ^{13}C of CO_2 and CH_4 produced in anoxic rice soil slurries at 37 and 50 °C during 90 days incubation. The arrow indicates increasing incubation time. The dotted lines give the apparent fractionation between CO_2 and CH_4 calculated by $\alpha = (\delta^{13}CO_2 + 1000)/(\delta^{13}CH_4 + 1000)$.

dependent methanogenesis could also have contributed to the large apparent fractionation factor. Therefore, we now studied the effect of methanol explicitly.

Addition of 0.1 μM $^{13}CH_3OH$ to anoxic soil slurry at 37 °C resulted in immediate production of $^{13}CH_4$ and linear increase of $\delta^{13}CH_4$ for about 1 h (Figure 2a). Production of $^{13}CH_4$ leveled off when a $\delta^{13}CH_4$ of about $-10‰$

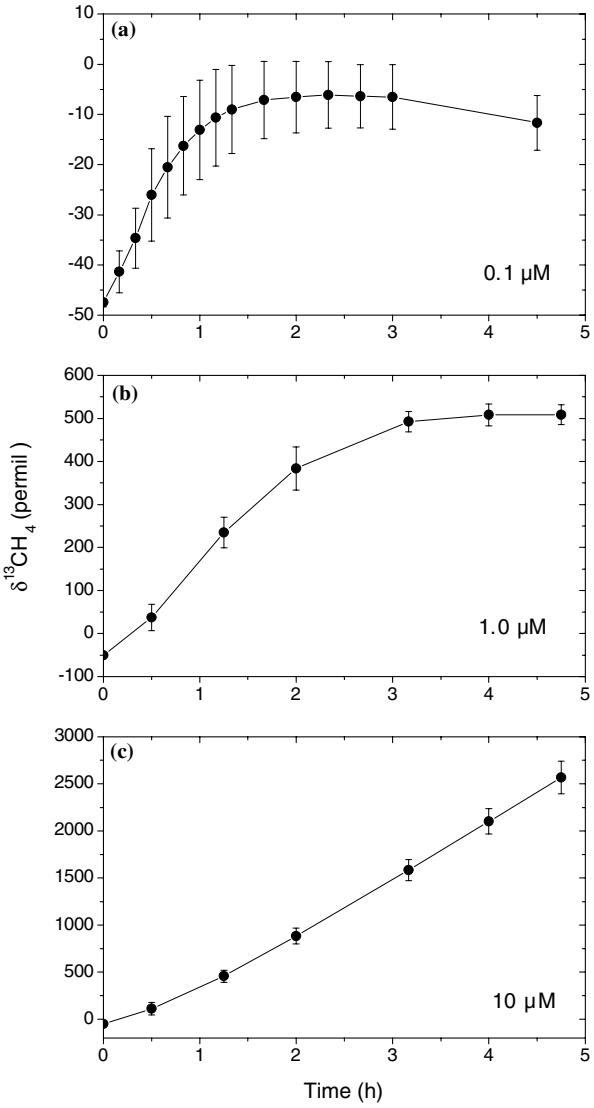

Figure 2. Temporal change of $\delta^{13}CH_4$ upon addition of different concentrations of $^{13}CH_3OH$ (99 at.%) to methanogenic rice field soil incubated at 37 °C.

was reached, and subsequently $\delta^{13}CH_4$ slowly decreased again (Figure 2a). A similar pattern was observed after addition of 1.0 μM, but $^{13}CH_4$ production lasted longer and $\delta^{13}CH_4$ reached at about $+500‰$ a much higher value (Figure 2b). Subsequently, $\delta^{13}CH_4$ again slowly decreased (not shown). This decrease indicates that $^{13}CH_3OH$ was exhausted and that the accumulated $^{13}CH_4$ was slowly diluted by $^{12}CH_4$ produced from unlabeled methanogenic precursors. When 10 μM $^{13}CH_3OH$ was added, accumulation of $^{13}CH_4$ was even stronger, and $\delta^{13}CH_4$ did not level off during the duration of the experiment (Figure 2c).

Using the maximum values of $\delta^{13}CH_4$ and the amounts of accumulated CH_4, the relative contribution of methanol-derived CH_4 to total CH_4 was calculated. The resulting values of $R_{^{13}CH_4}$ were up to 2, 5 and 17% after addition of 0.1, 1.0 and 10 μM $^{13}CH_3OH$, respectively. Increasing concentrations of methanol obviously resulted in an increasing relative contribution of methanol to total CH_4 production, at least at the peak of production of $^{13}CH_4$. This observation indicates that addition of $^{13}CH_3OH$ artificially

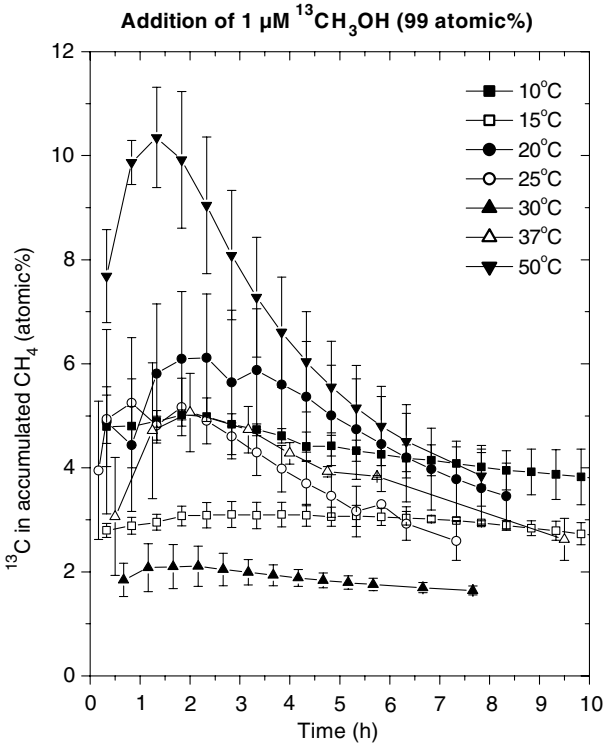

Figure 3. Temporal change of the ^{13}C content ($R_{^{13}CH_4}$) in the CH_4 accumulated upon addition of 1 μM $^{13}CH_3OH$ (99 at.%) to methanogenic rice field soil incubated at different temperatures. $R_{^{13}CH_4}$ was computed using Eq. (5).

Table 1. Balance of accumulated methanol against inhibited CH_4 production, using 200 μM chloroform as inhibitor (mean ± SD; $n = 4$)

Temperature, time of inhibition	Accumulation of CH_3OH[a] (μmol)	Inhibition of CH_4 (μmol)[b]	Percent contribution of CH_3OH
37 °C, 26 days	2.5 ± 0.4	340 ± 13	0.7 ± 0.1
37 °C, 46 days	3.0 ± 0.4	490 ± 33	0.6 ± 0.1
50 °C, 26 days	3.2 ± 0.8	269 ± 64	1.2 ± 0.4
50 °C, 46 days	4.3 ± 0.2	410 ± 96	1.1 ± 0.2

[a]Calculated from the concentration in the water phase of the soil slurry times total volume of soil water (40 ml).
[b]Calculated from the CH_4 concentration in the gas phase times total volume of headspace (94 ml).

increased the methanol pool concentration, thus stimulating methanol-dependent methanogenesis.

The experiment was repeated using anoxic soil slurries incubated at different temperatures to which 1 μM $^{13}CH_3OH$ was added. The resulting values of $\delta^{13}CH_4$ and of accumulated total CH_4 were used to calculate $R_{^{13}CH_4}$. A similar pattern was observed at all temperatures (Figure 3). After addition of $^{13}CH_3OH$, $R_{^{13}CH_4}$ increased, reached a maximum after 1–3 h, decreased again, and leveled off at values of about 2–4%. At this time, the added $^{13}CH_3OH$ was probably exhausted and CH_4 was produced at the actual *in situ* concentrations of methanogenic precursors, including methanol. There was no clear correlation between temperature and extent of methanol-dependent CH_4 production, except at 50 °C. At 50 °C, $R_{^{13}CH_4}$ increased faster and reached higher values than at the other temperatures. Ultimately, however, $R_{^{13}CH_4}$ also reached a value of up to 4% (Fig. 3), suggesting that methanol was only a marginal methanogenic substrate in anoxic Italian rice field soil.

Measurement of methanol by gas chromatography did not result in a signal, showing that the *in situ* concentration was lower than the detection limit of 10 μM. The experiment shown in Figure 1a suggests that the *in situ* concentration is probably < 0.1 μM. Addition of 200 μM chloroform resulted in complete inhibition of CH_4 production (for specificity of $CHCl_3$ see: (Chidthaisong and Conrad 2000; Scholten et al. 2000)). Instead CH_3OH accumulated reaching maximum concentrations of 110 μM in the pore water after 46 days of incubation. This concentration is equivalent to a total amount of 4.3 μmol methanol. The balance of CH_3OH against CH_4 produced in the presence and absence of chloroform, respectively, showed that CH_3OH contributed only marginally to total CH_4 production at both 37 (up to 0.7%) and 50 °C (up to 1.2%) (Table 1).

Discussion

Our results show that although methanol is rapidly converted to CH_4 in anoxic rice field soil, it contributes only little (about 1%) to total CH_4 production at

in situ conditions. Even at high temperature (50 °C) methanol-dependent methanogenesis did not play a major role, as shown by accumulation of only little methanol (up to1.2%) upon inhibition of CH_4 production. A temperature as high as 50 °C is probably never encountered under field conditions. Nevertheless, CH_4 is eventually produced at this temperature in many different rice field soils indicating the existence of a thermophilic microbial community (Yang and Chang 1998; Yao and Conrad 2000; Fey et al. 2001). Our results show that this community, as well as the communities active at lower temperatures, used methanol only to a marginal extent. However, contribution of methanol as methanogenic precursor increased, at least temporarily, when methanol was added to the soil. As little as 1 μM methanol was sufficient to increase the contribution of methanol-dependent methanogenesis up to 10% of total CH_4 production, *albeit* for only few hours. Obviously, methanol-utilizing methanogens are rapidly responding to addition of substrate by increase of activity.

The potential for methanol utilization is found within the families of *Methanosarcinaceae* and *Methanobacteriaceae*, members of which are active constituents of the archaeal community in anoxic rice field soils (Ramakrishnan et al. 2001; Lueders and Friedrich 2002). However, it remains unknown, which specific populations actually responded to the addition of methanol in the Italian rice field soil.

Since methanol-utilizing taxa of methanogens are found in many different anoxic soils and sediments, it is not surprising that addition of methanol to samples of such environments results in stimulation of CH_4 production (Naguib 1982; Nozhevnikova et al. 1997). However, such a potential for methanogenic utilization of methanol does not allow the conclusion that methanol is really an important precursor for CH_4 production *in situ*. Since pectin is, compared to cellulose and hemicellulose, a lesser constituent of plant cells, formation rates of methanol can be expected to be much lower than those of saccharide fermentation products, acetate in particular. Indeed, acetate, followed by H_2/CO_2, is usually the most important precursor of CH_4 formation in anoxic freshwater environments (Conrad 1999). The present study shows that methanol was only a marginal substrate for methanogenesis unless it was added at higher concentrations.

Also at 50 °C, the fraction of methanol-dependent methanogenesis (f_{CH_3OH}) was only about 1%. This low contribution could have only a marginal effect on the isotopic signature of the produced CH_4. However, it cannot be ruled out that methanol turnover in soil exhibits activity peaks on a microscale and/or during short periods, during which methanol may accumulate to relatively high concentrations. Analytical techniques hardly allow detecting these concentrations. Nevertheless, f_{CH_3OH} could then be on the order of 10%, *albeit* for a short time and/or on a microscale. Therefore, we calculated (using Eq. (4)) the effect of f_{CH_3OH} on the $\delta^{13}CH_4$ of the produced CH_4 against a background of $\delta^{13}C$ ($=\delta_x$) of CH_4 produced from other precursors, such as H_2/CO_2 and acetate (Figure 4). For background CH_4 production we assumed $\delta^{13}C$

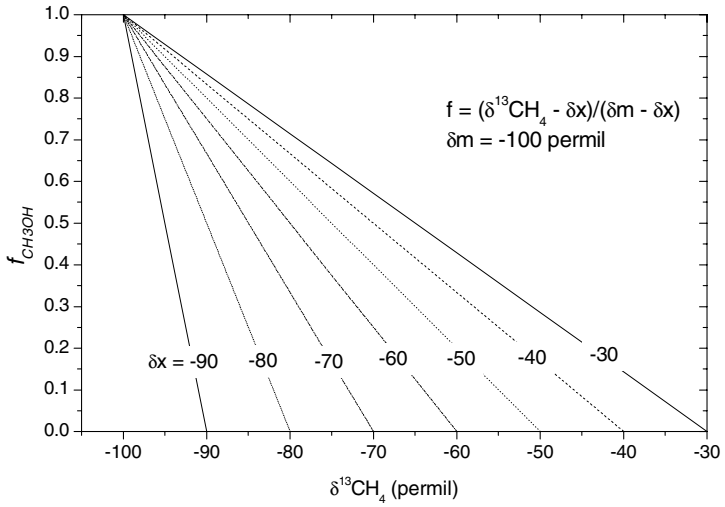

Figure 4. Relation between the fraction (f_{CH_3OH} of methanol-dependent methanogenesis and the $\delta^{13}C$ of the produced CH_4 against a background of other processes producing CH_4 with a $\delta^{13}C$ of δ_x and assuming that the $\delta^{13}CH_4$ of methanol-dependent mehanogenesis is $\delta_m = -100‰$.

published for CH_4 produced from acetate (-30 to $-50‰$) or CO_2 (-60 to $-95‰$) (Sugimoto and Wada 1993; Fey et al. 2003). The calculations show that a f_{CH_3OH} of 10% would decrease the $\delta^{13}CH_4$ of the produced CH_4 by 1–4‰, when background CH_4 production is due to CO_2 reduction, i.e. $\delta_x = -90$ to $-80‰$, and would decrease the $\delta^{13}CH_4$ by 5–7‰, when background CH_4 production is due to acetate utilization, i.e. $\delta_x = -30$ to $-50‰$. At a larger f_{CH_3OH}, the $\delta^{13}C$ of the produced CH_4 would become proportionally lower. These calculations assume that the $\delta^{13}C$ of the CH_4 produced from methanol is $\delta_m = -100‰$. In fact, if methanol is a limiting substrate for the methanogenic archaea, fractionation may be much smaller and thus, δ_m may be higher and the isotopic signal due to methanol-dependent methanogenesis may be smaller than calculated above.

In conclusion, methanol-dependent methanogenesis may have a significant effect on the isotopic composition of the produced CH_4, but only if the other methanogenic reactions produce CH_4 with a relatively high $\delta^{13}C$ (e.g. acetoclastic methanogenesis) and if f_{CH_3OH} is relatively large ($> 10\%$). In Italian rice field soil methanol turnover was apparently not important for the ^{13}C-isotopic signature of CH_4. Therefore, the rather high fractionation factors ($\varepsilon \approx -70‰$) determined for this soil (Conrad et al. 2002; Fey et al. 2003) cannot be biased by methanol-dependent methanogenesis. The value of f_{CH_3OH} under the actual field conditions, i.e. in the presence of plants that potentially release methanol into the soil, is unknown. However, a higher contribution of methanol to CH_4 production is unlikely, since methanol has never been detected in the soil and apparent fractionation factors for the conversion of CO_2 to CH_4 were relatively

low ($\varepsilon = -45$ to $-60‰$) under field conditions conditions (Krüger et al. 2002). However, this may be different in other methanogenic environments with rather low $\delta^{13}CH_4$, such as bogs and marine sediments (Whiticar et al. 1986; Lansdown et al. 1992; Hornibrook et al. 2000; Nakagawa et al. 2002). There, the low $\delta^{13}CH_4$ is usually ascribed to CH_4 production from CO_2. However, the possibility of CH_4 production from methanol should not be completely dismissed. Similarly, trimethyl amine (Summons et al. 1998), and possibly other methylated substrates, may also be a source for low $\delta^{13}CH_4$.

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