General Comments:

The authors present a large, detailed lipidomic dataset focusing on archaeal membrane lipids and identifying distinct lipidomic patterns across an extensive area across the Scotian Slope. To my knowledge, this is the first comprehensive lipidomic study in the deep marine Scotian Slope system. The study encompasses an impressive amount of sediment cores (32) with great geographic coverage and spatial resolution. They used hierarchical cluster analysis and principal component analysis to identify distinct lipidomes that reflect the local archaeal communities, with one highlighting a cold seep area. In general, this study highlights great spatial consistency in archaeal lipidome patterns, suggesting that lipostratigraphy is conserved in this deep marine setting.

The authors demonstrate great care in lipid analysis, such as the normalization approach using a TOC decay model to correct for degradation to improve the quantitative comparability of lipid data. The dual normalization approach using lipid concentration based on extracted sediment and TOC mass is novel and well executed to strengthen the source assignment of specific lipids (sedimentary vs. upper water column).

The use of two multivariate statistical approaches (PCA and HCA) for the identification of reproducible lipidome clusters adds depth and statistical strength and is appropriate given the large number of samples. The agreement between the two methods strengthens the classification of distinct lipid assemblages.

I like the dual statistical approach, but I would argue that the description of the methodology of the HCA and PCA in 2.7 is a bit short. I am not an expert, but I think that there are likely parameters (clustering thresholds, linkage methods [Ward, Average …]) that could be mentioned here to ensure reproducibility.

In my opinion, the manuscript could generally be shortened/condensed. In some places, the sentences feel wordy or repetitive. However, in general, condensing the manuscript would improve the clarity for the reader. In addition, when revising the manuscript, more "transitional phrases" could be incorporated to improve the flow.

There are some grammar, language, and spelling issues or errors, such as missing hyphens, that I believe can easily be fixed. I mentioned a few things I noticed in the "Technical issues" section; however, my list is incomplete.

Some figures are a bit crowded. In a later section, I made some suggestions to improve the clarity of some individual figures.

Line 280: "Resolving archaeal lipid source inputs is difficult and the subject of long-term disagreement." I think this sentence is important, and in my opinion, the difficulties in source attribution solely based on lipids deserve a bit more attention or acknowledgment. If DNA-based evidence (e.g., 16S rRNA gene sequencing, metagenomics) for these samples is unavailable, it should be explicitly acknowledged in the discussion. In the Introduction, you mention that genomic analyses, including 16S rRNA amplicon sequencing and metagenomic profiling, have been done in the area, highlighting several papers. For example, ANME-1 has been found in comparable sites within the Scotian Slope in previous papers using genomic evidence. You could further highlight this in the discussion and strengthen your lipid source attributions. The Dong et al. 2020 paper seems very interesting but is only cited in the Introduction.

In general, the authors put much emphasis on ANME and Thaumarchaeota, which is understandable given the lipids and proxies used. However, it would be great to acknowledge that marine sediment likely harbors other archaea as well (e.g., Bathyarchaea), which also contribute to the archaeal lipid pool.

δ13C values of lipids (e.g., archaeol) for further evidence of ANME-2-mediated methanotrophy could also help strengthen source attributions; however, I acknowledge that this analysis might not be possible and is beyond the scope of the manuscript.

Abstract:

The abstract currently has > 430 words and has a high density of information. I would consider shortening it to a maximum of 300 words. See examples below:

In lines 27–28, the phrases "which are largely sourced from living cells" or "…that collectively are sourced from different alteration stages following the death of the cell" are unnecessary in an abstract.

The description of the Scotian Slope at the very beginning could also be condensed. Some of the precise information, like the depth gradient from 400 to 5 km and the maximum sediment thickness of 24 km, could be saved for the site description in the methods or the Introduction.

Line 22–23: Precise numbers such as "40,000 km^2" or "3° of latitudinal change" could be mentioned in the method section instead to condense the abstract.

Introduction:

Line 43: Spelling error in hydrocarbon seep

Line 54: "dominant" instead of "dominate"

Line 57: "Indicator of" instead of "indicator to"

Line 73: Rephrasing "….lipids are formed from isoprenoidal…." to "…lipids consist of isoprenoidal…"

Line 75: Be careful with the phrasing. It sounds like only glycolipids and phospholipids exist; however, IPLs are much more diverse. For example, IPLs with amino-based head groups, such as ornithine lipids, are also quite common.

Line 75–78: There is a repeated definition of CL as degraded remnants of IPLs. That can be condensed.

Line 81–82: Consider rephrasing the sentence. "Significant" seems a bit vague in this sentence and could be interpreted in different ways. Making the connection to the following sentence clearer would help.

Line 83: "such as" instead of "like"

Line 84: Consider adding specific environmental conditions that can be reconstructed as examples. I think the two references focus only on sea-surface temperatures.

Line 89: Add an "and" between "geochemical, archaeal lipid proxy ratios"

Methods:

Line 106: Consider specifying the "additional geochemical evidence" that indicated hydrocarbon seepage

Table 1: The font size is quite small. Maybe consider putting this table in the supplements. It is a bit overwhelming. Alternatively, you could put the full table into the supplement but add a condensed version of the most important information here. I think that information like the coordinates, top and bottom core sample depth, and extracted sediment mass are better fit for the supplements.

Line 157: Spelling mistake in "1-alkyl-2-acetoyl-sn-glycero-3-phosphocholine" should be "1-alkyl-2-acetyl-sn-glycero-3-phosphocholine"

Line 159: Consider rephrasing "following additional protocols" to "following adaptations from Bentley et al. (2021)" or "following modifications from Bentley et al. (2021)." Otherwise, it sounds a bit like you are using additional extraction protocols other than the Bligh and Dyer protocol.

Line 161: Consider removing the phrase "living".

Results:

Line 225: You mentioned "plant-based" Chlorophyll a; however, could algae and cyanobacteria not also be contributing sources for chlorophyll?

Line 228: Consider rephrasing "0–3" to "GDGT-0 to GDGT-3" or "1G-GDGTs comprising 0–3 rings" for clarity.

Line 228–232: Glycerol dialkyl glycerol tetraether is spelled out multiple times. For better readability, consider using the abbreviation. For example, instead of "Hydroxyl diglycosidic glycerol dialkyl glycerol tetraethers," write "Hydroxylated 2G-GDGT" since 2G-GDGT was already defined before.

Discussion:

Line 257–260: This section seems somewhat redundant. The first sentence already states that isoprenoidal GDGTs are mainly produced by ammonia-oxidizing Thaumarchaeota, while the second sentence essentially repeats this, referring to specific GDGT distributions. Consider rephrasing or condensing this part. Also, to my knowledge, there are currently no confirmed thermophilic Thaumarchaeota, so the term "non-thermophilic" may be unnecessary.

Line 270: The phrase "methanogenic microorganism" may be misleading, as archaeol is generally produced exclusively by archaea, not other microorganisms.

Lines 273–275: These sentences may be more appropriate for the Results section.

Line 308: "GDGT moieties" instead of "GDGTs moieties."

Line 310: Consider rephrasing to "…distribution patterns of intact lipids from presumably viable sedimentary archaea differ significantly from …." or something similar.

Line 317: Consider using "reflect" instead of "arise from"

Line 318: A spelling error in "six group(s) of samples." Also, consider maintaining consistency in how numbers are presented. Sometimes they are written out (e.g., "six"), while other times they are shown as numerals (e.g., "6").

Line 322: Remove "analysis" as PCA already includes "analysis"

Line 393: Spelling error in ANME

Line 393: You mention that the increasing IPL-MI increases with depth, highlighting a higher proportion of ANME-1 and 2. However, I believe ANME-2 are not thought to be producers of GDGTs and instead produce diether lipids. Thus, I do not think mentioning ANME-2 specifically in this sentence is correct.

Conclusion:

Line 441: I think the suggestion that the increase in degradation products resulting from heterotrophy by microbial sulfate-reducing bacteria may be overly speculative for the conclusion as there is no direct evidence for this process.

Line 441 to 442: This sentence is also used in the abstract. Maybe consider rephrasing it slightly here to avoid exact repetition, especially since it is one of the main findings.

Line 443–445: The very end of the conclusion ends with you highlighting a limitation of this study and mentioning that these biomarker proxies did not fully resolve the existing biostratigraphy. While it is important to inform readers of limitations and room for future development, I would not put this as your closing statement. Maybe reorder the conclusion and end the manuscript with a strong statement emphasizing a key finding or novel aspect of your study.

Figures in general:

The font size should be increased for better readability, especially in Fig. 3, 4, and 8.

Figure 3:

Consider increasing the font size of the legend and the x-axis. While it may still be readable, I think it is overly small. I like that this figure gives the reader a great overview. All discussed lipids and the corresponding depth profiles are visualized in one image. However, it also looks a bit crowded. I want to suggest some modifications that may improve the figure:

1. Consider making it a bit more obvious where one quadrant starts and another begins, perhaps by highlighting quadrants B and D in light grey or another color of your preference.

2. In general, you could change the absolute abundance column to a line plot and make the column narrower to save space. Effectively, the relative abundances are shown twice. Once in the relative abundance plots and again in the bar plot, including absolute abundance. I think that might be redundant. This is particularly true for the photosynthetic pigments, where I recommend displaying only the absolute abundance plot.

Figures 6 and 7 may deserve more attention as they are somewhat underexplained. I assume they primarily serve to confirm the results shown in Figure 5, but providing more guidance for the reader when discussing these figures could be helpful.

In the caption of Figure 6, the SATZ is mentioned. Including a visual marker of this boundary would be helpful, as it appears to be a central geochemical transition. This would make it easier for readers to connect the SATZ to the lipidome shifts.

Figure 9:

If I did not miss it, you never explicitly acknowledge Figure 9 in the text. It should be put into the supplements, or you should acknowledge it in the text somewhere. If possible, I would include the core location in the figure. Also, it is unclear how exactly the boundaries of the specific lipidomes were determined. I assume it is interpolated based on the core location; thus, it would help to see them in the image. Since this image shows one of the main conclusions of the manuscript, I would elaborate a bit more on how it was created.

Supplements:

Table S2: In the references, you write Stuart et al., 2004. I assume you mean Sturt et al., 2004.