

RC1: 'Comment on bg-2021-353', Anonymous Referee #1, 09 Feb 2022

General comments:

White et al. collected soil samples and brought them to the laboratory for an observational mesocosm study under controlled conditions. They measured a suite of ecosystem processes including net ecosystem exchange, respiration, CH₄ flux, and stable isotope analysis. They performed captured metagenomics to examine the microbial community membership and gene content, including organisms directly implicated in CH₄-cycling processes. The paper does not in its current form seem to be driven by any particular hypothesis, but rather is focused on examining fluxes and microbial communities under laboratory conditions that approximate field conditions.

It seems that much of the focus of the paper relies on distinguishing the samples into 3 categories (HFM, MFM, and LFM), with the first and last category only coming from single samples (with 3 technical replicates each). In my opinion this makes the paper more about how two outliers differ from the rest of the samples than about the relationship between fluxes and communities in general. If distinguishing samples among three tiers is how you want to proceed, why not rank all samples by their fluxes and then divide them evenly into these three categories? In its current form this categorization seems to make your statistics very unbalanced.

In terms of the level of inference the authors make, there are several instances that I found problematic. For instance, the authors claim that HFM has higher B diversity than the rest, yet this relationship was not significant, and was also based on a mis-balanced design. The authors also state several times that just because they see several types of methanogens/methanotrophs that these communities ought to continue functioning under future climate scenarios. Without performing an experimental test of this hypothesis these types of speculation should not be in the paper, and especially not a main takeaway (e.g. in the conclusions).

Authors response: We would like to thank the reviewer for the comments supplied and we have made changes accordingly. With regards to the comment on how we distinguished categories we understand your concerns. The establishment of high, medium and low emitting mesocosms was a result we discovered after the measurement period and initially we did not have that study aim. However, after discovering that two mesocosms were acting significantly different from the others we decided to peruse whether the genomic data could help explain this variability. Rather than ranking all samples by their fluxes and then divide them evenly into these three categories we performed a randomization test to see whether these mesocosms could be separated and found that only mesocosm 4 and mesocosm 9 were giving statistically higher (mesocosm 4) and lower (mesocosm 9) fluxes. In addition, the statistical tests which we have performed have taken into account the uneven study design and low replication.

With regards to our conclusions made from the beta diversity section we have made considerable changes to account for your concerns. Rather than using such a definitive conclusion from our results we have highlighted that we observe a trend but further research is necessary to confirm what we propose. Our conclusions on how communities ought to continue under future climate scenarios has been made from reading existing literature. The literature states the environmental

conditions to what the microbes find tolerable. We have reflected this in our updated text in the comments below.

Thank you once again for your constructive criticism of our work and please don't hesitate to contact us with further questions if needed. Please see detailed responses to specific comments below.

Specific comments:

Line 45: add 'the': is the second most. Also important seems like it needs a qualifier: important for climate?

Authors response: This alteration has been made according to reviewer's suggestion

70-75: nice summary of controls.

Thank you. You don't often receive compliments in the peer review process.

86-88: "The targeting of...", this sentence may not be necessary for the scope of your paper. Just a suggestion.

Authors response: This sentence has been removed according to the reviewer's suggestion

105-108: Just as a comment, this reads a bit like an advertisement.

Authors response: Thank you for the comment. We have chosen to keep the original line as it emphasizes the reason why we chose to use the captured metagenomics approach opposed to 16s and whole metagenomics.

139-140: Do you mean to say that placement of the mesocosms was varied bi-weekly? "Rotating" could be interpreted as simply turning them.

Authors response: Thanks for the observation, we have added the following for clarification:

Original: The mesocosms were rotated bi-weekly to minimize the effect of spatial variations in growth conditions.

Edited: In response, the mesocosms were rotated to different positions on the table bi-weekly to minimize the effect of spatial variations in growth conditions.

147: Perhaps change section header to "Flux measurements of mesocosms" for clarity.

Authors response: This alteration has been made according to the reviewer's suggestion

171: I assume you mean 'stored at -20C', not '20C'. Please clarify.

Authors response: Yes, you are correct. An typo on our behalf. The change has been made accordingly

260: It becomes difficult to follow the text when there are so many abbreviations. Perhaps consider not abbreviating.

Authors response: This alteration has been made according to the reviewer's suggestion. HFM will become mesocosm 4, MFM will become medium emitting mesocosms and LFM will become mesocosm 9.

259-263: in a similar vein, there are a lot of abbreviations in this section that haven't been defined yet in the results section. Consider naming them here (or using the full words) for clarity.

Authors response: This alteration has been made according to reviewer's suggestion. See above comment.

302-303: you don't have to italicize the word phylum.

Authors response: Yes, you are correct. The alteration has been made accordingly

303-304: how do you know that this is due to environmental conditions? This sounds presumptuous without an explanation.

Authors response: the literature generally states that NC10 methanotrophs are mesophilic and neutrophilic, therefore we did not expect to find such methanotrophs in an acidic bog. We have added the following to justify the statement:

Original: Due to the environmental conditions no methanotrophs from the NC10 Phylum were detected

Edited: No NC10 methanotrophs were detected, as expected, since this functional group is generally only reported in mesophilic and neutrophilic conditions while the conditions at the Fäjemyr mire are acidic and cold.

315: bacteria can be lower case and not italicized here.

Yes, you are correct. The alteration has been made accordingly

321-329: I do not like that you can comparing Beta diversity among groups that have very uneven sample numbers. Remind the readers in this section how many samples are in each group.

Authors response: We have included the number of replicates in both methodology and also in the figure text of figure 4. For additional clarity we have added the number of replicates for each group within the text. In addition, we believe that renaming HFM to "mesocosm 4" and LFM to "mesocosm 9" will add further clarity to the reader. Furthermore, rather than plotting using the

boxplot we have change figure 4 to a NMDS plot. Here the reader can see the number of replicates clearer and interpret the results for Beta diversity more easily.

Original: β -diversity, which measures the change in diversity of species from one category to another, was measured as mean distance to the group centroid and highest in HFM (fig 4). HFM resulted in an average distance to median of 0.046, followed by MFM (0.042) and LFM (0.031).

Edited: β -diversity, which measures the change in diversity of genera from one category to another, was measured using dissimilarity indices. Furthermore, the dissimilarity indices were calculated using the average distance of group members to the group centroid which is shown in fig 4. In fig 4, we observe an overlap between mesocosm 4, medium emitting mesocosms and mesocosm 9, and a lack of distinct separation between clusters, indicating similar diversity and variation within all mesocosms. We observed the largest dissimilarity between mesocosm 4 ($n = 3$) to medium emitting mesocosms ($n = 21$) with a mean difference in dissimilarity of 0.023. The lowest difference in dissimilarity was observed between the medium emitting mesocosms and mesocosm 9 ($n = 3$) with a 0.005 difference in dissimilarity between groups. Due to a high variation and lack of spatial replications for mesocosm 4 and 9, this relationship was observed as non-significant. As the taxonomic data is a subset (i.e. only including methanogen / methanotroph taxa) of all the taxonomic sequences contributing to the whole metagenomic community, values for β -diversity are low. However, the differences between centroids indicates that communities of methanogens and methanotrophs become more similar to each other as the magnitude of flux decreases.

327-329: is this the Beta diversity of the whole community or just a subset of methanogens and methanotrophs? This sentence would lead me to believe it is the latter and if that is the case this sound be clarified in the section header as well as the text.

Authors response: The values for beta diversity shown in the paper are analysed of the subset of “captured” taxa. The taxa were filtered to only include methanogens and methanotrophs by removing off target taxa using the MG-RAST filter. This is written in the methods section 2.6 but may not be clear enough. We have made the alteration to the text to compensate for this.

Original: Although the values for β -diversity are low, the differences between centroids indicates that communities of methanogens and methanotrophs become more similar to each other as the magnitude of flux decreases.

Edited: As the taxonomic data is a subset (i.e. only including methanogen / methanotroph taxa) of all the taxonomic sequences contributing to the whole metagenomic community, values for β -diversity are low. However, the differences between centroids indicates that communities of methanogens and methanotrophs become more similar to each other as the magnitude of flux decreases.

Fig 4: show points on the same boxplot graph so readers can understand visually that you are not comparing equal sample numbers.

Authors response: We thank you for this observation. Upon reflection we decided that beta diversity is better displayed using NMDS rather than boxplots. With this alteration, the reader can clearly see the number of replicates and the variation between the groups. In addition, we have added clarification to the number of replicates in the text and figure text as stated above.

340: It is not clear in the text why you are doing this analysis three times and reporting three tables. Perhaps you could choose the one most important to your narrative and put the other two in the supplementary? The three tables have identical table legends so it really is not obvious what is distinguishing them and what the reader should take away.

Authors response: Table 1, 2 and 3 are the comparisons between each flux category. Within each table text and table headings the reader can observe which groups are being compared i.e. "Taxa are ranked according to their average contribution to dissimilarity between medium emitting mesocosms and mesocosm 4". This must not be as clear as we originally thought. In response, we have added a clearer table title to the table text.

Same comment for Tables 4, 5, and 6.

Please see comment above

433-436: We do not know anything about the environmental tolerances of these organisms. I think it is too speculative to make any inferences about the future prospects of these processes under climate change scenarios based on the sole observation that there are members of these different groups present.

Authors response: Our conclusions made on how communities may continue under future climate scenarios and have been made according to existing literature. The literature states taxa have the ability to exist within certain environmental boundaries.

Edited: (Discussion) Theoretically, if conditions were to shift within the peatland to favor acetoclastic or methylotrophic methanogenesis, the microbial community already holds the functional potential and specific environmental tolerances to continue producing CH₄.

(Discussion) This indicates a tolerance to the acid and cold conditions experienced within northern ombrotrophic peatlands. These results, similar to those observed in the methanogen community, indicate that the methanotroph community holds the ability to continue oxidizing CH₄ under alternate environmental conditions.

(Conclusion) This is important in terms of peatlands under future climate pressure where we may see altered nutrient status, hydrology or peat chemistry. If this shift in peatland status happens, our results indicate that the CH₄ producing and consuming microbial community hold the potential to be dominated by alternate functional groups (i.e. acetoclastic) than what we observe now, thus

holding the functional potential to continue the production and consumption of CH₄ at Fäjemyr mire under the correct environmental conditions.

489: “with little to no delay in transition period” – what are you basing this statement on?

We have removed this statement for clarity of the reader.

569-570, 574-575: but these differences were not statistically significant. This should not be in your conclusions.

Authors response: The statements regarding beta diversity has been removed from conclusion but remain in the discussion. After some thought, we agreed that even though the results are interesting the trend is not significant therefore we should not be included within the final conclusions.

581-584: If your study experimentally manipulated the environment of these mesocosms to examine future climate scenarios then you might have the data to back up this sentence. I think that just because you are seeing representatives of these different groups does not tell us anything about the future prospects of these microbes or the processes they perform.

We have revised this sentence to include less definitive wording, please see alterations below.

Original: This is important in terms of future climate scenarios where can expect altered nutrient status, hydrology or peat chemistry. If this happens, we can expect that there will be methanogen and methanotrophs present to continue to produce and consume CH₄ due to the potential for alternate metabolic pathways.

Edited: This is important in terms of peatlands under future climate pressure where we may see altered nutrient status, hydrology or peat chemistry. If this shift in peatland status happens, our results indicate that the CH₄ producing and consuming microbial community hold the potential to be dominated by alternate functional groups (i.e. acetoclastic) than what we observe now, thus the production and consumption of CH₄ may continue at Fäjemyr mire under the correct environmental conditions.