

## Response to Referee #1

This study aims to determine the intact lipids in suspended particles in the water column using samples collected from ocean oxygen minimum zones from the east/north pacific. OMZs are important marine ecosystems particularly with regard to oceanic N cycles. The comprehensive data presented in this study has significantly advanced our understanding of IPLs in this unique environment. This reviewer has no major concerns. Some specific comments are listed below for further improvement of the manuscript.

We thank Referee #1 for the positive comments.

L93, it would be very useful to specifically discuss previous IPL studies on OMZ samples, e.g ETNP, ETSP, Arabian Sea etc in discussions.

Most of the previous IPL studies performed in other OMZ regions have either focused on very specific IPLs for specific processes (e.g., ladderane lipids and HPH-crenarchaeol for ammonium oxidation in the Arabian Sea) or only discussed surface water IPL distribution of phototrophic organisms (in the ETSP). We therefore consider this study to be the first comprehensive study of IPLs in OMZs. Nevertheless, we made sure to refer to previous IPL studies in the discussion where appropriate, e.g. lines 564-565, lines 667-668.

L101 and throughout the MS, most previous studies have used MGDG, DGDG and SQDG to refer to mono- and di glycosyl- DAG and sulfoquinovosyl DAG, please change to these commonly used acronyms for the sake of consistency in literature.

We are aware that we use different abbreviations for these glycolipids than are often used in the literature. However, since we want to be consistent with our nomenclature, i.e. we are also calling mono- and diglycosyl GDGT 1G-GDGT and 2G-GDGT, we opted to stick to the currently used acronyms 1G-DAG and 2G-DAG. This nomenclature is also relevant when describing head groups with different core lipid structures, i.e. SQ-DAG and SQ-AEG.

We would also like to point out that there is plenty of literature in glycolipid research, particularly bacterial glycolipid research, where besides MGDG and DGDG (which are typically the acronyms for the specific thylakoid lipids monogalactosyl and digalactosyl diacylglycerol), the other sugars are referenced as Glc-DG, GlcGal-DG, depending on sugar type (Glc for glucose, Gal for galactose, etc.). We are therefore not that unique with our chosen nomenclature, which specifically highlights that we do not know the types of sugars (which surely change with the source organisms).

Since we are defining the used acronyms in the text (we also now added a footnote for clarification, page 6), we do not think this to be very problematic.

L104-5 , 1307-9; this may be a little misleading since DGTS has been found in a wide range of marine heterotrophic bacteria.

We noted here that DGTS has also been detected in marine heterotrophic bacteria in phosphate-limited environments (line 105-106).

L116, please refer to recent study of Hunter et al., AEM doi: 10.1128/AEM.02034-17 for novel diglycosylceramides found in *Thalassiosira*.

Done.

L214-215, the authors have referred to previous studies for mass spectral interpretation and IPL assignments. It would be very useful to summarize and synthesise these information in a table (or in the supplementary information) and to detail the criterial for IPL identification. Presumably IPL assignment is based on comparing to retention time of standards (where applicable) and characteristic MS/MS patterns, representative characteristic ions or characteristic neutral loss. How has DGTS but not DGTA been conclusively assigned in this study? Has DGTA been found in any samples?

There are on average between 600 and 800 compounds that are identified and quantified in each sample. Since IPL identification is quite complex, it will be difficult to provide all the necessary information in a comprehensive table that will explain each lipid identification. In spite of this, upon the reviewers' request, we included a table in the Suppl. Material (Suppl. Table 3) that provides examples how mass spectral assignment of lipids was conducted. Furthermore, we clarified in the text how we identified DGTS over DGTA (lines 220-223).

L223, for unknown aminolipids AL1 AL2, do the authors have any hypothesis of their structures based on MSn fragmentation patterns (suppl fig 4)? What are the possible amino head group structures? Have accurate ms of AL1, AL2 been obtained?

Unfortunately, we have not more insights or hypotheses on the headgroup structures of AL-I and AL-II other than the ones provided in the text and figure caption. We have accurate masses of AL-I and AL-II fragments up to the third decimal point, which is why we provide sum formulas for the potential headgroup fragments (see Suppl. Fig. 4), but unfortunately we cannot provide any further insights on their structures.

The authors mentioned CSRD FISH data in supplementary dataset but did not mention how this was done in the materials and methods.

This data is from Podlaska et al. (2012), the methods for the CARD-FISH analyses are also described in this paper. We made a reference to this paper in the respective figure caption.

My general impression for discussion is that it can be shortened significantly.

We revised and shortened the discussion substantially from the originally 19 (Word) pages to 13 pages.

It is a pity that no microbial diversity data were obtained in this study as one would like to see the correlation between specific microbial groups and IPLs, which may provide clues for the origin of these lipids, particularly w.r.t. to AL1 and AL2.

We agree that having microbial diversity data would have greatly improved this manuscript and we will ensure that such data will be available for future IPL studies. We did, however, try to correlate the IPL data with the available CARD-FISH data, but unfortunately did not see significant correlations (see section 4.2.2).

Section 4.1.1 two recent papers (Carini et al, Sebastian et al) have shown marine heterotrophic bacteria are also abundant in MGDG. These need to be discussed here in line with these new evidence.

We now added a sentence stating the potential for heterotrophic bacteria to be sources for these glycolipids (line 482) and then refer to the acyl side chains to further delineate if bacteria are indeed potential sources or not.