Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments

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Abstract. Methane is an important end product of degradation of organic matter in anoxic lake sediments. Methane is mainly produced by either reduction of CO2 or cleavage of acetate involving different methanogenic archaea. The contribution of the different methanogenic paths and of the diverse bacteria and archaea involved in CH4 production exhibits a large variability that is not well understood. Lakes in tropical areas, e.g. in Brazil, are wetlands with high potential impact on the global CH4 budget. However, they have hardly been studied with respect to methanogenesis. Therefore, we used samples from 16 different lake sediments in the Pantanal and Amazon region of Brazil to measure production of CH4, CO2, analyze the content of 13C in the products and in intermediately formed acetate, determine the abundance of bacterial and archaeal microorganisms and their community composition and diversity by targeting the genes of bacterial and archaeal ribosomal RNA and of methyl coenzyme M reductase, the key enzyme of methanogenic archaea. These experiments were done in the presence and absence of methyl fluoride, an inhibitor of acetoclastic methanogenesis. While production rates of CH4 and CO2 were correlated to the content of organic matter and the abundance of archaea in the sediment, values of 13C in acetate, CO2, and CH4 were related to the 13C content of organic matter and to the path of CH4 production with its intrinsic carbon isotope fractionation. Isotope fractionation was small (average 10‰) for conversion of Corg to acetate-methyl, which was hardly further fractionated during CH4 production. However, fractionation was strong for CO2 conversion to CH4 (average 75‰), which generally accounted for >50% of total CH4 production. Canonical correspondence analysis did not reveal an effect of microbial community composition, despite the fact that it exhibited a pronounced variability among the different sediments.

1 Introduction

Methane, an important greenhouse gas, is predominantly produced as an end product of the degradation of organic matter under anoxic conditions (Cicerone and Oremland, 1988). The actual CH4 formation is catalyzed by methanogenic archaea that mainly use acetate or H2 + CO2 as substrates, which in turn are the products of anaerobic organic matter degradation (Zinder, 1993). The relative contribution of acetotrophic and hydrogenotrophic methanogenesis to total CH4 production significantly affects the δ13C of the produced CH4, since hydrogenotrophic methanogenesis expresses a larger kinetic isotope effect than acetotrophic methanogenesis (Whiticar et al., 1986). Since computation of global budget is constrained by the δ13C of atmospheric CH4, knowledge of the processes involved in CH4 turnover is important (Quay et al., 1991; Tyler, 1992). The processes involved in microbial CH4 production during anaerobic degradation of organic matter constitute the most important sources in the global CH4 cycle and occur in natural wetlands, in rice fields and other artificial wetlands, in the intestine of ruminants and termites, and in waste treatment systems (sewage, landfills). Natural wetlands are one of the largest individual sources accounting for 20–30% of the total
atmospheric CH$_4$ budget (Lelieveld et al., 1998). The contribution of lake and river sediments to CH$_4$ emission was found to offset the continental carbon sink in terms of terrestrial greenhouse gas balance (Bastviken et al., 2011). Lakes within the floodplain of South American rivers (Amazon, Pantanal, Orinoco) have been found to be a particularly significant source of atmospheric CH$_4$ (Bastviken et al., 2010; Devol et al., 1990; Marani and Alvala, 2007; Smith et al., 2000). However, the processes involved in CH$_4$ formation and their effects on the $\delta^{13}$C of CH$_4$ have hardly been studied (Conrad et al., 2010b; Wassmann et al., 1992). Therefore, we decided studying lake sediments in tropical Brazil, and randomly selected sites within the Pantanal and Amazon region that were relatively easily accessible.

Methane production is achieved by a community of different microbial groups, which catalyze the stepwise degradation of organic matter (McInerney and Bryant, 1981; Zehnder, 1978). The process is initiated by the hydrolysis of polymers (e.g., polysaccharides) to monomers (e.g., sugars), which are then fermented by a variety of different bacteria to low-molecular-weight fatty acids, alcohols, CO$_2$, and H$_2$. Acetate is usually the most important primary fermentation product. Some bacteria can dissiplate sugars to acetate as sole product ( homoacetogenesis), two acetate being produced by fermentation of the sugar and one acetate by CO$_2$ reduction via the acetyl-CoA pathway (Dolfing, 1988; Drake and Küsel, 2003; Lever et al., 2010). However, acetate is also produced during secondary fermentation, during which primary fermentation products are further processed, or during chemolithoautotrophic homoacetogenesis (4H$_2$ + 2CO$_2$ → CH$_3$COO$^-$ + H$^+$ + 2H$_2$O) (Dolfing, 1988; Drake and Küsel, 2003; Lever et al., 2010). The final fermentation products are generally acetate, CO$_2$, and H$_2$. The last step in organic matter degradation is then the production of CH$_4$ from the dismutation of acetate (acetoelastic methanogenesis: CH$_3$COOH → CH$_4$ + CO$_2$) and from the reduction of CO$_2$ (hydrogenotrophic methanogenesis: 4H$_2$ + CO$_2$ → CH$_4$ + 2H$_2$O) (Schink and Stams, 2006; Zinder, 1993). Methane can potentially also be formed from methylandated precursors, such as methanol, but in freshwater systems such precursors have always been found to be of minor importance (Conrad and Claus, 2005; Lovley and Klug, 1983). Each step of organic matter degradation is crucial for the entire process and for CH$_4$ production. In systems with a dynamic input of organic matter, the degradation process may result in transient accumulation of intermediates, particularly acetate (Drake and Küsel, 2003). Thus, CH$_4$ production rates may be limited by each of the degradation steps involved. Only if the system eventually achieves steady state are CH$_4$ production rates limited by the first initiating reaction step, the hydrolysis of complex organic matter (Billen, 1982).

The $\delta^{13}$C of the produced CH$_4$ depends on several factors. One, mentioned above, is the different isotope fractionation during acetoelastic versus hydrogenotrophic CH$_4$ formation. This difference has been exploited for characterization of the predominant path of CH$_4$ formation (Conrad, 2005). This characterization can be done in a diagnostic way by comparing the $\delta^{13}$C in both CH$_4$ and CO$_2$ (Hornibrook et al., 2000; Whiticar, 1999), or by quantification of the percent contribution of each path using mass balance equations based on the measurement of $\delta^{13}$C in CH$_4$, CO$_2$ and acetate and on the knowledge of fractionation factors (Alperin et al., 1992). The latter approach, requiring a rather comprehensive analysis of $\delta^{13}$C has not very often been applied (Avery et al., 1999; Conrad et al., 2009; 2010b; Sugimoto and Wada, 1993). Quantification of the hydrogenotrophic versus acetoelastic methanogenic paths by radiotracer technique also has not been used very often (reviewed by Conrad, 1999). However, these studies showed that the path of CH$_4$ production can vary to a large extent between different environments. It is presently unclear which environmental factors, including microbiological data, control the path of CH$_4$ production. Therefore, we decided to investigate these parameters in several different lake sediments.

The $\delta^{13}$C of the produced CH$_4$ is not only dependent on the fractionation during CH$_4$ production but also on the $\delta^{13}$C of the substrate from which it is produced. A classical example of this interdependence is the use of $\delta^{13}$C of CO$_2$ and CH$_4$ for calculation of an apparent fractionation factor $\alpha_{C_{app}}$ that is diagnostic for whether CH$_4$ is produced predominantly by hydrogenotrophic or acetoelastic methanogenesis. This diagnosis was pioneered by Whiticar et al. (1986) who used the synonym terms carbonate reduction and methyl fermentation, respectively. However, CO$_2$ is not only a substrate for methanogenesis, but is first of all a product of organic matter degradation. The same is the case with acetate, which is consumed by acetoelastic methanogenesis, while is produced by fermenting or chemolithoautotrophic homoacetogenic bacteria (Heuer et al., 2010). The CO$_2$ and acetate may also have undergone stable isotope fractionation during the course of formation. Natural organic matter, the primary substrate, in turn is the product of photosynthesis, which fractionates stable carbon isotopes during assimilation of atmospheric CO$_2$ depending on the enzymatic mechanism (Zhang et al., 2002). Little is known how environmental conditions affect the isotopic composition of the substrates involved in methanogenesis. We therefore studied $^{13}$C stable isotopes in several different lake sediments.

All the conversions and isotope fractionations during methanogenic degradation of organic matter are achieved by the microbial community in the respective environment. The microbial community is composed of a large diversity of bacteria and archaea which potentially may change from site to site. Most of these microorganisms have never been isolated so that their physiological characteristics are unknown (Pace, 1997; Rappe and Giovannoni, 2003). However, it is possible to gain information about diversity and structure of the microbial community by molecular fingerprinting and sequencing techniques (Amann et al., 1995). Thus it is possible
to analyse the diversity of bacterial and archaeal communities by targeting the gene coding for synthesis of ribosomal RNA, which is ubiquitous in all life (Woese, 2000). Furthermore, it is possible to analyze the diversity of particularly the methanogenic archaea by targeting the mcrA gene coding for a subunit of the methyl coenzyme M reductase, the key enzyme involved in CH4 production (Lueders et al., 2001). However, microbial communities in tropical or subtropical lake sediments have hardly been studied in the context of biogeochemical activities, such as CH4 production and 13C-isotopic conversion (Conrad et al., 2010b; Nüsslein et al., 2001; 2003).

We studied 16 different lake sediments from the world largest tropical freshwater wetlands by measuring production rates of CH4 and CO2, analyzing many different variables and parameters involved in CH4 production, analyzing the δ13C of CH4, CO2, acetate (carboxyl and methyl group), and organic matter, determining isotopic fractionation factors, and analyzing the abundance and composition of microbial communities. The objective was to find out which environmental variables control (1) the path of CH4 production, (2) its rate, and (3) the δ13C of CH4, and its precursors.

2 Methods

2.1 Sampling

Sediment cores (6 cm diameter, 40 cm length) of floodplain lakes from the Amazon and Pantanal regions located in Brazil were taken using a corer sampler. The upper 0–3 cm sediment were placed into plastic bottles and completely filled. The bottles were shipped by air freight to Marburg (Germany) and processed immediately. The sites, sampling time and main characteristics of the sediments are shown in Table 1 and are consecutively numbered (#1–#16). Some of the sites are identical to those described by Bastviken et al. (2010). The sediment of Pantanal Lake 1 was sampled in 2007 during the beginning of the wet season from the center (#8) and the margin (#9) of the lake, and sampled again in 2008 (#7) during the dry season from the center of the lake.

2.2 Incubation experiments

The incubation procedure was basically the same as described by Conrad et al. (2007, 2010b). About 9-mL aliquots of the sediment were transferred in triplicate into 27-mL sterile tubes, flushed with N2, closed with butyl rubber stoppers and incubated over night at 25 °C. The exact amount of sediment was determined gravimetrically. Then, the bottles were flushed again with N2 and further incubated at 25 °C. The gas headspace of some of the bottles was supplemented with 2.0% CH3F (Fluorochrome company) to specifically inhibit acetoclastic methanogenesis (Janssen and Frenzel, 1997).

Gas samples were taken repeatedly during the course of incubation and analyzed for CH4, CO2, and δ13C of CH4 and CO2. At the end of incubation, the bottles were sacrificed for sampling of the liquid phase. Aliquots of the sediment slurry were centrifuged and the supernatants were filtered through 0.2-µm polytetrafluoroethylene (PTFE) membrane filters and stored frozen (−20°C) for later analysis of concentration and δ13C of acetate. The δ13C of organic carbon (δ13Corg) was analyzed after air drying of the sediment at room temperature, removal of carbonate carbon by addition of HCl, followed again by air drying of the sediment slurry at room temperature. All values are given per gram dry sediment (g dw−1).

2.3 Molecular analysis of the methanogenic archaeal community

The desoxyribonucleic acid (DNA) of the sediment samples was extracted with the Soil DNA Isolation Kit (MP) following the manufacturer’s instructions as described in detail by Kolb et al. (2005). The abundance of archaeal and bacterial 16S ribosomal ribonucleic acid (rRNA) gene copies was determined by real-time polymerase chain reaction (PCR) as described before (Conrad et al., 2008). Terminal restriction length polymorphism (T-RFLP) analysis of the 16S rRNA and mcrA genes were done as described previously in detail (Conrad et al., 2008; Noll et al., 2010). The mcrA gene codes for a subunit of the methyl coenzyme M reductase, the key enzyme of CH4 production that is unique to methanogenic archaea. Correspondence analysis and canonical correspondence analysis was done as described before (Conrad et al., 2008; Noll et al., 2010). Diversity indices richness (S), evenness (E) and Shannon index (H) of T-RFLP profiles were calculated. Richness was defined as the number of T-RFs with a relative abundance >1% within a given T-RFLP profile. To compare the dominance structure of the respective community structures as reflected by T-RFLP analysis, the evenness of the community fingerprint patterns was calculated as $E = \frac{-\Sigma A_p \ln A_p}{\ln S}$, where $A_p$ is the fluorescence intensity of the $i$-th T-RF relative to the total fluorescence intensity, and $S$ is the total number of T-RFs. The Shannon index was calculated as $H = -\Sigma A_p \log A_p$.

2.4 Chemical analyses

Methane, CO2, and H2 were analyzed by gas chromatography, acetate (and other fatty acids) by high pressure liquid chromatography (HPLC) as described by Conrad et al. (2007). The C and N content of the sediments were quantified on a CHNS-elemental analyzer by the Analytical Chemical Laboratory of the University of Marburg. Sulfate was analyzed by ion chromatography and total iron by the ferrozin method (Conrad and Klose, 2006). The δ13C (in units of permil) of CH4 (δ13CCH4) and CO2 (δ13CCO2) was analyzed by gas chromatography combustion isotope
Table 1. Sites of the lakes and main characteristics; mean ± SE, n = 3.

<table>
<thead>
<tr>
<th>#</th>
<th>Lake</th>
<th>Region</th>
<th>Sampling time</th>
<th>Coordinates</th>
<th>pH</th>
<th>Corg (%)</th>
<th>δ¹³C Corg</th>
<th>Ntot (%)</th>
<th>Fe (µmol g-dw⁻¹)</th>
<th>Sulfate (µM)</th>
</tr>
</thead>
</table>
| 1  | Arrozal   | Pantanal| July 2007     | S19°00'50" W 57°31'27"
| 2  | Sinbu     | Pantanal| July 2007     | S19°00'57" W 57°24'17" | 6.3  | 6.3 ± 0.2 | -25.2 ± 0.1 | 0.63 ± 0.03 | 99 ± 1         | 6            |
| 3  | Iatoba    | Pantanal| July 2007     | S19°02'2" W 57°22'7"  | 6.4  | 5.8 ± 0.5 | -27.1 ± 0.1 | 0.49 ± 0.05 | 159 ± 0        | 3            |
| 4  | Anzol de Ouro | Pantanal| July 2007     | S19°00'58" W 57°37'29" | 6.5  | 5.8 ± 0.3 | -29.3 ± 0.1 | < 0.05    | 38 ± 1         | 3            |
| 5  | Teresa    | Pantanal| November 2008 | S18°57'38" W 57°26'28" | 6.6  | 5.7 ± 0.1 | -28.0 ± 0.1 | 0.56 ± 0.04 | 286 ± 1        | 15           |
| 6  | Presa     | Pantanal| November 2008 | S18°59'0" W 57°25'0"   | 6.6  | 5.7 ± 0.1 | -27.9 ± 0.3 | 0.51 ± 0.02 | 105 ± 0        | 0            |
| 7  | Lobo      | Pantanal| November 2008 | S18°57'7" W 57°36'54"  | 6.7  | 5.9 ± 0.1 | -26.9 ± 0.1 | 0.55 ± 0.02 | 46 ± 0         | 0            |
| 8  | Lake 1, central | Pantanal| November 2008 | S19°1'45" W 57°32'54"  | 7.7  | 8.5 ± 0.2 | -29.2 ± 0.1 | 0.69 ± 0.01 | 49 ± 1         | 0            |
| 9  | Lake 1, margin | Pantanal| January 2007 | S19°1'45" W 57°32'54"  | 6.5  | 11.7 ± 1.4 | -30.1 ± 0.1 | 0.97 ± 0.08 | 150 ± 2        | 60           |
| 10 | Lake 1, central | Pantanal| January 2007 | S19°1'45" W 57°32'54"  | 7.0  | 11.4 ± 0.2 | -31.3 ± 0.2 | 0.92 ± 0.01 | 208 ± 1        | 96           |
| 11 | Lake 2    | Pantanal| January 2007  | S19°1'50" W 57°28'24"  | 6.9  | 3.1 ± 0.0 | -29.5 ± 1.0 | 0.26 ± 0.01 | 136 ± 1        | 36           |
| 12 | Belmont   | Amazonia| August 2007   | S08°39'3" W 63°50'23"  | 5.9  | 5.7 ± 0.4 | -28.6 ± 0.0 | 0.44 ± 0.00 | 106 ± 1        | 4            |
| 13 | Maravilha | Amazonia| August 2007   | S08°43'40" W 63°55'52" | 6.5  | 2.6 ± 0.0 | -29.1 ± 0.0 | 0.18 ± 0.01 | 78 ± 0         | 4            |
| 14 | Laguninho | Amazonia| August 2007   | S08°26'47" W 63°28'28" | 6.8  | 1.6 ± 0.2 | -29.5 ± 0.2 | 0.14 ± 0.00 | 234 ± 1        | 5            |
| 15 | Paca      | Amazonia| August 2007   | S08°29'46" W 63°27'56" | 6.7  | 4.1 ± 0.0 | -32.4 ± 0.1 | 0.35 ± 0.00 | 126 ± 1        | 9            |
| 16 | Purusinho | Amazonia| November 2008 | S07°22'19" W 63°23'22" | 7.6  | 1.9 ± 0.0 | -32.8 ± 2.5 | 0.24 ± 0.01 | 279 ± 3        | 2            |

ratio mass spectrometry (GC-C-IRMS), the δ¹³C of acetate (δ¹³C ac) by HPLC-C-IRMS as described before (Conrad et al., 2007). The δ¹³C of the methyl group of acetate (δ¹³C ac–methyl) was determined after off-line pyrolysis (Conrad et al., 2007). The δ¹³C of the carboxyl group of acetyl (δ¹³C ac–carbonyl) was calculated by δ¹³C ac = δ¹³C ac–methyl + δ¹³C ac–carbonyl. Analysis of the δ¹³C in organic matter was done at the Institute of Soil Science and Forest Nutrition (IBW) at the University of Göttingen (courtesy of Heinz Flessa) with an elemental analyzer (Fisons EA 1108) coupled to an IRMS. The precision of repeated analysis was generally better than ±0.3‰.

2.5 Calculations

Fractionation factors for a reaction A → B are defined after Hayes (1993):

\[ \delta_{A,B} = (\delta^{13}C_A + 1000) / (\delta^{13}C_B + 1000) \]  

(1)

sometimes expressed as isotopic enrichment factor ε ≡ 1 - α (in units of permil). The δ¹³C for a newly formed CH₄ (δ¹³C new) was calculated from the δ¹³C at two time points t = 1 (δ¹³C₁) and t = 2 (δ¹³C₂) by the following mass balance equation:

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

(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 CO₂ to CH₄ is given by

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

(3)

where δ¹³C CH₄-mc is the newly formed CH₄ Eq. (2) derived from H₂ + CO₂, i.e., the CH₄ produced in the presence of 2% CH₃F, assuming that acetoclastic methanogenesis was then completely inhibited.

Relative contribution of H₂ + CO₂-derived CH₄ to total CH₄ was determined using the following mass balance equation (Conrad, 2005):

\[ f_{CO_2,CH_4} = (\delta^{13}C_{CH_4} - \delta^{13}C_{CH_4-ma}) / (\delta^{13}C_{CH_4-mc} - \delta^{13}C_{CH_4-ma}) \]  

(4)

where \( f_{CO_2,CH_4} \) is the fraction of CH₄ formed from H₂ + CO₂, \( \delta^{13}C_{CH_4} \) the δ¹³C of total produced methane, \( \delta^{13}C_{CH_4-mc} \) the δ¹³C of CH₄ derived from H₂ + CO₂, and \( \delta^{13}C_{CH_4-ma} \) the δ¹³C of CH₄ derived from acetate determined by:

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

(5)

where \( \delta^{13}C_{ac-methyl} \) is the δ¹³C of the methyl group of acetyl accumulated and \( \varepsilon_{ac,CH_4} \) is the isotopic enrichment factor (equivalent to \( \alpha_{ac,CH_4} \)) during acetoclastic methanogenesis. It is assumed, that the methyl group of acetate was either converted to CH₄ without fractionation, i.e., \( \varepsilon_{ac,CH_4} = 0 \), or that acetate fractionation was due to Methanoseta species with \( \varepsilon_{ac,CH_4} = -10\%e \) (Penning et al., 2006; Valentine et al., 2004).

The concentrations of dissolved CO₂ and bicarbonate were calculated using Henry’s law and the dissociation constant of bicarbonate at 25°C, i.e., pK = 6.352 (Stumm and Morgan, 1981). Regression analysis was done using Origin 6.1 (Microcal, Northampton, MA, USA).

3 Results

Sediments were sampled from 16 different lake sites in Brazilian Pantanal and Amazonia in 2006, 2007 and 2008. The geographical coordinates of the sites and some of the
Table 2. Production rates (nmol h\(^{-1}\) g-dw\(^{-1}\)) of CH\(_4\) and CO\(_2\), values of δ\(^{13}\)C (‰), and enrichment factor for CH\(_4\) production from CO\(_2\) in the incubations of different lake sediments with and without inhibitor (2% CH\(_3\)F); mean ± SE, n = 3. Details of lake sites are explained in Table 1.

<table>
<thead>
<tr>
<th>Lake sites</th>
<th>CH(_4)production</th>
<th>CO(_2)production</th>
<th>δ(^{13})C-CH(_4)</th>
<th>δ(^{13})C-CO(_2)</th>
<th>α(_{CO_2,CH_4}) mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>#</td>
<td>control +CH(_3)F</td>
<td>control +CH(_3)F</td>
<td>Control +CH(_3)F</td>
<td>Control +CH(_3)F</td>
<td>mean</td>
</tr>
<tr>
<td>1</td>
<td>3.7 ± 0.2</td>
<td>2.8 ± 0.1</td>
<td>−59.0 ± 0.5</td>
<td>−3.7 ± 0.8</td>
<td>1.066 ± 0.003</td>
</tr>
<tr>
<td>2</td>
<td>6.0 ± 0.6</td>
<td>4.0 ± 0.1</td>
<td>−59.1 ± 0.2</td>
<td>−7.2 ± 0.3</td>
<td>1.073 ± 0.002</td>
</tr>
<tr>
<td>3</td>
<td>2.3 ± 0.3</td>
<td>1.1 ± 0.1</td>
<td>−65.3 ± 0.6</td>
<td>−11.0 ± 0.3</td>
<td>1.072 ± 0.003</td>
</tr>
<tr>
<td>4</td>
<td>14.3 ± 0.8</td>
<td>7.3 ± 0.2</td>
<td>−62.3 ± 0.9</td>
<td>−9.8 ± 0.1</td>
<td>1.074 ± 0.003</td>
</tr>
<tr>
<td>5</td>
<td>0.6 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>−83.7 ± 1.7</td>
<td>−16.3 ± 0.3</td>
<td>1.083 ± 0.008</td>
</tr>
<tr>
<td>6</td>
<td>1.5 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>−73.7 ± 1.1</td>
<td>−17.6 ± 0.7</td>
<td>1.085 ± 0.002</td>
</tr>
<tr>
<td>7</td>
<td>2.5 ± 0.5</td>
<td>1.6 ± 0.3</td>
<td>−64.5 ± 1.8</td>
<td>−8.2 ± 0.3</td>
<td>1.077 ± 0.005</td>
</tr>
<tr>
<td>8</td>
<td>2.9 ± 0.3</td>
<td>2.0 ± 0.3</td>
<td>−76.4 ± 1.2</td>
<td>−9.1 ± 1.1</td>
<td>1.082 ± 0.004</td>
</tr>
<tr>
<td>9</td>
<td>9.8 ± 0.7</td>
<td>3.7 ± 0.3</td>
<td>−66.2 ± 0.5</td>
<td>−9.2 ± 1.3</td>
<td>1.074 ± 0.010</td>
</tr>
<tr>
<td>10</td>
<td>8.0 ± 1.2</td>
<td>5.3 ± 0.5</td>
<td>−80.9 ± 1.2</td>
<td>−29.7 ± 1.1</td>
<td>1.076 ± 0.014</td>
</tr>
<tr>
<td>11</td>
<td>2.9 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>−82.8 ± 0.7</td>
<td>−26.8 ± 1.4</td>
<td>1.077 ± 0.015</td>
</tr>
<tr>
<td>12</td>
<td>4.5 ± 0.4</td>
<td>1.9 ± 0.1</td>
<td>−57.7 ± 0.3</td>
<td>−14.0 ± 0.6</td>
<td>1.057 ± 0.006</td>
</tr>
<tr>
<td>13</td>
<td>1.1 ± 0.0</td>
<td>0.7 ± 0.0</td>
<td>−78.0 ± 1.3</td>
<td>−21.1 ± 0.7</td>
<td>1.079 ± 0.007</td>
</tr>
<tr>
<td>14</td>
<td>0.9 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>−64.1 ± 0.7</td>
<td>−25.4 ± 0.3</td>
<td>1.060 ± 0.004</td>
</tr>
<tr>
<td>15</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.0</td>
<td>−94.5 ± 3.7</td>
<td>−30.9 ± 0.1</td>
<td>1.074 ± 0.013</td>
</tr>
<tr>
<td>16</td>
<td>2.0 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>−80.2 ± 1.7</td>
<td>−15.9 ± 0.4</td>
<td>1.085 ± 0.006</td>
</tr>
</tbody>
</table>

Mean ± SD 4.0 ± 3.8 2.2 ± 2.0 3.8 ± 3.3 3.1 ± 2.5 −71.8 ± 11.0 −88.8 ± 10.4 −17.0 ± 8.2 −20.8 ± 7.8 1.075 ± 0.008

The Amazonian lakes were located close to the city of Porto Velho and the Pantanal lakes were located close to the city of Ladario. In most of the sampled environments in the Pantanal there is a daily change in temperature values that can vary up to 5°C, and oxygen concentrations can vary from 120% saturation (day) to anoxic (midnight) right over the sediment within 12 h (data not shown). The Amazonian lakes were mostly sampled during high water level, with a temperature average of 28°C during sampling and with anoxia over the sediment. The pH values of the lake sediments were in a range of pH 5.9 to 7.7 (average pH 6.75); the contents of organic carbon ranged between 0.8 and 12.5% (average 5.3%); δ\(^{13}\)C\(_{org}\) between −32.8 and −25.2‰; N\(_{org}\) between <0.05% and 1.06%; total iron between 46 and 286 μmol g\(^{-1}\) dw\(^{-1}\); and porewater sulfate concentrations between 0 and 96 μM. Hence, the chemical conditions of the lake sediments covered a rather broad range. The highest sulfate concentrations were found in samples taken in 2007 from Lake 1 and Lake 2.

The sediment samples were incubated in triplicate under anoxic conditions at 25°C in the absence and presence of 2% CH\(_3\)F. These standard conditions guaranteed that data obtained from the different incubations were comparable and represented their methanogenic potentials. Methyl fluoride served as inhibitor of acetoclastic methanogenesis (Janssen and Frenzel, 1997). A typical experiment is shown in Fig. 1 for Lake Jatoba sediment. The experimental results of all the lake sediments are shown in supplementary Fig. S1. In Lake Jatoba as in all the other lake sediments both CH\(_4\) and CO\(_2\) were produced during the course of the experiment. Production of CO\(_2\) generally and production of CH\(_4\) in most of the lake sediments started right from the beginning of the incubation, with rates gradually decreasing with time (Fig. 1a, b). Only the sediments sampled in Lake 1 and Lake 2 in 2007, which contained relatively much sulfate (Table 1), exhibited a lag phase before CH\(_4\) production started (Fig. S1). This lag was probably due to inhibition of the methanogenic microbial community by the sulfate-reducing microbial community competing for the same substrates (Ward and Winfrey, 1985). Addition of CH\(_3\)F resulted in a lower CH\(_4\) production due to inhibition of acetoclastic methanogenesis, but usually had a much smaller effect on production of CO\(_2\). The rates are summarized in Table 2.

The δ\(^{13}\)C values of the accumulating CH\(_4\) and CO\(_2\) were also measured during the course of incubation (Fig. 1c). The time period for which values of δ\(^{13}\)C\(_{CH_4}\) and δ\(^{13}\)C\(_{CO_2}\) were averaged is indicated by arrows (Fig. 1c). The average values are summarized in Table 2. Values of δ\(^{13}\)C\(_{CH_4}\) of newly formed CH\(_4\) were calculated from those of accumulated CH\(_4\) using mass balance Eq. (2). The δ\(^{13}\)C\(_{CH_4}\) values across all the different lake sediments (−71.8‰) were generally much lower than values of δ\(^{13}\)C\(_{CO_2}\) (−17.0‰). The values of δ\(^{13}\)C\(_{CH_4}\) decreased even further (−88.8‰) when CH\(_3\)F was added to inhibit acetoclastic methanogenesis. However, CH\(_3\)F had only a relatively small effect on values (−20.8‰) of δ\(^{13}\)C\(_{CO_2}\) (Table 2). Assuming that the CH\(_4\) in the presence of CH\(_3\)F was exclusively formed from reduction of CO\(_2\) (CH\(_4\)-mc), the fractionation factor α\(_{CO_2,CH_4}\) for hydrogenotrophic methanogenesis was calculated from the
acetate samples had to be pooled to obtain sufficient acetate for analysis of δ^{13}C_{ acetate}. The δ^{13}C values of total acetate across all the different lake sediments (−26.8‰) were similar in the presence (−26.7‰) and the absence (−26.8‰) of CH\(_3\)F, but individual lake sediments had both lower and higher δ^{13}C values (Table 3). The δ^{13}C values of acetate-methyl were generally by about 10‰ lower than those of total acetate, both in the presence and absence of CH\(_3\)F. Individual lake sediments, however, exhibited both higher and lower δ^{13}C values when CH\(_3\)F was added (Table 3).

We also used the sediment samples to analyze the abundance, diversity and composition of the microbial community by using quantitative PCR and T-RFLP of the genes coding for the ribosomal rRNA of archaea and bacteria and of the mcrA gene coding for a subunit of the methylcoenzyme M reductase, the key enzyme of methanogenic archaea. The numbers of archaea and bacteria (given as 16S rRNA gene copies) are shown in Table 4. In T-RFLP analysis a total of 17, 109, and 37 different terminal restriction fragments (T-RFs) of archaean 16S rRNA genes, bacterial 16S rRNA genes and mcrA genes, respectively, were detected. On the average 9, 29, and 10, respectively, of these different T-RFs were found in each individual lake sediment. Thus, the richness of T-RFs in the combined lake sediments was larger than in the individual ones. The values of richness (S), Shannon diversity index (H), and evenness (E) for the different lake sediments and genes are summarized in Table 5.
Table 4. Copy numbers of bacterial and archaeal 16S rRNA genes and relative abundance of the most dominant archaeal T-RFs, putatively representing different methanogenic phyla, i.e., *Methanobacteriales* (92 bp), *Methanosarcinales* (186 bp), *Methanosaetaeae* (284 bp), *Methanomicrobiales* or *Methanocellales* (393 bp); mean ± SE, n = 3. Details of lake sites are explained in Table 1.

<table>
<thead>
<tr>
<th>Lake sites</th>
<th>Bacteria gdw⁻¹</th>
<th>Archeaea gdw⁻¹</th>
<th>92 bp (%)</th>
<th>186 bp (%)</th>
<th>284 bp (%)</th>
<th>393 bp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(1.5±0.6)×10⁸</td>
<td>(1.5±0.7)×10⁸</td>
<td>22.3 ± 2.5</td>
<td>20.0 ± 1.2</td>
<td>14.3 ± 1.0</td>
<td>30.5 ± 0.6</td>
</tr>
<tr>
<td>2</td>
<td>(2.6±0.7)×10⁸</td>
<td>(1.8±0.5)×10⁸</td>
<td>28.1 ± 4.6</td>
<td>12.7 ± 1.1</td>
<td>20.1 ± 0.1</td>
<td>26.0 ± 1.2</td>
</tr>
<tr>
<td>3</td>
<td>(4.0±1.3)×10⁷</td>
<td>(1.5±0.2)×10⁷</td>
<td>23.2 ± 0.4</td>
<td>7.6 ± 0.6</td>
<td>28.5 ± 0.7</td>
<td>27.7 ± 1.4</td>
</tr>
<tr>
<td>4</td>
<td>(6.7±0.5)×10⁸</td>
<td>(6.2±0.7)×10⁸</td>
<td>33.1 ± 1.5</td>
<td>19.5 ± 0.5</td>
<td>21.4 ± 0.7</td>
<td>20.9 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>(4.4±1.7)×10⁸</td>
<td>(1.5±0.2)×10⁷</td>
<td>41.5 ± 11.4</td>
<td>4.5 ± 0.0</td>
<td>20.8 ± 1.0</td>
<td>15.0 ± 3.6</td>
</tr>
<tr>
<td>6</td>
<td>(6.5±0.6)×10⁸</td>
<td>(2.7±0.5)×10⁷</td>
<td>33.2 ± 1.3</td>
<td>5.7 ± 1.0</td>
<td>17.3 ± 2.3</td>
<td>22.4 ± 0.6</td>
</tr>
<tr>
<td>7</td>
<td>(6.7±1.4)×10⁸</td>
<td>(3.9±1.1)×10⁷</td>
<td>18.3 ± 4.0</td>
<td>9.3 ± 1.7</td>
<td>11.2 ± 0.1</td>
<td>38.1 ± 3.0</td>
</tr>
<tr>
<td>8</td>
<td>(4.7±1.8)×10⁸</td>
<td>(4.5±2.2)×10⁷</td>
<td>16.3 ± 1.7</td>
<td>9.7 ± 0.2</td>
<td>22.0 ± 1.0</td>
<td>24.6 ± 1.8</td>
</tr>
<tr>
<td>9</td>
<td>(9.4±1.7)×10⁸</td>
<td>(5.5±0.7)×10⁸</td>
<td>8.4 ± 0.7</td>
<td>4.5 ± 0.1</td>
<td>23.0 ± 0.9</td>
<td>36.1 ± 0.6</td>
</tr>
<tr>
<td>10</td>
<td>(1.4±0.2)×10¹¹</td>
<td>(1.9±0.1)×10⁹</td>
<td>10.4 ± 0.8</td>
<td>6.1 ± 0.3</td>
<td>16.9 ± 0.2</td>
<td>35.0 ± 0.4</td>
</tr>
<tr>
<td>11</td>
<td>(1.4±0.2)×10¹⁰</td>
<td>(1.1±0.2)×10⁸</td>
<td>7.5 ± 1.7</td>
<td>16.9 ± 0.8</td>
<td>13.4 ± 0.8</td>
<td>33.2 ± 1.1</td>
</tr>
<tr>
<td>12</td>
<td>(1.8±0.3)×10⁸</td>
<td>(2.9±0.4)×10⁷</td>
<td>10.9 ± 0.8</td>
<td>35.9 ± 0.8</td>
<td>11.4 ± 0.3</td>
<td>37.2 ± 0.5</td>
</tr>
<tr>
<td>13</td>
<td>(1.1±0.1)×10⁸</td>
<td>(5.7±0.5)×10⁷</td>
<td>7.9 ± 0.4</td>
<td>10.3 ± 0.3</td>
<td>32.7 ± 0.9</td>
<td>35.8 ± 2.1</td>
</tr>
<tr>
<td>14</td>
<td>(1.0±0.3)×10⁸</td>
<td>(2.1±0.6)×10⁷</td>
<td>18.2 ± 0.4</td>
<td>14.5 ± 0.4</td>
<td>28.5 ± 0.5</td>
<td>22.3 ± 0.4</td>
</tr>
<tr>
<td>15</td>
<td>(1.5±0.5)×10⁸</td>
<td>(2.6±0.4)×10⁷</td>
<td>8.3 ± 1.0</td>
<td>29.4 ± 0.8</td>
<td>12.5 ± 0.2</td>
<td>30.6 ± 0.8</td>
</tr>
<tr>
<td>16</td>
<td>(7.9±1.5)×10⁸</td>
<td>(3.2±0.4)×10⁷</td>
<td>20.6 ± 4.6</td>
<td>2.1 ± 0.2</td>
<td>39.3 ± 5.2</td>
<td>16.0 ± 3.6</td>
</tr>
</tbody>
</table>

Mean ± SD (1.6±4.0)×10¹⁰ (2.6±5.0)×10⁸ 19.3 ± 10.5 13.0 ± 9.4 20.8 ± 8.1 28.2 ± 7.5

Table 5. Diversity indices (species richness S, evenness E, and Shannon index H) for T-RFLP patterns of the archaeal 16S rRNA, bacterial 16S rRNA and mcrA genes (mean ± SE). Details of lake sites are explained in Table 1.

<table>
<thead>
<tr>
<th>Lake sites #</th>
<th>S</th>
<th>E</th>
<th>H</th>
<th>S</th>
<th>E</th>
<th>H</th>
<th>S</th>
<th>mcrA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.3</td>
<td>0.82</td>
<td>1.73</td>
<td>23.0</td>
<td>0.91</td>
<td>2.84</td>
<td>10.7</td>
<td>0.69</td>
</tr>
<tr>
<td>2</td>
<td>10.7</td>
<td>0.76</td>
<td>1.79</td>
<td>33.7</td>
<td>0.91</td>
<td>3.22</td>
<td>9.7</td>
<td>0.69</td>
</tr>
<tr>
<td>3</td>
<td>9.0</td>
<td>0.78</td>
<td>1.71</td>
<td>37.0</td>
<td>0.96</td>
<td>3.46</td>
<td>9.7</td>
<td>0.70</td>
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<tr>
<td>4</td>
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<td>0.82</td>
<td>1.54</td>
<td>26.3</td>
<td>0.95</td>
<td>3.11</td>
<td>10.0</td>
<td>0.67</td>
</tr>
<tr>
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<td>0.78</td>
<td>1.57</td>
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<td>0.89</td>
<td>2.71</td>
<td>10.7</td>
<td>0.79</td>
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<tr>
<td>6</td>
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<td>0.80</td>
<td>1.79</td>
<td>23.3</td>
<td>0.89</td>
<td>2.80</td>
<td>9.0</td>
<td>0.83</td>
</tr>
<tr>
<td>7</td>
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<td>0.82</td>
<td>1.79</td>
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<td>0.86</td>
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</tr>
<tr>
<td>8</td>
<td>12.0</td>
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<td>2.04</td>
<td>30.0</td>
<td>0.94</td>
<td>3.18</td>
<td>10.7</td>
<td>0.84</td>
</tr>
<tr>
<td>9</td>
<td>9.3</td>
<td>0.83</td>
<td>1.85</td>
<td>24.7</td>
<td>0.91</td>
<td>2.91</td>
<td>8.7</td>
<td>0.87</td>
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<tr>
<td>10</td>
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<td>0.85</td>
<td>2.00</td>
<td>23.8</td>
<td>0.96</td>
<td>3.50</td>
<td>8.7</td>
<td>0.81</td>
</tr>
<tr>
<td>11</td>
<td>10.3</td>
<td>0.85</td>
<td>1.99</td>
<td>26.7</td>
<td>0.91</td>
<td>2.96</td>
<td>9.3</td>
<td>0.82</td>
</tr>
<tr>
<td>12</td>
<td>7.0</td>
<td>0.73</td>
<td>1.40</td>
<td>25.0</td>
<td>0.94</td>
<td>3.02</td>
<td>12.7</td>
<td>0.86</td>
</tr>
<tr>
<td>13</td>
<td>8.0</td>
<td>0.78</td>
<td>1.61</td>
<td>33.0</td>
<td>0.95</td>
<td>3.30</td>
<td>12.7</td>
<td>0.75</td>
</tr>
<tr>
<td>14</td>
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<td>0.83</td>
<td>1.82</td>
<td>37.0</td>
<td>0.94</td>
<td>3.38</td>
<td>12.3</td>
<td>0.82</td>
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<tr>
<td>15</td>
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<td>1.81</td>
<td>32.3</td>
<td>0.97</td>
<td>3.37</td>
<td>9.7</td>
<td>0.88</td>
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<tr>
<td>16</td>
<td>8.0</td>
<td>0.75</td>
<td>1.55</td>
<td>27.0</td>
<td>0.88</td>
<td>2.90</td>
<td>10.3</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Mean ± SD 9.1 ± 1.6 0.80 ± 0.04 1.75 ± 0.18 28.5 ± 6.0 0.92 ± 0.03 3.07 ± 0.29 10.4 ± 1.2 0.79 ± 0.07 1.84 ± 0.19

The T-RFLP patterns of the different genes were also analysed by canonical correspondence analysis (Fig. 2). The T-RFLP patterns of the lake sediments did not show a consistent clustering across the different genes indicating that bacterial and archaeal communities did not follow a geographical pattern. The T-RFLP patterns of the individual lakes were also not consistently correlated to one of the different vectors that indicate the chemical/physiological parameters measured, such as Corg, Δ¹³Corg, CH₄ production, abundance of archaea and bacteria, Δ¹³CCH₄, Δ¹³CCH₄₄—me₄, Δ¹³Cac, and Δ¹³Cac—methyl (Fig. 2).

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Fig. 1. Production of CH₄ and CO₂ in anaerobically incubated sediment of Lake Jatoba in the presence and absence of 2% methyl fluoride: (A) accumulation of CH₄ in the headspace; (B) accumulation of CO₂ in the headspace; (C) δ¹³C of the accumulated CH₄ and CO₂; average ± SE; n = 3. The arrows indicate the period used for averaging data for determination of production rates or average δ¹³C.

Fig. 2. Canonical correspondence analysis ordination plot for the effect of Corg, δ¹³Corg, CH₄ production, abundance of archaea and bacteria, δ¹³CC₄H₄, δ¹³CO₂, δ¹³CCH₄-methyl, δ¹³Cac, and δ¹³Cmethyl on the composition of the (A) archael 16S rRNA, (B) bacterial 16S rRNA, and (C) mcrA gene community in lake sediments, based on the relative abundances of respective T-RFs from the sediments of three replicate measurements. Symbol legend and eigenvalues of the first and second axes are included.

Most of the T-RFs in the T-RFLP patterns of archael 16S rRNA gene could be assigned to groups of methanogenic archaea on the basis of published clone sequences (e.g., Conrad et al., 2010b): Methanomicrobiales (84 bp); Methanobacteriales (92 bp); Methanosarcinaeae (186 bp); Methanosacetaceae (284 bp); Methanomicrobiales and Methanocellales (393 bp). These methanogenic groups contributed on the average 81% to the total archael community (Table 4).
4 Discussion

Incubation experiments with samples from 16 different lake sediments in tropical Brazil allowed estimating the activity and isotope fractionation involved in CH₄ production from organic matter. In particular, the data gave insight into the control of CH₄ production rate, the role of the microbial community, the path of CH₄ production, and the carbon isotope fractionation during acetate production.

4.1 Control of CH₄ and CO₂ production

The rates of CH₄ in the different lake sediments linearly increased with the content of Corg (Fig. 3a). The lake sediments with the highest organic carbon contents were from the Pantanal. Production rates of CH₄ almost doubled (relative slope = 0.83) with doubling of the organic matter content. These data show that organic matter content was a rate-limiting factor for CH₄ production. This is a reasonable conclusion, since the initial mobilization of organic substances is believed to be the rate-limiting step for further degradation and eventual CH₄ production (Billen, 1982). The dependency on organic matter content furthermore indicates that the CH₄ production process was under quasi steady state conditions. This is a reasonable conclusion, since acetate, or other organic intermediates apparently did not accumulate during the incubation but were below or close to the detection limit of the analysis. Acetate only accumulated, when its consumption by acetoclastic methanogens was inhibited with CH₃F. The observation that CH₄ production scales with the sediment organic matter content is in agreement with an analysis of lake characteristics determining CH₄ emission by Bastviken et al. (2004). These authors found that lake area, along with total phosphorous and dissolved organic carbon concentrations were the most useful variables for describing methane emissions on a global basis. Ebullication is the most likely fate (>50%) of the produced CH₄ especially in relatively shallow lakes. In lakes of the Pantanal, ebullition was found to account on the average for 91% of the total CH₄ flux (Bastviken et al., 2010). Generally, only a small percentage of the total organic matter present in anoxic sediments is used up for production of CH₄ and CO₂. Therefore, total sediment organic matter is only a proxy for the organic matter that is actually available for microorganisms and eventually degraded on the time scale used for our experiments. However, in the Brazilian lake sediments, sediment Corg was apparently a good predictor for potential CH₄ production.

Production rates of gaseous CO₂ also increased linearly with organic matter content (data not shown). The production rates of gaseous CO₂ versus CH₄ were almost proportional to each other (y = 0.74 + 0.77 x; r² = 0.83; P < 0.0001) but not quite equimolar. According to complete degradation of organic matter should actually produce equimolar amounts of CO₂ and CH₄ (i.e., relative slope = 1.0). However, the measured CO₂ production rates covered only the gaseous portion of CO₂ in the incubations. Rates of total CO₂ production were determined by accounting for dissolved CO₂ and bicarbonate, which were calculated from Henry’s law, the pH and the bicarbonate dissociation constant. In fact, total CO₂ production rates were almost two times higher than CH₄ production rates (Fig. 3b). Such behaviour is frequently observed in acidic bogs (Galand et al., 2010; Heitmann et al., 2007; Keller et al., 2009; Yavitt and Seidmann-Zager, 2006), but also in lake sediments (Conrad et al., 2010a, 2010b). It has been explained by the assumption that organic substances may act to some extent as electron acceptors for oxidation of organic matter, so that Eq. (6) is not rigorously valid. Nevertheless, this effect seemed to be rather small when considering the range of CH₄ and CO₂ production in the different lake sediments investigated.

Not only the production rates of CH₄ and CO₂ were related to the organic matter content of the sediment, also the δ¹³C of CH₄, CO₂ (and also acetate, see discussion below) were all correlated to the δ¹³C of organic matter (Fig. 4). This is reasonable since all are eventually produced from organic matter. However, the δ¹³C values were neither correlated with the content of organic matter nor the production rates of CH₄ and CO₂.

4.2 Role of the microbial community

The numbers of archaea (given as 16S rRNA gene copies) were generally one order of magnitude lower than those of the bacteria, similarly as observed in other lake sediments (Chan et al., 2005; Koizumi et al., 2003; Schwarz et al., 2007a; Zepp-Falz et al., 1999). Both numbers were correlated with each other (Fig. 5a). Production rates of CH₄ increased linearly with the logarithm of numbers of archaea (Fig. 5b) and bacteria (not shown). This is plausible since microorganisms are catalyzing the biogeochemical processes, including degradation of organic matter to CO₂ and CH₄. Vice versa, the transformation of organic matter provides the energy for growth and maintenance of the microorganisms. Production rates of CH₄ more than quadrupled (relative slope = 4.4) when the abundance of archaea increased by an order of magnitude. The increase of microbial numbers with CH₄ production rates is reasonable since microorganisms can proliferate when energy supply increases, i.e. the rate of methanogenic organic matter degradation increases. The number of methanogenic archaea that can be maintained by a particular CH₄ production rate can be calculated from the microbial maintenance requirement which at 25 °C is constant at 4.5 kJ h⁻¹ C-mol⁻¹ biomass (Tijhuis et al., 1993). Using this constant parameter, theoretical calculations indicate that a CH₄ production rate of 100 nmol h⁻¹ g⁻¹ could maintain about 6 × 10⁷ g⁻¹ methanogenic archaea (Conrad,
(B) Methanogenic and fermentative archaeal ribosomal genes per gram dry sediment (Fig. 5). Since methanogenic archaea typically contain 3 ribosomal gene copies per cell (Klappenbach et al., 2001), the lake sediments correspondingly contained between 3 × 10^6 and 7 × 10^6 methanogenic archaea per gram sediment. However, the CH4 production rates were only between 0.5 and 14 nmol h^-1 g^-1 dw, thus theoretically allowing the maintenance of about only 3 × 10^5 to 8 × 10^6 g^-1 methanogens, which is 1–2 orders of magnitude lower than the actually measured values. This is a large discrepancy even when considering uncertainties in the theoretical calculation. We hypothesize that much of the microbial DNA, i.e., the ribosomal genes detected, may be preserved in dead cells and no longer take part in the metabolism and substrate turnover in the lake sediment.

If this is true, the analysis of DNA and of genes in the sediment would not reflect the active microbial community but mainly give an impression about the history of microbial activity. In sediments, in which metabolism is in a stable steady state, these two aspects would not be much different, but sediments which undergo seasonal and daily changes might well result in strong differences. For the Brazilian lake sediments we have to expect the latter case, since these lakes are part of river floodplains which exhibit flooding pulses.

Such effect was probably the reason why sediment taken from the central of Lake 1 in 2008 (#8) behaved completely different from samples taken in 2007 (#10). Sample #8 was taken at a time when the lake was just at the beginning of the wet season, while the samples #9–11 were taken during the time when water from the Paraguay River inundated the lakes. At this time, the sulfate content of the sediment (#8) was much larger than in #10, and CH4 production exhibited a lag phase, probably because sulfate reducers outcompeted methanogens.

Analysis of the relative composition of the communities of bacteria, archaea and mcrA-containing methanogens showed quite diversity in each of the different Brazilian lake sediments. The Shannon index of bacterial community patterns ranged between 2.5 and 3.9, whereas that for archaea and methanogens was a bit lower between 1.4 and 2.0 (Table 5). The evenness of the bacterial community patterns were generally higher than 0.67 and reached maximum values of 0.97. These diversity indices should not be taken as absolute numbers, since molecular fingerprinting techniques only reveal the relatively abundant taxa but cannot cover the less common microbes (Bent and Forney, 2008). However, the indices and the T-RFLP patterns can be compared across the different lakes. The comparison shows that the composition of the communities was different for each lake and exhibited no pattern with respect to geographical location, organic matter content, microbial abundance, activity or isotope fractionation. The composition patterns were also different with respect to bacteria, archaea and methanogens. Neither of the diversity indices exhibited a significant correlation to the CH4 production rates. The failure to detect a microbial community pattern that would be related to the microbial functionality of the lake sediments may have several reasons: (1) the number of lake sediments tested was still too limited to detect such a relationship; (2) such a relationship does not exist on the level of microbial genes detected but might exist on the level of microbial transcripts (expression of genes) or microbial proteins (transcription and translation of genes); (3) such a relationship is not visible on the level of genes.

Fig. 3. Linear regression of production rates of CH4 and CO2 determined in 16 different lake sediments; (A) CH4 production against the content of Corg; (B) Total CO2 production against CH4 production; numbers of data points refer to the identity of the lake sediment as shown in Table 1; error bars give standard errors of n = 3; the result of linear regression is given by a thin straight line with small-dotted curved lines as 95% confidence limit; the dashed straight line in (B) indicates the 1:1 proportionality line between the two production rates.
of global genes, such as ribosomal RNA or mcrA genes, but only when targeting genes that allow differentiating between different peculiar functions; (4) there is no such relationship

at all, since diverse microbial species can replace each other to fulfill a particular functionality.

Although the data give an impression of the diversity of the bacterial, archaeal and methanogenic communities, they do not give insight into their phylogenetic and taxonomic structure. Such information can usually only be obtained after extensive sequencing of 16S rRNA and mcrA genes. The T-RFLP patterns of bacterial 16S rRNA and methanogenic mcrA genes are usually complex, and affiliation of phylogenetic groups to individual T-RFs is difficult without sequence information from the samples studied. However, such affiliation may be feasible with respect to archaeal T-RFLP patterns, which generally show a highly reproducible and relatively simple pattern (e.g., Conrad et al., 2010b). Thus, we are confident that the methanogenic groups identified by T-RFLP analysis of archaeal 16S rRNA genes (Table 4) are trustworthy. The methanogens comprised both putatively acetoclastic methanogens (Methanosarcinae and Methanosaetaeae) and hydrogenotrophic methanogens (all the groups except Methanosaetaeae). The family Methanosarcinae may also comprise species that are potentially methylotrophic (e.g., consuming methanol). However, the relative abundance of the different methanogenic groups in the different lake sediments did not correlate with biogeochemical characteristics (compare 4.3 below).

4.3 Path of CH₄ production

The fractionation factors (αCO₂,CH₄) of hydrogenotrophic methanogenesis, which were calculated from δ¹³C of CO₂ and CH₄ in the presence of CH₃F, did not reveal correlation with any other variable in the individual lake sediments, e.g., Corg or δ¹³Corg. However, the δ¹³C of CH₄ and CO₂ were all correlated to each other and to the δ¹³C or organic matter (Fig. 4). The relative increase of δ¹³CCH₄ and δ¹³CCO₂ was larger than that of δ¹³Corg with slopes of 2.9–3.5 (Fig. 4). On the average, the δ¹³C of newly formed CH₄ was 43‰ lower and that of CO₂ was 12‰ larger than δ¹³Corg. These data show that the δ¹³C of CH₄ and CO₂ were not only determined by the δ¹³Corg but also by the fractionation during the degradation process. In the presence of CH₃F, the difference between δ¹³CCH₄−CH₃F and δ¹³Corg was 60‰, which is about 17‰ more than with δ¹³CCH₄ in the absence of CH₃F. This increase is due to the inhibition of acetoclastic methanogenesis by CH₃F and the subsequently exclusive formation of CH₄ by hydrogenotrophic methanogenesis, which exhibits a larger fractionation factor than acetoclastic methanogenesis. The difference of 60‰ lower CH₄ and 12‰ larger CO₂ results in a difference of about 72‰ between δ¹³C of CO₂ and of hydrogenotrophically formed CH₄, which corresponds to αCO₂,CH₄ = 1.075 determined from the measured δ¹³CCO₂ and δ¹³CCH₄ measured in the presence of CH₃F. Thus, fractionation had the larger effect on δ¹³CCO₂ and δ¹³CCH₄ than the δ¹³Corg. The fractionation factors (αCO₂,CH₄) for the 16 different tropical lake sediments

![Graph](image-url)
Fig. 5. Linear regression of (A) numbers of archaea against numbers of bacteria in the sediment (given as copies of the 16S rRNA gene); (B) CH₄ production rates against numbers of archaea; other explanations see Fig. 3.

Fig. 6. Linear regression of the percent fraction ($f_{CO_2, CH_4}$) of hydrogenotrophic methanogenesis in 16 different lake sediments against (A) the average $\delta^{13}$C of newly formed CH₄ in the absence of CH₃F; (B) the $\delta^{13}$C of sediment organic matter; $f_{CO_2, CH_4}$ determined from Eq. (4) using $\varepsilon_{ac, CH_4} = 0$; other explanations see Fig. 3.

(Table 2) ranged between 1.050 and 1.092, also covering the $\alpha_{CO_2, CH_4}$ values of about 1.085 recently determined for two lake sediments in Amazonia (Conrad et al., 2010b). These data are consistent with literature data on aquatic sediments in which the energy provided by H₂ is limiting CH₄ production (Penning et al., 2005; Takai et al., 2008; Valentine et al., 2004).

The values of $\delta^{13}$C of newly formed CH₄ were used for calculation of the fraction ($f_{CO_2, CH_4}$) of CH₄ production by hydrogenotrophic methanogenesis using Eq. (4). The equation uses $\delta^{13}$C of CH₄, of CH₄-mc and of CH₄-ma, the latter being the CH₄ formed from aceticlastic methanogenesis. The values of $f_{CO_2, CH_4}$ are summarized in Fig. 6 and Fig. 7. First, it was assumed that $\delta^{13}$C(CH₄–ma) was identical to the $\delta^{13}$C of the acetate-methyl ($\delta^{13}$Cac–methyl). This assumption means that acetate-methyl was not further fractionated during aceticlastic methanogenesis Eq. (5), which is the case if acetate is consumed as rapidly as it is produced. This was most likely the case, since acetate concentrations were very low in all the lake sediments studied and only increased when aceticlastic methanogenesis was inhibited by CH₃F. Values of $f_{CO_2, CH_4}$ were generally higher than 50% (50–90%) demonstrating that hydrogenotrophic methanogenesis was more important than aceticlastic methanogenesis in all the lake sediments. The error imposed by neglecting a putative fractionation during formation of $\delta^{13}$C(CH₄–ma) is relatively small since the theoretical fractionation factor for the conversion of acetate-methyl to CH₄ is at the maximum $\varepsilon_{ac, CH_4} = 1.025$ ($\varepsilon_{ac, CH_4} = -25\%$) when Methanosarcina species are the active methanogens (Gelwicks et al., 1994; Goevert and Conrad, 2009) or $\varepsilon_{ac, CH_4} = 1.010$ (ac, CH₄ = $-10\%$) when Methanosaeta species are the active methanogens (Penning...
became increasingly larger in those lake sediments that had unspecific inhibition of hydrogenotrophic methanogenesis. In the present study we found that the small changes in CH$_3$CH$_3$ that hydrogenotrophic methanogenesis is indeed sensitive to. Therefore, we have to assume that CH$_4$F inhibition of acetoclastic methanogenesis by CH$_3$F (Fig. 7b). The $f_{CO_2,CH_4}$ values showed a significant negative correlation with the $\delta^{13}C_{CH_4-mc}$ (Fig. 6a), which was expected from Eq. (4), but no significant correlation with $\delta^{13}C_{ac-methyl}$ ($y = 74.5 + 0.26 x; r^2 = 0.05; P = 0.41$). The $f_{CO_2,CH_4}$ values also showed no correlation with $\alpha_{CO_2,CH_4}$ ($r^2 = 0$) and CH$_4$ production rates ($r^2 = 0.03$), and the decreasing tendency with $\delta^{13}C_{org}$ ($r^2 = 0.11$) was not significant (Fig. 6b).

The values of $f_{CO_2,CH_4}$ also correlated positively, but not proportionally, with the residual CH$_4$ production after inhibition of acetoclastic methanogenesis by CH$_3$F (Fig. 7). Such correlation is expected, as the residual rates are the CH$_4$ production rates diminished by those of acetoclastic methanogenesis and should be equal to the rates of hydrogenotrophic methanogenesis. However, $f_{CO_2,CH_4}$ was not proportional to the residuals and increased with a relative slope of much smaller than one. The result was principally the same whether fractionation during acetoclastic methanogenesis was assumed to be zero or $\varepsilon_{ac,CH_4} = -10$ (Fig. 7). Therefore, we have to assume that CH$_3$F inhibited not only acetoclastic methanogenesis but also to some degree hydrogenotrophic methanogenesis. Previous studies had shown that hydrogenotrophic methanogenesis is indeed sensitive to CH$_3$F if applied at a too high concentration (Conrad and Klose, 1999). It is hardly possible to optimize inhibition by CH$_3$F for each individual sediment, in particular, since quite small changes in CH$_3$F concentration can result in change of the inhibition pattern. In the present study we found that the unspecific inhibition of hydrogenotrophic methanogenesis became increasingly larger in those lake sediments that had a relatively low $f_{CO_2,CH_4}$. To check whether the deviation between $f_{CO_2,CH_4}$ and the residual CH$_4$ production might be due to interference by methylotrophic methanogenesis, the difference between the two data was plotted against the relative abundance of the Methanosarcinaceae (Table 5), which may contain methylotrophic species, but the plot showed no significant correlation ($r^2 = 0.02$).

The determination of $f_{CO_2,CH_4}$ assumed that the CH$_4$ produced in the presence of CH$_3$F was exclusively due to hydrogenotrophic methanogenesis, as acetoclastic methanogenesis was completely inhibited (Conrad, 2005). Previous studies with other lake sediments have shown that this assumption is robust (Conrad et al., 2007, 2009, 2010b). The observation of accumulation of some propionate in addition to acetate indicates that secondary fermentation was also partially inhibited, most probably by accumulating acetate and H$_2$ causing thermodynamic inhibition (Rothfuss and Conrad, 1993). Although H$_2$ was not measured, we assume increased H$_2$ partial pressures in the presence of CH$_3$F due to partially inhibition of hydrogenotrophic methanogenesis (Conrad et al., 2010b).

It is noteworthy that the determined values of $f_{CO_2,CH_4}$ were relatively large, independently of the $\varepsilon_{ac,CH_4}$ chosen. This observation is consistent with determinations in other lake sediments (Conrad, 1999; Conrad et al., 2009; 2010a; 2010b), but cannot be explained by the complete degradation of organic matter according to Eq. (6), since

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

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

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

where hydrogenotrophic methanogenesis can only achieve a maximum contribution of 33% (Conrad, 1999). Conceivable explanations are (1) that organic matter in the lake sediments is incompletely degraded with the preferential production of H$_2$ and the accumulation of residual organic substances having a higher oxidation state than the original C$_{org}$. 

![Fig. 7. Linear regression of the percent fraction ($f_{CO_2,CH_4}$) of hydrogenotrophic methanogenesis against the residual rate (percent of the uninhibited rate) of CH$_4$ production measured in the presence of CH$_3$F using (A) $\varepsilon_{ac,CH_4} = 0$, and (B) $\varepsilon_{ac,CH_4} = -10$‰; other explanations see Fig. 3.](image-url)
Another possible explanation is (2) that intermediate acetate is converted by syntrophic acetate oxidation instead of acetoclastic methanogenesis (Conrad et al., 2010a). Syntrophic acetate oxidation is the conversion of acetate to CO₂ plus H₂

\[
\text{CH}_3\text{OOH} + 2\text{H}_2\text{O} \rightarrow 4\text{H}_2 + 2\text{CO}_2
\]  

(10)

followed by hydrogenotrophic methanogenesis Eq. (9). Such a pathway was detected in sediment of subtropical Lake Kinneret Nüsslein et al., 2001, 2003), but it disappeared again later on being replaced by acetoclastic methanogenesis (Schwarz et al., 2007b). We cannot completely exclude syntrophic acetate oxidation in the Brazilian lake sediments, but note that syntrophic acetate oxidizers would then have to be sensitive to CH₃F inhibition similarly as acetoclastic methanogens. Otherwise the accumulation of acetate in the presence of CH₃F could not be explained. Furthermore, all the lake sediments contained archaean 16S rRNA sequences of putative acetoclastic Methanosaeta (Table 4), so that acetate could well have been consumed by methanogens rather than syntrophic acetate oxidizers.

As further possible explanation (3) some of the CH₄ may have been produced not only from H₂/CO₂ and acetate but also from methanol.

\[
4\text{CH}_3\text{OH} \rightarrow 3\text{CH}_4 + \text{CO}_2 + 2\text{H}_2\text{O}
\]  

(11)

Methanol is a product of microbial pectin degradation (Schink and Zeikus, 1982). However, the amount of methanol produced from pectin to that of acetate and H₂, which are also produced, would account for less than 25% of pectin-derived CH₄ production. In fact, whenever methylotrophic methanogenesis was quantified in freshwater environments by tracer experiments, it was found to contribute less than 5% and thus was considered to be negligible (Conrad and Claus, 2005; Lovley and Klug, 1983). Conversion of methanol to CH₄ exhibits a large isotopic enrichment factor (ε(CH₃OH,CH₄) = −74‰) similar to that of hydrogenotrophic methanogenesis (Krzyczki et al., 1987). Therefore, contribution of methanol to CH₄ formation can hardly be detected against a background of other processes that also produce relatively ¹³C-depleted CH₄ (Conrad and Claus, 2005). Thus, hydrogenotrophic methanogenesis could be mistaken as methylotrophic methanogenesis and vice versa. However, since methanol is probably as rapidly consumed as it is produced, isotope fractionation is unlikely. Only a limited number of methanogenic species within the family Methanosarcinaceae are able to convert methanol to CH₄. Recently, such methanogens were found to exhibit a remarkable potential of CH₄ production from methanol in sediment from the Tibet plateau, but it is still unclear whether this potential is also relevant at in-situ conditions (Zhang et al., 2008). Considering all these arguments we believe that methylotrophic methanogenesis and syntrophic acetate oxidation are unlikely to explain a major percentage of CH₄ production. Instead, we think that incomplete degradation of organic matter with preferential production of H₂ is the more likely explanation for the relatively high values of fCO₂–CH₄ determined.

Values of fCO₂–CH₄ were not significantly correlated with the relative abundance of any of the methanogenic groups determined by T-RFLP analysis (Table 4). However, fCO₂–CH₄ in the different lake sediments showed a slightly increasing tendency with the percentage abundance of Methanosarcinaceae (Fig. 8a) and slightly decreasing one with the Methanosaetaceae (Fig. 8b), albeit both being statistically not significant. These tendencies are consistent with the assumption that Methanosarcinaceae and Methanosaetaceae were involved in hydrogenotrophic and acetoclastic methanogenesis, respectively, which is a reasonable assumption. Values of fCO₂–CH₄ also increased with the percentage...
of all putatively hydrogenotrophic methanogens (i.e., T-RFs of 92, 186, and 393 bp), but again far below the level of statistical significance. Nevertheless, these observations are encouraging for future research to eventually reveal a significant relation between microbial community patterns and microbial functionality if sufficient lake sediments are tested for relevant microbial genes and their transcription.

4.4 Carbon isotope fractionation during acetate production

The isotopic signature of the acetate may be indicative for the processes being involved in acetate production and/or consumption (Heuer et al., 2010). While the kinetic isotope effect during acetate consumption would always result in the residual acetate being \( ^{13} \)C-enriched, that during acetate production would either result in only little fractionation (fermentation) or in a significant \( ^{13} \)C-depletion (homoacetogenesis) of the produced acetate with respect to its substrate (Heuer et al., 2010).

Acetate consumption most likely occurred by acetoclastic methanogenesis because of acetate accumulation in the presence of \( \mathrm{CH}_3 \mathrm{F} \), an inhibitor of acetoclastic methanogens (Janssen and Frenzel, 1997). Syntrophic acetate oxidation may also have contributed to acetate consumption, but substantial contribution was only likely if this process was sensitive to \( \mathrm{CH}_3 \mathrm{F} \) inhibition, which is not known. Acetate oxidation by sulfate reduction or reduction of other electron acceptors (e.g., ferric iron) is unlikely for the same reasons. Furthermore, \( \mathrm{CH}_4 \) production should then have started with a lag phase, which was not observed (except lake #8; see 4.2 above). Assimilation of acetate into biomass can be neglected, since microbial growth was most probably negligible during the time sediment was incubated (see 4.2 above).

The \( ^{13} \)C values of total acetate in the presence of \( \mathrm{CH}_3 \mathrm{F} \) were sometimes lower and sometimes higher than those in the absence (Table 3). Across all the different lake sediments, however, the two data sets of \( ^{13} \)C-ac were correlated to each other in an almost proportional way (Fig. 9a), indicating that on the average, acetate was not fractionated during consumption by \( \mathrm{CH}_3 \mathrm{F} \)-sensitive processes like acetoclastic methanogenesis. The same was true for the \( ^{13} \)C values of acetate-methyl, albeit exhibiting a larger variability. This observation does not exclude that acetate is fractionated in individual lake sediments. Indeed, a higher \( ^{13} \)C value in the absence versus the presence of \( \mathrm{CH}_3 \mathrm{F} \), as theoretically expected for ongoing fractionation, was observed in several sediments (Figs. 9a, 10, Table 3), indicating \( \varepsilon_{\text{ac,CH}_4} \geq -10 \%e \). Such fractionation is consistent with the operation of acetoclastic \textit{Methanosetaea} species, which we also used as a possible condition for the determination of the fraction \( f_{\text{CO}_2,\text{CH}_4} \) of hydrogenotrophically produced \( \text{CH}_4 \) (see 4.3 above). Syntrophic acetate oxidation was recently found to fractionate acetate similarly \( (\varepsilon_{\text{ac,CH}_4} \geq -10 \%e) \) (Conrad and Klose, 2011). Therefore, the \( ^{13} \)C values of acetate do not allow discrimination between the operation of acetoclastic methanogenesis versus syntrophic acetate oxidation.

We may assume that the \( ^{13} \)C values of acetate accumulated in the presence of \( \mathrm{CH}_3 \mathrm{F} \) were largely due to \( ^{13} \)-isotope fractionation during acetate production. Acetate may be produced by either fermentation of organic substrates (primary or secondary fermentation) or by homoacetogenesis. Homoacetogenesis is achieved by either chemolithoautotrophic homoacetogenesis, organoautotrophic or organoheterotrophic homoacetogenesis (Lever et al., 2010). While fermentative production of acetate is accompanied by little isotope fractionation of only a few permil (Blair et al., 1985; Penning and Conrad, 2006; Rinaldi et al., 1974), chemolithoautotrophic acetate production exhibits a large fractionation with acetate being \( ^{13} \)C-depleted relative to \( \mathrm{CO}_2 \) by about 59\%e (Gelwicks et al., 1989). The acetate produced during organoautotrophic or organoheterotrophic homoacetogenesis is most likely also \( ^{13} \)C-depleted relative to the substrate, albeit this has not yet been determined explicitly using microbial cultures (Lever et al., 2010).

Values of \( ^{13} \)C\textsubscript{ac–CH\textsubscript{3}F} were positively correlated to \( ^{13} \)C\textsubscript{org}, albeit with a slope larger than 2 (Fig. 9b), indicating that organic carbon was a source of acetate. Heuer et al. (2010) investigated two German lake sediments and found that \( ^{13} \)C\textsubscript{ac–CH\textsubscript{3}F} was almost equal to \( ^{13} \)C\textsubscript{org}. Lever et al. (2010) investigated the vertical profile of a marine sediment and found that \( ^{13} \)C\textsubscript{ac} values were lower than \( ^{13} \)C\textsubscript{org}. In our samples, five out of 16 lake sediments exhibited a \( ^{13} \)C\textsubscript{ac–CH\textsubscript{3}F} lower than \( ^{13} \)C\textsubscript{org}, but most of the samples exhibited a higher \( ^{13} \)C\textsubscript{ac–CH\textsubscript{3}F}. Possibly, the carboxyl group of acetate partially exchanged with the environmental \( \mathrm{CO}_2 \) (DeGraaf et al., 1996), thus affecting the \( ^{13} \)C of total acetate. However, it is unclear whether such an exchange reaction operates, since \( ^{13} \)C values of total acetate seem to discriminate well between the different processes involved (Heuer et al., 2010).

The \( ^{13} \)C of acetate-methyl was generally lower than \( ^{13} \)C\textsubscript{org}. Furthermore, \( ^{13} \)C\textsubscript{ac–methyl} increased at the same rate (relative slope = 1.1) as \( ^{13} \)C of \( \text{C}_{\text{org}} \) (Fig. 9c) as theoretically expected. However, it should be noted that the linear regression was not statistically significant for the number of data available. Nevertheless, the \( ^{13} \)C of acetate-methyl was on the average by 10\%e lower than that of \( \text{C}_{\text{org}} \) indicating some fractionation, possibly during fermentative acetate production. Previous studies in lake sediments also found that acetate-methyl was more negative than \( ^{13} \)C\textsubscript{org}, the difference ranging between 0 and 10\%e (Conrad et al., 2007, 2009, 2010b). Here, however, we observed a much larger range of 4–40\%e. The smaller values can be explained from experiments with pure cultures of fermenting bacteria, in which \( ^{13} \)C of acetate was only slightly different from \( ^{13} \)C of the substrate (Blair et al., 1985; Penning and Conrad, 2006; Rinaldi et al., 1974). However, for \( ^{13} \)C\textsubscript{ac–methyl} being 40\%e more negative than \( ^{13} \)C\textsubscript{org} there is no immediate explanation, except that the effective substrate from
Fig. 9. Linear regression of the $\delta^{13}$C of total acetate and acetate-methyl against the $\delta^{13}$C of sediment organic matter determined in 16 different lake sediments: (A) average $\delta^{13}$C of total acetate formed in the presence of CH$_3$F against that formed in the absence of CH$_3$F; (B) average $\delta^{13}$C of total acetate formed in the presence of CH$_3$F against $\delta^{13}$C of sediment organic carbon; (C) average $\delta^{13}$C of acetate-methyl formed in the presence of CH$_3$F against $\delta^{13}$C of sediment organic carbon; other explanations see Fig. 3.

Fig. 10. Linear regression of the difference between the $\delta^{13}$C of acetate or acetate-methyl measured in the absence and presence of CH$_3$F (Difference = $\delta^{13}$C$_{ac} - \delta^{13}$C$_{ac-CH_3F}$) in 16 different lake sediments; (A) regression against the $\delta^{13}$C of sediment organic matter using $\delta^{13}$C of total acetate; (B) regression against the $\delta^{13}$C of sediment organic matter using $\delta^{13}$C of acetate-methyl; (C) regression against the acetate-intramolecular difference of $\delta^{13}$C (Difference = $\delta^{13}$C$_{ac} - \delta^{13}$C$_{ac-methyl}$); other explanations see Fig. 3.
which acetate was produced may have been more negative than the $\delta^{13}C_{\text{org}}$ measured or the isotope effect involved was much larger than expected, perhaps including chemolithoautotrophic homoacetogenesis in some of the lake sediments.

We also found in some of the sediments that the $\delta^{13}C$ of accumulated acetate was higher in the presence than in the absence of CH$_3$F. The difference was only $<10‰$, and was larger for total acetate (Fig. 10a) than for acetate-methyl (Fig. 10b), but it was the same lake sediments where acetate carbon was fractionated this way. This way of fractionation is not expected. An analysis of the difference between the $\delta^{13}C_{\text{ac}}$ and $\delta^{13}C_{\text{ac−CH}_3F}$ showed that it was negatively correlated with $\delta^{13}C_{\text{org}}$ and changed from a positive to a negative value at a $\delta^{13}C_{\text{org}}$ of about $−30‰$ (Fig. 10a). A similar relationship was obtained using acetate-methyl instead of total acetate but was not significant (Fig. 10b). The difference between $\delta^{13}C_{\text{ac}}$ and $\delta^{13}C_{\text{ac−CH}_3F}$ was also negatively correlated with the intramolecular isotopic difference between carboxyl and methyl groups of acetate (as seen from the difference between $\delta^{13}C_{\text{ac}}$ and $\delta^{13}C_{\text{ac−methyl}}$) (Fig. 10c). However, the difference between $\delta^{13}C_{\text{ac}}$ and $\delta^{13}C_{\text{ac−CH}_3F}$ exhibited no significant correlation with other parameters, such as CH$_4$ production rate, fraction of hydrogenotrophic methanogenesis, fractionation factors, microbial numbers, etc. Such a counterintuitive fractionation of acetate carbon had also been observed in the sediment of a German lake (Conrad et al., 2009).

In chemolithoautotrophic homoacetogenesis, acetate is synthesized from CO$_2$ (i.e., reversal of Eq. (10). The acetate synthesized in this way exhibits only a small isotopic difference between methyl and carboxyl groups being on the order of $<2‰$ (Gelwicka et al., 1989). The acetate synthesized during fermentation, on the other hand, may have a much larger intramolecular isotopic difference being on the order of 24‰ (Blair et al., 1985). In the Brazilian lake sediments, the difference between $\delta^{13}C_{\text{ac}}$ and $\delta^{13}C_{\text{ac−methyl}}$ was between 5‰ and 17‰ (except Lake #15, Paca, Fig. 5) which corresponds to an intramolecular isotopic difference of 12–30‰. We hypothesize, similarly as before (Conrad et al., 2009), that acetate was mainly produced by fermentation production and that fermentation was partially inhibited by CH$_3$F in such a way, that carbon isotope fractionation was affected. This could happen when different fermenting microorganisms exhibit different isotope fractionation and different susceptibility to inhibition by CH$_3$F. There are hardly any studies of isotope fractionation during fermentative acetate production (Blair et al., 1985; Penning and Conrad, 2006) although numerous different fermentation pathways exist in nature. However, there may be alternative explanations, e.g., acetate production by organoautotrophic or organoheterotrophic homoacetogenesis from which we do not know the intramolecular isotopic difference between carboxyl and methyl of acetate (Lever et al., 2010), or exchange reactions (DeGraaf et al., 1996).

5 Conclusions

The study of various tropical lake sediments from Brazil demonstrated that production rates of CH$_4$ and CO$_2$ increased with the content of organic matter showing that the availability of substrate may limit the methanogenic degradation process.

The isotopic data were a function of both the $\delta^{13}C$ of the primary substrate (organic matter) and the path of CH$_4$ production. In the tropical lake sediments the path of CH$_4$ production seems to be dominated by hydrogenotrophic methanogenesis that exhibited relatively strong isotope fractionation with $\delta^{13}C_{\text{CH}_4}$ being by about 43‰ lower than $\delta^{13}C_{\text{org}}$, and acetate was probably produced mainly by fermentation. For the time being it remains unclear which environmental factors determine the relative importance of hydrogenotrophic versus acetoclastic methanogenesis. The overall abundance of bacteria and archaea correlated well with the production rates of CH$_4$ and CO$_2$ showing that methanogenic degradation of organic matter is dependent on and may be limited by microbial activity. However, the study revealed no apparent correlation of the CH$_4$ production rates, the $^{13}C$ stable isotope discrimination, or the path of CH$_4$ production with the diversity or the composition of the resident methanogenic microbial communities. Although this result does not exclude that not-measured microbial parameters, e.g., the extent of gene expression or protein synthesis in the different groups of bacteria and archaea, do have a decisive effect on CH$_4$ production, it nevertheless indicates that the relative abundance of a particular microbial population is of little importance.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/8/795/2011/bg-8-795-2011-supplement.pdf.

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