**Review of Verret et al.**

Verret et al. reports microbial organic matter degradation of a mid-Miocene age permafrost soil column from the McMurdo Dry Valleys (MDV), especially near the surface in the 'dry permafrost', which likely occurred during warm periods post-deposition. In addition, the lower 'icy permafrost' presents evidence of higher plants from a tundra ecosystem analogous to present-day southern Greenland. In order to study the degradation/preservation of organic matter, the author utilises bulk sediment TOC and $\delta^{13}C$, and biomarker distributions. A hierarchical clustering of this dataset highlights three distinct depth intervals that are attributed to increasingly lower levels of organic carbon degradation downcore. Moreover, the author uses two calibrations (i.e. MBT'$5_{ME}$ and Bay$MBT_0$) to acquire temperature reconstructions from brGDGTs. Both present a similar range of values and provide insight on the warming required to "unlock" the carbon stored in the permafrost for microbial activity.

Overall, this paper is articulately written with only a few incorrect spelling and/or grammar mistakes. The figures are also very clear and easy to interpret. The Introduction (section 1) and the Study Site (section 2) provide a succinct overview of the locality (i.e. MDV) and the rationale behind the research undertaken. It is nice to see a study that takes advantage of a multi-lipid approach, and not just the compounds most commonly applied (such as *n*-alkanes and fatty acids). The data also provides insight into high-latitude systems, which are considerably under-studied. The potential impact of carbon released from permafrost, as a response to future warming, is still a major gap in our knowledge, and fingerprinting the biogeochemistry of Antarctic soils would be a crucial step towards developing a better understanding.

Although written eloquently, I believe that the readers would benefit from the author providing geographical context in the introduction (see mostly minor comments for suggestions on improvements). In addition, please find suggestions for how the abstract (see **Section 4**) and results (see **Section 3**) could be developed slightly. Finally, there are a few interpretations that I find myself struggling to follow, and believe would benefit from more clarification (see **Section 1**). In particular, there are major questions pertaining to use of the term "degradation" that a slight reframing of the discussion may solve (see **Section 2.1**), and potential further exploration of hopanoids (see **Section 2.2**).

**Major comments:**

## 1) Interpreting the changes in biomarker distribution

### 1.1 A new source of organic carbon post-Miocene?

The author suggests that the overall biomarker distribution support a tundra ecosystem that was established during the mid-Miocene (section 5.1). This includes greater values in the SOCd, BIT index, and abundance of high-molecular weight compounds, phytosterols, and phytol. Although the macrofossils further confirm a tundra ecosystem, how confident is the author that the deposits are only composed of mid-Miocene sediments? As described in section 2, the dating relies on magnetostratigraphy constrained by a tie point at much lower depths (tephra layer at 5.13-5.18 m). Is this at a resolution that provides robust ages for the 140 cm section studied? Although the Py-GC-MS data provides evidence of plant material, could plants not have grown during warm intervals post-Miocene? Did a tundra ecosystem only exist during the Miocene or is the composition/species of macrofossils specific to the Miocene? If the latter, could the author cite some papers to support this? I ask for clarity on the age model as I wonder how the author ruled out the possibility that the distinct change in the biomarker distribution of unit 1 represent a different time interval with a shift

in the depositional environment, and thus lithology. Especially considering the $^{14}C$ analyses at ~10 cm yielded an age of ~42 kyrs (even in splits 4 and 5, that supposedly contain the "older material"; line 293), and the TOC is much higher in unit 1.

**1.2 Microbial organic matter degradation during deposition in the Miocene?**

In addition to clarifying why the change in the biomarker distribution of unit 1 does not represent a change in source, and instead is a degradation signal, how did the author determine that microbial organic matter degradation was less severe in the underlying icy permafrost? Besides the abundance of plant-derived degradation products (e.g. pristine and phytane), most of the evidence in the dry permafrost is indicative of a contribution of bacteria-derived organic carbon (e.g. low C:N ratios, high $\delta^{13}C_{org}$ values, and high relative abundance of iso-FAs, LMW *n*-alkanes, and hopanoids). I think inferring microbial organic matter degradation from multi-lipid evidence of microbial activity is not a bad assumption, however does this equate to better preservation of lipids in the icy permafrost? (see **Section 2.1** for further discussion and a potential solution). It seems unlikely that microbial organic matter degradation did not take place when the permafrost soil was accumulating during the Miocene. Especially considering that there is evidence of higher plants, which implies warmer and wetter conditions that would also favour microbial activity (Chorley et al., 2023). Therefore, is it more nuanced to state that the dry permafrost is influenced by a faster rate of microbial organic matter degradation compared to primary productivity?

**2) Gradient of organic carbon degradation that decreases downcore**

**2.1 Defining "degradation"**

Another reason for questioning the statement that there is lower levels of organic carbon degradation downcore, is that, although the Py-GC-MS data presents degraded organic matter in the dry permafrost, there is no Py-GC-MS data from the icy permafrost to compare this to. Potentially, it is actually comparatively much lower in concentration. Moreover, the CPI suggests degradation downcore. Typically, thermally mature organic matter exhibits low CPI values (~1), and high CPI values (>3–30) indicates relatively unaltered organic matter (Diefendorf & Freimuth, 2017). The dry permafrost coincides with CPI values >3. I think the solution here would be to differentiate between microbial degradation and abiotic degradation that has occurred over long timescales to the Miocene deposits. Reframing the paper to fit these definitions would massively help the confusion caused by what comes across as contradictory statements throughout. For example, "Beyond the dry pemafrost, $C_{org}$ is dominantly ancient and highly degraded" (line 444) and "Overall, the organic matter in the core appears to be compatible with a highly degraded signature of the mid-Miocene paleoenvironment" (line 455). This will improve what appears to be a lack of consistency between the abstract and conclusion (see more recommendations in **Section 4**).

As part of this recommendation, I would like to suggest that the author focuses on reframing the following section in the Introduction (section 1; line 53):

"Low-molecular weight organic compounds (e.g. sugars and amino acids) and lipids with double bonds or polar functional groups (e.g. fatty acids and alcohols) are typically susceptible to microbial decomposition. However, other lipid biomarkers, such as apolar, saturated hydrocarbons (e.g. alkanes), isoprenoids (e.g. phytane) and cyclic compounds (i.e. hopanes or steranes), are refractory compounds formed during diagenesis (Peters et al., 2007) and may be preserved for long geological timescales (e.g. Eigenbrode, 2008)."

In this section, the definitions can be introduced. Also, as it currently reads, since there is a dominance of easily degradable compounds in the dry permafrost and refractory compounds in the icy permafrost, this suggests lower levels of organic carbon preservation downcore.

**2.2 Exploring the hopanoids to determine abiotic degradation**

This is a recommendation that the author can chose to ignore, however the hopanoid dataset may provide interesting insight into abiotic degradation occurring at this site, further supporting the CPI results. I make this suggestion as the author has already identified and integrated the hopanoids, therefore it is information that would take little work to acquire. If hopanes and their various stereoisomers were discovered, there a multiple ratios that can be used for tracing thermal maturation. For example, the C-17 and C-21 position of hopanes change from a ββ configuration to a more stable βα configuration during early diagenesis (Farrimond et al., 1998).

**3) Developing the methods and results section**

The comment on the methods section is minor, but some of the indices which are eventually discussed in the results are not introduced in the methods. This includes the cholesterol to plant sterol ratio, 100*$C_{15\text{-}17}$ iso-FA+anteiso-FA/*n*-FA; Pr:Ph, and $C_{26\text{-}28}$ to $C_{22\text{-}24}$ *n*-alkanols. It would be useful for the readers to be able to refer back to the methods to understand the ratios and what they represent. Having said this, Pr:Ph (Figure 6 and 8) and $C_{26\text{-}28}$ to $C_{22\text{-}24}$ *n*-alkanols (Figure 5) are not actually discussed within the text anywhere…is it required in the figures? On the other hand, TN is discussed (line 269 and 298) but is not available in a figure.

For the results section, although I agree with the author's choice to first discuss the general background composition of biomarkers, prior to describing the data within each unit in section 4.2 and 4.3, I feel the first sentence is somewhat misleading. The author lists the compounds that show little variation, however then goes on to discuss their differences. For example, *n*-alkanes are stated as a biomarker that does not vary much, yet the CPI (which compares the odd vs. even *n*-alkanes) and SC:LC ratio (which compares short- vs. long-chain *n*-alkanes) is highest in unit 1. As done so for ACL, the author could instead focus on the averages or ranges of values. In addition, I would advise the author to be careful with not interpreting the data too much at this point. The fact that the cholesterol to plant sterol ratio and the BIT index indicates a terrestrial setting and the high-molecular weight compounds further support higher plant input is useful, however to infer that this reflects a "tundra-dominated ecosystem" and this agrees with interpretations made from "macrofossils" is something that could be saved for the discussion. Similarly, in section 4.2, I would remove the sentence starting with "Overall…" and maybe expand on what the biomarker indices described in the first paragraph indicate, as done so for HPFA on line 278. This also applies to section 4.3.

**4) Consistency between the abstract and conclusion**

In addition to being more cautious with the use of the term "degradation" in the conclusion, to avoid what could be interpreted as contradictory statements between the abstract and conclusion (see **Section 2.1**), the abstract could also benefit from providing a more 'complete summary' of the study.

The abstract appears to focus on the loss of the "legacy" mid-Miocene age carbon in the upper 'dry permafrost', however does not delve into the source of carbon that over-prints this signal, as done so in the conclusion on line 443, i.e "Although our study suggests that Holocene organic carbon is being introduced at high elevation sites such as the Friis Hills, modern $C_{org}$ contributions remain very low (<1%)." This would be useful to include, in addition to a sentence on line 20 that explicitly states what the evidence found within the 'dry permafrost' indicates, as done so for the icy permafrost on line 21,

i.e. 'the multiple proxies indicate contribution of bacteria-derived organic carbon, and thus microbial organic matter degradation.' Moreover, the discussion surrounding the brGDGT results and the significance of temperature on carbon availability / microbial activity is not mentioned in the abstract.

However, although I feel the abstract could better expand on the breadth of data and interpretations made, I also acknowledge that there is a word limit. If the addition of the source of carbon and the temperature reconstructions is difficult, I recommend the author prioritises honing the final sentence of the abstract to make it more impactful (line 25). What exactly is the key outcome that is novel to this study that the author would like to highlight here? Is it really that this record provides a nice archive to examine ecological changes in the past? How about the cautionary tale of needing to exclude more recent microbial activity prior to interpreting biomarkers in sedimentary deposits? Or even that these archives are vulnerable to future warming? Alternatively, what we can learn from "present-day organic processes" (line 63)?

**Minor comments:**

Line 15: "low input rates" of what exactly?

Line 15: replace "document" with explore or a verb that is more related to the aims of the study rather than results at this stage of the abstract

Line 17: define ages for mid-Miocene, e.g. '(xx–xx Ma)'

Line 18: define FA, e.g. 'branched fatty acids with an iso- configuration relative to *n*-fatty acids' (following how the author defined iso-FAs in line 249)

Line 19: go through the whole manuscript to check whether a hyphen is used or not used between "low/mid/high" and "molecular" and make it consistent

Line 24: define ages for late Neogene, e.g. '(xx-xx Ma)'

Line 24: Since this study specifically focuses on lipids, "Biomolecules" could be replaced with 'lipid biomarkers'

Line 27: it may be useful to clarify to the readers that "The hyperarid polar desert" refers to the MDV e.g. 'The MDV is a hyperarid polar desert, and amongst the coldest and driest environments on Earth (e.g. Horowitz et al., 1972). The MD can be divided into…'

Line 40: specify where the "Shackleton Glacier region" is in relation to MDV? e.g. 'further inland'

Line 42: could refer to Fig. 1a here, to place University Valley in geographical context

Line 43: the "3" needs to be in subscript, e.g. "$CaCO_3$"

Line 45: add r to 152 ky?

Line 46: Fig. 1a can again be referred to here, after "At Friis Hills…"

Line 47: the "oldest" what? permafrost?

Line 48: define ages for mid-Miocene again in introduction, e.g. '(xx–xx Ma)'

Line 56: could be reworded to, e.g. ‘and may be preserved on geological timescales’

Line 59: start new paragraph?

Line 59: could remove “diagenetic”

Line 60: for consistency within the text, use total organic carbon (TOC) rather than “total $C_{org}$”. In addition, technically speaking, total N is not part of “bulk organic carbon analysis”

Line 93: is there a map of Friis Hill with the three sites? Could be added as a supplementary figure?

Line 95: “metre” can be abbreviated

Line 117: this sentence could be reworded, e.g. ‘The total organic carbon (TOC) and total nitrogen (TN) were measured to determine: i) the soil organic carbon density (SOCd); and ii) if the C:N ratios in the bulk sediments follow the Redfield biological stoichiometry ratio (6:61, Redfield, 1934).’

Line 162: could a more specific word be used instead of “measurements”? e.g. ‘The concentrations or abundances of lipid biomarkers were used…’

Line 171: this sentence is repeating what was stated in line 166, and I would also remove “as odd chains get altered into smaller chain lengths” as it may confuse readers considering CPI does not capture changes in chain length. If this sentence is removed, line 169 could be incorporated into the paragraph beginning on line 165

Line 185: should “i” and “$C_i$” be in italics to match the Equation 3?

Line 195: although Strauss et al. (2015) uses the term “chemical degradation”, could the author clarify what this means? Is this an abiotic process not involving microbial activity?

Line 198: could reword “Since *n*-alkanes are typically preserved better…” to, e.g. ‘less labile’ or ‘more stable’ etc.

Line 198: is there a threshold or range for “low HPFA” values that represent “high degrees of organic carbon degradation.” I also wonder if it would be safer to say “relatively high” because a “high degree” could be interpreted as catagenesis or even metagenesis of organic matter, unless this is what the author intended? This also applies to line 278 and 361

Line 201: could the author define the BIT index, e.g. ‘branched/isoprenoid tetraether (BIT) index’

Line 201: is the equal sign most appropriate here? i.e. must the BIT value equal 1 to indicate a terrestrial signal and vice versa for 0 and a marine signal?

Line 210: a space is missing between the subsection 3.5 and paragraph below

Line 215: RPO is used here and in the captions for Figure 7 and Table 1, without it being defined anywhere in the text

Line 223: define OM, e.g. ‘organic matter’

Line 235: note here the variables. It is also not clear if the statistical analysis applied to the Py-GC-MS data? If not, this section (3.7) could be moved to below section 3.4.

Line 239: "which include TOC, C:N ratio, $\delta^{13}C_{org}$, lipid abundance and main biomarker indices" could be put into 3.7.

Line 240: stick to "Unit" rather than "Group" to make it clear that these refer to the Units seen in the figures? This also applies to line 241 and the subheadings of section 4.2 and 4.3

Line 242: could refer to Figure 2 here to allow readers to see a visual of the three Units and the outlier at 79 cm

Line 244: add following, e.g. 'The following results'

Line 252: link the sentence on GDGT analysis to the sentence prior, i.e. the BIT values also suggest a terrestrial setting

Line 271: correct to 'Fig. S1'

Line 271: what does "core" refer to?

Line 276: the "n" in "n-alkane" is not in italics

Line 281: change "Pr/Ph" to 'Pr:Ph' to be consistent with figures. This also applies to line 308

Line 298: could the author expand on "statistically lower"?

Line 340: "Soil organic carbon density" could be abbreviated here again, e.g. '(SOCd)'

Line 341: correct to 'Fig. S2'

Line 344: only time a hyphen is used before "age"

Line 378: how did the author get the <1% value?

Line 387: could this shift be explained by other factors?

Line 396: remove "the" in "Similar trends have been observed the in the Arctic…"

Line 437: typo "unti 2"

<u>Figures:</u>

Figure 1: is it possible to make the **a** and **b** more visible in the figure?

Figure 2: **a** to **e** is not easy to see, similar to Figure 1

Figure 3: change "SC/LC" in the caption to 'SC:LC' to be consistent with text

Figure 6: label "b." as phytosterols to make the figure caption consistent with the text? Also, since Pr:Ph is noted at the top of panel d, the same could be done for panels a-c, i.e. a. Hopanoids, b.

Phytosterols, c. Phytol and derivatives etc. However, this is a personal preference so feel free to ignore

Figure 7: this figure is not referred to in the text anywhere. Moreover, panel a appears to be missing an x-axis? I assume that each bar represents a split? It would be good to note the split number or temperature range below. Finally, "GC MS" in the caption is missing a hyphen

Figure 8: this figure is also not referred to in the text anywhere. Does this figure actually add anything new that Figure 3–6 does not present? If not, this could be added to the supplementary materials

Figure 9: define MAST in the caption

Supplementary materials:

A map of Friis Hill with the three sites could be added here and referred to on line 93.

Figure S3 is not referred to in the main manuscript. Is there an appropriate place in the text where this could be referred to?

**References**

Chorley, H. et al. (2023) 'East Antarctic Ice Sheet variability during the middle Miocene Climate Transition captured in drill cores from the Friis Hills, Transantarctic Mountains', *GSA Bulletin*, 135.

Diefendorf, A. F., & Freimuth, E.J. (2017) 'Extracting the most from terrestrial plant-derived n-alkyl lipids and their carbon isotopes from the sedimentary record: A review', *Organic Geochemistry*, 103.

Farrimond, P. et al. (1998) 'Biomarker maturity parameters: The role of generation and thermal degradation. *Organic Geochemistry*, 29.

Strauss, J. et al. (2015) 'Organic-matter quality of deep permafrost carbon–a study from Arctic Siberia', *Biogeosciences*, 12.

**Signed** Emily H Hollingsworth