**Associate Editor Decision: Publish subject to technical corrections** (29 Jan 2020) by Tina Treude

Comments to the Author:

Dear Ralf and Co-Workers,

the reviewer is very pleased with the revision and suggests acceptance of the manuscript. I agree with this suggestion. However, in a personal statement the reviewer pointed to me that the following relevant citations seem to be missing:

Nüüsslein et al. 2001 (Environmental Microbiology 3(7), 460–470)  
Beulig et al. 2018 (ISME Journal (2019) 13:250–262)  
Beulig et al. 2018 (PNAS vol. 115 | no. 2 | 367–372)

All three studies already documented that methyl-C of the 14C-labelled acetate is oxidized to CO2 rather than reduced to CH4. It would therefore be appropriate to give credit to these studies. I agree and suggest to place the respective citations at appropriate locations either in your introduction or discussion.

Let me know in case you have any questions.  
Best  
Tina

**Response**

Dear Tina,

Thank you for the hint to the references.

In fact, Nüüsslein et al. 2001 has been cited in the Introduction. We did not include the reference to Beulig et al. 2018, since the work is dealing with a seabed sediment rather than a lake sediment. Nevertheless, both references are now also included in the Discussion (see marked ms). However, we did not include the Beulig et al. reference in the ISME journal, since this work is mainly dealing with anaerobic methane oxidation, and the data on acetate oxidation are not thus obvious in this paper.

Best wishes

Ralf
Acetate turnover and methanogenic pathways in Amazonian lake sediments

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Abstract

Lake sediments in Amazonia are a significant source of CH$_4$, a potential greenhouse gas. Previous studies of sediments using $^{13}$C analysis found that the contribution of hydrogenotrophic versus aceticlastic methanogenesis to CH$_4$ production was relatively high. Here, we determined the methanogenic pathway in the same sediments (n = 6) by applying $[^{14}$C]bicarbonate or $[2-^{14}$C]acetate, and confirmed the high relative contribution (50-80%) of hydrogenotrophic methanogenesis. The respiratory index (RI) of $[2-^{14}$C]acetate, which is $^{14}$CO$_2$ relative to $^{14}$CH$_4 + ^{14}$CO$_2$, divided the sediments into two categories, i.e., those with an RI < 0.2 being consistent with the operation of aceticlastic methanogenesis, and those with an RI > 0.4 showing that a large percentage of the acetate-methyl was oxidized to CO$_2$ rather than reduced to CH$_4$. Hence, part of the acetate was probably converted to CO$_2$ plus H$_2$ via syntrophic oxidation, thus enhancing hydrogenotrophic methanogenesis. This happened despite the presence of potentially aceticlastic Methanosetaeae in all the sediments. Alternatively, acetate may have been oxidized with a constituent of the sediment organic matter (humic acid) serving as oxidant. Indeed, apparent acetate turnover rates were larger than CH$_4$ production rates except in those sediments with a R < 0.2. Our study demonstrates that CH$_4$ production in Amazonian lake sediments was not simply caused by a combination of hydrogenotrophic and aceticlastic methanogenesis, but probably involved additional acetate turnover.
1. **Introduction**

Acetate is an important intermediate in the anoxic degradation of organic matter and is produced by fermentation processes and chemolithotrophic homoacetogenesis. The contribution of these two processes to acetate production is difficult to determine, but seems to be quite different for different environments (Fu et al., 2018; Hädrich et al., 2012; Heuer et al., 2010; Lokshina et al., 2019; Ye et al., 2014). The degradation of acetate requires a suitable oxidant such as oxygen, nitrate, ferric iron or sulfate. If such oxidants are not or no longer available, such as in many freshwater environments (e.g., paddy fields, lake sediments, peat), acetate sometimes accumulates until suitable electron acceptors become again available. Temporal accumulation and subsequent oxidative consumption has for example been observed in peatlands during increase and decrease, respectively, of the water table (Duddleston et al., 2002). However, it is generally assumed that acetate degradation in the absence of inorganic electron acceptors is accomplished by aceticlastic methanogenesis (Zinder, 1993). If aceticlastic methanogenesis is operative, the methyl group of the acetate is converted to CH₄.

If methanogenesis is the exclusive final step in the anaerobic degradation of organic matter, polysaccharides (one of the most important compounds from primary production) will be dismutated to equal amounts of CH₄ and CO₂. Furthermore, acetate usually accounts for more than two third of total methane production, especially if polysaccharides are the predominant degradable organic matter (Conrad, 1999). However, CO₂ has often been found to be the main product in many anoxic environments despite the absence of inorganic electron acceptors (O₂, nitrate, ferric iron, sulfate) (Keller et al., 2009; Yavitt and Seidmann-Zager, 2006). Such results have been explained by the assumption that organic substances (e.g. humic acids) may also serve as electron acceptors (Gao et al., 2019; Keller et al., 2009; Klüpfel et al., 2014). Organic electron acceptors also allow the oxidation of acetate (Coates et al., 1998; Lovley et al., 1996). The role of organic electron acceptors during anaerobic degradation of organic matter is potentially important, but still not well known (Corbett et al., 2013).
There are also many reports that methane production in lake sediments is dominated by hydrogenotrophic rather than aceticlastic methanogenesis (Conrad, 1999; Conrad et al., 2011; Ji et al., 2016). Such observations were explained (1) by incomplete degradation of organic matter producing predominantly H₂ and CO₂ without concomitant acetate production (Conrad et al., 2010; Hodgkins et al., 2014; Liu et al., 2017), (2) by acetate oxidation coupled to the reduction of organic substances (see above), or (3) by syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (Lee and Zinder, 1988; Vavilin et al., 2017). If acetate oxidation is operative, the methyl group of the acetate is converted to CO₂. However, if acetate oxidation is syntrophic, it does not require a chemical compound (other than H⁻) as electron acceptors, since it is the hydrogenotrophic methanogenesis that eventually accepts the electrons released during acetate oxidation.

Syntrophic acetate oxidation can replace aceticlastic methanogenesis and thus, has been found when aceticlastic methanogenic archaea were not present in the microbial community of lake sediment (Nüsslein et al., 2001). This may also happen in other anoxic environments when conditions are not suitable for aceticlastic methanogens, e.g., at elevated temperatures (Conrad et al., 2009; Liu and Conrad, 2010; Liu et al., 2018), in the presence of high concentrations of ammonium (Müller et al., 2016; Schnürer et al., 1999; Zhang et al., 2014), or phosphate (Conrad et al., 2000). However, syntrophic acetate oxidation has also been found in lake sediments that contained populations of putatively aceticlastic methanogens (Vavilin et al., 2017). It is presently unkown under which conditions syntrophic acetate oxidizers can successfully compete with aceticlastic methanogens and co-occur with acetate oxidation that is coupled to the reduction of organic substances.

As a further step in understanding the ecology of acetate oxidizers (syntrophic or non-syntrophic ones) versus aceticlastic methanogens, we attempted to document their coexistence by studying lake sediments, which had been reported containing 16S rRNA genes of putatively aceticlastic Methanosaetaceae (Methanotrichaceae (Oren, 2014)) (Ji et al., 2016). We used these sediments and measured the fractions of hydrogenotrophic methanogenesis and of the methyl group of acetate being oxidized to CO₂ rather than reduced to CH₄, and compared the turnover of acetate to the production rate of CH₄.
2. Materials and Methods

The sediment samples were obtained from floodplain lakes in the Amazon region and have already been used for a study on structure and function of methanogenic microbial communities (Ji et al., 2016). In particular, these sediment have been assayed for the percentage of hydrogenotrophic methanogenesis and for the percentage contribution of putatively aceticlastic methanogens to the total archaeal community (Ji et al., 2016). Here, we used six of these sediments for incubation experiments with radioactive tracers. These are the same sediment samples as those listed in our previous publication (Ji et al., 2016). The identity of the lake sediments and the percentage content of putatively aceticlastic methanogens is summarized in Table 1. The experiments were carried out at the same time as those in our previous publication (Ji et al., 2016) and were basically using the same incubation techniques. However, the experimental approach to determine the fractions of hydrogenotrophic methanogenesis ($f_{H2}$) were different. In our previous experiment, values of $f_{H2}$ were determined from the $\delta^{13}C$ of CH$_4$ in the presence ($\delta^{13}C_{CH4-mc}$) and absence ($\delta^{13}C_{CH4}$) of methyl fluoride, an inhibitor of aceticlastic methanogenesis, and from the $\delta^{13}C$ of the methyl group of acetate ($\delta^{13}C_{ac-methyl}$):

$$f_{H2} = (\delta^{13}C_{CH4} - \delta^{13}C_{ac-methyl})/(\delta^{13}C_{CH4-mc} - \delta^{13}C_{ac-methyl})$$

(1)

The CH$_4$ production rates and $f_{H2}$ values from this experiment are shown in Fig. 1 for comparison.

In the present experiment, however, values of $f_{H2}$ were determined by addition of NaH$^{14}$CO$_3$ and measurement of the specific radioactivities in CH$_4$ and CO$_2$. Briefly, about 10-15 ml of each replicate (n = 3) were filled into 27-ml sterile tubes, flushed with N$_2$, closed with butyl rubber stoppers, and incubated at 25ºC. After preincubation for 12 days (in order to deplete eventually present inorganic oxidants), 0.5 ml of a solution of carrier-free NaH$^{14}$CO$_3$ (about 1 µCi; 50 Ci mol$^{-1}$) was added, the tubes flushed again with N$_2$, and incubation was continued at 25ºC for about 100 days. Partial pressures of CH$_4$ and CO$_2$ as well as their contents of $^{14}$C were measured at different time points after mixing the slurries by heavy manual shaking. The gas partial pressures were measured by gas chromatography with a
flame ionization detector (Ji et al., 2016), the radioactivities were analyzed with a radiodetector (RAGA) (Conrad et al., 1989). The data were used to calculate the fractions of hydrogenotrophic methanogenesis ($f_{\text{H}_2}$) from the specific radioactivities of gaseous CH$_4$ (SR$_{\text{CH}_4}$) and CO$_2$ (SR$_{\text{CO}_2}$): 

$$f_{\text{H}_2} = \frac{\text{SR}_{\text{CH}_4}}{\text{SR}_{\text{CO}_2}}$$  \hspace{1cm} (2)$$

For determination of acetate turnover, the same conditions were used, except that preincubation was for 25 days, 0.5 ml of a solution of carrier-free Na[2-14C]acetate (about 2 $\mu$Ci; 50 Ci mol$^{-1}$), equivalent to about 20 nmol acetate, was added, and incubation was continued for about 8 h. During this time gas samples were repeatedly taken and the radioactivities in CH$_4$ and CO$_2$ were analyzed in a gas chromatograph with a radiodetector (RAGA) (Conrad et al., 1989). In the end, the sediment samples were acidified with 1 ml of 1M H$_2$SO$_4$ to liberate CO$_2$ from carbonates, and the radioactivities in CH$_4$ and CO$_2$ were analyzed again. The data were used to calculate the acetate turnover rate constants ($k_{\text{ac}}$) and the respiratory index (RI) values from the radioactivities of gaseous CH$_4$ and CO$_2$ as described by Schütz et al. (1989). The RI is defined as:

$$\text{RI} = \frac{14\text{CO}_2}{14\text{CO}_2 + 14\text{CH}_4}$$  \hspace{1cm} (3)$$

Both $^{14}$CH$_4$ and $^{14}$CO$_2$ were measured at the end of the incubation after acidification. The acetate turnover rate constants were determined from the change of $^{14}$CH$_4$ and $^{14}$CO$_2$ with incubation time ($t$) and the maximal values of $^{14}$CH$_4$ and $^{14}$CO$_2$ at the end of the incubation before acidification:

$$k_{\text{ac}} = \frac{\ln(1 - (^{14}\text{CH}_4 + ^{14}\text{CO}_2)/(^{14}\text{CH}_4_{\text{max}} + ^{14}\text{CO}_2_{\text{max}}))}{t}$$  \hspace{1cm} (4)$$

The acetate turnover rates ($v_{\text{ac}}$) were calculated by:

$$v_{\text{ac}} = k_{\text{ac}} \cdot \text{ac}$$  \hspace{1cm} (5)$$

The acetate concentration (ac) was analyzed in the sediments at the end of the incubation using high pressure liquid chromatography. The acetate concentrations are summarized in Table 1. The rates of acetate-dependent CH$_4$ production ($P_{\text{ac}}$) were calculated from the acetate turnover rates and the RI:

$$P_{\text{ac}} = v_{\text{ac}} \cdot (1 - \text{RI})$$  \hspace{1cm} (6)$$
3. Results

Six different lake sediments from Amazonia were incubated in the presence of H\textsuperscript{14}CO\textsubscript{3}. Methane production started without lag phase indicating that the inorganic electron acceptors, which were present in the original sediment (Ji et al., 2016) had been depleted during the anaerobic preincubation and did not suppress methanogenesis. The CH\textsubscript{4} production rates were compared to those obtained in our previous experiments without addition of H\textsuperscript{14}CO\textsubscript{3} (Ji et al., 2016). Although the rates of CH\textsubscript{4} production were different in the two different incubations, the orders of magnitude were similar for the different lake sediments (Fig. 1A). The incubations in the presence of H\textsuperscript{14}CO\textsubscript{3} were used to follow the specific radioactivities of CH\textsubscript{4} (Fig. 2A) and CO\textsubscript{2} (Fig. 2B) over the incubation time. The specific radioactivities of CH\textsubscript{4} changed only little but were slightly different for the different lake sediments. The specific radioactivities of CO\textsubscript{2} decreased with time as expected due to the production of non-radioactive CO\textsubscript{2}. Both specific radioactivities were used to calculate the fractions of hydrogenotrophic methanogenesis (f\textsubscript{H2}), which increased with incubation time and eventually reached a plateau. The values of f\textsubscript{H2} averaged between 30 and 60 d of incubation are summarized in Fig. 1B. Only the incubations of sediment “Grande” did not reach a plateau but still increased after 260 d of incubation due to the continuously decreasing specific radioactivities of CO\textsubscript{2} (data not shown). Averaging these values over the 4 data points between 160 and 260 d resulted in f\textsubscript{H2} of about 60% (Fig. 1B). The thus determined values of f\textsubscript{H2} were comparable to those determined in the absence of H\textsuperscript{14}CO\textsubscript{3} using values of \(\delta^{13}C\), which have already been published (Ji et al. 2016) (Fig.1B).

The same sediments were used to determine the turnover of [2\textsuperscript{14}C]acetate by measuring the increase of radioactive CH\textsubscript{4} (Fig. 3A) and CO\textsubscript{2} (Fig. 3B). These data were used to determine the rate constants of acetate turnover (Fig. 3C), which ranged between 0.02 and 1.7 h\textsuperscript{-1}. The respiratory indices (RI) were generally larger than 0.2 except those of the sediments Tapari and Verde, which were smaller than 0.2 (Fig. 4B). The RI values and the acetate turnover rate constants were used to calculate the rates of CH\textsubscript{4} production from acetate in comparison to the rates of total CH\textsubscript{4} production (Fig. 4A). Interestingly, acetate-dependent
CH₄ production was always larger than total CH₄ production, except in those sediments exhibiting a RI <0.2.

4. Discussion

The RI value quantifies the fraction of the methyl group of acetate that is oxidized to CO₂ rather than reduced to CH₄. Since some oxidation of acetate methyl is also happening in pure cultures of aceticlastic methanogens (Weimer and Zeikus, 1978), and since a RI of around 0.2 has often been found in environments where acetate turnover was dominated by aceticlastic methanogenesis (Phelps and Zeikus, 1984; Rothfuss and Conrad, 1993; Winfrey and Zeikus, 1979), an RI value of 0.2 may in practice be used as the threshold for the change of methanogenic to oxidative acetate turnover. Based on this criterion, i.e. RI < 0.2, the lake sediments of Tapari and Verde behaved as when acetate turnover was exclusively caused by aceticlastic methanogenesis. The percentage of acetate-dependent CH₄ production was fairly consistent with the fraction of hydrogenotrophic methanogenesis, which made up the remainder of total CH₄ production. In conclusion, the acetate turnover and CH₄ production in these lake sediments behaved as expected as when aceticlastic methanogenesis was the sole process of acetate consumption (reaction 1 in Fig. 5).

However, the sediments of Jua and in particular those of Jupinda, Cataldo, and Grande exhibited RI values >0.2, showing that a substantial fraction of the acetate-methyl was oxidized to CO₂. Hence, acetate was not exclusively consumed by aceticlastic methanogenesis, but it was oxidized, for example by syntrophic acetate oxidation producing H₂ and CO₂. Similarly, RI values > 0.2 have been observed in the sediment of Lake Kinneret in Israel and interpreted as syntrophic acetate oxidation (Nüsslein et al., 2001). Also in the methanogenic zone of an anoxic seabed in the Baltic Sea, acetate has been shown to be degraded syntrophically (Beulig et al., 2018). The H₂ and CO₂ from acetate oxidation may subsequently be used as methanogenic substrates, thus supporting CH₄ production (reactions 2 and 3 in Fig. 5). Such support would be consistent with the relatively high fractions (f_H₂) of hydrogenotrophic methanogenesis observed in the sediments of Lakes Juas, Jupinda, Cataldo and Grande. However, it would not explain why acetate turnover rates were higher than
necessary for supporting the observed rates of total CH₄ production. A possible conclusion is that acetate was converted to CO₂ without concomitant production of H₂. Possibly, electrons from acetate were transferred to organic electron acceptors (reaction 4 in Fig. 5), such as suggested in the literature (Coates et al., 1998; Lovley et al., 1996). Alternatively, acetate may have first been converted to H₂ plus CO₂ followed by the oxidation of H₂ with organic electron acceptors (reactions 2 and 5 in Fig. 5) rather than syntrophic formation of CH₄ from H₂ plus CO₂ (reactions 2 and 3 in Fig. 5). In conclusion, these lake sediments behaved as when acetate consumption was accomplished not only by acetate-dependent methanogenesis, but also by oxidative consumption.

Our conclusions are mainly based on radiotracer measurements, which may be biased. For example, acetate turnover rate constants are calculated from acetate concentrations and turnover rate constants. Acetate concentrations were only measured at the end of incubation and thus, may not have been representative for the entire incubation time. Furthermore, acetate in the sediment may occur in several pools with different turnover (Christensen and Blackburn, 1982). Therefore, acetate turnover rates and acetate-dependent CH₄ production rates may be overestimated, if the actual acetate turnover depends on a pool size that is smaller than that analyzed. Overestimation may also result from too low RI values, such as when carbonate-bound radioactivity is neglected. However, such bias was avoided by acidification prior to determination of the RI. Finally, a potential bias may arise from the fact that the rates of CH₄ production and the acetate turnover rates were measured in two different sets of incubation, with different incubation times. While CH₄ production (and f_H₂) was measured over tens of days (Fig. 2), acetate turnover was determined within 8 h (Fig. 3). Nevertheless, the data in the lake sediments of Tapari and Verde resulted in CH₄ production and acetate turnover consistent with the operation of aceticlastic methanogenesis, which is the canonical acetate consumption pathway for methanogenic sediments. Therefore, we are confident that our results obtained from the sediments of Jua, Jupinda, Cataldo and Grande were also in a realistic range.

The determination of fractions of hydrogenotrophic methanogenesis (f_H₂) depends on the specific radioactivity of the dissolved CO₂ pool that is involved in CH₄ production. However,
it is the pool of gaseous CO₂ that is analyzed in the assay, assuming that its specific
radioactivity is identical to that of the active dissolved pool. Since non-radioactive CO₂ is
permanently produced from oxidation of organic matter, there may be disequilibrium.
Nevertheless, determinations of fH₂ using radioactive bicarbonate exhibited the same
tendencies as those based on δ¹³C values, and thus are probably quite reliable. Furthermore,
the fH₂ values were fairly consistent with the fractions of acetate-dependent methanogenesis
determined from the turnover of radioactive acetate.

Despite these reservations, our results collectively demonstrated that acetate turnover in
tropical lake sediments did not necessarily follow a canonical pattern with aceticlastic
methanogenesis as sole or predominant process of acetate turnover, despite the fact that all
these sediments contained populations of putative aceticlastic methanogenic archaea. Acetate
consumption in *Methanosaeta* species is known to have a relatively high affinity and a low
threshold for acetate (Jetten et al., 1992). Therefore, the question arises why oxidative
processes, including syntrophic acetate oxidation, could successfully compete with
aceticlastic methanogenesis.

5. **Author contribution**

RC designed the experiments, evaluated the data and wrote the manuscript; MK did the
experiments; AEP provided the samples and contributed to the discussion of the data.

6. **Competing interests**

The authors declare that they have no conflict of interest.
7. Acknowledgements

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8. References

Beulig, F., Roey, H., Glombitza, C., Joergensen, B. B.: Control on rate and pathway of anaerobic organic carbon degradation in the seabed, Proc. Natl. Acad. Sci. USA, 115, 367-372, 2018.

Christensen, D. and Blackburn, T. H.: Turnover of 14C-labelled acetate in marine sediments, Mar. Biol., 71, 113-119, 1982.

Coates, J. D., Ellis, D. J., Blunt-Harris, E. L., Gaw, C. V., Roden, E. E., Lovley, D. R.: Recovery of humic-reducing bacteria from a diversity of environments, Appl. Environ. Microbiol., 64, 1504-1509, 1998.

Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments [review], FEMS Microbiol. Ecol., 28, 193-202, 1999.

Conrad, R., Claus, P., Casper, P.: Stable isotope fractionation during the methanogenic degradation of organic matter in the sediment of an acidic bog lake, Lake Grosse Fuchskuhle, Limnol. Oceanogr., 55, 1932-1942, 2010.

Conrad, R., Klose, M., Claus, P.: Phosphate inhibits acetotrophic methanogenesis on rice roots, Appl. Environ. Microbiol., 66, 828-831, 2000.

Conrad, R., Klose, M., Noll, M.: Functional and structural response of the methanogenic microbial community in rice field soil to temperature change, Environ. Microbiol., 11, 1844-1853, 2009.

Conrad, R., Mayer, H. P., Wüst, M.: Temporal change of gas metabolism by hydrogen-syntrophic methanogenic bacterial associations in anoxic paddy soil, FEMS Microbiol. Ecol., 62, 265-274, 1989.

Conrad, R., Noll, M., Claus, P., Klose, M., Bastos, W. R., Enrich-Prast, A.: Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments, Biogeosciences, 8, 795-814, 2011.

Corbett, J., Tfaily, M. M., Burdige, D. J., Cooper, W. T., Glaser, P. H., Chanton, J. P.: Partitioning pathways of CO2 production in peatlands with stable carbon isotopes, Biogeochem., 114, 327-340, 2013.

Duddleston, K. N., Kinney, M. A., Kiene, R. P., Hines, M. E.: Anaerobic microbial biogeochemistry in a northern bog: Acetate as a dominant metabolic end product, Global Biogeochem. Cycles, 16, 1063-1078, doi:10.1029/2001GB001402, 2002.
Fu, B., Conrad, R., Blaser, M.: Potential contribution of acetogenesis to anaerobic degradation in methanogenic rice field soils, Soil Biol. Biochem., 119, 1-10, 2018.

Gao, C., Sander, M., Agethen, S., Knorr, K. H.: Electron accepting capacity of dissolved and particulate organic matter control CO2 and CH4 formation in peat soils, Geochim. Cosmochim. Acta, 245, 266-277, 2019.

Hädrich, A., Heuer, V. B., Herrmann, M., Hinrichs, K. U., Küsel, K.: Origin and fate of acetate in an acidic fen, FEMS Microbiol. Ecol., 81, 339-354, 2012.

Heuer, V. B., Krüger, M., Elvert, M., Hinrichs, K. U.: Experimental studies on the stable carbon isotope biogeochemistry of acetate in lake sediments, Org. Geochem., 41, 22-30, 2010.

Hodgkins, S. B., Tfaily, M. M., McCalley, C. K., Logan, T. A., Crill, P. M., Saleska, S. R., Rich, V. I., Chanton, J. P.: Changes in peat chemistry associated with permafrost thaw increase greenhouse gas production, Proc. Natl. Acad. Sci. USA, 111, 5819-5824, 2014.

Jetten, M. S. M., Stams, A. J. M., Zehnder, A. J. B.: Methanogenesis from acetate - A comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp., FEMS Microbiol. Rev., 88, 181-197, 1992.

Ji, Y., Angel, R., Klose, M., Claus, P., Marotta, H., Pinho, L., Enrich-Prast, A., Conrad, R.: Structure and function of methanogenic microbial communities in sediments of Amazonian lakes with different water types, Environ. Microbiol., 18, 5082-5100, 2016.

Keller, J. K., Weisenhorn, P. B., Megonigal, J. P.: Humic acids as electron acceptors in wetland decomposition, Soil Biol. Biochem., 41, 1518-1522, 2009.

Klüpfel, L., Piepenbrock, A., Kappler, A., Sander, M.: Humic substances as fully regenerable electron acceptors in recurrently anoxic environments, Nature Geoscience, 7, 195-200, 2014.

Lee, M. J. and Zinder, S. H.: Isolation and characterization of a thermophilic bacterium which oxidizes acetate in syntrophic association with a methanogen and which grows acetogenically on H2-CO2, Appl. Environ. Microbiol., 54, 124-129, 1988.

Liu, F. H. and Conrad, R.: *Thermoanaerobacteriaceae* oxidize acetate in methanogenic rice field soil at 50°C, Environ. Microbiol., 12, 2341-2354, 2010.

Liu, P. F., Klose, M., Conrad, R.: Temperature effects on structure and function of the methanogenic microbial communities in two paddy soils and one desert soil, Soil Biol. Biochem., 124, 236-244, 2018.

Liu, Y., Conrad, R., Yao, T., Gleixner, G., Claus, P.: Change of methane production pathway with sediment depth in a lake on the Tibetan plateau, Palaeogeogr. Palaeoclimatol. Palaeoecol., 474, 279-286, 2017.
Lokshina, L., Vavilin, V., Litti, Y., Glagolev, M., Sabrekov, A., Kotsyurbenko, O., Kozlova, M.: Methane production in a West Siberian eutrophic fen is much higher than carbon dioxide production: incubation of peat samples, stoichiometry, stable isotope dynamics, modeling, Water Resources, 46, S110-S125, 2019.

Lovley, D. R., Coates, J. D., Blunt-Harris, E. L., Phillips, E. J. P., Woodward, J. C.: Humic substances as electron acceptors for microbial respiration, Nature, 382, 445-448, 1996.

Müller, B., Sun, L., Westerholm, M., Schnürer, A.: Bacterial community composition and fhs profiles of low- and high-ammonia biogas digesters reveal novel syntrophic acetate-oxidising bacteria, Biotechnol. Biofuels, 9, doi:10.1186/s13068-016-0454-9, 2016.

Nüsslein, B., Chin, K. J., Eckert, W., Conrad, R.: Evidence for anaerobic syntrophic acetate oxidation during methane production in the profundal sediment of subtropical Lake Kinneret (Israel), Environ. Microbiol., 3, 460-470, 2001.

Oren, A.: The family Methanotrichaceae, in: The Prokaryotes, edited by: Rosenberg, E., DeLong, E. F., Lory, S., Stackebrandt, E., Thompson, F., Springer, Berlin, 298-306, 2014.

Phelps, T. J. and Zeikus, J. G.: Influence of pH on terminal carbon metabolism in anoxic sediments from a mildly acidic lake, Appl. Environ. Microbiol., 48, 1088-1095, 1984.

Rothfuss, F. and Conrad, R.: Vertical profiles of CH4 concentrations, dissolved substrates and processes involved in CH4 production in a flooded Italian rice field, Biogeochem., 18, 137-152, 1993.

Schnürer, A., Zellner, G., Svensson, B. H.: Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors, FEMS Microbiol. Ecol., 29, 249-261, 1999.

Schütz, H., Seiler, W., Conrad, R.: Processes involved in formation and emission of methane in rice paddies, Biogeochem., 7, 33-53, 1989.

Vavilin, V., Rytov, S., Conrad, R.: Modeling methane formation in sediments of tropical lakes, focusing on syntrophic acetate oxidation: dynamics and static isotope equations, Ecol. Modeling, 363, 81-95, 2017.

Weimer, P. J. and Zeikus, J. G.: Acetate metabolism in Methanosarcina barkeri, Arch. Microbiol., 119, 175-182, 1978.

Winfrey, M. R. and Zeikus, J. G.: Anaerobic metabolism of immediate methane precursors in Lake Mendota, Appl. Environ. Microbiol., 37, 244-253, 1979.

Yavitt, J. B. and Seidmann-Zager, M.: Methanogenic conditions in northern peat soils, Geomicrobiol. J., 23, 119-127, 2006.
Ye, R., Jin, Q., Bohannan, B., Keller, J. K., Bridgham, S. D.: Homoacetogenesis: A potentially underappreciated carbon pathway in peatlands, Soil Biol. Biochem., 68, 385-391, 2014.

Zhang, C., Yuan, Q., Lu, Y.: Inhibitory effects of ammonia on methanogen mcrA transcripts in anaerobic digester sludge, FEMS Microbiol. Ecol., 87, 368-377, 2014.

Zinder, S. H.: Physiological ecology of methanogens, in: Methanogenesis. Ecology, Physiology, Biochemistry and Genetics, edited by: Ferry, J. G., Chapman & Hall, New York, 128-206, 1993.
Table 1: Identity of sediment samples (same as those in Ji et al. (2016)), percentage content of putatively aceticlastic methanogens (*Methanosaetaceae*) relative to total archaea, and concentrations of acetate; mean ± SE.

| Lake # | Name   | Type       | *Methanosaetaceae* (%) | Acetate (nmol g⁻¹ dry weight) |
|--------|--------|------------|-------------------------|--------------------------------|
| P1     | Jua    | clear water| 21 ± 1                  | 93 ± 5                         |
| P8     | Tapari | clear water| 19 ± 3                  | 261 ± 39                       |
| P9     | Verde  | clear water| 19 ± 11                 | 126 ± 12                       |
| P10    | Jupinda| clear water| 27 ± 4                  | 110 ± 6                        |
| A1     | Cataldo| white water| 42 ± 1                  | 50 ± 3                         |
| A2     | Grande | white water| 36 ± 3                  | 35 ± 1                         |
Figure captions

Fig. 1: Methane production in sediments of different Amazonian lakes: (A) rates of CH₄ production, and (B) fractions of hydrogenotrophic methanogenesis, both determined in the absence and the presence of radioactive bicarbonate. The data in the absence of radioactive bicarbonate are the same as published in Ji et al. (2016), when f_H₂ was determined from values of δ¹³C; mean ±SE.

Fig. 2: Conversion of radioactive bicarbonate in sediments of different Amazonian lakes: (A) specific radioactivities in CH₄; (B) specific radioactivities in gaseous CO₂; and (C) fractions (f_H₂) of hydrogenotrophic methanogenesis; mean ±SE.

Fig. 3: Conversion of [2-¹⁴C]acetate in sediments of different Amazonian lakes: (A) accumulation of radioactive CH₄; (B) accumulation of radioactive gaseous CO₂; and (C) acetate turnover rate constants; mean ±SE.

Fig. 4: (A) Rates of total and acetate-derived CH₄ production in sediments of different Amazonian lakes and (B) respiratory indices (RI) of the turned over [2-¹⁴C]acetate; mean ±SE.

Fig. 5: Scheme of the pathways involved in acetate turnover in sediments of Amazonian lakes; (1) aceticlastic methanogenesis; (2) syntrophic acetate oxidation; (3) hydrogenotrophic methanogenesis; (4) acetate oxidation with organic electron acceptors.