

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

**Anonymous Referee #1**

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The manuscript “Acetate turnover and methanogenic pathways in Amazonian lake sediments” presents data from radioactive C isotope tracer incubations with the aim to identify the pathways responsible for consumption of acetate in freshwater lake sediments under methanogenic conditions. The radiotracer data are compared to results from previously published stable C isotope tracer incubations (Ji et al. 2016) that were performed in parallel to those incubations described in the presented manuscript. The studied lake sediments have been well characterized previously by Ji et al. (2016) and differ in their biogeochemistry, microbial community composition, their rates of CH<sub>4</sub> production, as well as their apparent contribution of different methanogenic pathways to overall CH<sub>4</sub> production.

In the presented manuscript, Conrad et al. find that an unusually large fraction of

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methanogenesis is apparently attributed to CO<sub>2</sub>-reduction, despite a high acetate turnover and the presence of putative acetoclastic methanogenic microorganisms. Similar to previous studies on methanogenesis in sediments, this is explained by the authors with a potential coupling between acetate oxidation and subsequent consumption of electrons and CO<sub>2</sub> by hydrogenotrophic methanogenesis. Additionally, the potential of organic C as a subterminal electron acceptor is briefly discussed. Overall, the paper is short and concise, conclusions are carefully formulated, but findings have little novelty. There are a few concerns that I would like to see addressed in a revised version of the manuscript:

The fraction of methanogenesis from CO<sub>2</sub> and/or acetate is estimated via calculation of the parameters “fH<sub>2</sub>” (calculated either from <sup>14</sup>C or <sup>13</sup>C incubations), the respiratory index “RI”, or the “methane production with <sup>14</sup>C-bicarbonate”. These parameters differ in their calculation, and the data that goes into these calculations. Even for a reader familiar with the applied methods, the text can be confusing at times, especially with regards to comparability between the methods/incubations. Aggravating the confusion, the manuscript presents several conflicting findings, e.g. higher production of methane from acetate than total methane production or major differences in CH<sub>4</sub> production with/without radiotracer, that are not well discussed by the authors. It seems that the authors disregard inconsistencies in their experimental procedures (e.g. different incubation times, different preincubation times) that might very well explain the before-mentioned discrepancies. Maybe the authors assume that the methanogenic communities stabilized over the course of the incubation time. Several studies have shown that this is most likely not the case and even under apparently stable incubation conditions, processes and associated microbial communities can be quite dynamic.

Some minor issues:

General – the authors should think about diversifying their references. About half of the references in the manuscript stem from the author’s lab. Some of these are appropriate, others not.

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Methods: CH<sub>4</sub> production without radiotracer addition is shown in Fig. 1 but not described in the Methods section. How much did the authors change the acetate concentration by the addition of radiotracer?

Fig. 2: Why is only the specific radioactivity over time shown?

L. 32: “Normally” is not appropriate here. I suggest “It is generally assumed that,”

L. 86: Please specify “identical”. Same sediment? Same sampling location? Same sampling time? Homogeneous mixture?

L. 106: How much radioactivity was found in the form of carbonates?

L. 124: I don’t understand why an average was chosen here.

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