

Reviewer 2

We thank the reviewer for the insightful comments. We have addressed the comments one by one in the following section. *Reviewer comments are written in red and italics.* Our responses are written in blue.

The weakness of the study is that the temporal coverage and frequency of flux observations is relatively scarce despite the well-known high variability associated with methane flux. The latter means both that drivers such as temperature are found not to be important drivers of CH₄ – because the seasonal temperature gradients may not have been captured – and that generally many environmental variables show weak/no relationship to methane

- The primary objective of our study was to understand controls on spatial (intra-bog) variation in CH₄ fluxes and concentrations. We agree that the lack of temporal variation is a limitation but this does not limit our ability to address biotic and abiotic drivers of differences in methane within our site. The lack of relationships between temperature and CH₄ flux is likely a function of our focus on variation within the late spring and summer. That approach is justifiable as spatial differences are likely to be most apparent during periods of maximum microbiological activity. It would certainly be interesting to assess intra- and inter-annual temporal variation but that would require further study and was not our aim. The spatial representation of different land covers we have investigated provides new insights into how heterogeneous CH₄ fluxes can be.

While the authors are correct to point out that a wide variety of factors influence fluxes, statistical power may have been low enough to limit the outcome of those analyses. Furthermore, what is measured is net flux, and concurrent production, oxidation and transport processes regulate methane flux, making interpretation more difficult

- Given the lack of temporal representativeness, we are cautious with our interpretation of how environmental factors affect methane fluxes and instead have focused on understanding the relationships with the microbiology and the pore-water concentrations. Through his analysis we can attempt to understand a little more about the processes of methane production and oxidation within the profile.

Figure 8. I am concerned about this plot. The relationship appears to be driven by the low CH₄ exchange velocities for TMW-S (dark blue dots) however, looking at Appendix Fig B1, TMW-N has very high and variable exchange velocities which, if they were plotted, might undermine the reported relationship. If you remove the outliers from TMW-N and maintain TMW-N, do you then retain the relationship? How would this affect the results?

- The CH₄ exchange velocity in the Tamarack zone was very different between transect south and transect north. Unfortunately, microbial samples were taken only at the Tamarack south transect, so the high CH₄ exchange velocity of the north transect does not have microbial data to compare with. If our relationships holds true for this data point we would expect to see a very high ratio of methanogens vs methanotrophs at this northern section.

- We have now clarified this by including some of the information above in the caption of the methane transfer velocity figure

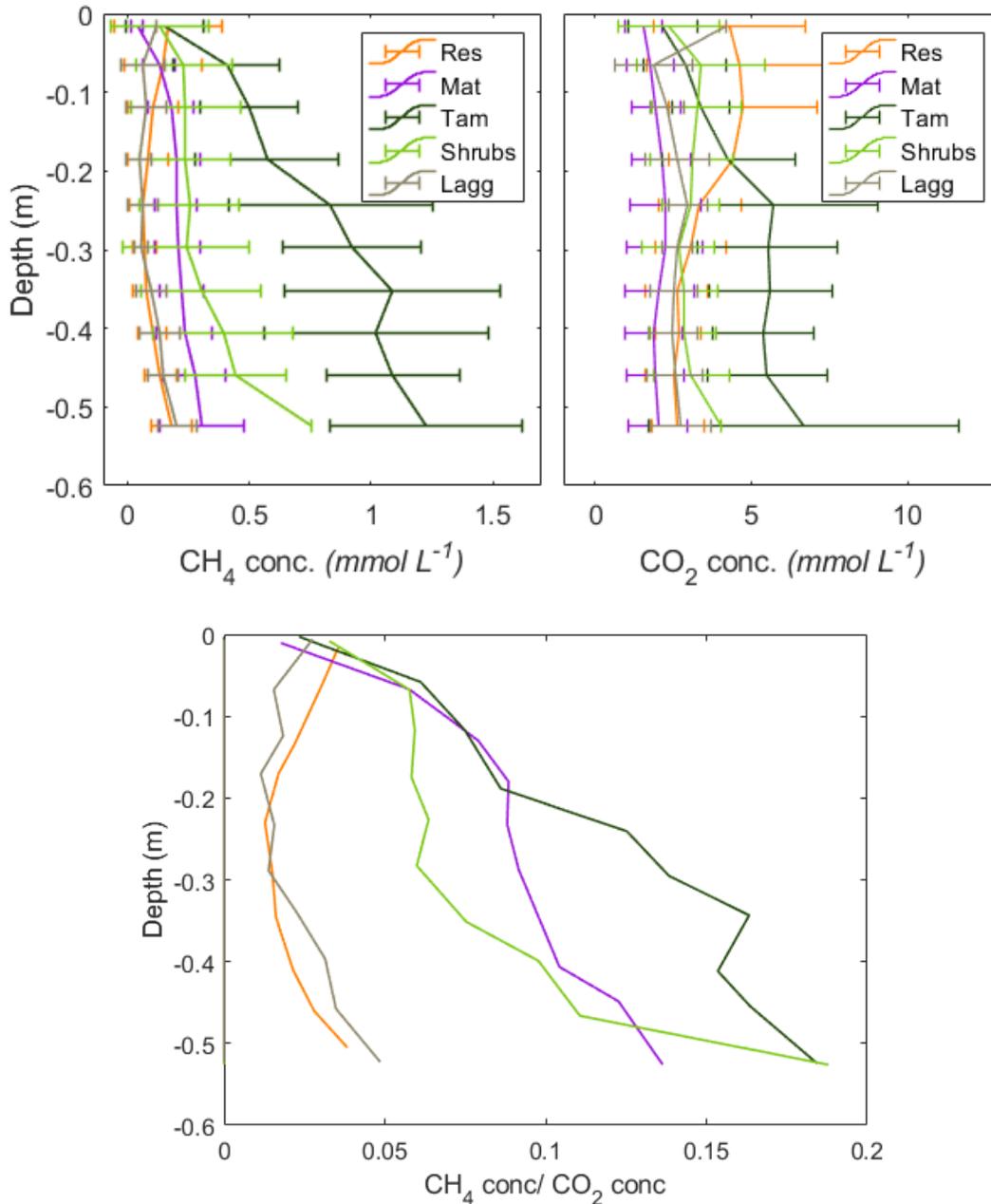
The conclusions are currently just a summary of the results that have already been reported. I think here there should be a greater attempt to zoom back out and generalize from the results or return to the global change context of the work.

- We have now added a paragraph in the conclusion that attempts to generalize patterns in methane release from the findings of the current studies:
 - Why would two locations with similar near-surface CH₄ concentrations have different fluxes if they also have similar diffusivities and negligible ebullition and plant transport? Our results show the answer is that they have different transfer velocities for CH₄. Transfer velocities are normally a function of wind speed, but beneath the shrub and tree canopy of peat bogs wind speeds are very low so something else is affecting this transfer velocity. The upper layer of the bog's peat mass is a dynamic region with both methanotrophs and methanogens living within the oxic layer (Angle et al., 2017). Within this layer higher abundance of methanogens drive higher transfer velocities if the concentration of CH₄ is assumed to be at quasi-steady state. At the same time, however, methanotrophs consume much of the methane produced. Therefore, methanogen abundance, when normalized by methanotroph abundance, can explain CH₄ transfer velocity differences in a peat bog where diffusive transport from porewater in saturated layers is dominant. We conclude that microbial communities, and their control by variation in water table depth, are the key drivers of variability in CH₄ fluxes across multiple hydro-biological zones in kettle-hole peat bogs. Future research should examine whether such patterns can be confirmed in other ecosystems where plant-mediated transport of CH₄ is low.

Minor Comments

Do you have concurrent CO₂ observations? It appears you don't, but if you did, evaluating the CH₄:CO₂ ratio can provide insight into whether CH₄ emissions are being limited by overall carbon flow (i.e., low CO₂ respiration overall) or competing respiration processes (i.e., low CH₄ in spite of high CO₂)

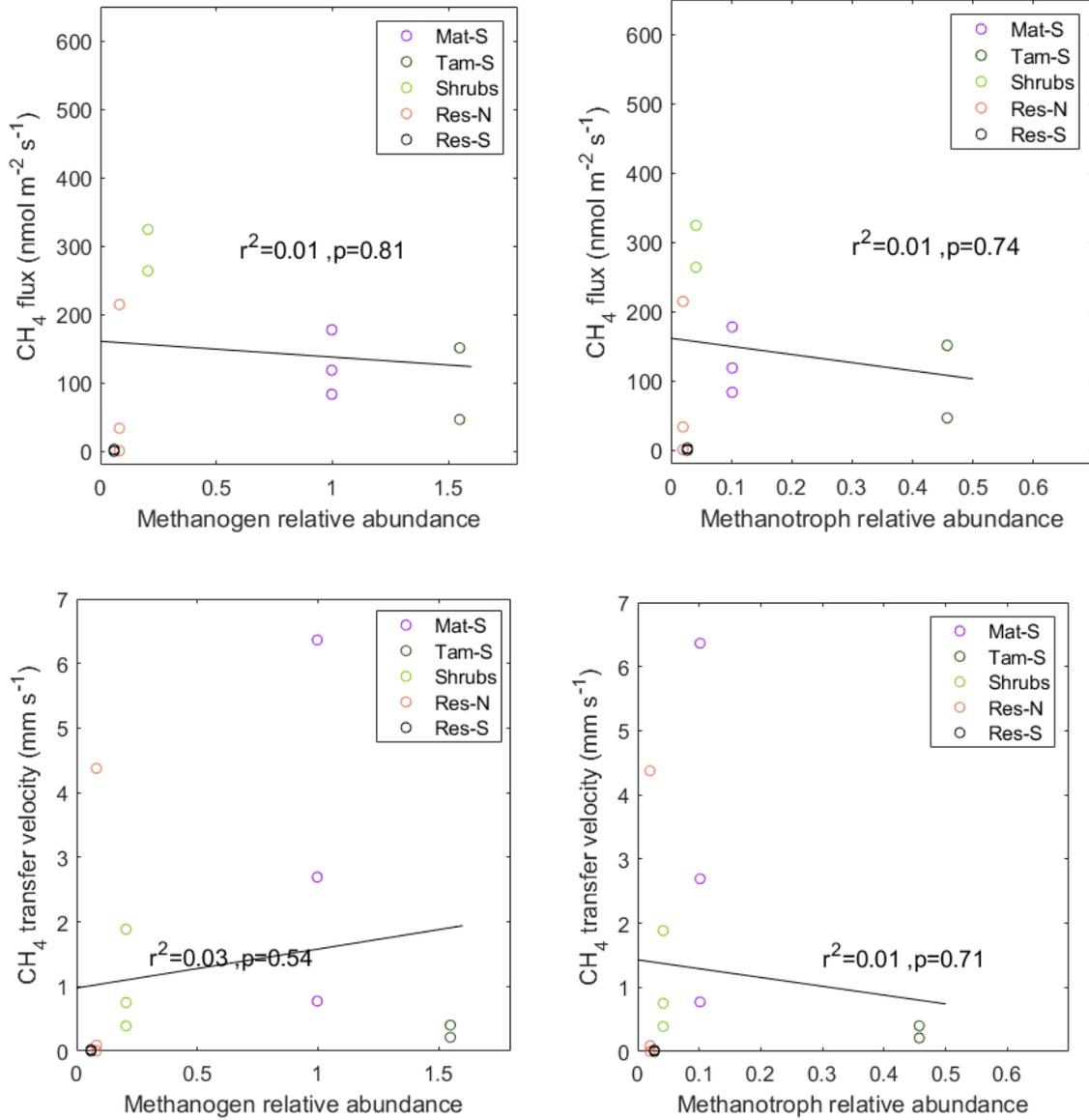
- We do have concurrent CO₂ observations and have had a look at them (see fig below). To your original question, I think the lower ratios in the restored and lagg zones indicate that there is an overall low carbon availability in these zones, which is in accordance with the expectations of the level of organic matter oxidation in these zones. I think it is interesting that the ratios are all very similar at the top of the profiles but then there is a differentiation of undisturbed bog versus restored bog (RES) with depth. This probably is an indication of how the high carbon content of the bog favors methanogens at the deepest sections. We have added this information to the supplementary information.



Is the methanogen/methanotroph ratio calculated from absolute abundance or relative? In either case, is variability in just one or the other driving the ratio variability? Is it primarily shifts in importance of methanotrophs or methanogens? If so, can this permit a more specific interpretation, e.g., variation in methanotrophy explains variation in net flux.

- The ratios are calculated from the relative abundances as displayed in Fig 7. Since the relative abundance of methanotrophs is overall lower than the relative abundance of methanogens, one could expect that the variability in CH₄ exchange velocity is mostly driven by methanotrophs relative abundance, but that is not the case. Here are some plots

showing how the differences are not quite explained by a methanotrophs alone or methanogens alone.



I suggest authors could make the zone names more specific/obvious as it is hard to recall which the acronyms refer to. Perhaps: OW = Water, FSL = Mat or Sphagnum, TMW = Tamarack, MES = Shrubs, Lagg is OK. Or Zone 1,2,3,4,5 (corresponding to concentric rings). I think this more closely ties to the central objective of the study which was to evaluate spatial heterogeneity.

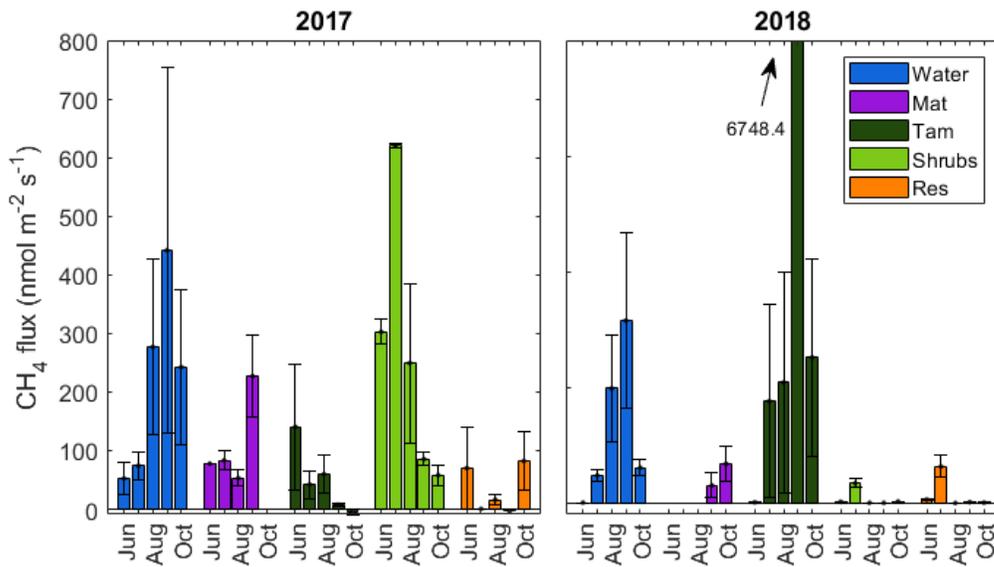
- We have adopted the first suggested change. We agree that the new labels makes the units easier to recall. Thank you for the suggestion.

Transpose table 1. Columns should be variables, rows should be entries.

- Done

Figure 3. Try grouping by wetland zone rather than month, That way you can show the full timeseries in one block, easily compare among blocks and easily see the singleblock dynamics.

- We have done it. Thank you for your suggestion.



Line-by-Line Comments Page 13, Line 26: Check units (g m-3)? I think it should be Mg m 3.

There was a typo. It was Mg m3, thank you.

Page 15, Line 16: Mean day-time air temp?

It is full day temperature as taken by the stations mentioned in the methods. We have clarified this in the text.

Page 15, Line 18: These range from negative to positive.

Fixed

Page 19, Line 22: fluxes

Fixed

Page 26, Line 27: can you comment on how much we can interpret from Genus level differences?

- Thank you, we believe the reviewer is asking specifically about the difference among the acetoclastic genera and their ecology. We have added a final clause to this paragraph commenting on the differing present of *Methanosarcina*; new text is bolded here for clarity: **“When acetoclasts were present, *Methanosaeta* dominated their community, consistent with observations of *Methanosaeta* in nutrient-poor acidic sites (Godin et al. 2012). However, in the inundated zones, *Methanosarcina* was also present. This is actually the opposite pattern we would have expected based purely on likely oxygen concentrations, as *Methanosaeta* typically dominates anaerobic environments while *Methanosarcina* can produce methane under partially oxic conditions (Angle et al 2011). We therefore interpret *Methanosaeta*’s presence in FSL-S and TMW-S to arise from its greater metabolic versatility – in addition to acetate, it can also use CO₂ or methylated compounds (Liu and Whitman, 2008) – and thus that these sites may have distinct substrate profiles.”**
- It is also possible that the reviewer is asking what genus-level differences in general imply vis-à-vis e.g. function, or what the differences in these particular genera are, and so address both here. For the former: metabolism follows relatedness to varying degrees for different types of metabolism and microbes. For example, antibiotic resistance is a well-known example of a trait (sometimes metabolic) that can move dynamically among many microbial lineages, such that two closely related strains can have quite different susceptibilities to antibiotics. Other traits, such as methanogenesis, are more narrowly phylogenetically distributed and their specific methanogenic metabolisms tend to be inherited vertically (i.e. from parent to progeny cell, not acquired from other unrelated cells in the environment) and reliably. This heterogeneous relationship between metabolism and phylogeny has been reviewed for example in Martiny et al, 2015, “Microbiomes in light of traits: a phylogenetic perspective”, *Science*. In the case of this research, we are examining methanogens (as noted above, for which metabolic traits do follow phylogeny in a fairly consistent way), and in addition these lineages have a large number of cultured representatives whose physiology is well-studied, and have been ecologically characterized over decades in a variety of habitats. So, while much of microbiome science is still charting unknown waters, in the case of these dominant acetoclastic and hydrogenotrophic methanogenic genera, much is known.

References

Angel R, Matthies D, Conrad R. 2011. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. PLoS One 6:e20453. doi:10.1371/journal.pone.0020453.

Liu Y, Whitman WB. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Ann N Y Acad Sci 1125:171–189.doi:10.1196/annals.1419.019