Biogeosciences Discuss., 7, 5497–5515, 2010
www.biogeosciences-discuss.net/7/5497/2010/
doi:10.5194/bgd-7-5497-2010

Biogeosciences Discussions

Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems

P. E. Galand[1,2], K. Yrjälä[3], and R. Conrad[4]

[1]UPMC Univ Paris 06, Observatoire Océanologique, 66651 Banyuls-sur-Mer, France
[2]CNRS, FRE 3350, Laboratoire d'écogéochimie des environnements benthiques (LECOB), Observatoire Océanologique, 66651 Banyuls-sur-Mer, France
[3]Department of Biological and Environmental Sciences, General Microbiology, University of Helsinki, 00014 Helsinki, Finland
[4]Max-Planck-Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, 35043 Marburg, Germany

Received: 24 June 2010 – Accepted: 29 June 2010 – Published: 16 July 2010

Correspondence to: R. Conrad (conrad@mpi-marburg.mpg.de)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The degradation of organic matter to CH_4 and CO_2 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 CO_2 and CH_4, but the two nutrient-poor peatlands (OLI and OMB) produced in general more CO_2 than CH_4. $\delta^{13}C$ analysis of CH_4 and CO_2 in the presence and absence methyl fluoride (CH_3F), an inhibitor of acetoclastic methanogenesis, showed that CH_4 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 CH_4 and CO_2 production rates by CH_3F, 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 km^2 (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 CH_4 is produced is, however, still limited. Anaerobic degradation of organic matter eventually results in the production of acetate, CO_2 and H_2 as end products of fermentation (Zinder, 1993). Degradation of cellulose, for example, would result in the production of 2 acetate, 2 CO_2 and 4 H_2 from each hexose molecule, which are then further converted by acetoclastic and hydrogenotrophic methanogenesis to 3 CH_4 and

BGD
7, 5497–5515, 2010

3 CO_2 (Conrad, 1999). Under these conditions, 2 CH_4 are derived from acetate and 1 CH_4 from H_2/CO_2. In fact, this path of CH_4 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 CH_4 is mainly produced from H_2/CO_2 (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 CH_4 (Duddleston 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* 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 CH_4 production from H_2/CO_2 (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 CH_4 production (Chanton et al., 2008).

The methanogenic path is crucial for the extent of carbon isotope fractionation, as methanogenesis by CO_2 reduction exhibits a much stronger fractionation factor than acetoclastic methanogenesis (Whiticar et al., 1986). Vice versa it is principally possible to use values of $\delta^{13}C$ measured in CH_4, CO_2 and acetate to compute the relative contribution of each pathway to total CH_4 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

BGD
7, 5497–5515, 2010

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 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 CH_4 production at different concentrations of methyl fluoride (CH_3F), an inhibitor of acetoclastic methanogenesis, we also determined the $\delta^{13}C$ of CH_4, CO_2 and acetate. We report these data and quantify the relative contribution of hydrogenotrophic and acetoclastic methanogenesis to CH_4 production. We hypothesized that the different peat ecosystems differ in the extent of isotope fractionation due to different paths of CH_4 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 exhibited the highest potential CH_4 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).

BGD
7, 5497–5515, 2010

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 (CH_3F) (99%, ABCR, Karlsruhe, Germany) was added to the gas phase to give a final mixing ratio of 0.5–2.0% CH_3F. Aliquots of the gas phase were regularly analyzed for CH_4 and CO_2. Methane was analyzed by gas chromatography using a flame ionization detector; CO_2 was analyzed after conversion to CH_4 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 and Conrad, 1991). The $\delta^{13}C$ of CH_4 and CO_2 were analyzed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS), and the $\delta^{13}C$ of acetate was analyzed by HPLC-C-IRMS as described before (Conrad et al., 2007). Analysis of $\delta^{13}C$ 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 equation:

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

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

BGD
7, 5497–5515, 2010

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

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

where $\delta^{13}C_{CH_4\text{-}CH_3F}$ 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 equation (Conrad, 2005):

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

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 $\delta^{13}C_{CH_4\text{-ac}}$ and $\delta^{13}C_{CH_4\text{-}CO_2}$ are the $\delta^{13}C$ of CH_4 derived either from acetate or $H_2 + CO_2$, which were determined by:

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

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

In general, calculations were done using the averaged data (± 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

BGD
7, 5497–5515, 2010

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

BGD
7, 5497–5515, 2010

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 ($\delta^{13}C_{CH_4\text{-}CO_2}$) 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 produced from H_2/CO_2. The calculation further assumed that the $\delta^{13}C$ of acetoclastically produced CH_4 ($\delta^{13}C_{CH_4\text{-ac}}$) was similar to $\delta^{13}C_{org}$. Previous studies have found that the $\delta^{13}C$ of the acetate-methyl from which CH_4 is formed is less than 9‰ smaller than $\delta^{13}C_{org}$ (Conrad et al., 2007, 2009a, b, 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 CH_4. 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 $\delta^{13}C_{CH_4\text{-ac}}$ being 5–10‰ smaller than $\delta^{13}C_{org}$. The resulting f_{CO_2,CH_4} showed that CH_4 production in MES was predominantly by acetoclastic methanogenesis, whereas CH_4 production in OMB and even more in OLI was predominately due to hydrogenotrophic methanogenesis.

BGD
7, 5497–5515, 2010

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 $\delta^{13}C$ values of organic matter, the primary substrate, were similar (−27 to −26‰) in all three peatlands, the $\delta^{13}C$ values of CH_4, the end product of degradation, were quite different. Rates of organic matter degradation, as shown by CH_4 and CO_2 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.

Production rates of CH_4 and CO_2 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). Methanogenic degradation of organic matter normally expects the production of equimolar amounts of CH_4 and CO_2. In OLI and OMB, the rates of CO_2 production were higher than those of CH_4 production. The rates of CO_2 production only consider the gaseous CO_2 measured in the headspace of the incubation vessels. While bicarbonate concentrations were negligible in the acidic peat samples, the concentrations of dissolved CO_2 as calculated from Henry's law (Stumm and Morgan, 1981) were not negligible. Thus, rates of total CO_2 production (gaseous plus dissolved CO_2) were about 50% higher than those of gaseous CO_2 alone. Hence, only MES produced CH_4 and CO_2 in the expected equimolar amounts, while OMB and OLI produced much more CO_2 than CH_4. 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

BGD
7, 5497–5515, 2010

degradation of organic matter, e.g. certain humic compounds that are reduced while others are concomitantly oxidized to CO_2 (Heitmann et al., 2007; Keller et al., 2009). Based on our observations, we hypothesize that organic 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 CH_3F. 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 CH_4 production, which would predominantly be formed by CO_2 reduction. Indeed, isotopic mass balance calculations indicate that CH_4 production in OMB and OLI was mainly due to hydrogenotrophic methanogenesis accounting for more than 75% of total CH_4 production. In MES, on the other hand, CH_4 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

BGD
7, 5497–5515, 2010

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; Conrad et al., 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.,

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow 2\ CH_3COOH + 2\ CO_2 + 4\ H_2 \quad (7)$$

$$2\ CH_3COOH \rightarrow 2\ CH_4 + 2\ CO_2 \quad (8)$$

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \quad (9)$$

$$\text{net:} \quad C_6H_{12}O_6 \rightarrow 3\ CO_2 + 3\ CH_4 \quad (10)$$

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

$$C_6H_{12}O_6 + 2\ H_2O \rightarrow C_4H_8O_4 + 2\ CO_2 + 4\ H_2 \quad (11)$$

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O \quad (12)$$

$$\text{net:} \quad C_6H_{12}O_6 \rightarrow C_4H_8O_4 + CO_2 + CH_4 \quad (13)$$

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

$$C_6H_{12}O_6 + C_4H_8O_4 + H_2O \rightarrow CO_2 + C_5H_{10}O_4 + C_4H_{10}O_4 \quad (14)$$

Our data concerning f_{CO_2,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 Eqs. (13) and (14), while in MES being mainly degraded by process Eq. (10).

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

BGD
7, 5497–5515, 2010

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 $\delta^{13}C_{CH_4}$ 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 $\delta^{13}C_{CH_4}$. A strong decrease is expected when most of the CH_4 is produced by acetoclastic methanogenesis, which exhibits a much lower fractionation factor ($\alpha_{ac,CH_4} \approx 1.009$–1.025) (Valentine et al., 2004; Penning et al., 2006; Goevert and Conrad, 2009) than hydrogenotrophic methanogenesis (as much as $\alpha_{CO_2,CH_4} \approx 1.090$) (Conrad, 2005; Penning et al., 2005). In OMB and even more so in OLI, $\delta^{13}C_{CH_4}$ 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, $\delta^{13}C_{CH_4}$ 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 $\alpha_{CO_2,CH_4} \approx 1.067$–1.078, or $\varepsilon_{CO_2,CH_4} \approx -78$ 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 Eq. (8). Inhibition of process Eq. (12), 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

BGD
7, 5497–5515, 2010

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.

Acknowledgements. We thank Eeva-Stiina Tuittila and Jukka Laine (Forest Ecology, Helsinki, Finland) for their advice and support with sample taking and Melanie Klose (MPI Marburg) for giving technical instructions during the tracer and inhibition experiments. Pierre Galand's work was funded by the Academy of Finland and by a FEMS fellowship.

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 $\delta^{13}C$ 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., et al.: 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.

BGD

7, 5497–5515, 2010

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. 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., 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., 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., 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., doi:10.4319/lo.2010.55.5.0000, in press, 2010a.

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 Hinesm, M. E.: Anaerobic microbial biogeochemistry in a northern bog: Acetate as a dominant metabolic end product, Global Biogeochem. Cy., 16, 1063, doi:10.1029/2001GB00140, 2002.

BGD

7, 5497–5515, 2010

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. Applications, 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. Acta, 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 13C isotopic composition of dissolved carbon and pathways of methanogenesis in a fen soil exposed to experimental drought, Biogeosciences, 5, 1457–1473, doi:10.5194/bg-5-1457-2008, 2008.

BGD
7, 5497–5515, 2010

Kotsyurbenko, O. R., Chin, K. J. , Glagolev, M. V., et al.: 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., et al.: Shift from acetoclastic to H_2-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.: CH_4 production via CO_2 reduction in a temperate bog: a source of ^{13}C-depleted CH_4, Geochim. Cosmochim. Acta, 56, 3493–3503, 1992.

Lovley, D. R., 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 alasses in eastern Siberia: implications from its ^{14}C and stable isotopic compositions, Global Biogeochem. Cy., 16, 14-1–14-15, 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, Glob. 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.

BGD

7, 5497–5515, 2010

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., Leuenberger, M., and Burns, S. J.: Stable carbon isotope composition and concentrations of CO_2 and CH_4 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: CO_2 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. In: Ferry JG (Ed) Methanogenesis. Ecology, Physiology, Biochemistry and Genetics, pp. 128–206, Chapman & Hall, New York, 1993.

BGD

7, 5497–5515, 2010

Table 1. Production rates of CH_4 and CO_2, concentrations of acetate, values of $\delta^{13}C$, isotopic enrichment factors and fractions of CH_4 produced from CO_2 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_3F	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_3F	113 ± 5 (68%)	25 ± 1 (86%)	27 ± 1 (60%)
Acetate (µM)	800 ± 490	85 ± 25	30 ± 20
Acetate (µM), + 2% CH_3F	2420 ± 1290	125 ± 125	50 ± 10
$\delta^{13}C_{org}$(‰)	−27.3 ± 0.1	−27.4 ± 0.1	−26.5 ± 0.2
$\delta^{13}C_{ac}$(‰), ± 0.5-2% CH_3F	−18.8 ± 1.3	−22.3 ± 0.6	−24.3 ± 1.4
$\delta^{13}C_{CH_4}$(‰)	−58.4 ± 0.9	−88.9 ± 4.8	−65.6 ± 3.7
$\delta^{13}C_{CH_4}$(‰), + 2% CH_3F	−78.8 ± 0.3	−86.4 ± 25.0	−73.1 ± 9.6
$\delta^{13}C_{CO_2}$(‰)	−16.8 ± 0.2	−16.9 ± 0.3	−11.5 ± 0.4
ε_{CO_2,CH_4} (‰)	−72.6 ± 7.3	−78.5 ± 29.3	−66.8 ± 11.2
f_{CO_2,CH_4}(%), A[1]	46 ± 2	89 ± 9	78 ± 4
f_{CO_2,CH_4}(%), B[1]	41 ± 2	88 ± 10	76 ± 4

[1] f_{CO_2,CH_4} was calculated using Eq. (4) assuming
(A) $\delta^{13}C_{CH_4\text{-}ac} = \delta^{13}C_{org} - 5$, and
(B) $\delta^{13}C_{CH_4\text{-}ac} = \delta^{13}C_{org} - 10$.

BGD
7, 5497–5515, 2010

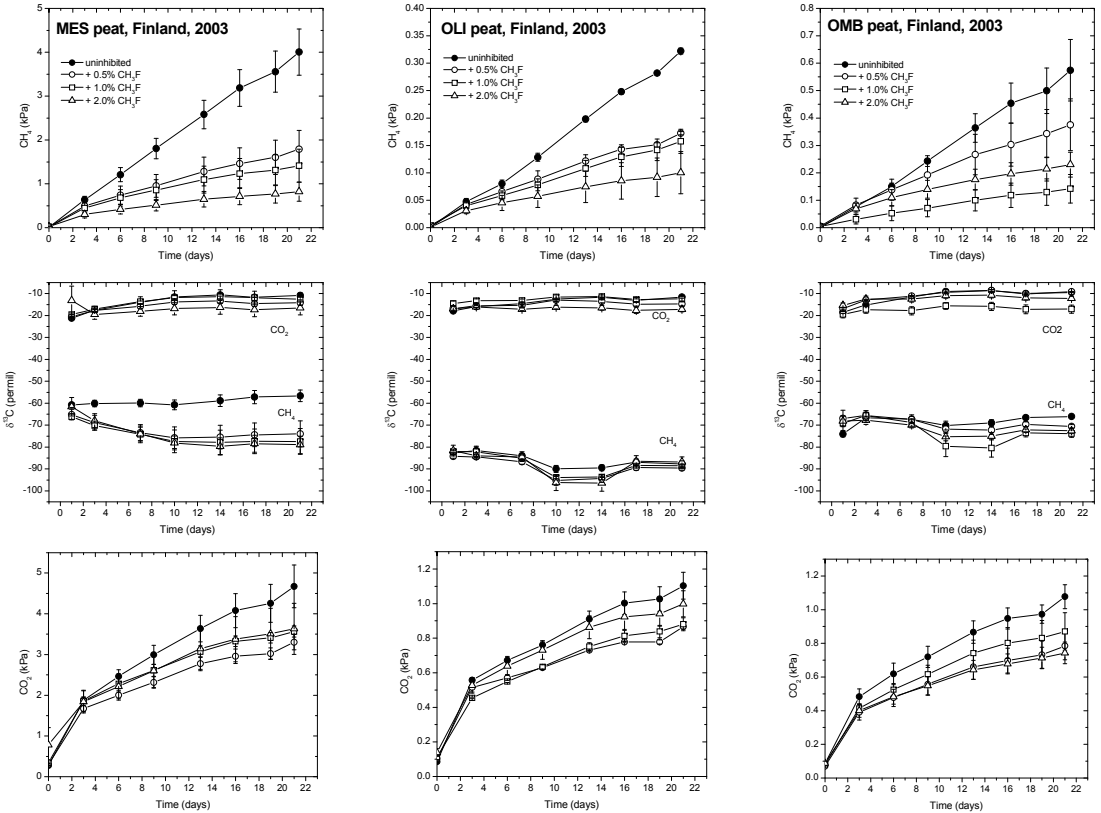

Fig. 1. Time course of accumulation of CH_4 and CO_2, and of $\delta^{13}C$ of the accumulated CH_4 and CO_2 in the absence and presence of different concetrations of CH_3F, an inhibitor of acetoclastic methanogenesis (CH_3F) using samples from three different peatland ecosystems in Finland, i.e., mesotrophic fen (MES), oligotrophic fen (OLI), and ombrotrophic bog (OMB); mean ± SE, $n = 3$.

BGD
7, 5497–5515, 2010

Carbon isotope fractionation in methanogenic peatlands

P. E. Galand et al.