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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.

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Dear reviewer

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

In their manuscript "Methane production correlates positively with methanogens, sulfate-reducing bacteria and pore water acetate at an estuarine brackish-marsh landscape scale" Tong et al. present data of several geochemical and genetic analysis performed at 3 different site in a Chinese estuarine. They are interested in factors controlling methanogenic activity in such an environment, an interesting topic where literature data does not give a conclusive answer. Unfortunately, the authors focus mostly on statistical analysis and not so much on understanding of the processes and interpretation of the trends seen in the measurements. For example the sulfate profiles in the 3 different sites show very different trends, indicating differences in the importance of bioturbation/oxygen pumping by plants vs. microbial activity in the sediment. Thanks sincerely for the valuable comment. We had added some analysis on the process and interpretation of the trends seen in the measurements. For the sulfate profiles in the 3 different sites show very different trends, indeed, SO42ïĂ■concentrations in the top soil was significantly higher than that in the deeper layer (25-30 cm) in the P. australis and S. alterniflora marshes, however, in the C. malaccensis marsh, the SO42ïĂ■concentrations in the top soil was not significantly higher than that in the deeper layer (25-30 cm), this may be caused by bioturbation/oxygen pumping by plants vs. microbial activity in the sediment. We considered that: (1) the distribution of the roots of C. malaccensis is lower than that of P. australis and S. alterniflora, which indicating that that roots of P. australis and S. alterniflora can pump more oxygen and to the deeper soil. Sulfate-reducing bacteria are generally anaerobic, but recent some studies have shown that some varieties are capable of O2 use, are able to withstand several hours of full aeration (Cypionka, 2000);ïijĹ2ïijĽthe abundance of SRB in the 0-10 cm soil was significantly higher than that in the deeper soil (20-30 cm) in the P. australis marsh, however, in the C. malaccensis marsh, the difference in abundance of SRB in three soil layers was not significant, the more SRB abundance would promote sulfate reduction.

Statistically comparing the average or 30cm concentrations of the different site does not help very much to understand the biogeochemistry. For the correlation between parameters, all single data points are used. The different parameters, however, have not been measured in the same sample and no information is given about the lateral heterogeneity. With the data not showing a clear trend, it seems more arbitrary then on how to pair the data. In Figure 5 for example, there is only 1 data point with increased methanogenic activity. Depending of the pairing with the other 2 acetate measurements from the same site, the corresponding acetate concentration will vary over at least 30% of its value. As this point is the only data point significantly different from all the others, the position of it has huge implications on the slope of the trend line. Ignoring this single data point as an outlier will most likely give no slope significantly different from 0 and thus no dependence of methane production on acetate concentrations. Thanks sincerely for the valuable comment. The sore cores for measuring methane production, physical and chemical properties, soil methanogens were in close proximity to each other. 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 (R2 = 0.0034, P= 0.688), we deleted the Fig.5 in the revision.

While this might be surprising at first, as the substrate concentrations should affect the microbial activity, the measured concentrations are substantially higher than what is usually reported for marine and estuarine sediments. This would also easily explain the co-occurrence of methanogenesis and sulfate reduction, as there is no competition at these high substrate concentrations. The ability of sulfate reducers to outcompete methanogens for substrates, as mentioned by the authors, (though this has only really been proven for hydrogen) implies that the sulfate reducers lower the substrate concentrations to values too low for the methanogens. This is obviously not the case at these high concentrations. Thanks sincerely for the valuable comment. We added the explanation in the discussion.

The authors also report a correlation between sulfate reducers and methane production. While the correlation again is very week, no information on total bacterial abundance is given. Population sizes of sulfate reducers and methanogens correlate, and actually show the highest R2 for any of the correlations, suggesting that there actually is a factor controlling microbial abundance as such and not that abundance of sulfate reducers influences activity of methanogens and thus rate of methanogenesis. Thanks sincerely for the valuable comment. We had deleted the regression analysis between methanogens and SRB and the Fig. 8.

Additionally, not abundance of the different groups was measured but the copy numbers of certain genes. No information is given, why they should be equal to population size, or why the correlation between copy number and population should be the same for both groups. Thanks sincerely for the valuable comment. We had changed the “population” to “abundance” in the revision.

The statistical relationships are done on linear, power and logarithmic functions. While these are probably the functions that give the highest R2, an explanation for the use of the different functions based on biological, chemical or physical properties are needed to justify. Thanks sincerely for the valuable comment. We had changed power and logarithmic functions to linear function in the Fig. 5 in the revision.

Additionally, an R2 of for example 0.2621 or 0.306 as presented in figures 5 and 7 do not indicate a close correlation between the 2 parameters. Thanks sincerely for the valuable comment.

The methods used for the experiments are not very well described. For example it is not mentioned, if the pore water was sampled, and if so, if it was kept under anoxic conditions until then. The high Fe3+ concentrations, however, indicate that the water was either not filtered, or partly oxidized before acidification. If the acetate samples were not sterile filtered, post sampling microbial activity will have influenced the concentrations changing the values. Were the soil cores collected at different depths, or

were the cores split into different depth intervals after recovery? What kind of rubber was the hose made of that was used on the gas tight syringe? Thanks sincerely for the valuable comment. We added some more detailed description for the method in the revision.

Specific comments: P 18245, L6: What do you mean with “semi-diurnal tides on the diurnal scale”? Semi-diurnal tides on the diurnal scale mean the soil surface is generally submerged for two times over a 24 h cycle. We had changed the “semi-diurnal tides on the diurnal scale”to “semi-diurnal tides over a 24 h cycle” in the revision

P 18246, L18: Why do you measure CO2 concentrations, if the dissolved inorganic carbon is what is important for the microbes. How did you calculated the CO2 concentrations? Thanks sincerely for the valuable comment. We deleted the information of CO2 concentrations in the revision.

P 18247, L2: Did you test if the sediment was actually dry after 24h by reweighing it after extended drying? Yes we did.

P 18247, L7: What was the injector temperature at the GC? Injector temperature at the GC was about 20 °C.

P 18248, L6: How big was the headspace, what is the sample size to gas phase ratio? Did you prepare any dead controls, mainly to account for desorption of methane from clays and such? The headspace was about 125 cm3. In our study, chambers with a headspace (125 cm3, volume of chamber 231 cm3, volume of soil core 106 cm3) for the anoxic incubation of soil cores with intact structures were constructed using polyoximethylene as a material tight for soil-gases and inert for methane.

P 18248, L11: In marine sciences production rates usually reported in mol/vol of wet sediment. Without information about the porosity and density, it is hard to compare ug d-1 g-1(dw) with the uM concentrations reported for the dissolved species. We had measure the soil moisture content, and calculated the methane production rates (μg

d-1 g-1(dw))

P 18250, L9: You averaged the values of the methane production rates before comparing it with the microbial abundance data. I assume that you did not sample the whole 10cm section, but took a sample either at the top or bottom of the core. Thus it would be more appropriate to use the rate from this section instead of averaging it. Each fresh soil sample in each 10 cm soil core for extracting the total DNA was taken from the middle section of the 10 cm soil core.

P 18251, L8: You write that there was “not a significant interaction”. The only thing you measured was statistics of the correlation. That does not prove that there was no interaction. Thanks sincerely for the valuable comment. We had changed to “they were not significant for vegetation types$\times$depths for all terminal substrates and electron acceptors”.

P 18252, L19: There is a clear variation of the microbial communities with depth. In P australis, abundance decreases with depth for both groups, in the other SRB seem to increase, but it is not clear if that is significant. Thanks sincerely for the valuable comment. The result in Table 3 was analyzed by two-way ANOVA across three marsh zones together at the landscape scale. Indeed, for single marsh zone, the abundance of SRB had some variation with depth, we added the information in the revision.

P 18253, L22: Acetate concentrations are low, if the system is in kind of a steady state and the production is balanced by the consumption. If, like in Duddlestons case, conditions change, it is not surprising, that concentrations increase, as this balance is disrupted. This has also been reported by Hoehler et al. 1999 Limnology and Oceanography. Under unbalanced conditions, the competition between for example sulfate reducers and methanogens does not work, maybe because of sulfate limitation or because the sulfate reducers can not keep up with an increase production. The concentrations in your marsh are high, indicating that there is no steady state. This could be due to diel flooding. Thanks sincerely for the valuable comment. We had added the

new interpretation of the relatively higher acetate concentrations in our study based on your suggestion. We also had added the paper of Hoehler et al (1999) as a reference and added the data of acetate concentrations of this new reference of in the revision.

P 18254, L8: ïA■M g(dw) is not a valid unit. ïA■M is equal to ïA■mol l-1 and thus has a volume term in there. If the concentrations you report are ïA■mol g-1(dw) you need to write it like this, but also explain, how you determined the concentrations as your measurements will most likely give you ïA■M. Thanks sincerely for the valuable comment. We missed a "-1", we had changed to ïA■M g-1 (dw) .

P 18254, L15: The concentrations reported by Sorensen were similar to your measurements in the 2 other environments. Thanks sincerely for the valuable comment. Indeed, the highest concentration of about 0.1 ïA■M DMS reported by Sorensen was similar to our measurement in the P. australis and C. malaccensis marsh zones, we added this result in the revision.

P 18254, L29: "26.2%" is too many significant digits, reduce (also at other places) to significance in line with the precision of your measurements. Thanks sincerely for the valuable comment. 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 (R2 = 0.0034, P= 0.688).

P 18255, L13: Do you have chloride data as conservative tracer for sea water? We did not measure the chloride concentrations of porewater in our study.

P 18255, L14ff: I do not understand the importance of this paragraph. Thanks sincerely for the valuable comment. We deleted this paragraph.

P 18255, L19ff: The slight difference in pH will not have an effect on the oxidation of Fe, but Fe3+ will not be soluble anyways. Thanks sincerely for the valuable comment. We deleted this paragraph.

P18257, L1: If you consider the vertical profile, there is a difference between the different sediments. Thanks sincerely for the valuable comment. Indeed, see from the vertical profile of the mean value of the abundance of methanogens in the P. australis and S. alterniflora marsh zones, there was a difference between some sediment layers, however, the difference was not significant.

P18258, L17: As mentioned before there are vertical variations. Thanks sincerely for the valuable comment. Response is same with before.

Figure 2: What exactly do the different letters (a, b, c) in the graph stand for? Different letters indicate significant differences at $P < 0.05$.

Yours sincerely!

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