

We thank the reviewer for their helpful comments and constructive suggestions which have resulted in a much-improved manuscript overall. Below you can find our full response to each point raised.

Response to Anonymous Referee #2

The study of Wen et al on the "Predominance of methanogens in rewetted fans" is very well written and presented in a clear way. In this study the abundance and community structure of methanogenic and methanotrophic microorganisms in two rewetted fans is related to geochemical parameters. However, the study has in my opinion to major drawbacks: In the title and within the text the authors refer to methane emissions of the two fans, however no data on methane emission are presented. With so many authors involved there certainly should be data on this important factor?? The relation / explanation how the environmental parameters influence the abundance or community structure of the methane related organisms is not convincing; it seems to be rather biased. I know it is not easy to explain microbial patterns with geochemical ones, but I would suggest a more serious statistic here.

With regards to the first drawback mentioned by the reviewer, we agree that the paper would profit from including actual methane emission data. To this end, we have added the most recent published values for average methane flux rates for both fans to the revised manuscript to lines 104-108.

With regards to the second major drawback mentioned by the reviewer, we believe 2-dimensional non-metric multidimensional scaling (NMDS) is a robust statistical method. The environmental fit to the NMDS is a statistical approach based on a Monte Carlo permutation that shows which variables are significantly related to the community structure of the microorganisms. For this reason, we feel that additional statistics are not necessary to support our overall conclusions based on the NMDS. We performed correlation analyses on methanotroph abundance versus oxygen and dissolved methane concentrations, however the relationships were not significant. Nevertheless, we failed to mention this in the original manuscript and have adjusted the text in lines 362-363.

Line 201 "PCR products of three individual runs per sample were combined." – why this?

The PCR products were combined to reduce amplification bias. A short phrase was added to the manuscript to make this clear to the reader in lines 220-221.

Line 292 "I suggest to start the results section with the geochemical description of the study site"

Thank you for the suggestion. In an earlier version of the manuscript, we had the geochemical section first but decided later that the main message of the paper is supported better when the microbial data were presented first. We tried both approaches, and still came to this conclusion. So, we hope it is acceptable that we keep the order of the presentation of our results.

Line 295 "as you refer later in the discussion to salinity, it would be nice to have these values converted to PSU, for comparison with other studies"

We agree that it would be better to have salinity values for comparability. However, for brackish waters the calculation is unreliable as salinity in low-salt waters is not well-defined. This is an issue that is unresolved among hydrogeologists and chemical oceanographers alike, thus conversions from EC to psu are generally not performed for brackish systems. We would therefore suggest that we present our original EC data, which is more scientifically sound, but provide the information needed for conversion from EC to psu for the reader (in the methods section).

Line 322-323 "I do not think that "depth" is a suitable parameter here. It should be seen as enveloping parameter which is characterized in itself by NO₃, SO₄, O₂ ...Also it makes figure 6 rather confusing. Maybe you could try to do the analysis without "depth", by pooling all the data? also, the parameter "site" could be omitted....

We did not want to include depth as a parameter but rather as a proxy for other parameters. We think including depth is important as it may stand for a proxy for other parameters which were not measured in this study. We further believe that site is an important parameter here as comparison of the two fens is a main point of the paper. The inclusion of site in the NMDS reinforces our findings that both geochemistry and microbial community composition were much more variable in the Hütelmoor than in Zarnekow.

Line 347 "where does the emission data come from? Are there any data available??"

Data on methane exchange was recorded in both fens by us and other colleagues with chambers and eddy covariance in the past and still today. Since we have no measurements that are directly associated with the core samplings and the porewater sampling used here, we first decided to go without CH₄ exchange data. As two reviewers have criticized this point, we have updated the manuscript to include the most recent publish values for methane fluxes from the two fens in lines 104-108.

Line 362 "thus CH₄ concentration had no influence?? At least for the methanotrophs this should be an important factor. If not, this should at least be stated so"

You are right, a correlation between methanotrophs and CH₄ concentrations is an important factor. According to our analyses CH₄ concentrations did not correlate with methanotroph abundance nor with the abundance of other microbes. Because many studies have found it to be an important factor influencing methanotroph populations, we should have nevertheless mentioned that we found no correlation in our study. Therefore, we now describe this in lines 362-363.

Line 365 "for comparison it would be nice to have EC converted to salinity"

We agree that it would be better to have salinity values for comparability. However, for brackish waters the calculation is unreliable as salinity in low-salt waters is not well-defined. This is an issue that is unresolved among hydrogeologists and chemical oceanographers alike, thus conversions from EC to psu are generally not performed for brackish systems. We would therefore suggest that we present our original EC data, which

is more scientifically sound, but provide the information needed for conversion from EC to psu for the reader (in the methods section).

Line 380 “I do not see any significant shifts in the figure, but only a scatter of data”

The sentence was adjusted in lines 406-409 to help the reader follow the specifically mentioned shift in the isotopic data of HC 1. The delta signature of HC 1 (open circles) shifts from ~-65 to ~60 which could be the result of oxidation processes.

Line 401 “however, it is not clear to me, why the abundance of methanotrophs is so low. Shifting O₂ regimes should be no problem, as this is often the case in other environments, tidal sediments...”

The argument for the low abundance of methanotrophs was strengthened in the revised manuscript. Specifically, in lines 461-467 we suggest that competition for oxygen with heterotrophic organisms rather than fluctuations in oxygen are likely a reason for the low abundance of methanotrophs. In fact, our data support this as our bubble plot for bacteria shows hyphomicrobiaceae dominated the bacterial community, a family of which the large majority are aerobic heterotrophs.

Line 402 “The heading is not suitable here, as you only discuss the low abundance of methanotrophs here. Data on methane emissions would be helpful here....”

The authors agree that the headline was not suitable. We thus changed the headline in line 429 to better represent the section – “Low methanotroph abundances in rewetted fens”

Line 423 “but these disturbances in O₂ regime would be also inhibitory for the methanogens on the other side....”

Though recent studies show that methanosaeta, which was the most abundant methanogen in this study, thrive even in oxic layers, it is also likely additional factors are affecting the methanotroph populations that were not thoroughly discussed. In our revised manuscript we expand on the discussion regarding the absence of methanotrophs. Specifically, we suggest in lines 461-467 that competition by heterotrophs which also utilize oxygen may ultimately be preventing methanotroph establishment.

Line 428 “what about methane availability??”

Substrate (i.e. methane) availability has indeed been shown to correlate with methanotroph populations. We have added this to our revised manuscript in lines 362-363 as previously mentioned. As mentioned above, in our study methanotroph abundance and methane concentrations did not correlate, though. Also, methane concentrations in the pore water were high throughout all sites so the availability of methane is unlikely to constrain methanotroph abundance in the two rewetted fens of our study.