



EGUsphere, referee comment RC2
<https://doi.org/10.5194/egusphere-2022-65-RC2>, 2022
© Author(s) 2022. This work is distributed under
the Creative Commons Attribution 4.0 License.

Comment on egusphere-2022-65

Anonymous Referee #2

Referee 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-RC2>, 2022

This manuscript explores the biogeochemistry and microbiology of peat cores collected from across a "brackishness" gradient near the Baltic Sea. Cores were collected in a rewetted coastal fen following an inflow of brackish water and compares the response to data from similar cores collected in both a baseline year and a drought year (presented in previous publications). Responses were measured in peat bacterial and archaeal communities using both DNA and RNA amplicon sequencing as well as group-specific qPCR). In both peat and water, data collected included gas data (CH₄, 13CH₄, and CO₂) and sulfate, chloride, and inorganic C. The authors demonstrate the importance of sampling across the entire environment (peat-water-atmosphere) as well as knowledge of the biogeochemical legacy of a site to more fully understand GHG fluxes and associated microbial communities. My only major comment is regarding the description of the methods associated with microbiology: please make it abundantly clear that the methods used to compare the microbiology are the exact same across the three used data sets (including extractions, primers, conditions, etc) and indicate if the data were normalized somehow across the sampling time points (to gene copy number, etc). if the sequence data from previous studies was used here, please make it clear that the data were re-analyzed to be comparable across studies and that the older data were reclassified using the same taxonomic data base across all studies. Also please indicate if sequence data are deposited in a publicly available database.

Line comments:

Line 33: I find this sentence a bit confusing to read. Perhaps remove "also"?

Line 46: do the authors intend "loose" instead of "lose"?

Line 112-114: sentence a bit confusing to read, do the authors intend "Thus" instead of "This"? In particular the " and, therefore can explain" is causing some befuddlement.

Line 198: Can you please clarify how samples were kept cold and if they were kept anoxic? I would be concerned that collecting the soils, cooling them on ice (in a cooler?), then later placing the samples in a -80 freezer would not preserve the RNA as it was in the field, especially as there may have been an influence of O in the previously anoxic depths as the cores were stored in falcon tubes. Please indicate the length of time between sampling and freezing, if greater than a few hours, then the RNA data may be more

reflective of the storage conditions and not the in-situ conditions.

Line 274: which PCR buffer? What was the final reaction volume? Was the same amount of DNA added to each reaction?

Line 292: Was the same amount of DNA added to each reaction? How was this normalized? What is the final reaction volume? What was the primer concentration?

Line 296: standard curve was based on a series of dilutions of what? Please indicate brief methods, even though detailed in the sister studies.

Line 296: How were gene copy numbers normalized to the amount of soil used for DNA extractions? Based on the x-axes in Figure 5 this appears to be the case, but please clarify in the text.

Line 330: how were ordination vectors constructed?

Line 386 "Drought"

Line 371 (and elsewhere): please indicate if these are average +/- standard error, perhaps in the methods? Or indicate the first time mentioned.

Line 425 (editorial comment, can be ignored): to my eye, the colours appear more yellow than orange in the online version.

Line 475: 16S rRNA gene

Line 475: as mentioned above, please provide details on how these comparisons were made (i.e. were all of the same methods followed? Comparison of reactions efficiencies, same extraction kits, normalization to gene copy number to g of soil, etc.)

Line 492: were the ordinations made only on the DNA data? Were ordinations of cDNA data similar? Perhaps these could be presented in the supplemental data if they add to the story?

Figure 1: I did not find a reference to this figure in the manuscript? It's an excellent figure and should be included.