

Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems

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Abstract. The degradation of organic matter to CH₄ and CO₂ was investigated in three different boreal peatland systems in Finland, a mesotrophic fen (MES), an oligotrophic fen (OLI), and an ombrotrophic peat (OMB). MES had similar production rates of CO₂ and CH₄, but the two nutrient-poor peatlands (OLI and OMB) produced in general more CO₂ than CH₄. $\delta^{13}\text{C}$ analysis of CH₄ and CO₂ in the presence and absence methyl fluoride (CH₃F), an inhibitor of acetoclastic methanogenesis, showed that CH₄ was predominantly produced by hydrogenotrophic methanogenesis and that acetoclastic methanogenesis only played an important role in MES. These results, together with our observations concerning the collective inhibition of CH₄ and CO₂ production rates by CH₃F, indicate that organic matter was degraded through different paths in the mesotrophic and the nutrient-poor peatlands. In the mesotrophic fen, the major process is canonical fermentation followed by acetoclastic and hydrogenotrophic methanogenesis, while in the nutrient-poor peat, organic matter was apparently degraded to a large extent by a different path which finally involved hydrogenotrophic methanogenesis. Our data suggest that degradation of organic substances in the oligotrophic environments was incomplete and involved the use of organic compounds as oxidants.

1 Introduction

Northern peatlands cover about 400 million km² (Gorham, 1991) and are important emitters of the greenhouse gas methane (Matthews and Fung, 1987; Bartlett and Harriss, 1993). Our knowledge about the methanogenic substrates and the pathway by which CH₄ is produced is, however, still limited. Anaerobic degradation of organic matter eventually results in the production of acetate, CO₂ and H₂ as end products of fermentation (Zinder, 1993). Degradation of cellulose, for example, would result in the production of 2 acetate, 2 CO₂ and 4 H₂ from each hexose molecule, which are then further converted by acetoclastic and hydrogenotrophic methanogenesis to 3 CH₄ and 3 CO₂ (Conrad, 1999). Under these conditions, 2 CH₄ are derived from acetate and 1 CH₄ from H₂/CO₂. In fact, this path of CH₄ production has been demonstrated in various peat bogs ranging from Michigan (Avery et al., 1999), western Siberia (Kotsyurbenko et al., 2004) to the permafrost region of northwestern Siberia (Metje and Frenzel, 2007). In some peat ecosystems, however, acetoclastic methanogenesis is apparently impeded and CH₄ is mainly produced from H₂/CO₂ (Lansdown et al., 1992; Horn et al., 2003; Metje and Frenzel, 2005; Prater et al., 2007). In Alaskan peatland acetate was found to accumulate instead of being further converted to CH₄ (Duddlestone et al., 2002). In a Finnish peat bog part of the acetate was found to be further converted to butyrate (Metje and Frenzel, 2005). Later studies indicated that a decreasing pH resulted in decreasing acetate turnover and in the relative dominance of hydrogenotrophic methanogenesis (Kotsyurbenko et al., 2007), and that the type of vegetation, i.e., dominance of *Sphagnum*



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over vascular plants, coincides with the occurrence of acetate accumulation (Hines et al., 2008). When acetoclastic methanogenesis operates, it seems to occur preferably in the upper peat layers, whereas the deep layers are dominated by CH₄ production from H₂/CO₂ (Popp et al., 1999; Chasar et al., 2000; Kotsyurbenko et al., 2004). These observations indicate that the quality of the degradable organic substances may affect the path of CH₄ production (Chanton et al., 2008).

The methanogenic path is crucial for the extent of carbon isotope fractionation, as methanogenesis by CO₂ reduction exhibits a much stronger fractionation factor than acetoclastic methanogenesis (Whiticar et al., 1986). Vice versa it is principally possible to use values of $\delta^{13}\text{C}$ measured in CH₄, CO₂ and acetate to compute the relative contribution of each pathway to total CH₄ production (Conrad, 2005). This approach has also been used for peat ecosystems (Lansdown et al., 1992; Avery et al., 1999; Hornibrook et al., 2000; Nakagawa et al., 2002; Prater et al., 2007; Steinmann et al., 2008; Knorr et al., 2008). Many systems have been studied without having information on the methanogenic microbial community. The operation of the acetate-dependent path requires the presence of acetoclastic methanogenic archaea which only occur in the genera *Methanosarcina* or *Methanosaeta* (Zinder, 1993), which are not always present in peat ecosystems (Horn et al., 2003; Kotsyurbenko et al., 2007; Rooney-Varga et al., 2007). Hydrogenotrophic methanogenesis, on the other hand, occurs in almost every methanogenic taxon (Zinder, 1993), which are always present at more or less diversity in peat bogs.

Recently, we have studied three different peat ecosystems (a mesotrophic fen, an oligotrophic fen, and an oligotrophic ombrotrophic bog) in Finland, which differed in composition of the methanogenic archaeal community and also exhibited hydrogenotrophic and acetoclastic methanogenesis to different extent (Galand et al., 2005). While measuring CH₄ production at different concentrations of methyl fluoride (CH₃F), an inhibitor of acetoclastic methanogenesis, we also determined the $\delta^{13}\text{C}$ of CH₄, CO₂ and acetate. We report these data and quantify the relative contribution of hydrogenotrophic and acetoclastic methanogenesis to CH₄ production. We hypothesized that the different peat ecosystems differ in the extent of isotope fractionation due to different paths of CH₄ production with the nutrient poor ombrotrophic and oligotrophic systems exhibiting larger isotope fractionation than the mesotrophic fen.

2 Methods

Samples – Three replicate peat profiles were taken with a box sampler (8×8×100 cm) in August 2003 from the Lakkasuo mire complex in central Finland (61°48′ N, 24°19′ E). The samples were taken from a mesotrophic fen (MES), an oligotrophic fen (OLI) and an ombrotrophic bog (OMB) at a depth of 10–20 cm below the water level. These layers ex-

hibited the highest potential CH₄ production rates (Galand et al., 2002). The hydrological conditions and vegetation cover of the sites have already been described in detail (Juottonen et al., 2005). Briefly, MES is a mesotrophic fen, the vegetation of which is a mosaic of lawn and minerotrophic hollow level communities with high diversity. The field layer in both communities is characterized by sedges (*Carex rostrata*, *C. lasiocarpa*) and some herbaceous species, such as *Potentilla palustris* and *Menyanthes trifoliata*. In the drier lawn surfaces, the bottom layer is dominated by *Sphagnum* mosses (*S. fallax*, *S. flexuosum*, *S. magellanicum*), whereas in wetter hollow surfaces *Sphagnum subsecundum* is found together with *Warnstorfia exannulata* and *Utricularia intermedia*. Study site OLI is an oligotrophic fen, which consists of a fairly homogenous lawn level vegetation, dominated by *C. lasiocarpa* with some *Betula nana* in the field layer, and *Sphagnum papillosum*, *S. fallax* and *S. flexuosum* in the moss layer. Water table in both fen sites MES and OLI is near the surface and has small spatial and seasonal variation. Site OMB is an ombrotrophic bog. It is a mosaic of ecohydrological gradients shown as changing plant communities from wet hollows to intermediate lawns and finally to drier hummock communities. In addition to spatial variation, water level has large seasonal variations. *Eriophorum vaginatum*, together with *Andromeda polifolia* and *Rubus chamaemorus*, is the most abundant field layer species; *Sphagnum cuspidatum* dominates in the bottom layer of the hollows, *S. balticum* in the lawns and *S. fuscum* in the hummocks.

Incubation experiments – Peat samples were incubated anaerobically at 10 °C in 100-mL infusion bottles as described before (Galand et al., 2002). For inhibition of acetoclastic methanogenesis methyl fluoride (CH₃F) (99%, ABCR, Karlsruhe, Germany) was added to the gas phase to give a final mixing ratio of 0.5–2.0% CH₃F. Aliquots of the gas phase were regularly analyzed for CH₄ and CO₂. Methane was analyzed by gas chromatography using a flame ionization detector; CO₂ was analyzed after conversion to CH₄ with a methanizer. At the end of incubation, the pore water was recovered by centrifugation and filtration through 0.2- μm pore size membrane filters (SRP 15; Sartorius, Göttingen, Germany). The pH was measured using a glass electrode. Acetate (and other fatty acids) was analyzed by high pressure liquid chromatography (HPLC) (Sykam, Gilching, Germany) equipped with both refraction index detector and UV detector (Krumböck & Conrad 1991). The $\delta^{13}\text{C}$ of CH₄ and CO₂ were analyzed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS), and the $\delta^{13}\text{C}$ of acetate was analyzed by HPLC-C-IRMS as described before (Conrad et al., 2007). Analysis of $\delta^{13}\text{C}$ in organic matter was done at the Institute of Soil Science and Forest Nutrition (IBW) at the University of Göttingen using an elemental analyzer coupled to an IRMS.

Calculations – Fractionation factors for a reaction $A \rightarrow B$ are defined after Hayes (Hayes 1993):

$$\alpha_{A,B} = (\delta^{13}C_A + 1000)/(\delta^{13}C_B + 1000) \quad (1)$$

sometimes expressed as isotopic enrichment factor $\varepsilon \equiv 1 - \alpha$ (in units of permil). The $\delta^{13}C$ for a newly formed CH_4 ($\delta^{13}C_{new}$) was calculated from the $\delta^{13}C$ at two time points $t = 1$ ($\delta^{13}C_1$) and $t = 2$ ($\delta^{13}C_2$) by the following mass balance Reaction:

$$\delta^{13}C_2 = f_{new} \delta^{13}C_{new} + (1 - f_{new}) \delta^{13}C_1 \quad (2)$$

with f_{new} being the fraction of the newly formed C-compound relative to the total at $t = 2$.

The fractionation factor for conversion of H_2/CO_2 to CH_4 is given by

$$\alpha_{CO_2,CH_4} = (\delta^{13}C_{CO_2} + 1000)/(\delta^{13}C_{CH_4-CH_3F} + 1000) \quad (3)$$

where $\delta^{13}C_{CH_4-CH_3F}$ is the $\delta^{13}C_{CH_4}$ produced in the presence of CH_3F , i.e., with acetoclastic methanogenesis inhibited.

Relative contribution of $H_2 + CO_2$ -derived CH_4 to total CH_4 was determined using the following mass balance Reaction (Conrad, 2005):

$$f_{CO_2,CH_4} = (\delta^{13}C_{CH_4} - \delta^{13}C_{CH_4-ac}) / (\delta^{13}C_{CH_4-CO_2} - \delta^{13}C_{CH_4-ac}) \quad (4)$$

where f_{CO_2,CH_4} is the fraction of CH_4 formed from $H_2 + CO_2$, $\delta^{13}C_{CH_4}$ the $\delta^{13}C$ of total produced methane, and $\delta^{13}C_{CH_4-ac}$ and $\delta^{13}C_{CH_4-CO_2}$ are the $\delta^{13}C$ of CH_4 derived either from acetate or $H_2 + CO_2$, which were determined by:

$$\delta^{13}C_{CH_4-ac} = \delta^{13}C_{org} + \varepsilon_{org,CH_4} \quad (5)$$

$$\delta^{13}C_{CH_4-CO_2} = \delta^{13}C_{CH_4-CH_3F} \quad (6)$$

In general, calculations were done using the averaged data (\pm standard error) from triplicate incubations. Total amounts of gases in the headspace of the incubation vessels were calculated from the partial pressures using the volume of the gas space and the gas constant.

3 Results

Production rates of CH_4 were much higher in peat samples from the mesotrophic fen (MES) than from the ombrotrophic peat (OMB) and the oligotrophic fen (OLI) (Table 1). The same was found for CO_2 production (Table 1). The extent of inhibition of CH_4 production by CH_3F was larger in MES > OMB > OLI (Table 1). Production of CH_4 was progressively inhibited with increasing concentration of CH_3F reaching maximum inhibition at 2% CH_3F (Fig. 1), except in OMB where it was already reached at 1% CH_3F

(Fig. 1). By contrast, maximum inhibition of CO_2 production was already reached at 0.5% CH_3F . However, CO_2 production was generally much less inhibited than CH_4 production (Table 1). The concentration of acetate was also highest in MES (Table 1). Those in OLI and OMB were at least one order of magnitude lower. Inhibition of acetoclastic methanogenesis should result in accumulation of acetate. Indeed acetate accumulated in MES, on the average to about 3-fold higher concentrations. However, in OLI and OMB acetate accumulated only marginally (Table 1). In MES, caproate (<700 μM), propionate (<500 μM), butyrate (<200 μM), isopropanol (<100 μM) and valerate (<60 μM) also accumulated, but in OLI and OMB accumulation of these compounds was mostly not detectable.

The $\delta^{13}C$ of the organic matter of the peat samples was similar in the different peat ecosystems, ranging between -27.4% and -26.5% (Table 1). An effect of CH_3F on the $\delta^{13}C$ of acetate could not be discerned. Therefore, all acetate data were averaged. The $\delta^{13}C$ of the averaged acetate in OMB and OLI was only by 2‰ and 5‰ larger than that of C_{org} . However, that of MES was by almost 9‰ larger than that of C_{org} .

The $\delta^{13}C$ of CO_2 was relatively constant with incubation time (Fig. 1). It was similar for MES and OLI (i.e., about -17%) but was larger for OMB (-11%) (Table 1). Addition of CH_3F had only a slight effect on $\delta^{13}C_{CO_2}$, decreasing the values by a few permil only (Fig. 1). However, the $\delta^{13}C$ of CO_2 were generally much higher (on average 15‰) than those of C_{org} , (on average -27%), indicating that CO_2 was fractionated during its further conversion to CH_4 . Such fractionation was apparent since the $\delta^{13}C$ of CH_4 was quite negative with values around -58% in MES, -66% in OMB and -89% in OLI (Fig. 1, Table 1). Since CH_4 can be produced from both hydrogenotrophic and acetoclastic pathways, the latter was inhibited by addition of CH_3F so that $\delta^{13}C$ of CH_4 was only affected by CO_2 reduction. Under these conditions, $\delta^{13}C_{CH_4}$ indeed further decreased already at the lowest CH_3F concentration (Fig. 1). Interestingly, addition of CH_3F resulted only a comparatively small decrease of $\delta^{13}C_{CH_4}$ when added to OMB and OLI, indicating that acetoclastic methanogenesis did not contribute much to CH_4 production in these peat ecosystems.

Assuming that any acetoclastic methanogenesis was inhibited completely by the presence of CH_3F , it is possible to calculate the fractionation factor of hydrogenotrophic methanogenesis (α_{CO_2,CH_4} or ε_{CO_2,CH_4}) from the difference between the $\delta^{13}C_{CH_4}$ in the absence and the presence of CH_3F . The fractionation factor was largest in OLI > MES > OMB, i.e., ε_{CO_2,CH_4} ranging between -78.5% and -66.8% (Table 1).

The fraction (f_{CO_2,CH_4}) of hydrogenotrophically produced CH_4 to total CH_4 production was calculated from Eq. (4). The calculation assumed that the $\delta^{13}C$ of hydrogenotrophically produced CH_4 ($\delta^{13}C_{CH_4-CO_2}$) was identical to the $\delta^{13}C_{CH_4}$ measured in the presence of CH_3F , when acetoclastic methanogenesis was inhibited and CH_4 was exclusively

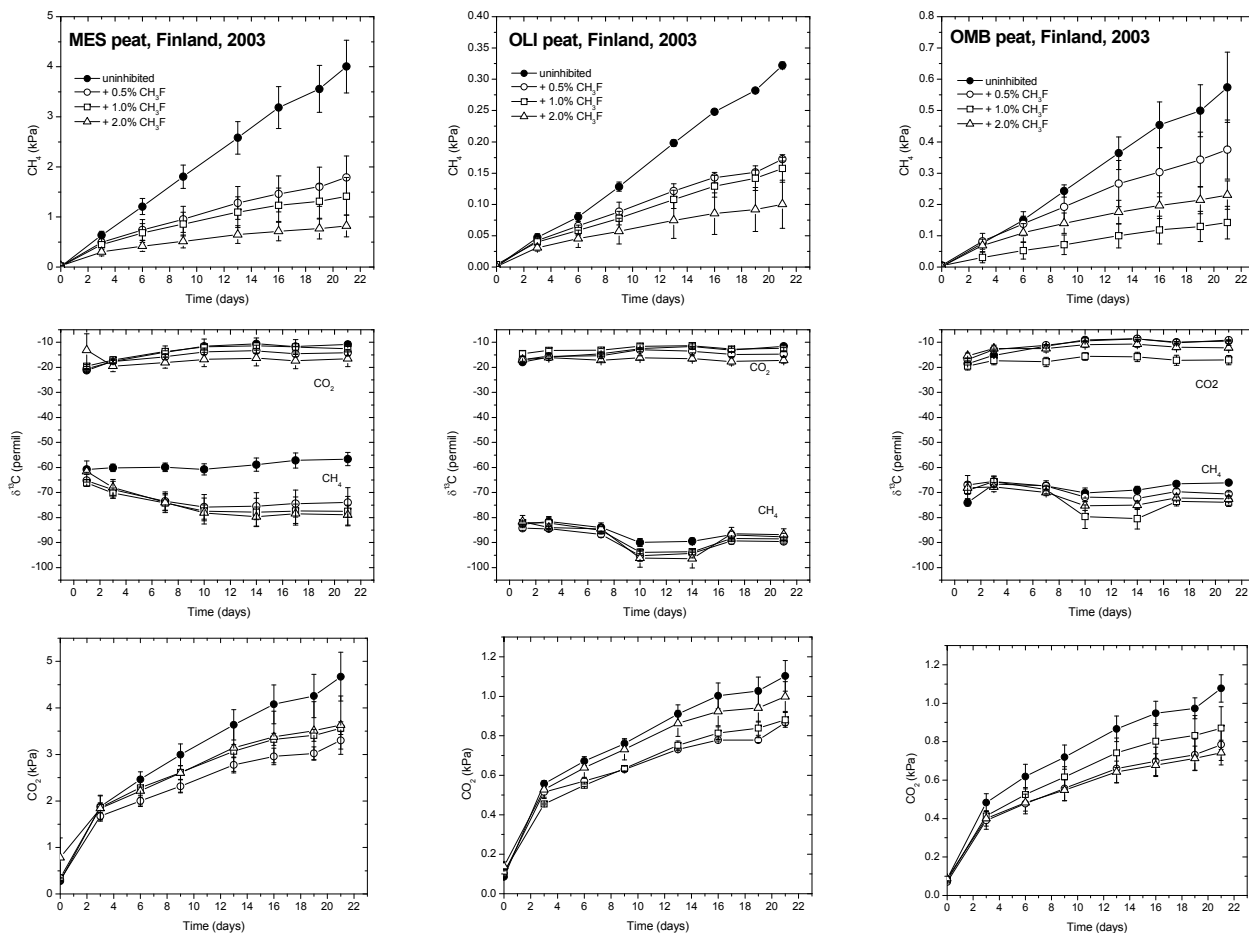


Fig. 1. Time course of accumulation of CH₄ and CO₂, and of δ¹³C of the accumulated CH₄ and CO₂ in the absence and presence of different concentrations of CH₃F, an inhibitor of acetoclastic methanogenesis (CH₃F) using samples from three different peatland ecosystems in Finland, i.e., mesotrophic fen (MES), oligotrophic fen (OLI), and ombrotrophic bog (OMB); mean ± SE, *n* = 3.

produced from H₂/CO₂. The calculation further assumed that the δ¹³C of acetoclastically produced CH₄ (δ¹³C_{CH₄-ac}) was similar to δ¹³C_{org}. Previous studies have found that the δ¹³C of the acetate-methyl from which CH₄ is formed is less than 9‰ smaller than δ¹³C_{org} (Conrad et al., 2007, 2009a, 2009b, 2010b). In OMB and OLI acetate concentrations were so low that acetate was probably utilized as it was produced so that there was no further carbon isotope fractionation during the conversion of acetate-methyl to CH₄. In MES, acetate concentrations were larger, so that further fractionation is feasible. This fractionation should be on the order of less than 10‰ as typical for *Methanosaeta* (Valentine et al., 2004; Penning et al., 2006), which was the prevailing acetoclastic methanogen in MES (Juottonen et al., 2005) (Galand et al., 2005). Therefore, we assumed values of δ¹³C_{CH₄-ac} being 5–10‰ smaller than δ¹³C_{org}. The resulting *f*_{CO₂,CH₄} showed that CH₄ production in MES was predominantly by acetoclastic methanogenesis, whereas CH₄ production in OMB and even more in OLI was predominantly due to hydrogenotrophic methanogenesis (Table 1).

4 Discussion

Our study demonstrated that different peatlands in Finland exhibited different carbon isotope fractionation during degradation of organic matter under anaerobic conditions. These differences were obvious from the fact that while δ¹³C values of organic matter, the primary substrate, were similar (−27 to −26‰) in all three peatlands, the δ¹³C values of CH₄, the end product of degradation, were quite different. Rates of organic matter degradation, as shown by CH₄ and CO₂ production, and concentrations of the degradation intermediate acetate were also quite different among the three peatlands. The differences in stable carbon isotope fractionation were explained by different paths of organic matter degradation and different prevalence of the acetoclastic versus hydrogenotrophic methanogenesis.

Table 1. Production rates of CH₄ and CO₂, concentrations of acetate, values of δ¹³C, isotopic enrichment factors and fractions of CH₄ produced from CO₂ in samples from different boreal peatland ecosystems, i.e., mesotrophic fen (MES), oligotrophic fen (OLI), and ombrotrophic bog (OMB).

Variables	MES peat	OLI peat	OMB peat
pH	5.3 ± 0.1	5.2 ± 0.1	3.9 ± 0.2
CH ₄ production (nmol h ⁻¹ gdw ⁻¹)	210 ± 77	15 ± 4	40 ± 13
CH ₄ production (nmol h ⁻¹ gdw ⁻¹), + 2% CH ₃ F	38 ± 7 (18%)	4.2 ± 4.2 (28%)	14.6 ± 3.3 (36%)
CO ₂ production (nmol h ⁻¹ gdw ⁻¹)	167 ± 99	29 ± 2	45 ± 6
CO ₂ production (nmol h ⁻¹ gdw ⁻¹), + 2% CH ₃ F	113 ± 5 (68%)	25 ± 1 (86%)	27 ± 1 (60%)
Acetate (μM)	800 ± 490	85 ± 25	30 ± 20
Acetate (μM), + 2% CH ₃ F	2420 ± 1290	125 ± 125	50 ± 10
δ ¹³ C _{org} (‰)	-27.3 ± 0.1	-27.4 ± 0.1	-26.5 ± 0.2
δ ¹³ C _{ac} (‰), ± 0.5–2% CH ₃ F	-18.8 ± 1.3	-22.3 ± 0.6	-24.3 ± 1.4
δ ¹³ C _{CH₄} (‰)	-58.4 ± 0.9	-88.9 ± 4.8	-65.6 ± 3.7
δ ¹³ C _{CH₄} (‰), + 2% CH ₃ F	-78.8 ± 0.3	-86.4 ± 25.0	-73.1 ± 9.6
δ ¹³ C _{CO₂} (‰)	-16.8 ± 0.2	-16.9 ± 0.3	-11.5 ± 0.4
ε _{CO₂,CH₄} (‰)	-72.6 ± 7.3	-78.5 ± 29.3	-66.8 ± 11.2
f _{CO₂,CH₄} (%), A ¹	46 ± 2	89 ± 9	78 ± 4
f _{CO₂,CH₄} (%), B ¹	41 ± 2	88 ± 10	76 ± 4

¹ f_{CO₂,CH₄} was calculated using Eq. (4) assuming (A) δ¹³C_{CH₄-ac} = δ¹³C_{org} - 5, and (B) δ¹³C_{CH₄-ac} = δ¹³C_{org} - 10.

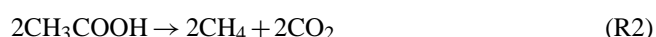
Production rates of CH₄ and CO₂ were highest in peat from a mesotrophic fen (MES). The rates in the other peat samples were less than 25% of those in MES. Rates were slightly higher in peat from the ombrotrophic bog (OMB) than the oligotrophic fen (OLI). Rates of CH₄ production were higher than those previously reported by Juottonen et al. (2005), who sampled the peat in October whereas our samples were from August. Methanogenic degradation of organic matter normally expects the production of equimolar amounts of CH₄ and CO₂. In OLI and OMB, the rates of CO₂ production were higher than those of CH₄ production. The rates of CO₂ production only consider the gaseous CO₂ measured in the headspace of the incubation vessels. While bicarbonate concentrations were negligible in the acidic peat samples, the concentrations of dissolved CO₂ as calculated from Henry's law (Stumm and Morgan, 1981) were not negligible. Thus, rates of total CO₂ production (gaseous plus dissolved CO₂) were about 50% higher than those of gaseous CO₂ alone. Hence, only MES produced CH₄ and CO₂ in the expected equimolar amounts, while OMB and OLI produced much more CO₂ than CH₄. Such imbalance has frequently been observed in methanogenic peat samples, and has even been observed when great care was taken that potential inorganic oxidants such as oxygen, nitrate, sulphate, iron(III) etc. had been completely reduced (Yavitt and Seidmann-Zager, 2006). The reasons for such imbalance are unclear at the moment, but one possible answer is the use of organic oxidants for the degradation of organic matter, e.g. certain humic compounds that are reduced while others are concomitantly oxidized to CO₂ (Heitmann et al., 2007; Keller et al., 2009). Based on our observations, we hypothesize that or-

ganic oxidants are more important in the more oligotrophic than the mesotrophic peatlands.

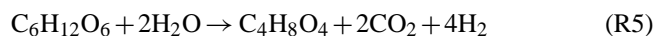
The mesotrophic peat (MES) also exhibited much higher (more than 10 times) acetate concentrations at the end of incubation than the oligotrophic peat samples (OMB, OLI). These acetate concentrations were further increased when acetoclastic methanogenesis, the only conceivable acetate degradation process, was inhibited by CH₃F. This stimulation was again more strongly expressed in MES than in OMB or OLI. Hence, MES behaved as expected for an environment in which organic matter is first fermented to acetate as the major fermentation product. Interestingly, MES also contained other potential fermentation products, i.e., caproate, propionate, butyrate, isopropanol, and valerate, albeit at much lower concentrations than acetate. Such compounds are frequently observed in methanogenic lake sediments or flooded soils (Lovley and Klug, 1982; Phelps and Zeikus 1985; Chin and Conrad, 1995), but were not detected in OMB and OLI. There, acetate and other fermentation products seemed to play a comparatively minor role in the degradation of organic matter.

If degradation produces only little acetate, then acetoclastic methanogenesis should be comparatively less important for CH₄ production, which would predominantly be formed by CO₂ reduction. Indeed, isotopic mass balance calculations indicate that CH₄ production in OMB and OLI was mainly due to hydrogenotrophic methanogenesis accounting for more than 75% of total CH₄ production. In MES, on the other hand, CH₄ was mainly (about 54–59%) produced by acetoclastic methanogenesis. These data are consistent with an earlier study in which the percentage contribution

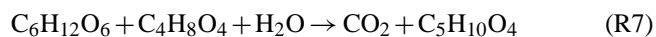
of hydrogenotrophic versus acetoclastic methanogenesis was determined by measuring the conversion of ^{14}C -labelled bicarbonate to CH_4 (Galand et al., 2005). Theoretically, one would expect that >66% of the CH_4 is produced by acetoclastic methanogenesis, if organic matter, such as polysaccharides, proteins, lipids etc., is completely degraded (Conrad 1999; Conrad et al., 2010a). Hence, it appears that even in MES part of the organic matter is degraded in a non-canonical way. We assume that in peatlands organic substances are only partially degraded rather than completely. This speculation is consistent with recent studies in lake sediments (Conrad et al., 2009a; 2010b), in particular with a study in the sediment of an acidic bog lake (Conrad et al., 2010a). Thus the complete degradation of an organic substance, e.g.,



would contrast with incomplete degradation of an organic substance, e.g.,



and the oxidation of one organic substance by using another one as oxidant, e.g.



Our data concerning $f_{\text{CO}_2, \text{CH}_4}$ and relative production rates of CH_4 versus CO_2 would be consistent with organic matter in OMB and OLI being mainly degraded by processes Reactions (R6 and R7), while in MES being mainly degraded by process Reaction (R4).

This interpretation is also consistent with the effect of CH_3F , which showed the strongest inhibition (18% residual activity) for CH_4 production in MES, which was presumably caused by complete inhibition of acetoclastic methanogenesis and in addition by partial inhibition of hydrogenotrophic methanogenesis. Although acetoclastic methanogenesis is more sensitive, hydrogenotrophic methanogenesis was found to be also inhibited at increasing concentrations of CH_3F (Conrad and Klose, 1999). Hence the observed decrease of CH_4 production with increasing CH_3F (Fig. 1) is not unexpected. Acetoclastic methanogenesis was probably completely inhibited at 1% CH_3F , since values of $\delta^{13}\text{C}_{\text{CH}_4}$ did not decrease further when more CH_3F was added (Fig. 1). Only

in MES, but not in OMB or OLI, did CH_3F result in a strong decrease of $\delta^{13}\text{C}_{\text{CH}_4}$. A strong decrease is expected when most of the CH_4 is produced by acetoclastic methanogenesis, which exhibits a much lower fractionation factor ($\alpha_{ac, \text{CH}_4} \approx 1.009\text{--}1.025$) (Valentine et al., 2004; Penning et al., 2006; Goevert and Conrad, 2009) than hydrogenotrophic methanogenesis (as much as $\alpha_{\text{CO}_2, \text{CH}_4} \approx 1.090$) (Conrad 2005; Penning et al., 2005). In OMB and even more so in OLI, $\delta^{13}\text{C}_{\text{CH}_4}$ exhibited very low values already when CH_3F was not applied and decreased only a bit further upon application. In MES, on the other hand, $\delta^{13}\text{C}_{\text{CH}_4}$ decreased only in the presence of CH_3F to values comparable to those found in OLI and OMB (note that data in Table 1 are from newly formed CH_4). The isotopic fractionation factors determined were on the order of $\alpha_{\text{CO}_2, \text{CH}_4} \approx 1.067\text{--}1.078$, or $\epsilon_{\text{CO}_2, \text{CH}_4} \approx -78$ to -67% ; Table 1). Partial inhibition of hydrogenotrophic methanogenesis by CH_3F is also consistent with the observation that CO_2 production was less inhibited by CH_3F than CH_4 production. Inhibition of only acetoclastic methanogenesis would result in equal inhibition of CO_2 and CH_4 production because of Reaction (R2). Inhibition of process Reaction (R3), however, would inhibit CO_2 consumption and thus result in more net CO_2 production.

A previous study found that the MES, OLI and OMB peatlands can also be distinguished on the basis of their methanogenic archaeal communities (Galand et al., 2005). Interestingly, the most abundant group of methanogens in MES was related to putatively acetoclastic *Methanosaeta* spp. On the other hand, OMB had a completely different methanogenic community composition dominated by the Fen cluster of *Methanomicrobiales*, while OLI contained a more diverse community including different clades of the Fen Cluster and Rice Cluster I (now *Methanocellales* (Sakai et al., 2008)). These microbial community differences between peatlands probably explain the presence of different paths for organic matter degradation. Noteworthy, a second study, found similar proportions of putatively acetoclastic *Methanosaeta* spp. in both OLI and MES (Juottonen et al., 2005). That study was, however, done later during the year (October vs. August).

In summary, our experiments showed that methanogenesis in peatlands was driven by two fundamentally different processes. Canonical fermentation followed by acetoclastic and hydrogenotrophic methanogenesis was a major process only in the mesotrophic fen. In the oligotrophic peat, however, organic matter was apparently degraded to a large extent by a different path which finally involved hydrogenotrophic methanogenesis as the major process while acetate formation and acetoclastic methanogenesis played only a minor role. The exact path of methanogenesis in such oligotrophic peatlands is not completely clear, but probably involves incomplete degradation of organic substances and use of organic compounds as oxidants so that CO_2 rather than CH_4 is the major degradation product. Generally, however, H_2/CO_2 and acetate were both used for CH_4 production thus contrasting

the degradation process at sites where acetoclastic methanogenesis is completely lacking and acetate accumulates over the season (Dugglestone et al., 2002; Hines et al., 2008).

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References

- Avery, G. B., Shannon, R. D., White, J. R., Martens, C. S., and Alperin, M. J.: Effect of seasonal changes in the pathways of methanogenesis on the $\delta^{13}\text{C}$ values of pore water methane in a Michigan peatland, *Global Biogeochem. Cy.*, 13, 475–484, 1999.
- Bartlett, K. B. and Harriss, R. C.: Review and assessment of methane emissions from wetlands, *Chemosphere*, 26, 261–320, 1993.
- Chanton, J. P., Glaser, P. H., Chasar, L. S., Burdige, D. J., Hines, M. E., Siegel, D. I., Tremblay, L. B., and Cooper, W. T.: Radiocarbon evidence for the importance of surface vegetation on fermentation and methanogenesis in contrasting types of boreal peatlands, *Global Biogeochem. Cy.*, 22, GB4022, doi:10.1029/2008GB003274, 2008.
- Chasar, L. S., Chanton, J. P., Glaser, P. H., Siegel, D. I., and Rivers, J. S.: Radiocarbon and stable carbon isotopic evidence for transport and transformation of dissolved organic carbon, dissolved inorganic carbon, and CH_4 in a northern Minnesota peatland, *Global Biogeochem. Cy.*, 14, 1095–1108, 2000.
- Chin, K. J. and Conrad, R.: Intermediary metabolism in methanogenic paddy soil and the influence of temperature, *FEMS Microbiol. Ecol.*, 18, 85–102, 1995.
- Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments [review], *FEMS Microbiol. Ecol.*, 28, 193–202, 1999.
- Conrad, R.: Quantification of methanogenic pathways using stable carbon isotopic signatures: a review and a proposal, *Org. Geochem.*, 36, 739–752, 2005.
- Conrad, R., Chan, O. C., Claus, P., and Casper, P.: Characterization of methanogenic *Archaea* and stable isotope fractionation during methane production in the profundal sediment of an oligotrophic lake (Lake Stechlin, Germany), *Limnol. Oceanogr.*, 52, 1393–1406, 2007.
- Conrad, R., Claus, P., and Casper, P.: Characterization of stable isotope fractionation during methane production in the sediment of a eutrophic lake, Lake Dagow, Germany, *Limnol. Oceanogr.*, 54, 457–471, 2009a.
- Conrad, R., Claus, P., and Casper, P.: Stable isotope fractionation during the methanogenic degradation of organic matter in the sediment of an acidic bog lake, Lake Grosse Fuchskuhle, *Limnol. Oceanogr.*, 54, 1932–1942, 2010a.
- Conrad, R. and Klose, M.: How specific is the inhibition by methyl fluoride of acetoclastic methanogenesis in anoxic rice field soil? *FEMS Microbiol. Ecol.*, 30, 47–56, 1999.
- Conrad, R., Klose, M., Claus, P., and Dan, J.: Activity and composition of the methanogenic archaeal community in soil vegetated with wild rice versus cultivated rice, *Soil Biol. Biochem.*, 41, 1390–1395, 2009b.
- Conrad, R., Klose, M., Claus, P., and Enrich-Prast, A.: Methanogenic pathway, ^{13}C isotope fractionation, and archaeal community composition in the sediment of two clearwater lakes of Amazonia, *Limnol. Oceanogr.*, 55, 689–702, 2010b.
- Duddleston, K. N., Kinney, M. A., Kiene, R. P., and Hines, M. E.: Anaerobic microbial biogeochemistry in a northern bog: Acetate as a dominant metabolic end product, *Global Biogeochem. Cy.*, 16, 1063, doi:10.1029/2001GB001402, 2002.
- Galand, P. E., Fritze, H., Conrad, R., and Yrjälä, K.: Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems, *Appl. Environ. Microbiol.*, 71, 2195–2198, 2005.
- Galand, P. E., Saarnio, S., Fritze, H., and Yrjälä, K.: Depth related diversity of methanogen *Archaea* in Finnish oligotrophic fen, *FEMS Microbiol. Ecol.* 42, 441–449, 2002.
- Goevert, D. and Conrad, R.: Effect of substrate concentration on carbon isotope fractionation during acetoclastic methanogenesis by *Methanosarcina barkeri* and *M. acetivorans* and in rice field soil, *Appl. Environ. Microbiol.*, 75, 2605–2612, 2009.
- Gorham, E.: Northern peatlands – Role in the carbon cycle and probable responses to climatic warming, *Ecol. Appl.*, 1, 182–195, 1991.
- Hayes, J. M.: Factors controlling ^{13}C contents of sedimentary organic compounds: principles and evidence, *Mar. Geol.*, 113, 111–125, 1993.
- Heitmann, T., Goldhammer, T., Beer, J., and Blodau, C.: Electron transfer of dissolved organic matter and its potential significance for anaerobic respiration in a northern bog, *Glob. Change Biol.*, 13, 1771–1785, 2007.
- Hines, M. E., Duddleston, K. N., Rooney-Varga, J. N., Fields, D., and Chanton, J. P.: Uncoupling of acetate degradation from methane formation in Alaskan wetlands: Connections to vegetation distribution, *Global Biogeochem. Cy.*, 22, GB2017, doi:10.1029/2006GB002903, 2008.
- Horn, M. A., Matthies, C., Küsel, K., Schramm, A., and Drake, H. L.: Hydrogenotrophic methanogenesis by moderately acid-tolerant methanogens of a methane-emitting acidic peat, *Appl. Environ. Microbiol.*, 69, 74–83, 2003.
- Hornibrook, E. R. C., Longstaffe, F. J., and Fyfe, W. S.: Evolution of stable carbon isotope compositions for methane and carbon dioxide in freshwater wetlands and other anaerobic environments, *Geochim. Cosmochim. Ac.*, 64, 1013–1027, 2000.
- Juottonen, H., Galand, P. E., Tuittila, E. S., Laine, J., Fritze, H., and Yrjälä, K.: Methanogen communities and Bacteria along an ecohydrological gradient in a northern raised bog complex, *Environ. Microbiol.*, 7, 1547–1557, 2005.
- Keller, J. K., Weisenhorn, P. B., and Megonigal, J. P.: Humic acids as electron acceptors in wetland decomposition, *Soil Biol. Biochem.*, 41, 1518–1522, 2009.
- Knorr, K.-H., Glaser, B., and Blodau, C.: Fluxes and ^{13}C isotopic composition of dissolved carbon and pathways of methanogenesis in a fen soil exposed to experimental drought, *Biogeochemistry*, 5, 1457–1473, doi:10.5194/bg-5-1457-2008, 2008.
- Kotsyurbenko, O. R., Chin, K. J., Glagolev, M. V., Stubner, S., Simankova, M. V., Nozhevnikova, A. N., and Conrad, R.,

- R.: Acetoclastic and hydrogenotrophic methane production and methanogenic populations in an acidic West-Siberian peat bog, *Environ. Microbiol.*, 6, 1159–1173, 2004.
- Kotsyurbenko, O. R., Friedrich, M. W., Simankova, M. V., Nozhevnikova, A. N., Golyshin, P. N., Timmis, K. N., and Conrad, R.: Shift from acetoclastic to H₂-dependent methanogenesis in a West Siberian peat bog at low pH values and isolation of an acidophilic *Methanobacterium* strain, *Appl. Environ. Microbiol.*, 73, 2344–2348, 2007.
- Krumböck, M. and Conrad, R.: Metabolism of position-labelled glucose in anoxic methanogenic paddy soil and lake sediment, *FEMS Microbiol. Ecol.*, 85, 247–256, 1991.
- Lansdown, J. M., Quay, P. D., and King, S. L.: CH₄ production via CO₂ reduction in a temperate bog: a source of ¹³C-depleted CH₄, *Geochim. Cosmochim. Ac.*, 56, 3493–3503, 1992.
- Lovley, D. R. and Klug, M. J.: Intermediary metabolism of organic matter in the sediments of a eutrophic lake, *Appl. Environ. Microbiol.*, 43, 552–560, 1982.
- Matthews, E. and Fung, I.: Methane emission from natural wetlands: Global distribution, area, and environmental characteristics of sources, *Global Biogeochem. Cy.*, 1, 61–86, 1987.
- Metje, M. and Frenzel, P.: Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a northern wetland, *Appl. Environ. Microbiol.*, 71, 8191–8200, 2005.
- Metje, M. and Frenzel, P.: Methanogenesis and methanogenic pathways in a peat from subarctic permafrost, *Environ. Microbiol.*, 9, 954–964, 2007.
- Nakagawa, F., Yoshida, N., Nojiri, Y., and Makarov, V. N.: Production of methane from allasses in eastern Siberia: Implications from its ¹⁴C and stable isotopic compositions, *Global Biogeochem. Cy.*, 16, 3, doi:10.1029/2000GB001384, 2002.
- Penning, H., Claus, P., Casper, P., and Conrad, R.: Carbon isotope fractionation during acetoclastic methanogenesis by *Methanosaeta concilii* in culture and a lake sediment, *Appl. Environ. Microbiol.*, 72, 5648–5652, 2006.
- Penning, H., Plugge, C. M., Galand, P. E., and Conrad, R.: Variation of carbon isotope fractionation in hydrogenotrophic methanogenic microbial cultures and environmental samples at different energy status, *Global Change Biol.*, 11, 2103–2113, 2005.
- Phelps, T. J. and Zeikus, J. G.: Effect of fall turnover on terminal carbon metabolism in Lake Mendota sediments, *Appl. Environ. Microbiol.*, 50, 1285–1291, 1985.
- Popp, T. J., Chanton, J. P., Whiting, G. J., and Grant, N.: Methane stable isotope distribution at a Carex dominated fen in north central Alberta, *Global Biogeochem. Cy.*, 13, 1063–1077, 1999.
- Prater, J. L., Chanton, J. P., and Whiting, G. J.: Variation in methane production pathways associated with permafrost decomposition in collapse scar bogs of Alberta, Canada, *Global Biogeochem. Cy.*, 21, GB4004, doi:10.1029/2006GB002866, 2007.
- Rooney-Varga, J. N., Giewat, M. W., Duddleston, K. N., Chanton, J. P., and Hines, M. E.: Links between archaeal community structure, vegetation type and methanogenic pathway in Alaskan peatlands, *FEMS Microbiol. Ecol.*, 60, 240–251, 2007.
- Sakai, S., Imachi, H., Hanada, S., Ohashi, A., Harada, H., and Kamagata, Y.: *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order *Methanocellales* ord. nov., *Int. J. Syst. Evol. Microbiol.*, 58, 929–936, 2008.
- Steinmann, P., Eilrich, B., Leuenerberger, M., and Burns, S. J.: Stable carbon isotope composition and concentrations of CO₂ and CH₄ in the deep catotelm of a peat bog, *Geochim. Cosmochim. Ac.*, 72, 6015–6026, 2008.
- Stumm, W. and Morgan, J. J.: *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters*, Wiley, New York, 1981.
- Valentine, D. L., Chidthaisong, A., Rice, A., Reeburgh, W. S., and Tyler, S. C.: Carbon and hydrogen isotope fractionation by moderately thermophilic methanogens, *Geochim. Cosmochim. Ac.*, 68, 1571–1590, 2004.
- Whiticar, M. J., Faber, E., and Schoell, M.: Biogenic methane formation in marine and freshwater environments: CO₂ reduction vs. acetate fermentation – isotopic evidence, *Geochim. Cosmochim. Ac.*, 50, 693–709, 1986.
- Yavitt, J. B. and Seidmann-Zager, M.: Methanogenic conditions in northern peat soils, *Geomicrobiol. J.*, 23, 119–127, 2006.
- Zinder, S. H.: *Physiological ecology of methanogens*, edited by: Ferry, J. G., *Methanogenesis, Ecology, Physiology, Biochemistry and Genetics* (128–206), Chapman & Hall, New York, 1993.