

Biogeosciences Discuss., author comment AC1
<https://doi.org/10.5194/bg-2021-232-AC1>, 2021
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Reply on RC1

Edgart Flores et al.

Author comment on "Bacterial and eukaryotic intact polar lipids point to in situ production as a key source of labile organic matter in hadal surface sediment of the Atacama Trench" by Edgart Flores et al., Biogeosciences Discuss.,
<https://doi.org/10.5194/bg-2021-232-AC1>, 2021

Response to Reviewer 1 Comments (RC1):

We thank the reviewer for the constructive comments

Specific comments

RC1 – 1: The lipid extraction and analytical methods are appropriate for the sample types in the study. Please report the type of chromatographic column in the methods section.

Response RC1 – 1: We will add details of the chromatographic column in the methods section: "We used Waters Acquity BEH Amide column (2.1 × 150 mm; 1.7 μm particle size) that enables class-specific separation of polar lipids based on their hydrophilic head group (Wörmer et al., 2013)."

RC1 – 2: I appreciate the use of deuterated standards to account for matrix effect on quantifying lipids. Would it be possible to include an assessment of the importance of this treatment for the benefit of planning future studies on sedimentary IPLs? How was the correction applied? What was the overall impact of including this step on the final data reported? Were there different effects observed for the different lipid classes? Since deuterated standards were added just for PC, PG, PE and DGTS, was there a way to correct the concentration calculations for the other lipid classes as well? If not, how does this affect quantitative comparisons between lipid classes?

Response RC1 – 2: We agree that this is an important aspect to highlight in more detail for future studies. We corrected for the matrix effect on ionization efficiency by comparing the loss of signal observed between deuterated standards (PC, PG, PE and DGTS) analyzed both pure and spiked to samples. While we did not have deuterated standards available for other IPL classes, we found that the matrix effect was consistent among the four standards we tested and accounts for a $\sim 7 \pm 0.6$ % loss in ionization efficiency. Therefore, since the matrix effect appears to have a similar impact on these 4 different lipids classes, our overall results are unlikely to be significantly biased by the lack of other deuterated standards. We plan to acknowledge these observations more explicitly in the revised version of the manuscript.

"Unfortunately, we could not find more deuterated standards to test for other IPL classes. However, on average, we observed that the matrix effect accounts for a $\sim 7 \pm 0.6\%$ loss in ionization efficiency for the four available deuterated standards. Therefore, it is reasonable to assume a similar loss for other IPL classes, although this remains to be tested in future studies. We highlight the importance of using as many IPLs classes as possible to account for both differences in ionization and matrix effect when performing IPL quantification in environmental samples."

RC1 – 3: Figure 3 is very helpful for understanding the clusters described in Figure 2. It shows which specific IPLs are the main controls on the differences in lipid class composition that defines clusters. The font size in figure 3 is very small, though, so could that figure be rotated 90 degrees to increase its size?

Response RC1 – 3: We will correct this figure in the revised version.

RC1 – 4: In Table 2 and in the text, how did the authors decide to use the DAG designation for PE and PG abbreviations at some places and not others? PC also has AEG species, so should PC-DAG be used? It also appears that SQ and SQDG are used interchangeably. I'm mostly thinking about using consistent naming, so the reader isn't looking for a distinction between different abbreviations used for the same compound.

Response RC1 – 4: Thank you for this remark. We use DAG to designate a diacylglycerol and AEG to designate an acyletherglycerol; thus, we will use this same nomenclature for PCs. Also, we refer to SQ throughout the manuscript as SQDG. We will standardize all compound abbreviations to ensure consistency.

RC1 – 5: In sections 4.2, 4.3, and 4.4, I really like the introduction paragraphs for each IPL class describing possible biological sources.

Response RC1 – 5: We are glad to hear that the inclusion of this text is meaningful.

RC1 – 6: How do the authors define IPLs with short vs. long-chain fatty acids? Is there are consistent definition, or does it vary by IPL class? In lines 472-473 and 575-577 there is overlap in the ranges.

Response RC1 – 6: We describe carbon ranges following previous studies (i.e. Rêzanka et al., 2009; Schubotz et al., 2009a; Brandsma., et al., 2011), where short- and long-chains refer to combined alkyl chains of C_{28} - C_{36} and C_{36} - C_{44} , respectively. We will add this information in the revised version.

RC1 – 7: Figures 7, 8, and 9 are very difficult to read at the size of a printed page. Would it be possible to provide a simpler summary figure in the manuscript and include the three figures as nine individual figures in the supporting information?

Response RC1 – 7: We agree with the reviewer about the benefits of presenting a simpler summary of these three different figures while moving their detailed individual versions to the supporting information. We will change this in the revised version.

RC1 – 8: The conclusions section could better represent the work. Conclusion 1 (801-804) is well supported. The middle part of conclusion 2 (806-814) repeats part of conclusion 1. The five IPLs listed on line 808 are in such low abundance that I don't think they warrant their own conclusion as possible distinctive biomarkers of trench communities. They could very well be in other biota but unreported because they are quantitatively insignificant in

an organism's lipidome. Why not focus conclusions more on drawing specific conclusions based on the significant differences in PG, PC, MGDG, DGDG, SQDG, DGCC, DGTS, and DGTA that are so clear in Figures 7-9? That's really interesting and should be a major part of the conclusions.

Response RC1 – 8: We agree with the referee's comment regarding a revised conclusions section that better highlights the most important contributions of our work. We will add this in the revised version. Please see a more detailed reply to a similar comment by Reviewer 2 below (RC2 – 4).

Technical Corrections:

RC1 – 9: Line 596: Add heading for Potential Sources of Betaine Lipids.

Response RC1 – 9: We will correct this.

RC1 – 10: Line 649: Are the 21-38 carbons just on the fatty acid or also on the ceramide chain?

Response RC1 – 10: We report both, the total number of carbons including the ceramide chain and the fatty acids. We will add this sentence: "[...] shows Glycer with ceramide chain, and polyunsaturated fatty acids with C₂₁₋₃₈ [...]"

RC1 – 11: Line 694-696: Correct incomplete sentence.

RC1 – 11: We will correct this in the revised version.