Response to Reviewer #1's Comments:

Reviewer #1 comments are in grey

Responses are in black

This manuscript characterizes the macromolecular composition of organic matter (OM) in seven lowland tropical and two temperate peatlands, using pyrolysis–gas chromatography–mass spectrometry (Py-GC-MS) as the primary method. Through analysis of vegetation, leaf litter, and downcore peat profiles, the authors show a consistent enrichment of aromatic (lignin-derived) pyrolysates relative to polysaccharides with depth, following a logarithmic decline that is most pronounced in anoxic horizons of the tropical sites. They also find that a pyrolytic fingerprint of the original vegetation persists in highly degraded deep peat, and discuss what this means for peatland carbon accumulation modelling and the home-field advantage (HFA) concept. The geographic and ecological breadth of the dataset is a real strength, and Py-GC-MS provides molecular-level detail that FTIR alone cannot. That said, a number of issues need to be addressed before the manuscript is ready for publication.

We thank the reviewer for the constructive and balanced comments. We will address all below comments in detail.

1. The role of FTIR should not be dismissed

In Section 3.1, the authors show that FTIR is affected by silicate mineral interference at the 1030 cm^{-1} band in several hydrologically active tropical sites (Pantano, Inírida, Mpologoma), producing apparent carbohydrate enrichments that the Py-GC-MS data do not support. This point is well taken. But the manuscript goes on to frame FTIR as more or less inapplicable for tropical peatland systems, which overstates things.

The downcore trends revealed by the Py-GC-MS-derived PPA ratio (preferential loss of polysaccharides, relative enrichment of aromatics with depth, logarithmic decline in OM lability) are in fact very similar to what Hodgkins et al. (2018, *Nature Communications*) found using FTIR-based ratios on a broadly comparable set of tropical and temperate peatlands. That the two methods converge on similar conclusions, despite FTIR's known limitations in mineral-rich settings, says something worth noting: FTIR is still a useful screening tool. It is fast, cheap, and works well where mineral input is low. A more balanced treatment would acknowledge the two methods as complementary rather than presenting FTIR as a method that 'could be inappropriate.'

We agree that FTIR is a valuable screening tool in many peatland settings and the 'could be inappropriate' wording is carefully chosen to reflect that. The point the reviewer made is exactly what we tried to avoid, so we will better articulate that here and in future work. We note that a main point here is similar to a comment from reviewer #2 which argues that the FTIR vs Py-GC-MS discussion requires more depth than the scope of this paper allows. Therefore, we have decided to omit the FTIR and EA data here (as suggested by Reviewer #2) and include it in a future paper that can do justice to the comparison and allow more depth and discussion of the mismatches, especially in the plant material, while maintaining the narrative and focus of this manuscript.

Removed the following lines: 27-28, 95:", complemented by FTIR", 211-226; 269-331; 832

2. Section 3.2.3: The argument for aliphatics as an "early degradation" signal needs clarification

Section 3.2.3 argues that aliphatic compounds are enriched in peat relative to vegetation and that this enrichment "signals early degradation." As written, the section is ambiguous and could easily be misread. The authors should clarify their reasoning. Specific concerns:

The text moves between several mechanisms (condensation of biological alkyl compounds during humification, selective preservation of aliphatic-rich macromolecules such as cutin and cutan, preferential degradation of polysaccharides and lignin relative to aliphatics) without specifying which diagnostic features are actually being used to infer early degradation. Is it the enrichment of aliphatics relative to the parent vegetation? A shift in chain-length distribution? The n-alkane/n-alk-1-ene ratio? It is not clear from the text.

To improve the clarity of this section, we have rewritten and restructured it. We have also numbered the mechanisms (i/ii/iii) and linked these directly to the observations made in the text. See fully revised version at the end.

Plant pyrolysis products themselves contain abundant aliphatics (leaf waxes, cutin, suberan, etc.). The authors note that "both peat and plants have a similar pattern among the HMW components" (lines 524–526) and that the C14/C15 doublets "probably derived from cutin, which is common in plants." So a key question remains: how much of the aliphatic enrichment in surface peat is simply inherited from the parent vegetation (aliphatic-rich roots and leaves), and how much reflects genuine diagenetic change?

The authors should spell out the diagnostic criteria used to identify early degradation, distinguish between inherited and diagenetically enriched aliphatic pools, and revise the section title and text so that aliphatic enrichment alone is not presented as sufficient evidence of early degradation.

The strongest argument for aliphatic enrichment of the peat is perhaps understated in our original manuscript: the peat has higher proportions of aliphatic pyrolysates than *any* of the parent plant material, and must have been enhanced by selective degradation of other plant components. We have highlighted this at the start of the rewritten section: *"The aliphatic pyrolysates are enriched (higher proportion) in peat relative to the accompanying parent material ($p < 0.05$; Fig. 6a) to the degree that they are the primary mathematical distinguisher between plant material and all peat samples in our database, which includes a range of tropical peatlands as well as a temperate fen and bog. Aliphatic pyrolysates form the primary discriminant in our statistical analysis, represented through PC1 (Fig. 4) and the first two branches of our HCA dendrogram (Fig. 5). As an example, the proportion of aliphatics in all plant material is highest in Papyrus leaves (~ 20% akin to A. americana), but all peat samples (except one) from the Papyrus swamp have higher proportions of aliphatics (Fig. 6a). The peat's aliphatic character, therefore, can only partially arise from components inherited from the plants and requires other mechanisms."*

The remaining rewritten section articulates the mechanisms more clearly and concisely:

"*This enrichment has been observed prior in pyrolysis-GC-MS studies, and* those *invoked several mechanisms: (i) production of aliphatic-rich macromolecules by the condensation of biological alkyl*

compounds, such as n-alkanes, fatty acids, or alcohols into a larger macromolecule during humification (Lichtfouse et al., 1995); (ii) selective preservation of larger aliphatic-rich macromolecules such as cutin, cutan, or suberan (Deshmukh et al., 2005; Nierop, 1998; Gupta et al., 2006; Gupta et al., 2007b) as aliphatic macromolecules are energetically difficult to break down, either aerobically or anaerobically (Gunina and Kuzyakov, 2022; Lorenz et al., 2007); and (iii) non-extractable, entrained or bound leaf waxes released through volatilization of odd n-alkanes or even decarboxylation / dehydration of fatty acids/alcohols during pyrolysis (Nierop and Van Bergen, 2002; Eglinton and Hamilton, 1968).

The n-alkane/n-alk-1-ene ratio is close to 1 in most of our plant and peat material, especially from C16 to C18 (Fig. 6b). This suggests that the n-alkane/alk-1-ene doublets originate from pyrolytic cracking and therefore an aliphatic macromolecule (mechanisms i/ii) (Schellekens and Buurman, 2011). However, the n-alkane/n-alk-1-ene ratio in the HMW doublets increases and exhibits a subtle odd-over-even distribution (n-alkane/alk-1-ene ratio ~ 2) in the chain length, which indicates an additional contribution from leaf waxes (mechanism iii; Fig. 6b). We suggest that the in-situ condensation mechanism (i) is unlikely to be at play given the abundance of aliphatic macromolecules at the peat surface and the high temperatures (>300 °C) required to significantly produce condensed aliphatic macromolecules (Gupta et al., 2007a; Stankiewicz et al., 2000). Furthermore, although the distribution in plant samples is variable, both peat and plants have a similar pattern among the HMW components and both feature a higher proportion C14/C15 n-alkane/n-alk-1-ene doublets, probably derived from cutin, which is common in plants and supportive of selective preservation (mechanism ii; Schellekens and Buurman, 2011). Even most of the shallow peat (1–10 cm) is enriched in all aliphatic pyrolysates compared to its plant material (including roots; Fig. 6a), which implies that the parent material could undergo selective degradation (ii) before entering the peat horizon, enriching the proportion of the aliphatic macromolecules relative to polysaccharides and aromatics.

We also detect more aliphatics with increasing depth in some peatlands (Fig. 6a), which suggests that such preferential degradation (mechanism ii) persists under anaerobic conditions, although this differs among sites. In the Everglades, aliphatic pyrolysates are particularly abundant, both at the surface and significantly enriched at depth ($R2 = 0.67$, $p < 0.05$). The lily pad roots feature a high proportion of aliphatics, making them a likely source for the aliphatics detected in these peat samples. The Everglades site is also permanently inundated, such that significant aerobic degradation might occur during transport and sinking, prior to reaching the peat profile. The Mpologoma papyrus swamp also contains a uniquely high proportion of aliphatics (up to 60% akin to A. americana), but especially at depth (< 50 cm), which is likely sourced from both the aliphatic-rich Papyrus leaf and subsequent aerobic and anaerobic degradation enrichment (Fig. 6a). The deeper Tor Royal core (UKTR_2) also shows a significant enrichment of aliphatics at depth ($R2 = 0.82$, $p < 0.05$), also more enriched than any measured plant litter, suggesting a continuous anaerobic preferential preservation (ii) of these aliphatic macromolecules (Schellekens and Buurman, 2011). The proportion of aliphatics at other sites, however, does not increase with depth (Fig. 6a), suggesting that their

aliphatic enrichment relative to the peat-forming plants was caused by aerobic selective degradation prior to reaching anoxic horizons.

Regardless of the mechanism, the higher proportion of aliphatics represents a large proportion of the peat macromolecular signal, which could be an underappreciated recalcitrant carbon pool. In the next section we dissect the effect of downcore degradation on plant biopolymers."

3. Vegetation signal preservation at depth needs refinement

The authors state repeatedly that "a pyrolytic fingerprint of the original vegetation persists" in deep peat (abstract; lines 41, 760–762, 1270–1272). This is an important and well-supported finding, but the current formulation is too general. Two points need refining:

The HCA (Section 3.2.2) and PCA (Section 3.2.1) results show that the vegetation fingerprint is not retained uniformly. Some signals, such as 4-vinylphenol (P3) as a sedge biomarker, S-lignin as a hardwood indicator, and the polysaccharide signature of *Sphagnum*, are detectable in shallow peat but fade with depth as degradation progresses.

What persists most robustly seems to be tied to the relative stability of certain pyrolysis products, particularly lignin-derived guaiacols and syringols, and to a lesser extent high-molecular-weight n-alkanes from leaf waxes.

This is a valuable comment that will require additional data analyses and that we think will significantly improve the manuscript. As such we briefly address it here and will fully address it in our revised manuscript. The PCA and HCA communicate different approaches to understand big datasets. We are using the same fractional abundance dataset for both analyses, which indeed show the downcore degradation to be the major driver of pyrolysate abundances, i.e. increased aromatic and aliphatic contents. However, the later branching in the HCA do reveal the same discriminant pyrolysates as the plants. So while the overall macromolecular pool is changing, the subtle variations, within the lignin/polysaccharide/aliphatic pool maintain, i.e. 4-vinylphenol (P3) is more abundant in a sedge-derived peat, S-lignin in hardwood-derived peat, and polysaccharide signatures in *Sphagnum*-derived peat, even if each of those components represents a smaller proportion of the total pyrolysates. This is why pyrolysates still distinguish peat even after significant degradation (Section 3.2.2). We will incorporate more distinct statistical elucidation (by splitting the dataset on aromatic/aliphatic/polysaccharides etc.) and visualization to expand on these arguments and clarify our conclusions in the revised manuscript.

Their resistance to microbial degradation is what allows a chemotaxonomic signature to survive even in highly decomposed peat. The authors should state explicitly which classes of pyrolysis products carry the persistent fingerprint and why (their chemical recalcitrance), rather than implying that the entire vegetation signal is preserved.

Due to manuscript length we had only a limited discussion on the pyrolysate composition of plant tissues in the original submission. That already confirmed or revealed high level differences, such as the lack of lignin-associated components in *Sphagnum* and the greater proportions of S-lignin in trunks, P3 in sedges, and

phenolic moieties in palms - with those same features preserved in the deeper peat (Fig. 5). However, we agree that we can explore this in greater depth. Given the encouragement of both reviewers and the space afforded by removing the FTIR comparison, we can now expand that discussion in our revised manuscript. We do not intend to have in an in depth discussion on every species or plant type and their associated peat, and will instead focus on the major taxonomic groups of peat-forming plants as examples (i.e. Sphagnum, sedges and palms), and identify the suite of features that are retained in peat and play a key role in distinguishing peat types (noting that the inclusion of all of our raw data in the SI will allow future workers to examine any of our investigated vegetation).

4. Section 3.3.3: The HFA discussion is overextended and should be substantially condensed

Section 3.3.3 has no microbiological data of any kind: no 16S surveys, no metagenomics, no enzyme assays, no decomposition experiments. The section is therefore speculative and should be cut back substantially. The manuscript

We agree that this section would benefit from cutting back to enhance the focus of the paper, as was also suggested by reviewer #2. We omitted lines 771-806.

The home-field advantage hypothesis, as originally formulated and as the authors themselves cite (Dehaen et al., 2025; Hoyos-Santillan et al., 2017), concerns the differential decomposition of autochthonous versus allochthonous organic matter. That is, microbial communities are adapted to decompose litter from their local vegetation more efficiently than litter imported from elsewhere. The authors extend this concept to argue that microbial communities at different *depths* within a single peatland are specialized to degrade the OM at that depth (lines 1252–1254). These are not the same claim.

Agreed. This is a hypothesised novel extension to the homefield advantage theory. We explain in the section the basis for the home-advantage theory, but we adjusted the text to make it more explicit that this depth extension builds on but is distinct from the HFA hypothesis .

Line 824: "*Our HCA analysis detects a macromolecular fingerprint of the vegetation from which that peat formed: at the surface, at depth and, even in highly degraded peat.* Therefore, we suggest an extension to the *HFA hypothesis: that aspects of past microbial communities could persist even in deep (> 50 cm) peat, reflecting the molecular composition of the ancient peat-forming vegetation.*"

And added "and" to line 827: "***We hypothesise** that the microbial community is optimized to degrade the litter from its specific peatland vegetation, **and that includes** the most labile material at the top of the profile as well as the most recalcitrant "leftovers" at the basement.*"

Deep peat in tropical systems can be centuries to millennia old (the authors' own age models span "decades to millennia"; line 1143). The vegetation, climate, and hydrology under which that deep peat formed may have been very different from conditions at the surface today. Attributing the persistence of a vegetation fingerprint at depth to a specialized, depth-adapted microbial community overlooks the possibility that the deep microbial community has itself shifted over time in response to changing conditions.

We did not intend to imply that the current microbial community is driving the organic matter composition at depth. As the reviewer argues, the organic matter composition will reflect the entire history of microbial alteration from shallow to deep burial and over the associated timespan. What we had intended to argue here is that the OM composition is a driver of the current microbial community that persists – at least in part – over time.

To clarify that, we have added "active" to line 818: "*HFA theory argues for an intimate interaction between the **active** microbial community and OM composition*" and line 820: "*such that subtle differences in OM character cause specialisation in the **active** microbial community and their capacity to degrade that specific peat substrate*." We also note that in response to both reviewers, we have trimmed this discussion section. We agree that it is overly speculative and that a shorter discussion still allows us to introduce the idea as the basis for future work.

Without compositional or functional data on the microbial community at different depths, the authors cannot distinguish between "the microbial community is adapted to the deep OM" and "the deep OM is simply more recalcitrant, and the microbial community is whatever happens to be there."

We agree. And we did not intend to suggest that we had proven this, rather than the persistence of OM signatures at depth could have implications for the microbial that can be tested in future work.

Adjusted line 827 prior, which addresses this comment as well.