Stable carbon isotope fractionation during methanogenesis in three boreal peatland ecosystems

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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$_4$. δ$^{13}$C analysis of CH$_4$ and CO$_2$ in the presence and absence methyl fluoride (CH$_3$F), an inhibitor of aceticlastic methanogenesis, showed that CH$_4$ was predominantly produced by hydrogenotrophic methanogenesis and that aceticlastic 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$_3$F, 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 aceticlastic 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 aceticlastic and hydrogenotrophic methanogenesis to 3 CH$_4$ and 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, aceticlastic methanogenesis is apparently impeded and CH$_4$ is mainly produced from H$_2$/CO$_2$ (Landsdown 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 aceticlastic 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 aceticlastic methanogenesis (Whiticar et al., 1986). Vice versa it is principally possible to use values of δ¹³C 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 aceticlastic methanogenic archaea which only occur in the genera Methanosarcina or Methanothrix (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 aceticlastic methanogenesis to different extent (Galand et al., 2005). While measuring CH₄ production at different concentrations of methyl fluoride (CH₃F), an inhibitor of aceticlastic methanogenesis, we also determined the δ¹³C of CH₄, CO₂ and acetate. We report these data and quantify the relative contribution of hydrogenotrophic and aceticlastic 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 x 8 x 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 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 alpina 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 ecophysiological 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 aceticlastic 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 δ¹³C of CH₄ and CO₂ were analyzed by gas chromatography combustion isotope ratio mass spectrometry (GC-C-IRMS), and the δ¹³C of acetate was analyzed by HPLC-C-IRMS as described before (Conrad et al., 2007). Analysis of δ¹³C 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 → B are defined after Hayes (Hayes 1993):

\[ \alpha_{A,B} = \frac{\delta^{13}C_A + 1000}{\delta^{13}C_B + 1000} \]  

(1)

sometimes expressed as isotopic enrichment factor \( \varepsilon = 1 - \alpha \) (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:

\[ \delta^{13}C_2 = f_{new} \delta^{13}C_{new} + (1 - f_{new}) \delta^{13}C_1 \]  

(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

\[ \alpha_{CO_2,CH_4} = \frac{\delta^{13}C_{CO_2} + 1000}{(\delta^{13}C_{CH_4} - CO_2) + 1000} \]  

(3)

where \( \delta^{13}C_{CH_4-CO_2} \) is the \( \delta^{13}C \) of 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:

\[ \delta^{13}C_{CH_4-ac} = \delta^{13}C_{org} + \varepsilon_{org,CH_4} \]  

(5)

\[ \delta^{13}C_{CH_4-CO_2} = \delta^{13}C_{CH_4-CH_3F} \]  

(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 fen (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 (\( \alpha_{CO_2,CH_4} \) or \( \varepsilon_{CO_2,CH_4} \)) from the difference between the \( \delta^{13}C \) of \( CH_4 \) in the absence and the presence of \( CH_3F \). The fractionation factor was largest in OLI > MES > OMB, i.e., \( \varepsilon_{CO_2,CH_4} \), 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 \( \delta^{13}C \) of \( CH_4 \) measured in the presence of \( CH_3F \), when acetoclastic methanogenesis was inhibited and \( CH_4 \) was exclusively
produced from H2/CO2. The calculation further assumed that the $\delta^{13}C$ of acetyl-lactically produced CH4 ($\delta^{13}C_{CH4-ac}$) was similar to $\delta^{13}C_{org}$. Previous studies have found that the $\delta^{13}C$ of the acetate-methyl from which CH4 is formed is less than 9‰ smaller than $\delta^{13}C_{org}$ (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 Methanoseta (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 $\delta^{13}C_{CH4-ac}$ being 5–10‰ smaller than $\delta^{13}C_{org}$. 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 $\delta^{13}C$ values of organic matter, the primary substrate, were similar (−27 to −26‰) in all three peatlands, the $\delta^{13}C$ values of 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.

Fig. 1. Time course of accumulation of CH4 and CO2, and of $\delta^{13}C$ 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.
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 peat (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, 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 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 aceticlastic 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 aceticlastic 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 aceticlastic methanogenesis. These data are consistent with an earlier study in which the percentage contribution

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).

<table>
<thead>
<tr>
<th>Variables</th>
<th>MES peat</th>
<th>OLI peat</th>
<th>OMB peat</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.3 ± 0.1</td>
<td>5.2 ± 0.1</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>CH₄ production (nmol h⁻¹ gdw⁻¹)</td>
<td>210 ± 77</td>
<td>15 ± 4</td>
<td>40 ± 13</td>
</tr>
<tr>
<td>CH₄ production (nmol h⁻¹ gdw⁻¹) + 2% CH₃F</td>
<td>38 ± 7 (18%)</td>
<td>4.2 ± 4.2 (28%)</td>
<td>14.6 ± 3.3 (36%)</td>
</tr>
<tr>
<td>CO₂ production (nmol h⁻¹ gdw⁻¹)</td>
<td>167 ± 99</td>
<td>29 ± 2</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>CO₂ production (nmol h⁻¹ gdw⁻¹) + 2% CH₃F</td>
<td>113 ± 5 (68%)</td>
<td>25 ± 1 (86%)</td>
<td>27 ± 1 (60%)</td>
</tr>
<tr>
<td>Acetate (µM)</td>
<td>800 ± 490</td>
<td>85 ± 25</td>
<td>30 ± 20</td>
</tr>
<tr>
<td>Acetate (µM) + 2% CH₃F</td>
<td>2420 ± 1290</td>
<td>125 ± 125</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>δ¹³C_{org}(‰)</td>
<td>-27.3 ± 0.1</td>
<td>-27.4 ± 0.1</td>
<td>-26.5 ± 0.2</td>
</tr>
<tr>
<td>δ¹³C_{ac}(‰), ± 0.5-2% CH₃F</td>
<td>-18.8 ± 1.3</td>
<td>-22.3 ± 0.6</td>
<td>-24.3 ± 1.4</td>
</tr>
<tr>
<td>δ¹³C_{CH₄}(‰)</td>
<td>-58.4 ± 0.9</td>
<td>-89.8 ± 4.8</td>
<td>-65.6 ± 3.7</td>
</tr>
<tr>
<td>δ¹³C_{CO₂}(‰)</td>
<td>-78.8 ± 0.3</td>
<td>-86.4 ± 25.0</td>
<td>-73.1 ± 9.6</td>
</tr>
<tr>
<td>e_{CO₂,CH₄}(‰)</td>
<td>-16.8 ± 0.2</td>
<td>-16.9 ± 0.3</td>
<td>-11.5 ± 0.4</td>
</tr>
<tr>
<td>f_{CO₂,CH₄}(%, A¹)</td>
<td>46 ± 2</td>
<td>89 ± 9</td>
<td>78 ± 4</td>
</tr>
<tr>
<td>f_{CO₂,CH₄}(%, B¹)</td>
<td>41 ± 2</td>
<td>88 ± 10</td>
<td>76 ± 4</td>
</tr>
</tbody>
</table>

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.
of hydrogenotrophic versus acetoclastic methanogenesis was
determined by measuring the conversion of $^{14}$C-labelled bi-
carbonate to CH$_4$ (Galand et al., 2005). Theoretically, one
would expect that $>$66% of the CH$_4$ is produced by acetoc-
lastic methanogenesis, if organic matter, such as polysac-
charides, proteins, lipids etc., is completely degraded (Con-
rad 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 sub-
stances are only partially degraded rather than completely.
This speculation is consistent with recent studies in lake sed-
iments (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 sub-
stance, e.g.,

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (R1)$$

$$2CH_3COOH \rightarrow 2CH_4 + 2CO_2 \quad (R2)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (R3)$$

net: $C_6H_{12}O_6 \rightarrow 3CO_2 + 3CH_4 \quad (R4)$

would contrast with incomplete degradation of an organic sub-
stance, e.g.,

$$C_6H_{12}O_6 + 2H_2O \rightarrow C_6H_8O_4 + 2CO_2 + 4H_2 \quad (R5)$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (R3)$$

net: $C_6H_{12}O_6 \rightarrow C_4H_8O_4 + CO_2 + CH_4 \quad (R6)$

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

$$C_6H_{12}O_6 + C_4H_8O_4 + H_2O \rightarrow CO_2 + C_5H_{10}O_4 \quad (R7)$$

$+C_4H_{10}O_4$

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

This interpretation is also consistent with the effect of
CH$_3$F, which showed the strongest inhibition (18% residual
activity) for CH$_4$ production in MES, which was presumably
caused by complete inhibition of acetoclastic methanogene-
sis 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$_3$F
(Conrad and Klose, 1999). Hence the observed decrease of
CH$_4$ production with increasing CH$_3$F (Fig. 1) is not un-
expected. Acetoclastic methanogenesis was probably com-
pletely inhibited at 1% CH$_3$F, since values of $\delta^{13}$C$_{CH_4}$ did not
decrease further when more CH$_3$F was added (Fig. 1). Only
in MES, but not in OMB or OLI, did CH$_3$F 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 methano-
genesis (as much as $\alpha_{CO_2,CH_4} \approx 1.090$) (Conrad 2005; Pen-
ning et al., 2005). In OMB and even more so in OLI $\delta^{13}$C$_{CH_4}$
exhibited very low values already when CH$_3$F was not ap-
plied and decreased only a bit further upon application. In
MES, on the other hand, $\delta^{13}$C$_{CH_4}$ decreased only in the pre-
ceence of CH$_3$F 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$_3$F is also consistent with the obser-
vation that CO$_2$ production was less inhibited by CH$_3$F than
CH$_4$ production. Inhibition of only acetoclastic methanogen-
esis would result in equal inhibition of CO$_2$ and CH$_4$
production because of Reaction (R2). Inhibition of process Re-
tion (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 differ-
ent 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 be-
tween peatlands probably explain the presence of different
paths for organic matter degradation. Noteworthy, a sec-
ond study, found similar proportions of putatively acetoclas-
tic 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 pro-
cesses. 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 peat-
lands is not completely clear, but probably involves incom-
plete 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

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

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