

Interactive comment on “Acetate turnover and methanogenic pathways in Amazonian lake sediments” by Ralf Conrad et al.

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Reply to anonymous referee #1

We thank the referee for the helpful comments. We are happy to address them in the following and in a revised version of the manuscript:

The referee briefly remarks that the findings have little novelty. This may be the case with the conclusions, which emphasize the potential importance of acetate oxidation coupled either syntrophically to hydrogenotrophic methanogenesis or to the reduction of organic substances. Such conclusions have indeed been made before. However, to our knowledge they have so far not been supported by showing that the methyl group of acetate is indeed oxidized to CO₂ rather than reduced to CH₄. Here, we addressed

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this point by determining the fate of radioactively labeled acetate-methyl, and indeed found that in some of the lake sediments a large percentage of the acetate methyl was oxidized to CO₂. In our opinion, this is a crucial and novel experimental result.

The fraction of methanogenesis from either CO₂ (hydrogenotrophic) or from acetate (aceticlastic) was determined in three different ways. Two of them determined the fraction of hydrogenotrophic methanogenesis. The first used the fractionation of ¹³C. This approach had been used in our previous publication (Ji et al. 2016), and the method and the results are described there in detail. This method basically depends on the abundance of ¹³C in CH₄ and in acetate after anaerobic incubation without any amendments or with methyl fluoride, an inhibitor of aceticlastic methanogenesis. The second method was to add ¹⁴C-labelled bicarbonate and determine upon incubation the specific radioactivities in the substrate (i.e., CO₂) and the product (i.e., CH₄) of hydrogenotrophic methanogenesis. This approach was used in the present study and the results were compared to those using the natural abundance of ¹³C.

However, the measurement of the RI from ¹⁴C-labeled acetate-methyl was not done for the determination of the fraction of hydrogenotrophic versus aceticlastic methanogenesis. Instead it served a different purpose, i.e., to determine the fraction of acetate-methyl being oxidized to CO₂ or being reduced to CH₄. This analysis was important to prove that acetate-methyl had indeed been oxidized. We regret that the text of the methods section confused the purpose of the different determinations. We will revise the text for improvement.

Finally, the rate constants of acetate turnover were determined from the conversion of ¹⁴C-labeled acetate-methyl. Together with the RI coefficient, the turnover rate constant allows the calculation of acetate-dependent methanogenesis, which basically is the third way to determine the fractions of methanogenesis from either CO₂ or acetate. In the sediments of Tapari and Verde, which have low RI values, the fractions of acetate-dependent CH₄ production were consistent with the fH₂ values measured by the natural ¹³C and ¹⁴C-bicarbonate methods (see above).

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However, in the other sediments with high RI, the determination of acetate turnover resulted in rates of aceticlastic methanogenesis that were actually larger than the rates of total methanogenesis. We agree with the referee that this is a conflicting result. The first idea for explanation is of course the assumption of experimental bias. We discussed a few possibilities, but agree that this discussion was probably not exhaustive (we will do better in the revised version). In particular, we should have also emphasized the fact that total rates of CH₄ production were measured in different incubations (extending of days) than rates of acetate turnover (extending over hours). The different incubation conditions might give conflicts. However, we still can safely conclude that the fractions of hydrogenotrophic methanogenesis were comparable irrespectively of whether using natural 13C or 14C-bicarbonate, and that in most sediments acetate-methyl was to a large extent oxidized rather than reduced. Hence, the only ambiguity is whether acetate oxidation was coupled to the reduction of organic substrates (processes 4 and 5 in Fig. 5), or was simply the result of syntrophic oxidation coupled to the production of CH₄ (processes 2 and 3 in Fig. 5).

Minor issues:

They will all be addressed in a revision.

General: We will work on the reference list as suggested by the referee.

Methods: The CH₄ production rates without addition of radiotracers (Fig. 1A) are basically those from our previous study (Ji et al., 2016), analogously to the values of fH₂ shown in Fig. 1B.

The addition of 14C was almost carrier free, i.e., the specific radioactivities of [2-14C]acetate and 14C-bicarbonate were on a range of about 50 Ci/mol. Thus, the addition of 1 μ Ci acetate-methyl was equivalent to an amount of about 20 nmol acetate. Hence, the addition of radioactive acetate was small (<5%) compared to the in-situ concentration of acetate. The same is true for bicarbonate.

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Fig. 2: The specific radioactivity is the relevant number for determining fH₂. Therefore, we preferred showing these values instead of showing only Bq of CO₂ and CH₄ without reference to total CH₄ and CO₂.

L.32: ok

L.86: ok

L.106: There was a significant amount of radioactivity in the sediment carbonates, which has to be considered for the correct determination of the RI. In some of the sediments the released radioactive CO₂ more than doubled upon acidification. Actually, we also calculated RI values using the final radioactivity in CO₂ before acidification. These RI values were (of course) generally smaller than those after acidification, ranging between RI = 0.05-0.30 compared to RI = 0.06-0.48. The use of the unacidified values of RI for calculation of acetate dependent CH₄ production would result in even higher rates than those given in our paper. Hence, the rates of acetate-dependent CH₄ production given in our paper were conservative. Nevertheless, the conclusions would be the same irrespectively of using the radioactive CO₂ before or after acidification.

L.124: Averages were calculated since the values of fH₂ were not constant (Fig. 2C) and we wanted to compare the values with those determined earlier using ¹³C (comparison in Fig. 1B).

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