

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

R. Conrad et al.

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We thank referee #3 for the helpful comments. There are so many points that are easily overlooked in a large manuscript. We appreciate the opportunity making the paper clearer. In the following we will consecutively address each point raised and, if appropriate, will make suggestions how to change the manuscript.

GENERAL COMMENTS

As stated in the end of the Introduction and in our reply to referee #2, the objective was studying CH₄ production in various tropical lakes, to find out which measurable variables of the lake sediment, such as content of organic matter, $\delta^{13}\text{C}$ of organic matter, C4912

microbial community abundance and composition may influence the rates and isotopic composition of products of organic matter decomposition, CH₄ and acetate in particular. Of course there are many more aspects that would be interesting, such as the possible response of the methanogenic microbial community to climate change and possible increase of temperature. However, this was not subject of the present investigation. We also did not determine the composition of the bacterial communities, only the diversity of the dominant terminal restriction fragments of ribosomal RNA and *mcrA* genes. We agree that some of the listed references are relevant for the Discussion and should be quoted, and are grateful for the hint.

SPECIFIC COMMENTS

P.8621, L.11: Correct; CH₄ consumption is also affecting the isotopic composition of atmospheric CH₄. We will change the term “CH₄ production” into “CH₄ turnover”.

P.8622, 8623: We will try to shorten this part of the Introduction.

P.8623, L.20-23: Thank you; we will rephrase.

P.8624, L.4-8: Thank you; we will rephrase.

P.8624, L.9-10: In fact, the entire Introduction presents the motivation of our study. The sentences in the end only summarize the essentials in form of bullets. The impact of wetland systems on climate cannot be one of our objectives, since we did not address the actual CH₄ emission into the atmosphere. However, we refer to relevant literature, e.g. that of Bastviken et al. (2010) conducted in the same region. The effect of climate on the biogeochemical pathways also cannot be an objective of our study, since we did no experiments on the effect of temperature. We addressed such effects in previous papers (e.g., Glissmann et al. 2004), similarly as Nozhevnikova et al. (Nozhevnikova et al. 2007) did. However, we believe that this subject would be out of context here.

P.8624, L.14-16: Thank you for this phrasing; it is much shorter and better.

P.8624, Sampling and Table 1: The sampling was done expedition-style. Water level, C4913

O₂ concentration, and temperature in lakes of the Pantanal and Amazonia are highly variable over the season. Although we could give these data for the particular time of sampling, they would not be a characteristic value. We are afraid that readers would not appreciate this fact and take such values as given if they would be tabulated. Therefore, we would rather not present these data in form of table columns, but rather add a descriptive sentence, such as: "In most of the sampled environments in the Pantanal there is a daily change in temperature values that can vary up to 5°C, and oxygen concentrations can vary from 120% saturation (day) to anoxic (midnight) right over the sediment within 12 hours (data not shown). The amazonian lakes were mostly sampled during high water level, with a temperature average of 28°C during sampling and with anoxia over the sediment." It is true that we incubated the sediments under anoxic conditions to ensure that methanogenic conditions were optimal. Thus, we studied potential CH₄ production. We also used 25°C as incubation temperature. By this type of experimental standardization, we ensured that we are able to compare parameters across the different lake sediments. However, this kind of treatment could hardly have affected the ratio of bacteria to archaea. First, without additional substrate the growth rates are most probably too low to be expressed over the relatively short incubation times. Second, there is no reason to assume that bacteria would grow faster under anoxic conditions than archaea. For CH₄ production, bacteria are as important as archaea. Finally, the fact that bacterial numbers are by 2 orders of magnitude larger than archaea is well known from literature including determinations in various sediment depths and in lakes with anoxic hypolimnion (Zepp-Falz et al. 1999; Chan et al. 2005; Koizumi et al. 2003; Schwarz et al. 2007).

P.8624, L.22-23: Agreed; we can compare CH₄ production in other tropical and sub-tropical lakes using the few literature data available.

P.8626, Chemical analyses: Agreed; we will give information on precision and detection limits. It is correct that the tabulated values pretend a too high precision. We are sorry for overlooking this point.

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P.8630, L20: Thank you; we overlooked. We will use fCO₂,CH₄ throughout the ms.

P.8630, L.27-28: Thank you; the paper by Heuer et al. (2010) is indeed relevant and we will reference it in our revision.

P.8632, L.11-14: Fig.7a is mentioned before Fig.8, while Fig. 7b is mentioned later. We think this is all right !?

P.8632, L.3-14: We would love having these data. However, we only have bacterial T-RFLP patterns, which show us how many different bacteria were present and how their relative abundance was (at least that of the more dominant ones). The individual T-RFs were not assigned to bacterial phyla, since this would have required generating and analyzing a clone library for each sediment. This is quite some work, in particular in case of the highly diverse Bacteria. We did not undertake such study so far.

P.8633, L.1-5: We agree; the paper by Heuer et al. (2010) is indeed relevant.

P.8633, L.16: Thank you; we will discuss in a revision the paper by Heuer et al. (2010) in this context.

P.8636, L.10-15: More positive delta¹³C are higher delta¹³C. Although we may rephrase the sentence according to the suggestion of the referee, we think that this is simply a matter of taste. In fact, for previous publications reviewers had just the opposite request.

P.8636, L.25 to P.8637, L.1: We agree that there are numerous hints in the literature (also own publications) indicating the importance of acetate production from CO₂. However, this process is notoriously difficult to quantify. The entire chapter is about acetate. This chapter will be revised anyway in the context of comments made by referee #1.

P.8637, L.8-14: We do not agree; we need an explanation for the effect of CH₃F on acetate isotope data (compare comment on Table 3 below). We think we cannot avoid presenting some speculation, as long as it is theoretically possible.

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P.8637/8638, Section 4.3: Thank you; this section indeed looks a bit crowded with data. Also some discussion in context with literature would be adequate. However, the Discussion section is also needed for data analysis and interpretation, not only for putting the interpretation into context with the literature.

P.8639: We will follow the suggestion and rename the section.

P.8639, L.23-28 to P.8640, L.1-4: We agree; this story is not urgently needed and can be deleted.

P.8640, L.25 to P.8643, L.8: Thank you for the suggestion; we think it is a matter of taste.

P.8642, L.6-27: The T-RFs of Archaea can be compared to some extent, since they are relatively few and since they are robustly assigned to the same phyla again and again. This is not the case with bacterial T-RFLP. Although we could show the T-RFs, this would be meaningless without assignment to cloned sequences. A comparison with the data of Schwarz et al. (2007) is not possible, since we did not determine the bacterial phyla in the Brazilian lake sediments.

P.8643, Conclusions: We agree; the Conclusions will be rephrased.

P.8644, L.5-7: We agree and will rephrase the sentence.

Tables 1-3: Thank you; the positions after the decimal point are indeed too many.

Table 2: Epsilon and alpha are synonymous, but we agree that that is more reasonable to use alpha as in most of the text. The same is true for fH₂, which we overlooked.

Table 3: The questions posed by the referee are all valid. We tried to address them in the Discussion. In fact, we will revise this part of the paper according to the comments of referee #1, so that the present comments should also be taken care of.

Reference List

C4916

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