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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.

Anonymous Referee #2

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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

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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. 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 trendline. 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. 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. The authors also report a correlation between sulfate reducers and methane production. While the correlation again is very weak, 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. Additionally, not abundance of the different groups

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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. 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. 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. 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?

Specific comments: P 18245, L6: What do you mean with “semi-diurnal tides on the diurnal scale”? 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? P 18247, L2: Did you test if the sediment was actually dry after 24h by reweighing it after extended drying? P 18247, L7: What was the injector temperature at the GC? 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? 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. 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

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of the core. Thus it would be more appropriate to use the rate from this section instead of averaging it. 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. 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 2 SRB seem to increase, but it is not clear if that is significant. 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 Duddleston's 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. P 18254, L8: $\mu\text{M g(dw)}$ is not a valid unit. μM is equal to $\mu\text{mol l}^{-1}$ and thus has a volume term in there. If the concentrations you report are $\mu\text{mol g}^{-1}(\text{dw})$ you need to write it like this, but also explain, how you determined the concentrations as your measurements will most likely give you μM . P 18254, L15: The concentrations reported by Sorensen were similar to your measurements in the 2 other environments. 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. P 18255, L13: Do you have chloride data as conservative tracer for sea water? P 18255, L14ff: I do not understand the importance of this paragraph. P 18255, L19ff: The slight difference in pH will not have an effect on the oxidation of Fe, but Fe^{3+} will not be soluble anyways. P 18257, L1: If you consider the vertical profile, there is a difference between the different sediments. P 18258, L17: As mentioned before there are vertical variations. Figure 2: What exactly do the different letters (a, b, c) in the graph stand for?

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