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Biogeosciences Discussions

Interactive comment on "Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments" *by* R. Conrad et al.

Anonymous Referee #1

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Review of "Stable carbon isotope discrimination and microbiology of methane formation in tropical anoxic lake sediments" by Conrad et al.

Overall, this study presents a unique and very comprehensive data set in which methane production, methane production pathways, and phylogenetic compositions of methane-cycling Archaea, Bacteria and Archaea from 16 tropical lakes are compared. Not only are the δ13C-isotopic compositions of methane and CO2 presented in the discussion of in situ pathways, but so are those of molecular acetate and methyl-acetate. The results are not as clear-cut as one might hope, however. Moreover, several assumptions are made early on in the manuscript that compromise the potential to draw

accurate and appropriate conclusions from the data. The main weaknesses are (1) the assumption of zero isotopic fractionation between methyl-acetate and methane in aceticlastic methanogenesis, and (2) the assumption that all methane is produced via CO2 reduction and aceticlastic methanogenesis. Both assumptions are unnecessary and not helpful. Furthermore, the discussion of δ13C-isotopic compositions of acetate needs to be reorganized, more substantiated, and clarified. It would be helpful if the discussion of mcrA gene community composition was more in-depth, showed actual phylogenetic information for the different lakes, and thereby allowed the reader to assess on their own, whether they agree with the authors' claim that there is no clear trend in mcrA community composition associated with environmental variables. In turn, the 16S data on bacterial and archaeal diversity and abundance can be removed, as it does not contribute any useful information to the manuscript.

GENERAL COMMENTS

Page 8626, line 23-25, Page 8629, line 23-26: The assumption that there is no fractionation between in δ13C between the methyl group of acetate and methane produced via aceticlastic MG seems questionable. As the authors will remember, the isotopic fractionation factor for this reaction has been shown experimentally to be –7 to -10‰ for Methanosaeta (Valentine et al. 2004, Penning et al. 2006) and -20‰ for Methanosarcina (Gelwicks et al. 1994). Hence, the authors need to explain why they assume this fractionation factor to be zero, OR be more precise and include previously determined fractionation factors in the calculations.

I do not think the data in Table 3 underscores this assumption of zero fractionation. First of all, we see differences in δ13C-ac-methyl of 1-7‰ ($\neq$ 0) between sediments amended with methyl fluoride and controls. Secondly, the difference in δ13C-ac-methyl between sediments amended with methyl fluoride and controls is only representative of the isotopic fractionation associated with aceticlastic MG IF all the acetate is consumed via aceticlastic methanogenesis. To conclude this, it is necessary to rule out additional, potentially important acetate sinks, such as syntrophic anaerobic acetate oxidation,

(cryptic) sulfate reduction, or biosynthesis. These reactions are likely to occur in the presence and absence of aceticlastic MG and will dilute any differences in δ13C-ac-methyl between methyl fluoride-treated and control sediments that can be attributed to the inhibition of aceticlastic MG by methyl fluoride. And they may even produce their own fractionations to complicate things further (Goevert and Conrad 2008). The vastly higher acetate concentrations at the end of incubations with methyl fluoride compared to controls could be due to the fact that other acetate consumers have not had sufficient time to increase in numbers to draw acetate concentrations back down (many acetate consumers are slow-growers). They do not demonstrate that other acetate consumers are insignificant. For these reasons, the differences in δ13C-ac-methyl of 1-7‰ between sediments amended with methyl fluoride and controls (Table 3) are not a reliable proxy for in situ fractionation between δ13C-ac-methyl and methane during aceticlastic MG.

The authors detect mcrA genes of Methanosaeta and Methanosarcina. Given the documented isotopic fractionations between δ13C-ac-methyl and methane in aceticlastic MG by these two genera, it would be important to include these isotopic fractionations in calculations of relative contributions of hydrogenotrophic and aceticlastic MG. If the effect of using 0‰ -10‰ or -20‰ as fractionation factors for the conversion of methyl-acetate to methane only has a minor effect on calculated relative contributions of hydrogenotrophic and aceticlastic methanogenesis, then this needs to be demonstrated by sensitivity analyses.

Finally, the authors only focus on hydrogenotrophic and aceticlastic MG. Methanosarcinacea are mentioned as possible aceticlastic methanogens, however, the acetate concentrations measured (Table 3) are below the threshold concentrations required by Methanosarcina, and in a range only known to be used by Methanosaeta. Hence, Methanosaeta are likely to be the only active aceticlastic methanogens (and they were present in all samples, if I read Fig. 7b correctly). Given that Methanosarcinaceae can use other substrates and given the likely presence of substantial amounts

of terrestrially-derived pectin in the lake sediments, how can the authors preclude a substantial contribution of methylated substrates, especially methanol, to biological methane production? What would the effect of a significant contribution of methylotrophic MG be on δ13C-methane, and how would this affect the calculated contributions of aceticlastic and hydrogenotrophic MG?

Table 3: How do the authors explain that the δ13C of total acetate in some cases decreases in methyl fluoride treatments (e.g. by 7‰ in sample 16), while it increases in others (e.g. by 8‰ in sample 8). The same trend can be observed in the δ13C of methyl-acetate, e.g. in sample 3 the δ13C decreases by 7‰ while it increases by 7‰ in sample 12. Moreover, how do the authors explain that decreases/increases in δ13C of total acetate between controls and methyl fluoride treatments are not reflected in changes in δ13C of methyl-acetate?

In the bottom row of Table 3, the authors show standard errors for each population of sample acetate concentration averages and sample δ13C-averages. Use of the standard error requires normal distribution of the data. Neither the acetate concentration averages nor the δ13C-averages look normally distributed. Hence, standard deviations should be used instead. These will more accurately reflect the undoubtedly high variability in δ13C values within each population of δ13C averages. The authors may also want to consider including the SD instead of the SE for the triplicate measurements of acetate concentrations and δ13C.

p. 8628, line 13: δ13C of acetate in controls and methyl fluoride treatments were not only proportional, but almost had a 1:1 relationship, meaning that there was no difference in δ13C-acetate between controls and methyl fluoride treatments. As argued earlier, the lack of difference, does not mean that aceticlastic methanogenesis is not producing any isotopic fractionations, however.

P. 8630, line 6-7: Could the lack of 1:1 proportionality between fCO2,CH4 and residual methane produced in methyl fluoride treatments be explained with a methylotrophic

contribution to MG and/or an overestimation of the hydrogenotrophic contribution to methanogenesis due to the assumption that there is no fractionation between δ13C-ac-methyl and methane during aceticlastic MG? If methylotrophic MG is important, perhaps the distribution of Methanosarcinaceae will provide a clue, e.g. if samples with the highest deviation from 1:1 proportionality harbor (relatively more) Methanosarcinaceae. If the contribution of aceticlastic MG to total MG is underestimated as a result of assuming no fractionation between the methyl group of acetate and methane, perhaps replotting this graph after recalculating fCO2,CH4 assuming -10‰ fractionation from methyl-acetate to methane will provide a clue.

p. 8632, line 15-21: According to this logic, we might expect to see the lowest/no isotopic differences between δ13C-acetate in controls and methyl fluoride treatments or between δ13C-ac-methyl in controls and methyl fluoride treatments in samples where acetate concentrations were below detection (<10μm). This was not the case (Table 3). Hence, the authors have yet to provide arguments why their assumption of 0‰ fractionation between δ13C-ac-methyl and methane is correct.

p. 8632, line 26-28: Good. Please change the entire manuscript to the assumption of -10‰ fractionation between δ13C-ac-methyl and methane. You have support for this case with (1) experimentally determined fractionations for Methanosaeta, and (2) the likely absence of aceticlastic MG by Methanosarcina given that the acetate concentrations are 1-2 orders of magnitude lower than known acetate threshold concentrations required by Methanosarcina.

p. 8634, line 1-8: methylotrophic MG is an additional explanation.

p. 8635, line 1-8: relative depletions of -10‰ in δ13C-ac-methyl relative to Corg are consistent with fermentative production of acetate from sediment organic matter, where acetate bears the δ13C of the Corg (Blair et al. 1987). This is due to intramolecular differences in δ13C between the methyl and carboxyl groups of acetate, where the methyl group is depleted by -10‰ and the carboxyl group enriched by +10‰ relative

to the acetate molecule. Moreover, C-discriminations associated with acetogenesis can explain δ13C-ac-methyl depletions of -40‰ relative to Corg (Gelwicks et al. 1989 on autotrophic acetogenesis; Lever et al. 2010 on possible isotopic discriminations associated with other acetogenesis reactions). Pure cultures of Acetobacterium woodii produce δ13C -isotopic depletions of -59‰ during autotrophic acetogenesis (Gelwicks et al. 1989).

p. 8635, line 9-17: there might be a fractionation associated with syntrophic acetate oxidation that produces heavier residual acetate, as has been shown for sulfate reducers using the reductive acetyl CoA pathway. Some, if not all, anaerobic acetate oxidizers are capable of acetogenesis, and all known acetogens use the reductive acetyl CoA pathway...

p. 8635, line 10-25, p. 8636, line 9-21: there is some repetition between these sections; perhaps they should be combined; fractionations associated with syntrophic acetate oxidation and acetate-oxidizing sulfate reduction could explain the increase in δ13C-acetate in the absence of aceticlastic MG. The vast increase in acetate concentrations may increase potential fractionations associated with these processes.

p. 8636, line 1-15: this discussion should be combined with the discussion on the isotopic compositions of δ13C-ac-methyl (one page earlier). Separating the two is confusing to the reader.

p. 8636, l. 25 to p. 8637, l. 14: The intramolecular difference may also vary between different acetogenesis pathways (lithoautotrophic, organoautotrophic, organoheterotrophic; see discussion in Lever et al. 2010). In organoautotrophic and organoheterotrophic acetogenesis, the methyl group of acetate is likely to derive directly from organic matter (and hence has an isotopic composition similar to the source compound), while the carboxyl group undergoes CO2 reduction (and hence strong isotopic fractionation). Hence, we would expect to see strong intramolecular variation in δ13C also with organoautotrophic and organoheterotrophic acetogenesis reactions.

Unlike in acetate produced by fermentation, however, the carboxyl would be heavily δ13C-depleted.

p. 8642, l. 6-27: It would be nice to have a table showing the actual community compositions at each site, especially of mcrA genes, which are highly relevant to this study. The 16S data is not crucial to this manuscript – perhaps an in-depth phylogenetic analysis of the 16S genes could be done in a separate publication. A possible further explanation is that the authors did not measure the environmental variables that were critical in determining microbial community composition.

TECHNICAL CORRECTIONS p. 8621, line 17: . . ., acetate in particular → particularly acetate

p. 8621, Line 18-20: change to: "Only if the system eventually achieves steady state are CH4 production rates limited by. . ."

p. 8628, line 8: "among themselves" → "with each other"; was their correlation significant? If yes, please include p and R2 values.

p. 8628, line 8-10: unclear; be more specific; which other correlations were checked?

Interactive comment on Biogeosciences Discuss., 7, 8619, 2010.