Final Response to Reviewer Comments:
From the Top: Surface-derived Carbon Fuels Greenhouse Gas Production at Depth in a Neotropical Peatland
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Dear Editor,

We would like to thank you, editor and referee, for your time and feedback on this manuscript. We appreciate the overall positive view of our manuscript.

In response to your overall comments, we agree that more background and introductory material improve the manuscript and have added new material to provide better context and improve clarity. We have included in this response to reviewers a new figure that presents a conceptual model for peat accumulation, peat C cycling, C source availability, and C source utilization in the peat profile at our study site, based on our understanding of our peatland system. If this figure is useful, we could include a more polished version in the revised manuscript as a supplemental or main text figure.

Specifically, we added more details about the pathways of methanogenesis, the importance of identifying which pathway is dominant in wetland ecosystems, and our approach to identify the dominant methanogenesis pathway at our study site.

Below are the specific responses to the individual comments and proposed corrections.

Thank you for your time and input,

Alexandra Hedgpeth and co authors

<u>Specific Responses (reviewer comments in bold, our responses in standard text)</u>
REVIEWER 1:

Minor comments for clarity:
21: measured to 2 meters, I do not understand this. Is this referring to the maximum depth studied, when in fact some samples reached 4 meters?

Yes, this is referring to maximum depth studied. Radiocarbon measurements of carbon dioxide (CO_2) and methane (CH_4) were conducted at two locations—the outer and inner peatland sites—both of which extend to 2 meters depth. We removed this specific depth description from the abstract, leaving it to be explained in the Methods as is more appropriate, together with other sampling details.

23: this line creates confusion when I go to line 318, when discussing lignin as a separate category to carbohydrates which did not change over depth, but lignin did, seemingly, as it is stated as storage over depth. Then in the end there was preferential preservation which means there is preferential decomposition as lignin is not decomposed, or is this premise only applicable to all carbohydrate categories? The back and forth between categories creates difficulties to follow.

To clarify, we added wording to emphasize that throughout the peat profile, the radiocarbon values for respiration products were more similar to radiocarbon values of dissolved organic carbon (DOC) than bulk peat. We found that deep peat respiration products had radiocarbon signatures that were more similar to surface DOC than deep solid peat, even though the proportion of carbohydrates in the solid-phase peat remained constant with depth. We will also add wording to clarify that carbohydrate and lignin are separate categories in the ^{13}C NMR results for the bulk peat.

78: if depths have high DOC with enriched 14C signature, does this not contradict the lower activity that is at risk if conditions change? Seems as though, there is fast decomposition based on the deeper levels of the signature.

This line is describing radiocarbon signatures of dissolved organic carbon (DOC), which is vertically transported from the surface, and is apprarently fueling microbial respiration at depth. If the DOC were from the peat located at the same depth, the radiocarbon signature of the DOC would be more depleted, similar to the solid material of peat at the same depth. Thus, our results point to younger surface DOC vertical transport through the soil profile in water. In contrast, the gas profiles across depths (here collected from dissolved gas in water) reflect instantaneous microbial activity in situ. These processes are happening on different temporal scales. We have included a figure with this response to better clarify this (New Figure).

95: The premise of changes in availability of labile materials in the peat profile even at depths of 2 meters, is supported by disturbances from vegetation? How does new

material deposits at depths to supply the protons needed and the carbon dioxide needed in the hydrogenotrophic pathway?

Yes, the premise regarding changes in the availability of labile materials in the peat profile, even at depths of 2 meters, could result from changes in the vegetation community. We will clarify this in the revised manuscript.

The solid peat is reflecting past decomposition processes as the peat accumulated. Our results show that microbes aren't actively decomposing that at depth, rather relying on surface fluxes. Vegetation introduces new organic matter through litterfall, root exudation, and other processes, which provide labile carbon sources and protons to support microbial activity including the production of methane. Tropical peatlands that have a greater presence of sedge or shrub species may show different preferences for source selection, influenced by changes in DOC, root exudates, and peat composition. When organic materials decompose, they release CO_2 and other nutrients that support microbial communities capable of utilizing hydrogen and CO_2 in their metabolic processes. Decomposition of plant litter also increases porewater acidity; We will provide additional background to clarify that this site is an acidic bog (pH = 4), which means the porewater is not limited by hydrogen. Carbon, hydrogen, and oxygen account for approximately 90% of the mass of peat, originating from the decomposition of dead plant and animal matter. The input of organic matter from vegetation, along with fermentation, further increases the acidity. This interaction between plants and the microbial community helps sustain hydrogenotrophic methanogenesis and provides resources to peat at deeper depths through vertical transport.

103: what is more advanced decomposition at depth? Advanced mechanisms or advanced rates of decomposition? Which brings me to the same question, if there is active decomposition at depths how does the lignin fit in this scenario where it accumulates meaning that this is the least degraded carbohydrate? Connects me to line 137.

We acknowledge this wording was vague and have replaced the phrase "more advanced decomposition" with "higher decomposition index".

135: Carbon Dioxide formula, has a 2 that should be subindex.

Thank you, this has been corrected.

137: do the authors mean to say that decomposition occurs at the surface, and then products are transported to deeper layers? To serve as feedstocks for methanogenesis? So there is initial decomposition above 30 cm, at lest partially, but is not lignin that was partially degraded? I think this is the common doubt I have, to really see what is the DOC that is being discussed. Later it seems it is lipids, while there is discrimination to carbs usage as feedstock. If lipids are the source of hydrogen, is this

decomposition happening in depths because methanogenic feedstocks would not migrate down, in particular VFAs and hydrogen.

Most decomposition takes place in the surface layers, where aerobic conditions are more favorable.

Peat forms vertically with new organic matter at the surface partially decomposed before being incorporated into the anaerobic zone. Our biochemical analysis using ^{13}C NMR of the bulk peat at our study sites reveals that it is predominantly composed of lignin and carbohydrates. The proportion of lignin in the solid-phase peat accumulated with depth (Appendix Fig A6; $p=0.002$), indicating that it was not being utilized by microbes in the surface layers as it was selectively preserved. In contrast, lipids show a significant decrease with depth ($p=0.029$), suggesting they were degraded when that layer of peat was accumulating at and near the surface. We do not have biomolecular composition of the DOC, however, we know that even as decomposition of solid peat is occurring at the surface, there is a simultaneous flushing downward of DOC. This DOC might result from direct leaching of plant material, and/or from incomplete decomposition byproducts. In either case, our results show what is being flushed down is quickly being respired by deep microbes, and does not appear to contribute to deep peat C storage.

Due to the lack of characterization of the chemical composition of the DOC, we can only infer that, based on the radiocarbon values, the DOC is a more likely source of the produced gases than the peat itself. The high proportion of carbohydrates in the peat indicates that the peat could be readily decomposed under conditions more favorable for decomposition. This suggests that the bioavailability of the peat makes it susceptible to decomposition if conditions change, such as if the supply of modern DOC from the surface is cut off or if the water table lowers and this labile peat is exposed to oxic conditions.

This line of the text was intended to inform the reader of past results showing similarities across the vegetation gradient within the peatland. To avoid confusion, we will remove the reference to decomposition, and just state that across all sites subsurface peat was similar in carbohydrate and aromatic-C content.

144: I do not understand: "Peat cores from were collected".

Thank you, this was changed to "Peat cores were collected."

295: Do the authors mean that the recent signatures from newer material then age to older peat? I think this needs clarity, is the materials found at depths old or not old.

At the same depth, there will be two source materials with two different ages, both old (peat) and not old (DOC produced from decomposition of surface litter). Organic material can enter the peat column gradually as new organic matter accumulates above it, or it can rapidly move down the peat column through vertical transport as DOC. These two routes—

rapid transport of C into deeper peat and the slower accumulation of peat—create two potential feedstocks for microbial activity. To clarify this further we have included a figure (New Figure) to better explain this concept of source availability and use, and add wording to the discussion to better explain.

299: to address a wider audience that perhaps is not expert in the complexity of the methods and result presentation, "While microbial utilization of surface DOC deep in the soil profile was seen at our sites" needs further discussion, what was the evidence from all the results and correlations reported. Somehow, a short synthesis of what is striking for DOC substrates and how this evidence looks like, could help enrich the discussion value of the results for the readers.

We appreciate the feedback and will revise this section to provide a more detailed presentation of the results. Our aim is to guide readers unfamiliar with peatland carbon dynamics through the potential influence of the biological origin of materials on source selection. Our findings indicate that surface-derived DOC significantly influences gas production deep within the soil profile, potentially linked to the high lignin content typical of wood-dominated peatlands. At our sites, contrasting surface vegetation types—*Raphia taedigera* and mixed hardwood—supply the organic matter for DOC. Enriched radiocarbon signatures in deeper DOC suggest that this organic carbon primarily originates from surface sources, emphasizing the critical role of DOC substrates throughout the peat profile. Even at greater depths, the influence of surface vegetation remains significant, underscoring the importance of surface-derived DOC for carbon dynamics in these ecosystems. Tropical peatlands with higher abundances of sedge or shrub species may exhibit different source selection preferences based on variations in DOC, root exudates, and peat composition.

313: "tree" instead of "treen"

Thank you, this will be corrected.

322: It would be enriching if the authors were specific, what specific support do these results give in relation to what Girkin and Hoyos found, the paragraph states there is support but the items being supported are not explicit and calls for reading other research. Perhaps naming what indicators were supported by your results could be straightforwardly stated.

Thank you for the suggestion, we agree this enriches the manuscript. To this end, we have added text to highlight the findings from the referenced studies. Our data suggest that under anaerobic conditions, lipids are decomposed more than other compounds, and/or microbial biomass production of lipids declines, or changes in carbon inputs over time. Taken together, our data support different decomposition rates and preservation processes of individual biomarkers that contribute to the accumulation of OC within tropical peatlands. Specifically, there is evidence of varying rates of biomarker degradation related to the composition of the organic matter in these sites, suggesting that certain compounds are more resistant to decomposition (Girkin et al., 2018a). Meanwhile, specific environmental conditions and microbial communities can also influence the preservation of these biomarkers, further supporting our findings that the interplay between biological origin and decomposition processes is critical for understanding OC dynamics (Hoyos-Santillan et al., 2016). Together, these studies reinforce the complexity of OC dynamics and the significance of individual biomarkers in shaping C storage within these ecosystems.

345: incomplete methane molecule.

Thank you, this will be corrected.

REVIEWER 2:

The isotope systematics of methanogenesis and carbon dating need clarification: the d13C value as such is meaningless to determine a source/process but rather the diffference of substrate and product in isotopic composiition. Make sure that you explain well which substrates and products you've measured and thus which processes you can resolve (d13C-CO2 vs d13C-CH4 can in principiple only resolve carbonate reduction but not acetoclastic Mog).

We agree with the reviewer that $\delta^{13}C-CH_4$ cannot be interpreted in isolation, and that was not our intent. We apologize that the substrate measurements were not clearly referenced in the text, and will revise the manuscript to better explain the origin of the $\delta^{13}C$ values used to calculate the apparent carbon isotope fractionation factor (α) in the manuscript. We will clarify this in both in the Methods and Results/Discussion.

We measured the stable carbon isotope signatures of CH_4 ($\delta^{13}C-CH_4$) and CO_2 ($\delta^{13}C-CO_2$) during the methane production process i.e. trapped gas dissolved in peat pore water. The $\delta^{13}CH_4$ value ranged between −89.2‰ and −90.0‰, and the $\delta^{13}CO_2$ value ranged between −13.7‰ and −21.2‰. Based on the measured $\delta^{13}CH_4$ and $\delta^{13}CO_2$, the apparent carbon isotope fractionation (α) for this methanogenic process was calculated according to formula: $\alpha = (\delta^{13}CO_2 + 1000)/ (\delta^{13}CH_4 + 1000)$. This formula has been used to determine CO_2 reduction vs. acetate fermentation in several previously published studies (Whiticar et al, 1986; Corbett et al, 2013; Zhang et al, 2019, among others; full references below). The α calculated for this study was consistently higher than 1.065 (1.076 – 1.083) for all sites and soil layers, indicating an environment dominated by CO_2 reduction (hydrogenotrophic methanogenesis). Our results are also consistent with Holmes et al. (2015), who completed a review of fractionation factors in peatlands around the world.

Both northern and tropical peatlands can be dominated by hydrogenotrophic methanogenesis, but due to differences in the precursor $\delta^{13}C$-CO_2, the $\delta^{13}C$-CH_4 signature varies widely. We have clarified this point in the manuscript.

Sources supporting the apparent carbon isotope fractionation factor:

Whiticar, M. J., Faber, E. & Schoell, M. Biogenic methane formation in marine and freshwater environments: CO 2 reduction vs. acetate fermentation—Isotope evidence. Geochim. Cosmochim. Acta 50, 693–709, https://doi.org/10.1016/0016-7037(86)90346-7 (1986).

Corbett, J. E., Tfaily, M. M., Burdige, D. J., Cooper, W. T., Glaser, P. H., and Chanton, J. P.: Partitioning pathways of CO2 production in peatlands with stable carbon isotopes, Biogeochemistry, 114, 327–340, https://doi.org/10.1007/s10533-012-9813-1, 2013.

Holmes, M. E., J. P. Chanton, M. M. Tfaily, and A. Ogram. CO2 and CH4 isotope compositions and production pathways in a tropical peatland, Global Biogeochem. Cycles, 29, 1–18, doi:10.1002/2014GB004951, 2015.

Zhang, Y., Ma, A., Zhuang, G., and Zhuang, X.: The acetotrophic pathway dominates methane production in Zoige alpine wetland coexisting with hydrogenotrophic pathway, Sci Rep, 9, 9141, https://doi.org/10.1038/s41598-019-45590-5, 2019.

Check throughout the manuscript that the use of D14C, d13C etc is used corectly (I've seen isolated uses of '14C').

This will be corrected.

I'm missing discussion RE pro/contra the differennt MOg pathways addressing the questions:
where is the H2 coming from (fermentation of... DOM? Or rahter solid state OM?).

We will give more background to explain that this site is an acidic bog (pH=4), as such the porewater would not be H_2 limited. Carbon, hydrogen, and oxygen form about 90% of the mass of peat, derived from the decomposition of dead plant and animal material. The vegetation OM inputs along with fermentation increase the acidity further.

It was also our intent to use the $\Delta^{14}C$ of CH_4 to determine the C source for methanogenesis. An old $\Delta^{14}C$ of CH_4 would indicate the solid state peat as a source material, while a young $\Delta^{14}C$ of CH_4 would indicate that the fresh DOM transported downwards from the surface is driving methanogenesis. We did not see any indication of solid state peat material as a precursor to methanogenesis at depth (Fig 2 within manuscript).

How does the DOM arrive at depth and why does that not disturb gas profiles (if it was via roots or other types of bioventilation processes).

We will clarify how DOC reaches the deep peat in the revised manuscript. We have also created a figure (New Figure) to serve as a conceptual diagram to better explain this.

Biological processes that could affect gases in the peat have been documented in studies of methane and CO_2 emissions in this peatland and include proximity to plants and animal burrowing. We will add text to include these studies in the site description. These samples were collected within the undisturbed peat, from wells situated at specific depths i.e. not along one single vertical profile. The DOC measured here is young, suggesting that it comes from plant litter leachates, root exudates, or decomposition of recent C inputs.

Tropical peatlands are highly porous and have a relatively high flow of water through them, which transports both DOM and dissolved gases, simultaneously. The C source for hydrogenotrophic methanogenesis is CO_2 produced from this younger material that has been delivered to the deep peat via vertical transport of porewater. This has been documented in other peatlands as a pathway for surface C to reach deeper layers of peat and provide microbes with a preferred alternative to the older peat at the same depth.

How can that be different at temperate zone peatlands? What are the implications for tropical peatlands in general (and how does that influence our view on GHG related questions)?

We agree that our study cannot definitely say that this data suggests tropical peatlands are different than temperate zone peatlands. The implications are that what is currently known from research in higher latitude peatlands is a starting place to explore how C is cycled in tropical peatlands , but more validation of the assumptions that they function similarly is needed. We will add more to the discussion section to expand on this.

Further detailed specific comments

L36 better specify C as org C (OC, orgC or the like), needs adjustment throughout the MS

Thank you, this will addressed for the results and discussion sections where we discuss the C that was measured.

L110 'Ramsar site..', define, is otherwishe meaningless for the broader community

This section will be revised to clarify the significance of Ramsar recognition for wetlands.

L144 sentence crippled

Thank you, this will be changed to “Peat cores were collected.”

L144 ‘Russian peat corer’ is misleading. Eijkelkamp is a Dutch company, the corer would be described as a gouge auger (also on Eijkelkamp’s web page).

We acknowledge that using the manufacturer's name for the equipment is appropriate. The Eijkelkamp peat sampler set includes a peat sampler (commonly referred to as the Russian Peat Corer) and an auger. However, the auger was not utilized during peat collection, as the sharp-coned peat sampler was adequate for the soft, wet soils. We will update the description to "peat sampler" as listed on the Eijkelkamp product website.

L177 referencing wrong (…outlined by McNicol et al (2020)… would be corect.

Thank you, this will be corrected.

L196 there is some unclarity regarding isotope systematics. Acetoclastic Mog follows: CH3COO- + H+ ◊ CH4 + CO2, hence the sentences IDing substrate d13C and prod d13C to calc alpha are not right (only fit to carb red).

The apparent fractionation factor for methane (CH_4) can be calculated using the ratio of the stable carbon isotope signatures ($\delta^{13}CO_2$ + 1000)/($\delta^{13}CH_4$ + 1000). We will clarify to indicate that ^{13}C of the gas products are used in the calculation of the apparent fractionation factor.

L245 ‘14C value’ is unclear, D14C?

Thank you, this will be changed to the Delta nomenclature.