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Reply on RC1

Janne Rinne et al.

Author comment on "Spatial and temporal variation of delta13C values of methane emitted from a temperate mire: Methanogenesis, methanotrophy, and hysteresis" by Janne Rinne et al., Biogeosciences Discuss., <https://doi.org/10.5194/bg-2022-76-AC1>, 2022

We thank the reviewer for his constructive and thoughtful comments. We will address the comments (in bolded italics) below.

General Comments

The authors present an interesting and valuable dataset showing temporal and spatial patterns of mire methane flux and its ^{13}C signature. They aimed to disentangle the relative importance of methanotrophy vs methanogenesis as well as the availability of substrates for methanogenesis for explaining temporal and spatial variability hypotheses in their data. Secondary goals were to describe the methane associated prokaryotic community and compare the mire-level ^{13}C signature from upscaled measurements (using their chambers and a land cover map for the mire derived from a previous study) to nocturnal boundary layer measurements. While the data itself are useful, and the upscaled ^{13}C method successful, there are substantial issues. Primarily that the data presented are insufficient to fully test their hypotheses.

The analysis and interpretation of our $\delta^{13}\text{C}$ data does indeed indicate that this data cannot fully solve the question between the hypotheses. We may have expressed some conclusions too strongly, and not stated the caveats of these results clearly enough. We will reformulate our discussion, as also discussed below in detail, to take the uncertainties and complexities better into account. However, in our opinion the hypotheses, as presented after the Introduction, do offer a useful framework for data analysis and interpretation.

Spatial (HS1 and HS2):

HS1 proposes that variation (in methane flux and ^{13}C signature) is due to spatial changes in methane consumption, while HS2 proposes variation is due to spatial shifts from hydrogenotrophic to acetoclastic methane production.

These are not mutually exclusive, which is not inherently an issue, although they are treated as such in the study. Without information on the spatial distribution

of methanotrophs or the respective groups of methanogens (or their substrates) or well-constrained values for the expected ^{13}C signatures from individual processes, any conclusions on their relative contribution to the data is conjecture.

As the reviewer states, the two hypotheses on spatial variation are not mutually exclusive and they are not intended to be such, as is implied e.g. by the discussion of behavior of the data from chamber 3. However, their purpose is to act as useful simplifications to help analyzing and interpretation of the data. We agree that the presentation of the hypotheses should stress the non-exclusivity better, and we will modify the revised manuscript to include a case where either of the two processes of HS1 and HS2 dominates. Following this "zero-hypothesis", no systematic relation between $\delta^{13}\text{C}$ and methane emission rate would be found. We will also re-title the "hypotheses" chapter as "Hypothetical framework", and in general make the role of the presented hypotheses as simplifications to aid data interpretation clearer.

We do not agree that all conclusions on the contribution of certain processes to the variation in $\delta^{13}\text{C}$ and methane emission rate (F_{CH_4}) are just conjecture, as the data goes some way into refuting some hypotheses. What we state is that the observation of positive correlation between $\delta^{13}\text{C}$ and F_{CH_4} does show that it is unlikely that methanotrophy would be the dominant cause of the spatial variation in F_{CH_4} .

Temporal (HT1 – HT3)

HT1 states that temporal variation (in methane flux and ^{13}C signature) is driven by temperature. HT2 proposes temporal shifts from hydrogenotrophic to acetoclastic methane production. HT3 wisely combines them and proposes there will be a time lag between temperature and production of substrate (presumably of acetate) in the ecosystem that produces a hysteretic out and back arc in the data.

The presence of HT3 resolves the issue of exclusivity there, however the issue of being able to ascribe ^{13}C signature changes to changes in microbial processes without any constraining values or direct measurement of those processes remains. Additionally, although the evidence from their data is evenly split between a temperature-driven response (see point clouds in Figure 9) and a response indicative of hysteresis, they conclude that HT3 is supported.

Also here, as in temporal hypotheses, the different hypotheses are simplifications designed to aid the analysis and interpretation of the data. It is true that we do not have data on temporal development of the microbial communities. As this would have required larger resources this is out of the scope of this study. The central aim of this study is to analyze the spatial and temporal variations of $\delta^{13}\text{C}$ and CH_4 emission rate, to find out which hypotheses they may refute or corroborate.

The chambers from which no hysteretic behavior of $\delta^{13}\text{C}$ - F_{CH_4} relation is observed are those with low methane emission. Thus, the random uncertainty of the measurements leads to a larger relative noise in the data, which can mask any relatively hysteretic behavior in these chambers. Therefore, we conclude that the hysteretic behavior is evident in the high-flux locations which dominate the mire-scale CH_4 emission, and its $\delta^{13}\text{C}$ value. We will add the above interpretation to the revised manuscript.

We agree that with our data we cannot distinguish e.g. possible shifts between hydrogenotrophic and acetoclastic methanogenesis on one hand and changes in the energetics of hydrogenotrophic methanotrophy on the other hand. Thus, we will modify the revised manuscript to make the terminology clearer.

While the dataset is strong, its strength is mainly in describing spatial and temporal variation in methane flux and signature. If data are available from the site on the ^{13}C signature of soil C, this might be enough to draw conclusions on microbial processes based on assumptions regarding fractionation rates. Otherwise, the framing of the study should shift to focus on its strengths; I can imagine an analysis of hotspots and hot moments, and/or the relationship between fluxes, vegetative cover, and water depth.

This description of spatial and temporal variation of $\delta^{13}\text{C}$ and its covariation with CH_4 emission rate are the central themes of this study. The different hypotheses, presented in the beginning, are to be treated as useful simplifications to be used as framework for data interpretation. We will emphasize this on the revised version of the manuscript.

We choose to present the hypotheses right after the introduction as separate chapter, as they influence not only interpretation of the data, but also analysis. The other option would be to present a more vague hypotheses in the end of the introduction, with more detail (incl. Figures) in the Discussion.

We are not sure what exactly you mean with "hotspots" and "hot moments". If they are to mean times and locations with considerably higher emission rate than in the near-by spatio-temporal environment, we do not really observe such events or locations (see Figures S1 and S2). What we observe is more like a continuum, with gradual changes overlaid by some variation.

We feel that an analysis of CH_4 emission rates in relation with vegetation cover and water depth would not be very novel as it has been conducted in many previous studies. Also, this data set has rather limited spatial coverage (six chambers) due to the instrumental requirement of isotope Keeling-plot approach that leads to 30 min chamber closure time.

Specific comments:

Additionally, there are a few issues that are reducing the clarity of the authors' message. One is the use of the phrase "trophic status", which is used in the manuscript to indicate both seasonal build-up of plant-derived carbon as well as the metabolic pathway of methanogenic archaea. Neither of these is likely the default interpretation that readers will be using when they first encounter the phrase. Distinct phrases should be used (and explained on first use) for these two phenomena and the link between them should be made explicit.

We agree that the terminology here is somewhat confusing. We used the term "trophic status" in the same as in Hornibrook and Bowes, 2007 and Hornibrook 2009 (both cited in the manuscript). We imply that the trophic status (= quality and quantity of available substrates) has an effect on the metabolic pathway. Furthermore, we discussed in places exclusively on shifts between acetoclastic hydrogenotrophic methanogenesis but did not mention the changes in energetics of the hydrogenotrophic methanogenesis, which can facilitate similar relations. "Substrate availability", both in quantity and quality (acetate vs CO_2/H_2), may be a better term than "trophic status" or "trophic level" in our analysis. Thus, we will revise the terminology and discussion, for them to be more exact.

Another issue is the description of the Keeling plot method, which currently leaves the reader to put together that the mixing ratio is based on the up-scaled land cover values, unless my interpretation is widely off-base (see L168 & 219). Please clarify this.

The Keeling-plot method itself does not need up-scaled land cover values. In the Keeling plot method, the best fit line between $\delta^{13}\text{C}$ and inverse of the CH_4 mixing ratio (X) is

extrapolated to $1/X=0$, as explained in the manuscript (lines 190-198). This is done separately for each chamber closure in chamber approach, and for each night in the nocturnal boundary-layer approach.

The land cover values are used in upscaling the chamber $\delta^{13}\text{C}$ values (Eq. 2) to represent the whole mire, and for comparison with NBL-A method.

Finally, the formatting for different taxonomic levels is none-standard and inconsistent throughout the manuscript.

We are not sure what the reviewer means by his comment. Is it that we need to italicize all the taxonomic levels or only the genus level and below?

Technical corrections:

66 Replace "mires" with "wetlands", as this statement applies to all wetlands ecosystems, rather than mires specifically

Yes, this is a good suggestion as it makes the sentence more general.

71 This implies the phase-change fractionation leads to biological (or kinetic) fraction, which is not true. It would be more accurate to describe biotic and abiotic fraction processes as just that, two separate chemical phenomena

Maybe this sentence is not exact enough. We did not refer to phase-changes here. We will reformulate the sentence.

74 Introduce the reader to what makes a mire ecosystem distinct from other wetland types here

In short, mires are wetlands with active peat formations. This definition of mires will be added to the text.

93 Alternative to what?

Alternative to each other.

175 Is this plot within the chamber itself or around the chamber?

With plot we mean the surface inside the chamber itself.

189 Define RMSE

Yes, this will be defined.

207-214 unit formatting, "spectrometer"

Thank you for noticing this. These will be corrected.

239 Totally fine to use gDNA as shorthand for "genomic DNA", but it should be defined on first use.

Yes, this will be defined.

268 Spatial and temporal fluxes/signatures as well? This is unclear because of the lack of a separate statistical analysis section

No, this refers to genetic analysis. The fluxes and isotopic signatures are analyzed with MatLab.

Figure 8 takes up a lot of space it is, without providing a lot of information. The 10 day periods could be further collapsed into 4 blocks throughout the growing season, perhaps with a trend line. Or a subset of representative panels could be shown and the remainder moved to a supplement.

You are right that this takes a lot of space. The suggestion to move the full figure to Supplement and to have a subset in main text is good and we will follow this.

374 Is this mire nearby, how different is the climate/vegetation? Some quantitative context for the comparison would be helpful. Also, is an overlap of one methanogenic genus meaningful? It seems likely be mere chance.

The Abisko-Stordalen mire is in Northern Sweden and thus very different in its climate. We have discussed these differences in lines 376-380. We can add coordinates to Abisko-Stordalen to make the geographic difference more obvious. Our statement is a bit misleading, as we observed the same genera of hydrogenotrophic methanogens and the same genus to be dominant. We found this similarity in spite of geographic and climatic difference interesting to warrant mentioning.