

Biogeosciences Review

We again thank the reviewers for the time and effort in improving our manuscript. Comments and a detailed account of the changes that have been made to the revised submission are presented below.

1. I thank the authors for clarifying how TOC concentrations in each sample were corrected to an expected value of the original TOC based on the “regionalized Scotian Slope sediment TOC depth profile”. I re-read the methods and still see the regionalized profile to be a key part of the way normalized lipid concentrations are calculated but also found that the method used to create this regionalized profile is not explained anywhere. Adding this explanation to the methods is needed.

I searched through the manuscript for evidence of how this regionalized profile was determined and the only clue is in the legend of the left-hand panel of figure 2 (no panel labels), a green line marked “Regression”. It is not easy to tell what kind of regression this is except that it is clearly not linear. And again, I note that the fit is poor between the regression and data, overestimating the TOC concentration for almost all actual samples at shallow burial depths and underestimating it at deeper sediment depths. This poor fit would result in systematic error with a depth-based bias when calculating lipid concentrations normalized to the “original” TOC concentration considering how the regression model deviates from your data.

There are established models for organic matter degradation and TOC concentrations in sediment profiles like the classic model proposed by Middelburg, 1989 or the version that includes methane cycling proposed by Wallmann et al, 2006. The review by Arndt et al., 2013 contains a summary of models (Fig. 11) that includes a couple of published models made very close to the study location. Although a suggestion to go back to the data analysis stage is not going to be particularly welcome during a manuscript review, changing the regionalized TOC depth profile to one that is generated by a better, published model should not take too much work but would significantly improve on the robustness and accuracy of the normalized lipid concentrations and comparisons between them.

Recommend adding panel letters to Figure 2. [This has been done.](#)

References:

Middelburg, J. J. 1989. “A Simple Rate Model for Organic Matter Decomposition in Marine Sediments.” *Geochimica et Cosmochimica Acta* 53 (7): 1577–81.

Wallmann, K., Aloisi, G., Haeckel, M., Obzhirov, A., Pavlova, G., Tishchenko, P., 2006. Kinetics of organic matter degradation, microbial methane generation and gas hydrate formation in anoxic marine sediments. *Geochimica et Cosmochimica Acta* 70, 3905–3927.

Arndt, S. , B. B. Jørgensen, D. E. LaRowe, J. J. Middelburg, R. D. Pancost, and P. Regnier. 2013. “Quantifying the Degradation of Organic Matter in Marine Sediments: A Review and Synthesis.” *Earth-Science Reviews* 123 (August): 53–86.

In light of the above comments, we have reviewed the provided references and found that they do not conflict but support our technique. As such, these have been added to the paper in line 168. We have also rewritten this section for better clarity. The section heading has been changed to "Calculation of adjusted TOC concentrations for lipid normalization." We have added additional text to the beginning of the section which better clarifies the intent and direction of how sediment TOC was adjusted to factor out expected losses from diagenetic processes. We also realized that the prior regression model did induce bias to the lipids normalized at both shallow and deep intervals of the sampled sediment depth intervals. The logarithmic regression statistic was applied to 82 core sediment samples as the best fit line to the merged downcore TOC profile. The statistic produced an R^2 value of 0.48. We have examined the data analysis again and find the regression technique still produces the most optimal fit. However, in our reanalysis, we realized that two TOC samples deeper in the profile behave as outliers. We have removed those (now $n=80$). This produces a tighter fit to the data (particularly at the deeper sediment depths) with a new R^2 value of 0.58. The merged TOC normalized data (Fig. 4) was then renormalized to the lipid data, but adjusted values did not change the normalization profiles, and no further edits have therefore been made to those parts of the text.

2. I understand that the authors feel Discussion Section 4.1 is necessary to educate the readers of Biogeosciences but I would suggest this is highly unusual, unnecessary for most readers with an interest in this topic and makes a long paper even longer. I have never seen a discussion that has an entire section with no mention of the actual data at all; a discussion section exists to discuss the data. This material could be used to expand the short sections describing "Lipidome" A1-A4, although I do not think this is necessary. Those paragraphs are clear enough. It could also be turned into a mini review separate from this paper.

We realize there is considerable push-back on this point. Given the paper is long we have moved this section to the supplemental information and have included the below statement to mark the existence of this information for those who have additional interest in knowing lipid sourcing.

4.1 Chemotaxonomic relationships

Details on the chemotaxonomic relationships of the resolved Scotian Slope archaeal lipids can be found the Supplemental Information.

3. I also include responses to the final author comment copied here in quotes with my response below:

"While older uses of term 'lipidome' have been quite narrow such as being the full characterization of lipid molecular species and of their biological roles with respect to expression of proteins involved in lipid metabolism and function, including gene regulation or within a cell. More recent descriptions of the term loosely define it as the entire spectrum of lipids in a biological system (e.g. Seppaneo-Laakso and Oresic, 2009; Swinnen and Dehairs,

2022). That implicitly extends to ecological systems such that the adjective ‘biological’ is often dropped within environmental lipidomic based discussions. The term is therefore correctly applied in this study”

The authors’ reply that their definition of “lipidome” (including both biological lipids and their degradation products), is different from many others that just include biological lipids including the references that were included. There is room for misunderstanding when implicitly extending this term to ecological systems when either dropping the “biological” or not dropping it are both done. Therefore, this use of the word should be defined at its first instance. For example at the end of the introduction on line 82, something like “distinct assemblages of archaeal lipids, referred to here as lipidomes”

The correction is done and the sentence now reads:

“The resolvable assemblages of archaeal lipids, referred to here as lipidomes, are examined across three spatial dimensions: sediment depth, distance down the continental slope, and along ~3° latitude change of the northwestern trend of the continental margin.”

Also,

“We would also argue that one of the primary purposes of producing lipidomic studies is to establish reliable inferences on what comprises existing, past, and ancient microbiomes when other ‘omics’-based approaches are either not available or are unsuited for use in a particular study. The longevity of how well such interpretations hold up is of course an omnipresent issue impacting all organic geochemistry biomarker-based studies.”

I will still maintain that attempting to infer archaeal community composition from lipid distributions when many important sedimentary bacteria (e.g. Bathyarchaeota) have uncharacterized lipid profiles pushes beyond the limits of current knowledge and is almost certainly inaccurate regardless of what we might wish lipids could tell us about existing, past, and ancient microbiomes. But, at this point, I’ll leave that up to the editor and potentially the readers of this manuscript to judge the wisdom of doing this.

This is a truly valid statement. It is a piece of the puzzle. Additionally, while archaea and bacteria form commensal and symbiotic associations, we cannot detail these relationships here and the ability for lipids as a standalone proxy to do this is contingent on the acquisition of additional data (namely their compound specific isotope compositions). Nonetheless, we have additional manuscripts in prep that entirely focus on the bacterial lipid assemblages of the Scotian Slope and will be working to understand slope sediment community dynamics using these targets.