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Interactive comment on "Vertical profiles of sediment methanogenic potential and communities in two plateau freshwater lakes" *by* Yuyin Yang et al.

Yuyin Yang et al.

xiesg@pku.edu.cn

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The manuscript "Vertical profile of sediment methanogenic potential and communities in two plateau freshwater lakes" is comprehensive written. The aim of the study is clearly stated and well supported with data. The authors describe the methanogenic potential (MPP) of sediment incubations, quantify the archaeal and methanogenic community and analyses the community structure using NGS. They can show that the two lakes exhibit different patterns for almost all analyzed parameters and show some changes along a depth profile of 20cm. The MPP measurements would benefit from a better time resolution and the additional measurement of the isotopic signal of the released methane. The quantification of the archaeal and methanogenic community

supports in large previous findings for other lake systems. The NGS gives some new insights into the community structure. Especially NGS data for the mcrA gene are currently still scarce in the literature. In addition they contrast the sediment of two lakes and show a well resolved depth profile of the top 20cm of the respective sediment.

ResponseïijŽThe authors appreciate the reviewer's positive comments. Revisions were made in response to the following points.

Specific comments: Introduction: Line 73: belonging to the archaeal. . . Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Line 74: Methanogens from seven archaeal orders Response: As suggested, the revision has been made.

Methods: Line 110: Were the five replicate cores taken at the same location or at different spots around the lake? Response: They were taken at the same location. Preliminary experiement has been done to test the methane production potential in surface (top 5 cm) sediment of several sites around the lake, and the site with a median MPP was chosen for the current study.

Line 112: What was the diameter of the columnar sediment sampler? Response: 10 cm.

Line 114: 14.8o C not 14.8C Response: As suggested, the revision has been made.

Line 130ff: Conrad et al. 2010 used a time series to estimate the methane production potential as maximal slope of the methane concentration over the time for several consecutive points. (Compare Liu et al 2016). Using endpoint values will largely underestimate the methanogenic potential since most incubations will have a lag phase in the beginning without any methane production (compare Liu et al. 2016 in your references). Likewise the time span of 28 days may be insufficient to establish the full potential of such samples at the low incubation temperatures (16oC).

Response: The authors appreciate the reviewer's interesting points. In the preliminary experiment, methane production was measured at an interval of one week, and result showed that the methane concentration could be detected at the end of the first week (although quite low), and remained nearly constant after 5 weeks. Hence, in the current study, methane concentration was measured at the end of the 4th week to get an approximate methane production potential. To avoid the inaccurate expression, we use total methane production instead of methane production potential in the new version of manuscript.

Line 134: a total of six sediment... Response: As suggested, the revision has been made.

Line 134: Why did you initially mix the five cores (line 118) and now redistribute into six replicates? Response: One sediment core was not enough for all the analysis. To attain a sufficient amount of sediment sample, we had to take several cores. Considering there might be difference between these cores, they were mixed and redistributed for physicochemical, activity and molecular analysis.

Line145 the quality of the DNA was checked... Response: As suggested, the revision has been made.

Line 151 mcrA and archaeal 16S rRNAgenes, respectively (change order!) Response: As suggested, the revision has been made.

Line 155: The range of the standards is rather small. The results for 16S rRNA are not covered! Response: We feel sorry that we made a mistake of the unit in the manuscript. It has been corrected as copies/μL.

Line 168: how was the quality filtering done? Response: By using the Sliding Window quality filtering (Trimmomatic). The window was set as 50 bp, and the threshold was set as 20.

Results Line 199: The hydrogenotrophic methanogenic potential is not equal to

the methane production under CH3F inhibition. CH3F partially inhibits the hydrogenotrophic methanogenesis as well; hence one has to use isotopic signals of the produced methane under both conditions together with dedicated fractionation factors to estimate the hydrogenotrophic contribution (Compare Conrad et al 201 as well as Liu et al 2016 in your referenences). Better use the term "inhibited samples" to describe the methanogenic potential of these samples. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Line 206: between these two...(delete: in) Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Discussion General: You tend to discuss several depth related changes by comparing your results to previous studies. You should carefully check (and quote) the respective sampling depth. You are doing a relatively well resolved profile, while many others use deeper cores. Response: The authors appreciate the reviewer's suggestions. Most studies we chose for comparison have a comparable sampling depth with ours, especially those for activity and abundance are all limited to less than 50 cm. To put it more clearly, we have added the respective sampling depth as well as interval to the manuscript as suggested.

Line 331: rate could differ drastically between the two... Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Line 350: How did you calculate the contribution of hydrogenotrophic methanogenesis? See comments to line 199? I would describe this more carefully! If you e.g. use the concentration values given in Conrad et al. 2010 for lake batata in Fig 1. (2.3 vs. 0.5 kPA) you would estimate a contribution of roughly 20%; using the isotope values you reach 30-50% (Table 5).

Response: The authors appreciate the reviewer's suggestions. Since isotope data was lacking, the contribution might not be accurately estimated. We had replaced AMP with IMP (inhibited methane production). Conrad et al (2010) reported the CH3F inhibited

about 90% of hydrogenotrophic methanogenesis, so we might estimate the contribution roughly.

Line357: How does the produced methane correlate with the organic carbon in your study? Response: The TMP showed a significant correlation with sediment TOC (Spearman rank correlation, $p<0.05$). However, methane production might be related to several parameters (TOC, ORP and so on), and most of these parameters were also related to depth. So a simple correlation might give misleading conclusion. Unluckily, data here was not enough for partial correlation. So correlation analysis was not shown here, and the correlation was not discussed in the manuscript.

Line 374 Mthanogen? Response: It has been corrected as methanogen.

Line 447 Methanombacteriales (last letter is currently not italic) Response: As suggested, the revision has been made.

References: Bastviken et al. 2009 and Conrad et al 2014: incomplete:missing pages! Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Tables and Figures Fig 1: The unit is nmol/g dry weight/day. However it is unclear how you have quantified the dry weight and it is very unlikely that your estimates using only endpoint values will give a meaningful estimate of the potential. I would rather show the amount of methane produced. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Fig 2b: check the x axis! It somehow has different scaling than Fig 2a. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

Fig 4: The tree has only very few reference strains incorporated. Where are the Methanocellales? (Close to OUT 10 OUT 11 I would guess??). Where is Methanobacterialles Response: The authors appreciate the reviewer's suggestions. Several

reference strains had been added to the tree. Methanocellales strain NC009464 (Methanocella arvoryzae) was related to OUT 23-28. And Methanobacterialles strains Methanobacterium beijingense and Methanobacterium congolense were also shown in the tree.

Fig 5: I am not good in statistics but you seem to feed in much more data in the 16S tree than sequences in the mcrA tree. How does that influence the tree structure? Response: This doesn't significantly change the tree structure. The mcrA libraries have a high coverage, indicating that the diversity has been well captured.

Fig4: OUT 7 and OUT 1 which have the most sequences originate from E4 and E6 respectively? Response: OTU 7 has the more sequences originate from both E4 and E6. The representative sequence was attained using Mothur command Get.oturep, and was not related to the relative abundance of different samples.

Supplementary Figures: Check Order: Fig S1 is first mentioned in line 341; while Fig S2 (Line 261) and S3 (Line 293) are mentioned much earlier. Response: As suggested, the revision has been made.

Fig S2: error bars missing. Response: Replicated samples were mixed and then analyzed, so here we only gave an average result.

Fig S2: you find a relative high relative contribution of methanogens in the top sediment sample; in contrast the activity there is apparently low (Fig. 1). Likewise you find some sequences associated with Methanosarcinales; while in your NGS data (Fig S3) you do not find any Methanosarcinales? Response: A high relative abundance might not always consist with activity. In our result, a high relative contribution of methanogens was found in the top layer of Erhai Lake, and the activity was high, too. In contrast, methanogen showed high abundance but low activity (still higher than the top layer of Erhai Lake) in the top layer of Dianchi Lake. In the NGS data, Methanosarcinales were found in all samples.

Fig S3: Give the cluster name or related organisms in the figure legend (or legend) as well. Response: The authors appreciate the reviewer's suggestions. As suggested, the revision has been made.

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