

***Interactive comment on “Methane production correlates positively with methanogens, sulfate-reducing bacteria and pore water acetate at an estuarine brackish-marsh landscape scale” by C. Tong et al.***

**Prof. chuan**

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Received and published: 10 January 2014

Dear Dr. Wegener

Ref.: BGD paper: Methane production correlates positively with methanogens, sulfate-reducing bacteria and pore water acetate at an estuarine brackish-marsh landscape scale

We thank you for your valuable comments and care edition on our paper. Based on the comments and editions, we have completed a careful revision to improve our paper. All

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the comments and editions were carefully considered and addressed in the revision. The following is a summary of the responses to the comments:

Tong et al., examined methane production and abundance of methanogens and sulfate reducers in marsh sediments of the Min River estuary. They support their data with chemical analyses (i.e.  $\text{NO}_3^-$ , Fe, DMS,  $\text{SO}_4^{2-}$ ,  $\text{CO}_2$ , Acetate, Corg) to find out what factors the abundance of methanogens, sulfate reducers and methanogenesis rates in this system. Interestingly methane production rates do only weakly correspond to community sizes (abundance of methanogens), or acetate concentrations – emphasizing this relationship in the title is misleading - maybe a different title (unfortunately I have no suggestion) might be suitable. I sympathize with the extensive statistical approval of the results here – however I am not sure about the variation partitioning done here. Thanks sincerely for the valuable comment. We had changed the title to “Weaker correlation between methane production and abundance of methanogens at a subtropical brackish marsh landscape in the Min River estuary, China”

In any case it would, for sure, need a better explanation i.e. in the M&Ms. Furthermore I am not satisfied with explaining production by the concentrations of a precursor, as turnover plays the more important role. Please also see the details below. Thanks sincerely for the valuable comment, we had done multi-factors stepwise regression analysis for the methane production rate and pore water concentrations of acetate, DMS,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$  and  $\text{Fe}^{3+}$  for the three vegetation zones together at the landscape scale. See in revision of page 20.

Another important point: Why do you compare qPCR data of ribosomal genes (16S or archaea) with functional genes (dsr of SRB). For me this weakens the comparability. The authors need to at least comment on this (i.e. abundance of 16S per archaea, coverage of primers) In total the study shows reasonable results, however at the end I wasn't sure what I might have learned here. Thanks sincerely for the valuable comment, we had deleted the regression analysis between methanogens and SRB and deleted the Fig. 8.

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Whereas introduction, methods and results are okay, the discussion needs some serious cleanup and a focus. Several points are contradictory or at least very fuzzy. And finally, why do the authors focus on acetate? The coupling to sediment organic carbon is much stronger. Thanks sincerely for the valuable comment, we had cleaned up the discussion part. We analyzed again the correlation between soil methane production rate and pore water acetate concentrations when ignoring that single data point as an outlier in the Fig. 5, and the result showed that there is no correlation ( $R^2 = 0.0034$ ,  $P = 0.688$ ), we deleted the Fig.5 in the revision.

Fig.5 to 8: Color code should be consistent – why are dots sometimes black, grey, or white. This implies an additional information layer. Typo in Fig 5 Acetate Thanks sincerely for the valuable comment, we had made the color in Fig.5 to 7 consistent.

Table 2/3: Please explain the table better – what is F, DF etc., if you are not familiar with statistics those factors have no meaning F is a statistics value in ANOVA, df is degree of freedom in ANOVA.

Table 5: mentioned in the text but it does not exist in the MS Thanks sincerely for your careful check, and we had deleted the Table 5 in the text.

p18243 Line 5: I think the term “terminal substrate“ is not commonly used or? Usually one uses the term “terminal electron acceptor” but not for the substrate. Better use something like “energy sources suitable for methanogens” Line 10ff: Please consider to include hydrogen as energy source in your introduction as it is the major energy substrate for methanogens in marine systems. Line 10ff: If you mention DMS, it should contain information, i.e. it is usually seen as a non-competitive substrate meaning it is rather used by methanogens than by SRB Line 20: What are “soil microbiological properties” – this means all or nothing. Line 23: “the findings are inconsistent” – please explain Thanks sincerely for the valuable comment, we had changed the term “terminal substrate” to “energy sources suitable for methanogens” or directly changed to “substrate”. We also added the information of hydrogen as energy source and DMS,

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and changed directly the “soil microbiological properties” to “soil microbe”.

p18244 Line 10 ff: Although some studies have determined the abundance of SRB in marine sediments and tidal flats in recent years : no published research has determined the spatial distribution of pore water concentrations of DMS among different brackish marshes along a gradient from dam to sea, and revealed their relationship with the methane production rate. (I) What is the contradiction here – meaning why the although ? (II) What is the relation between the two parts – please rephrase into 2 sentences Line 20ff: The objectives were (they are two) and better write “(1) to : (2)to” as it reads easier Line 25: easier “ landscape scale and vegetation types”. Thanks sincerely for your careful check in above sentence, we typed the SRB to DMS, it was wrong, and we had corrected to SRB. We also rewrote the sentences of Line 20ff and Line 25 as your suggestion.

p18245 Line 8ff: Please define the three habitat types a bit more – as a non-mangrove specialist I do not see differences. Line 10: Mean elevations – against sea level? Line 20ff: Why exactly did they were the cores stored in situ for a while ? Thanks sincerely for the valuable suggestion, we had added the information of the three marsh zones, changed the “mean elevations” to “mean elevations against sea level”

p18246 Line 12ff: “The pore water was sampled using 100mL gas-tight glass syringes connected to a rubber hose and immediately placed into different containers” Please explain better – I do not see how you sampled pore water using this approach – did you use the Rhizon-technique? Thanks sincerely for the valuable suggestion, we had gave a new description of pore water sampling and added a reference to support it.

p18248 It is a bit invidious that cell abundances derived by qPCR rely on functional genes (for dsr/ SRB) and ribosomal RNA (16S in archaea). This can be involve biases due to different numbers of 16S, or different (often lower) PCR efficiency in functional genes. Furthermore: Does the primer pair covers all (important) groups

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of methanogens. You might check this in silico. Thanks sincerely for the valuable comments. Indeed, cell abundances derived by qPCR rely on functional genes is often lower than ribosomal RNA. For methanogens archaea, at first we also try to use the functional genes, but the result is not good, finally we used the 16S rRNA for methanogens (Watanabe et al., 2006, 2009). The primer pair of 1106F/1378R used in methanogens in our study can cover all important groups of methanogens.

p18249 Line 22: All results were normalized on gram oven-dried soil Thanks sincerely for the valuable suggestion, we had changed as suggestion.

p18250 Line 17ff: "Soil moisture in the *P. australis* marsh was also significantly higher than that in the other two marsh zones." Why – sediment differences Thanks sincerely for the valuable suggestion, we guess that the difference in soil texture may be one reason. The sand percentage in the *P. australis* marsh is relatively lower than that in the other two marsh zones.

p18251: I wonder a bit about the concentration profiles in Fig. 2: I hardly see consumption of NO<sub>3</sub><sup>-</sup> or any other species. Why is that not the case, please comment. Fe<sup>3+</sup> is almost not dissolvable in water – here it seems it does Thanks sincerely for the valuable comment, indeed in our study, the vertical variation of pore water concentration of NO<sub>3</sub><sup>-</sup> in the sediment profile (0-30 cm) in almost three marsh zone is litter, and we know that in general the NO<sub>3</sub><sup>-</sup> can be absorbed by the plants and is easy in migration and transformation. We guess that the relatively shallow depth (30 cm) sampled may be one reason, if we sample to a deeper depth, it may show the difference in pore water concentration of NO<sub>3</sub><sup>-</sup> in the sediment profile. Our another study (not published) on the soil NO<sub>3</sub><sup>-</sup> content in the *P. australis*, *C. malaccensis* marshes in the same study area also showed that there was not significant difference in the soil NO<sub>3</sub><sup>-</sup> content in a depth of 30 cm, however with the increase in depth, the soil NO<sub>3</sub><sup>-</sup> content decreased in the *P. australis* marsh.

p18252: How is methane production measured – I do not see any method here. Hope-

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fully there was a multipoint measurement done – as the sediment would only slowly leak its methane. Please clarify. The information measuring methane production rate is in Page 10, we sampled the gas samples for 4–5 times over the incubation period (three days).

p18253 I do not see how multivariate statistics have been performed here such as PCA/ Multi factor analyses tests etc., are not mentioned at all in your statistic method section? Please clarify. It is fundamental to explain how you come to the conclusion that i.e. acetate concentration explains n% of the methane production rates. Thanks sincerely for the valuable suggestion, we had done multi-factors stepwise regression analysis on the methane production rate and the pore water concentrations of acetate, DMS, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup> and Fe<sup>3+</sup> for the three vegetation zones together at the landscape scale.

p18254 Line 9 "0.1 μMg (dw)" it is missing a "-1" Line 17: change to "The relationship:". Line 18: change to "In our study the methane production rate increased" or "In our study methane production increased:". Line 19f: "... linearly with the pore water concentration of acetate for the three vegetation zones together at the landscape scale (Fig. 5), however, it was not associated with concentrations of dissolved CO<sub>2</sub> and DMS at the landscape scale (P >0.05, n = 27)." – I don't understand this sentence, it makes no sense – why should there be an association or correlation between CO<sub>2</sub> and – what exactly? Thanks sincerely for your careful check, we had added "-1", and we changed the sentences of Line 17, 18 based on the suggestion. Because dissolved CO<sub>2</sub> and DMS are energy sources suitable for methanogens, we analyzed the relationship between methane production rate and them.

Line 22ff: The result indicated that the acetate fermentation path would explain more variation of methanogenesis than the methane production path via DMS in estuarine brackish marsh with lower salinity (< 1 mScm) . Change path to pathway- however I do not see the correlation between acetate concentrations and methane production, since you compare concentrations and production. The only thing that can be stated

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here is: Acetate concentrations correlate stronger with methane production rates than DMS concentrations. HOWEVER this does not deduce higher acetoclastic than methy lotrophic methane oxidation. A simple example: An alcoholic does not necessarily have more alcohol at home than a non-alcoholic. He simply has a higher turnover than the non-alcoholic. The same might be true for methanogens. Thanks sincerely for the valuable suggestion. We analyzed again the correlation between soil methane production rate and pore water acetate concentrations when ignoring that single data point as an outlier in the Fig. 5, and the result showed that there is no correlation ( $R^2 = 0.0034$ ,  $P = 0.688$ ), we deleted the Fig.5 in the revision.

P18255 Line 19ff: "Higher pH value in the S. alterni marsh zone may be one reason causing the higher  $Fe^{3+}$  concentration, since  $Fe^{2+}$  is easy to be oxidized to  $Fe^{3+}$  in relatively higher pH condition." This statement is not correct for the ambient pH – this slight pH difference would not make a difference for the kinetics Thanks sincerely for the valuable comment, we had deleted the statement.

P18256 Line 10ff: This repeats only results. It is also pretty hard to read for me – and misses any clear statement. And somehow you switched from SRB to SBR this page Thanks sincerely for the valuable suggestion and care check, we had deleted Line 10, and corrected the SBR to SRB.

P18258 Conclusion is starting kind of surprisingly, after that it kind of randomly repeats results. Line 6-7: if this is the central finding then it should be emphasized in the discussion "Our results suggest that, provided that substrates are available in ample supply, methanogens can continue to produce methane" Well this finding is not new – (if you add enough acetate to a sediment, both, SRB and methanogens can thrive until thermodynamics first inhibit methanogens. Other substrates are non-competitive for methanogens as mentioned above. Thanks sincerely for the valuable suggestion, we had rewrite the conclusion part.

Yours sincerely!

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Interactive comment on Biogeosciences Discuss., 10, 18241, 2013.

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