

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

**Anonymous Referee #4**

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This paper includes various and also many intriguing results for isotope ratios of CH<sub>4</sub>, acetate and organic matter, while for microbiology, which might be a new approach, their results were not clear. That's why the conclusions in this manuscript was negatively written, I guess. However, investigation on microbial community corresponding to the isotopes has only just started. In the conclusion section, the authors described only the correlation between CH<sub>4</sub> production and number of gene of Archaea, and nothing was concluded about their results on other experimental results. On the other hand, introduction was differently written. They described mainly about the isotopes and pathways of methane production. If the authors are interested in the relationship between methane production in the ecosystem and microbial community, both should be

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described, especially investigation on the basis of both parameters at once. There are sometimes discrepancies between isotope and microbiological results. They should emphasize that at least their isotope results showed or explain most results consistently but at this moment microbiological parameters such as Archaeal and bacterial 16S rRNA and mcrA gene, are not well corresponding to the CH<sub>4</sub> production. There are several weakness in isotope mass balance, however I was convinced mostly. For example, no-fractionation assumed for methyl group of acetate may be acceptable assumption, if they showed the error arisen from the assumption. Not so different value of d<sup>13</sup>C of acetate accumulated with and without inhibitor may also support this, although there is a small difference. Acetate is commonly produced metabolites, therefore there are many pathways and precursors and also mediating microbes. Change in the isotope ratio by the addition of inhibitor is not so surprising.

fH<sub>2</sub> or fCO<sub>2</sub>-CH<sub>4</sub>? Both may be the same! In the table epsilon was used, whereas fractionation factor alpha was used in the text. Why don't you add alpha in the table 2. 8635 L25-27: I did not understand it. Is this describing about the difference between carboxyl and methyl group?? d<sup>13</sup>C<sub>org</sub> showed correlation with various parameters. Is it possible to consider the soil (or sediment) characteristics such as contribution of C<sub>3</sub> and C<sub>4</sub> plants, and age or decomposability. Conrad et al., 2010a L&O vol 55 (not 54)

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