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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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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. NO3-, Fe, DMS, SO42-, CO2, 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. 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. Another important point: Why do you compare qPCR data of ribosomal genes (16S or archaea) with functional genes of (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. 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. Please find my detailed comments below:

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

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

Table 5: mentioned in the text but it does not exist in the MS

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

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".

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 the were the cores stored in situ for a while ?

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?

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 of methanogens. You might check this in silico.

p18249 Line 22: All results were normalized on gram oven-dried soil

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

p18251: I wonder a bit about the concentration profiles in Fig. 2: I hardly see consumption of NO3- or any other species. Why is that not the case, please comment.

Fe3+ is almost not dissolvable in water – here it seems it does

p18252: How is methane production measured – I do not see any method here. Hopefully there was a multipoint measurement done – as the sediment would only slowly leak its methane. Please clarify.

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.

p18254 Line 9 "0.1μMg (dw)" it is missing a "-1" Line 17: chage 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 CO2 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 CO2 and – what exactly ?

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 (< 1mScm$-$1). 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 here is: Acetate concentrations correlate stronger with methane production rates than DMS concentrations. HOWEVER this does not deduce higher acetoclastic than methylotrophic 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.

P18255 Line 19ff: "Higher pH value in the S. alterniflora marsh zone may be one reason

causing the higher Fe3+ concentration, since Fe2+ is easy to be oxidized to Fe3+ 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

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

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.

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