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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.***

**R. Conrad et al.**

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We thank referee #2 for the helpful comments. We are sure they will assist us to improve our manuscript. In the following we will consecutively address each point raised and, if appropriate, will make suggestions how to change the manuscript.

**GENERAL COMMENTS**

The objective was not just to determine the pathways of CH<sub>4</sub> production in various lakes, but to find out which measurable variables of the lake sediment, such as content of organic matter,  $\delta^{13}\text{C}$  of organic matter, microbial community abundance and composition may influence the rates and isotopic composition of products of organic

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matter decomposition, CH<sub>4</sub> and acetate in particular. The quantification of isotope fractionation and the path of CH<sub>4</sub> production is a further result that is derived from such data. The approach for pathway calculation is not new and has been used before for individual environments in which CH<sub>4</sub> is produced from anaerobic organic matter degradation. The novelty of the present study is the comparative application to a larger number of lake sediments with the goal to detect relations across different environmental conditions. This approach showed that the isotopic composition of products was indeed correlated to the isotopic composition of substrates, as theoretically expected, and allowed to evaluate the different fractionation steps from organic matter via acetate to CH<sub>4</sub> on a broader data basis.

We are grateful for the comment on structuring the Results and Discussion of the manuscript in a better way. We agree that the principle results are shown in the Tables and that the correlations shown in Fig.2-7 may be better presented in the Discussion section. In a revised manuscript, we will structure the Results and Discussion section accordingly.

### SPECIFIC COMMENTS

2) We tried to introduce the rationale for using tropical lakes on P.8621, L. 15-22, but will attempt to make this clearer in a revised version. We targeted a tropical zone in Brazil where lakes make up a substantial part of the landscape and CH<sub>4</sub> flux from lakes into the atmosphere is quite significant. For reference we can add a recent publication by Bastviken et al. (2011) showing that tropical areas are of particular significance.

3) The referee is correct that the technical approach to determine isotopic composition, fractionation, and process pathways is not new and has been applied before to individual lakes. Although repeating a study in a different system is not worthless, we agree with the referee that it needs to be well justified. However, our study is not the mere repetition of a previous study using another lake sediment. The objective of the present study was to provide data for a suite of different tropical lake sediments, in order to al-

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low correlation analysis across sediment characteristics. The results and conclusions of the present study could not have been obtained from published data, and the study of just another lake sediment would not have been sufficient.

4) The choice of the sites was based on our desire to study places within the Pantanal and the Amazonia regions, where lakes make up a substantial part of the landscape and CH<sub>4</sub> flux from lakes into the atmosphere is quite significant (Bastviken et al. 2011). After this choice, the selection of sampling sites and period of the year depended largely on the possibility of access (not trivial in these regions) and thus, sampling was to a certain extent indeed random selection. In other words, within Pantanal and Amazonia we had no a priori sampling scheme that would be based on geographic factors. We had also no a priori sampling scheme for season, but took most of the samples during dry season, since many of the sampling sites were only then accessible.

5) We did not attempt to determine vertical profiles in the sediment, although this would of course be very interesting. For logistic reasons we had to compromise on what to measure. Therefore, we also did not measure pore water concentrations in-situ.

6) We agree that CO<sub>2</sub> in the headspace does not quite represent the total amount of CO<sub>2</sub> produced. We mentioned and discussed this in the text (P.8640, L.5-19). Besides gaseous CO<sub>2</sub> there is also dissolved CO<sub>2</sub> and bicarbonate. We agree that bicarbonate may not be thus negligible as stated in our manuscript (see comment (10) below). We did not measure the dissolved inorganic carbon in the sediment, but can calculate the data from the gaseous CO<sub>2</sub>. Values of  $\delta^{13}\text{C}$  can also easily be calculated using published fractionation factors (Stumm & Morgan 1981). Thus  $\delta^{13}\text{C}$  of dissolved CO<sub>2</sub> will be by about 1‰ lower than gaseous CO<sub>2</sub>, while  $\delta^{13}\text{C}$  of bicarbonate will be by about 9‰ larger.

7) We measured fatty acids on our HPLC systems and detected only very low concentrations of acetate in the uninhibited (without methyl fluoride) sediment (see Table

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3 of our paper). In the samples, in which acetoclastic methanogenesis was inhibited with methyl fluoride and acetate accumulated (Table 3 of our paper), we also detected low concentrations of propionate, albeit only in 12 of the 16 lake sediments tested. The propionate concentrations were on the average  $61 \pm 57 \mu\text{M}$ , or  $4.5 \pm 2.8\%$  of the simultaneously accumulated acetate. Occasionally, also low concentrations of isovalerate (in 7 lake sediments) or butyrate (in 3 lake sediments) were detected under these conditions. However, other fatty acids were not detected on our HPLC systems, and other dissolved organic compounds were not analyzed. We would mention this in a revised manuscript.

8) We observed a difference (Fig. 6C) in the percentage hydrogenotrophic  $\text{CH}_4$  production by determining it either from the inhibition of total  $\text{CH}_4$  production with methyl fluoride or from pathway calculation using isotopic data. We interpret this difference as bias due to partial inhibition of hydrogenotrophic methanogenesis by methyl fluoride. Such partial inhibition has been shown before in systematic experiments (Conrad & Klose 1999). The problem is that methyl fluoride either does not inhibit acetoclastic methanogenesis completely or it inhibits not only acetoclastic methanogenesis but in addition also part of the hydrogenotrophic methanogenesis. It is hardly possible to establish an ideal methyl fluoride concentration that would result in both specific and complete inhibition. This is a general problem with so called “specific inhibitors” (Orem-land & Capone 1988). We therefore compromised somewhat on specificity and chose rather high methyl fluoride concentrations to make sure that acetoclastic methanogenesis was completely inhibited, so that the  $\delta^{13}\text{C}$  of the produced  $\text{CH}_4$  was representative for hydrogenotrophic methanogenesis. Part of the hydrogenotrophic methanogenesis was then probably also inhibited. Accordingly, the residual  $\text{CH}_4$  production was lower than expected from the isotopic data, especially when hydrogenotrophic methanogenesis accounted for a relatively low percentage. We suggest explaining the data in a less terse way for the revision.

9) We do not expect that biomass of microbes increased during the incubation of the

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sediment. In fact, many of the microbes present in the sediment must have been in a dormant state, as suggested in our discussion on P. 8641, L.2-20. Therefore, we dismiss an effect of biomass synthesis on acetate isotopic values. We will mention this in a revised manuscript.

10) We thank the referee for this comment. Bicarbonate may indeed play an important role in the CO<sub>2</sub> balance of our sediments, since their pH values were usually slightly above the pK of bicarbonate (6.352 at 25C). Therefore, total CO<sub>2</sub> production was larger than CO<sub>2</sub> production determined from gaseous CO<sub>2</sub> only. We now calculated total CO<sub>2</sub> production rates and used these data in a regression against CH<sub>4</sub> production rates similarly as in Fig. 2B of our paper. The regression then showed that total CO<sub>2</sub> production had on the average a 1.78 times larger rate than CH<sub>4</sub> production ( $r^2 = 0.69$ ,  $P < 0.0001$ ). The two lakes (#8, 16) with pH > 7 showed the largest deviation from the regression line. We may add a figure (Fig. 1) illustrating this new regression and amend the discussion in a revised manuscript.

#### Reference List

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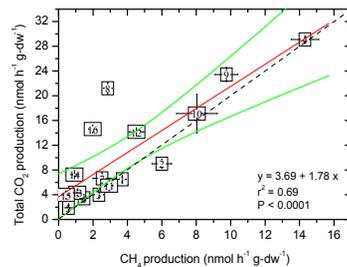
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**Fig. 1.** Linear regression of the production rates of CH<sub>4</sub> and total CO<sub>2</sub> (gaseous CO<sub>2</sub> + dissolved CO<sub>2</sub> + bicarbonate).

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