



EGUsphere, author comment AC2
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Reply on RC1

Cordula Nina Gutekunst et al.

Author comment on "Effects of brackish water inflow on methane-cycling microbial communities in a freshwater rewetted coastal fen" by Cordula Nina Gutekunst et al., EGU sphere, <https://doi.org/10.5194/egusphere-2022-65-AC2>, 2022

Dear Reviewer 1,

Many thanks for your very constructive feedback on our manuscript. We think, most of your comments mentioned will find their way into the manuscript and thus, you helped us very much in improving it. Yes, unfortunately we were not able to take a more detailed look into the microbial community other than we did. Thank you for acknowledging the broad range of data covered. To answer you comments in detail, we will re-post your comments and our reply right below.

Major comments:

- In the discussion, the possible reasons behind the geochemical patterns are considered in great detail and very well, but I was missing more broader context. What do these results mean outside this specific system and outside these specific sampling points? What new was learned that could be generalized to the effect of drought and brackish water inflow in other wetlands with brackish influence?

Reply: Thank you very much for this important comment. While discussing the issue among authors we realized that we were indeed somewhat missing the broader picture especially in the discussion. Therefore, we suggest to add the following paragraph after the sentence in l. 557:

"So, what does this mean for a broader context? The whole peatland was affected by a single storm surge and the resulting brackish water inflow. Such events are likely to happen more frequently and possibly more intensely in the future in the investigated site and in many low lying peatlands as a consequence of global warming induced sea level rise (Jurasinski et al., 2018). In parallel, as temperatures increase and weather patterns are getting more extreme, drought periods in peatlands may occur more often in the future. Thus, we were able to study possible future events, rendering the results exemplary for other coastal peatlands. The change from drought conditions to brackish water inflow might even trigger similar process chains in non-rewetted, still drained fens, since their normal is the dry situation.

Brackish water inflow is sometimes, also by some of the authors, discussed as a possible way to reduce methane emissions after rewetting of peatlands, even if they are not intentionally rewetted as a natural-based solution for climate change mitigation. However,

although the sulfate input and/or activation we have seen, seems to have been beneficial because it leads to lower methane emissions, salinization is also seen as a dangerous thread to many coastal ecosystems. In addition, since sulfate also acts as an alternative terminal electron acceptor, this might lead to higher decomposition rates (Zak et al., 2019), which, in the long run, could compensate for the positive effects of lower methane emissions. Therefore, further research in a variety of shallow coast peatland ecosystems is necessary to draw final conclusions. Since these complex ecosystem effects are hard to investigate in experimental studies, this calls for a network of long-term monitoring sites.”

In addition, as a reaction to specific comment no. 11, we will also change the sentence in lines 609-610, so that the changed role of the water column, is better acknowledged. Before conducting the study, we expected the water column to be a source for methane-production, but we suspect it to rather fulfill the function of methane oxidation. We think, that this has also wider meaning outside of the specific case study.

In the conclusion however, we consider the broader context sufficiently covered and would prefer not to change the text, also in order to avoid the concluding paragraph to get too long.

- Please mention the accession number for the nucleotide sequences in the main manuscript (now it is mentioned in Table S2). I can't find anything with the accession number PRJEB52161- are the data not public yet?

Reply: Yes, you are totally correct, the accession numbers will be included into the text. The reason for the incomplete statement in the manuscript under “Data availability” is that the manuscript submission was done before the uploading of the data. We are planning to include the following paragraph into the manuscript in l. 680 and replace the sentence, which is currently there:

“The data for all 97 post-inflow samples have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB52161 (with sample accession numbers ERS11559347-ERS11559443). Baseline2014 data can be found at EBI under the BioProject PRJNA356778 (accession numbers are SRR5118134 - SRR5118155 and SRR5119428 - SRR5119449) and Drought2018 data were deposited at ENA under BioProject accession number PRJEB38162 (sample accession number ERS4542720-ERS4542735, ERS4542752-ERS4542767, ERS4542784- ERS4542800 and ERS4542822- ERS4542837). Depth profile data can be provided by the corresponding authors upon request and will be uploaded to the Pangaea data base in the near future.”.

And yes, the data are not public yet, but were submitted on 6th April 2022. The predicted release date is 31th October 2022, please also see the attached record below. However, the data might be public even before that date.

Specific comments:

- line 156 What is meant with 'for better comparison' here? Same sampling time or something else?

Reply: This is mostly about a spatial effect. The drought sampling only took place at location HC2 and so did the Spring post-inflow sampling 2019. We wanted to say that at

location HC2 there is a higher temporal resolution, with two additional samplings (drought and spring 2019). Unlike the other stations, where sampling only took place in 2014 and Autumn 2019. But in addition, yes, this is also about the sampling time. The drought sampling took place in August 2018, so while including May and November 2019 sampling, we relativize the seasonal influence slightly.

To make it easier to follow our thoughts here, and to better reflect our intentions, we suggest to change the sentence to: "Soil cores and pore water samples were also taken on May 16th, 2019 ("Post-inflow Spring2019") at one of our sampling locations (HC2, see Fig. 2) for better comparison with the previous drought study (Unger et al., 2021) in order to increase the temporal resolution at this common location.". The following sentence ("This sampling was, however, only done at one of our sampling locations (HC2, see Fig. 2) ") will be deleted.

2. I. 274-275 Should the primer concentration be 0.5 μM instead of 0.5 mM ? 0.5 mM would be an unusually high concentration.

Reply: Yes, that is totally correct, it should be μM . Thank you very much for the careful read. We will change the unit accordingly.

3. I. 275 What was the final volume of the PCR reaction?

Reply: The final volume was 25 μl . We will change the sentence to reflect this detail to: "For the PCR (Thermal Cycler, T100, Biorad, Feldkirchen, Germany) we added PCR-Buffer, 1.25 U OpitTaq DNA Polymerase, 0.2 mM dNTP, 0.5 mM MgCl_2 and 0.5 μM of each primer to 5 μl purified sample and filled the mixture to a final volume of 25 μl using sterile water."

4. I. 292 Please mention the concentration of the primers.

Reply: Ok, we will change the sentence to: "According to the in-house protocol, we used 10 μl of SYBR Green, 0.08 μl of each primer (with a concentration of 100 μM each), 5.84 μl sterile water and 4 μl template per reaction."

5. I. 480-482 Please be careful when directly comparing the results of two different qPCR assays. We can't know if the abundances are affected by primer biases etc.

Reply: Well, actually the assays were especially prepared in a way that they are indeed comparable to one another, since we used standards with known gene copy numbers for each gene, so absolute abundances should be correct. Please see also Wen et al. (2018) and Unger et al. (2021) for method comparison. However, your comment makes us aware that a single comparison between *mcrA* and *pmoA* is not of great relevance for our manuscript, because we mainly focus on the comparison of the same gen within different time frames. So, we would also agree to delete the sentence or change the wording as the following: "After the brackish water inflow, absolute *mcrA* gene abundances of DNA-based analysis were substantially higher compared to *pmoA* abundances, which is also reflected in the cDNA-based abundances from location HC2 (Table S1)."

6. I. 496-498 I understand what is meant here, but please try to rephrase this sentence taking into account that the environmental variables are properties of the soil samples, not bacteria (for example that in the bacterial ordination, these samples were associated with higher EC etc.).

Reply: Thank you for this comment. We will change the sentence accordingly. It could read as following: "In the bacterial ordination, the Baseline2014 samples were associated with slightly higher EC and CO_2 concentrations and with more enriched ^{13}C in CH_4 (see

post-hoc fit arrow in Fig. 6a) compared to the other sampling campaigns.”

7. l. 522 Please consider reminding the reader here if the sampling point HC2 is closer to Baltic Sea or further inland.

Reply: This is a good point indeed. Thank you. To acknowledge this comment, we will change the sentence in the previous lines 518-519 to: “Instead, two zones of different brackish impact, separated by the main ditch, formed with higher EC concentrations close to the Baltic Sea (HC3 and HC4) and lower EC concentrations further inland (HC1 and HC2, see also Fig. 2).”

8. l. 539-541 I'm not sure why but I'm having difficulties following this sentence. Please consider if you can clarify the main point of sentence or its connections to what is said above.

Reply: To make clearer what we wanted to state here, we will try to separate the information into two sentences: “Despite the fact that the locations differed in pore water biogeochemistry, the shift from freshwater to brackish conditions was clearly visible. This is especially true, because sulfate, chloride and EC levels showed an approximation of the freshwater-influenced upper part and the partly brackish-influenced deeper pore water (HC2) after the inflow (Fig. 3b, c and d).”

9. l. 554 What is meant with 'drought-induced salinization'?

Reply: We refer to drought-induced salinization in the introduction in l. 99. It is a rather broad term, used e.g. in Chamberlain et al. (2020) to describe the increase of salinity (measured in PSU) during drought conditions. Presumably they used the term synonymously to sulfate to describe sulfate-enrichment during drought, resulting from the re-oxidation of sulfide under aerobic conditions. In the discussion, we wanted to draw a connection with the introduction, but this time emphasizing, that chloride cannot, like sulfate increase simply because of dry conditions, but was most likely transported from the sea. So, because sulfate is a difficult proof of the brackish-water inflow, we used chloride additionally to support our hypothesis that brackish water inflow indeed happened and that sulfate did not only increase because of the aerobic conditions during the drought.

10. l. 579 Please clarify here which time point has the lower values.

Reply: Yes, of course. We will change the sentence accordingly to: “The decrease of $\delta^{13}\text{C-DIC}$ between the baseline sampling in 2014 and autumn 2019 sampling post-inflow indicates an increase in non-methanogenic CO_2 production (Fig. 3I).”

11. l. 609-610 Please check if you can clarify this sentence. I'm especially having trouble with the word 'changes' on l. 610.

Reply: We tried to clarify what we mean by rephrasing the sentence. We suggest the following: “Therefore, carbon cycling might have changed after the complex impact of drought and subsequent brackish water inflow from well-known patterns, turning the usual role of the water column from a source of methane into a methane emission avoidance function in the investigated ecosystem.”

12. l. 1223-1224 I see from the R markdown file (thank you for including this file!) that the arrows for the environmental factors come from envfit, but this should be mentioned in the methods section too.

Reply: Yes, good point. We suggest to add the following sentence between the sentence in

l. 333 and l. 334: "We used the function envfit of package vegan (Oksanen et al., 2020) in order to add environmental variables on the NMDS ordination configurations."

Minor technical or language comments:

l. 48 Open the abbreviation 'GHG'.

Reply: Thanks, will be changed as suggested.

l. 151 Open the abbreviation 'EC'.

Reply: Thanks, will be changed as suggested.

l. 164 Open the abbreviation 'IC'.

Reply: Thanks, will be changed as suggested.

l. 169 0,45 -> 0.45

Reply: Thanks, will be changed as suggested.

l. 273 'Specific' could be a better word here than 'precise'?

Reply: Yes, you are right, "precise" alone may be a bit off here. However, we would like to change the wording to "more precise". We wanted to emphasize the fact that universal primers were less suitable to detect archaea relative abundances precisely enough and therefore archaeal primers were used.

l. 424 and elsewhere: Methanosarciniales -> Methanosarcinales

Reply: Thanks, will be changed as suggested.

Please also note the supplement to this comment:

<https://egusphere.copernicus.org/preprints/2022/egusphere-2022-65/egusphere-2022-65-AC2-supplement.pdf>