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

### **Anonymous Referee #3**

Received and published: 21 January 2011

The manuscript by Conrad et al. presents experimental data about the various pathways of methane formation in 16 different lake sediments. The authors combine stable carbon isotope measurements of precursors (organic matter), intermediates (acetate) and products (methane and carbon dioxide) with gene analyses of bacterial and archaeal ribosomal RNA. The readership of Biogeosciences would be suited for such kind of topic.

General comments:

The study is to some degree very descriptive and is circling around the data set. Often the authors exclude other important publications from the discussion covering the same

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or similar topic which I think should be avoided. Papers to name are: Heuer et al. (2010, OG); Nozhevnikova et al. (2007, FEMS); Schwarz et al. (2007, EM); Nüsslein et al. (2001, EM) ; Nüsslein et al. (2003, L&O). Especially those papers that deal with acetate and associated processes of production and consumption are missing. Interestingly, some of the papers I got from a quick ISI search are actually coming from the Marburg group itself

Specific comments:

Page 8621, Line 11: What about methane consumption? Isn't that important as well?

Page 8622, 8623: Very detailed introductory part. Can this be condensed?

Page 8623, Lines 20-23: Out of context. Please rewrite or delete.

Page 8624, Lines 4-8: Context gets lost here. Please rewrite.

Page 8624, Lines 9-10: Description of the motivation of the study is very short. Why choosing those lakes? What is the possible impact of the wetland system on climate? Does climate change, in turn, influence the biogeochemical pathways of methane formation? For example, high temperatures should favor hydrogenotrophic methanogenesis due to an enhanced production H<sub>2</sub> coming from organic matter degradation. See also Nozhevnikova et al. (2007, FEMS) for dependence of methanogenesis at high T.

Page 8624, Lines 14-16: Shorten to "...find out which environmental variables control (1) the path CH<sub>4</sub> production, (2) its rate, and (3) the d<sup>13</sup>C of CH<sub>4</sub> and its precursors."

Page 8624, Sampling and Table 1: The lake description is pretty short as are the numbers of parameters shown in Table 1. Are there other general environmental parameters available such as water depth, exact temperatures, oxygen content or nitrate concentrations. Oxygen contents are probably important since the authors used the upper 3 centimeters during incubation which at least include oxic or suboxic zones. Generally, this means the authors turned these sediments to be anoxic during incubation which thus may explain the low numbers of archaea versus those of bacteria

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(page 8631, Fig. 7a). Moreover, such parameters would give more insight into the different results the authors get from the incubated sediments sampled in different time periods of the year (see also discussion stretching from page 8641, Line21 to page 8642, Line 5).

Page 8624, Lines 22-23: The authors should compare their results with their own study from Lake Kinneret having the same focus (Nüsslein et al., 2003, L&O) at temperatures of 15°C and especially 30°C. I am missing that in the reference list and the discussion later on.

Page 8626, Chemical analyses: An information about analytical errors on concentration and isotopes should be given here. Generally, the precision of the isotopic measurements in the Tables is far too high. The real precision is probably not in the way that you can present two digits.

Page 8630, Lines 20: In the calculation section before  $f_{CO_2,CH_4}$  is defined as  $f_{H_2}$ . Why is it changed here and later on?

Page 8630, Lines 27-28: Similar proportions were reported by Heuer et al. (2010, OG) who studied the stable carbon isotope biogeochemistry of acetate in lake sediments.

Page 8632, Lines 11-14: Should Fig. 7b not be mentioned before Fig. 8? Thus, please move that statement and maybe also the whole paragraph up.

Page 8632, Lines 3-14: I am missing the results from the bacterial 16S rRNA analyses as described in the method section. Is there an explanation why those are not presented? Results on bacteria are for example in the Schwarz et al. (2007, EM) paper? Similar results and following discussion could be presented here.

Page 8633, Lines 1-5: There are probably more papers to cite than just those of Conrad and co-workers. For example, please compare your data with those from Heuer et al. (2010, OG).

Page 8635, Line 16: Although there are internal isotopic differences in the methyl

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and the carboxyl group within acetate the whole molecule generally mirrors in isotopic composition that of Corg. Thus, overall there seems no fraction associated with fermentation leading to acetate (Heuer et al., 2010, OG).

Page 8636, Line 10 and 15: Please exchange higher with more positive. Higher is very much irritating. Moreover, detailed numbers would help to guide the reader.

Page 8636 Line 25 to Page 8637, Line 1: The finding of acetate production from CO<sub>2</sub> could actually be pointed out even earlier in the paper and is supported by already existing data from the literature. The negative values given in Table 3 after CH<sub>3</sub>F addition are a clear hint.

Page 8637, Lines 8-14: The argumentation about inhibition of fermentation by CH<sub>3</sub>F is very vague and not proven by the data. I suggest to delete this section.

Page 8637/8638, Section 4.3: This section is very descriptive with a lot of details, has no references and is in most parts already presented in the results section. I suggest to remove this part or to rewrite in a way that it is really worth of discussion.

Page 8639: This section should be renamed. Because it also contains information about the produced CO<sub>2</sub> I would suggest to name it “Control of CH<sub>4</sub> and CO<sub>2</sub> production rate”.

Page 8639, Lines 23-28 to Page 8640, Lines 1-4: Is the side story of soil sediments of importance to this study? If there is no real reason, I would suggest to delete this paragraph.

Page 8640, Line 25 to Page 8643, Line 8: A very long part here. The interesting finding comes in the final paragraph that should actually be moved more to the beginning where it stands out more.

Page 8642, Lines 6-27: Why were T-RFLP results from other lake studies (e.g. Schwarz et al., 2007) not used for comparison here?

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Page 8643, Conclusions: Negative results are presented first. Why not starting the conclusions with Line 23? You may think about reorganization.

Page 8644, Lines 5- 7: What are easy measurable lake variables? Corg-content, temperature? I think d13C-CH4 measurements are easily performed nowadays.

References: Please update according to changes.

Tables 1 to 3: I cannot imagine that your isotopic measurements are that precise. Please give decimal places according to precision you get from the isotopic measurements. One seems reasonable to me.

Table 2: Why using the epsilon expression here? You dominantly use  $\alpha_{\text{CO}_2\text{-CH}_4}$  and  $f_{\text{CO}_2,\text{CH}_4}$  in the text. Moreover, the latter is actually introduced as  $f_{\text{H}_2}$  in eq. 4.

Table 3: Are the the more positive d13C-values of acetate after CH3F addition due to incomplete inhibition of acetoclastic methanogenesis or due to acetate oxidation? Why is the latter only occurring at some sites and not always? Are there different microbial communities involved? What is causing the strong depletions in acetate of samples 15 and 16? Autotrophic acetogenesis? Are these samples characterized by different microbial communities as well?

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**BGD**

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