Response to Reviewer 1 Comments (RC1):

We thank the reviewer for the constructive comments

RC1-1: In this work, Cantarero et al. investigated the lipid remodeling in phytoplankton in response to various environmental variables by mesocosm experiments, including oxygen concentration, temperature, pH, nutrient concentration, chl-a, and light availability. By combining multiple linear regression and random forest model, the main and secondary factors affecting lipid category and lipid distribution were identified. In general, the presented results are interpreted by suitable assessment methods and the novel and important conclusions are supported by the data. In addition, the paper is very well written and fits in quite well with the theme of this special issue, I recommend the manuscript for publication after the following several problems are addressed.

Response RC1–1: Thank you for your positive review of our work and for the valuable comments to improve this manuscript.

General comments

RC1-2: What substances can be called intact polar lipids, it is best to give some examples in the introduction section, and contain specific standardized definitions.

Response RC1–2: We agree and will provide more detail to their specific structural definitions that include the breadth of molecules analyzed in this manuscript.

We will add this sentence: "Intact Polar Lipids (IPLs) are a class of membrane lipid characterized by a polar head group typically attached to a glycerol backbone from which aliphatic chains are attached via ester and/or ether bonds (Sturt et al., 2004; Lipp et al., 2008; Schubotz et al., 2009; Van Mooy and Fredricks, 2010). Dominant planktonic lipid classes include phospholipids with a phosphate-bearing polar head group (e.g., phosphatidylcholine PC; phosphatidylethanolamine PE; and Phosphatidylglycerol PG), glycolipids featuring a sugar moiety in the polar head (e.g., monoglycosyldiacylglycerol MG; diglycosyldiacylglycerol DG; sulfoquinovosyldiacylglycerol SQ), and betaine lipids with a quaternary amine positively charged and attached to lipid chains (e.g. diacylglyceryl hydroxymethyl-trimethyl-β-alanine DGTA; diacylglyceryl trimethylhomoserine DGTS; and diacylglycerylcarboxy-N-hydroxymethyl-choline DGCC) (Kato et al., 1997; Rütters et al., 2001; Zink et al., 2003; Suzumura, 2005; Van Mooy et al., 2006)".

In addition to their corresponding references:

Lipp, J. S., Morono, Y., Inagaki, F., and Hinrichs, K.-U.: Significant contribution of Archaea to extant biomass in marine subsurface sediments, Nature, 454, 991–994, 2008

Kato, C., Masui, N., and Horikoshi, K.: Properties of obligately barophilic bacteria isolated from a sample of deep-sea sediment from the Izu-Bonin trench, Oceanogr. Lit. Rev., 1, 53–54, 1997

Rütters, H., Sass, H., Cypionka, H., and Rullkötter, J.: Monoalkylether phospholipids in the sulfate-reducing bacteria Desulfosarcina variabilis and Desulforhabdus amnigenus, Arch. Microbiol., 176, 435–442, 2001

Suzumura, M.: Phospholipids in marine environments: a review, Talanta, 66, 422–434, 2005

Van Mooy, B. A., Rocap, G., Fredricks, H. F., Evans, C. T., and Devol, A. H.: Sulfolipids dramatically decrease phosphorus demand by picocyanobacteria in oligotrophic marine environments, P. Natl. Acad. Sci. USA, 103, 8607–8612, 2006

Zink K-G, Wilkes H, Disko U, Elvert M, Horsfield B. Intact phospholipids—microbial "life markers" in marine deep subsurface sediments. Org. Geochem; 34: 755-769, 2003

RC1-3: Page 4, Line 36: What is the specific sampling depth of the surface and subsurface layers, and what is the difference between them? There doesn't seem to be an obvious definition.

Response RC1–3: We agree this information is a bit unclear in the methods sections and we will clarify the usage of terms surface/subsurface and the exact depth intervals they refer to.

We will add this sentence: "The samples were segregated into surface and subsurface layers, which were slightly modified over the course of the experiment to accommodate for changes in water stratification and the position of the chemocline (refer to Bach et al., 2020 for further details). The depths were 0–5 and 5–17 m from Day 1 to 2, 0–10 and 10–17 m from Day 3 to 28, and 0–12.5 and 12.5–17 m from Day 29 to 50.

RC1-4: How efficient is it to use the lipid extraction method described by the author, and has the author conducted relevant validation? Moreover, the analysis conditions of mass spectrometry need to be mentioned appropriately briefly in the methods section, rather than directly citing the literature. What was the detection limit for the various lipids in this study?

Response RC1–4: The modified Bligh and Dyer extraction method is considered comprehensive in the lipidomic community as it contains 3 different extraction buffers to facilitate dissolution across a large range of analyte polarities and pKa values. We also include a recovery standard (C_{16} PAF $C_{26}H_{54}NO_7P$) to account for potential inefficiencies in the extraction and dilution steps. These details along with the mass spectrometry analysis were originally referenced from a previous publication (Cantarero et al., 2020), but will be added to the methods section in this manuscript for clarity and completeness. We will also report the limit of detection for each lipid class based on individual calibration curves.

RC1-5: Since I am not an expert in this area, I would like to ask whether the sample number requirements of random forest can be met in this study, and how many sample number were used to conduct it?

Response RC1–5: We used a total of 72 samples to conduct the random forest analysis. Random forest utilizes a bootstrap aggregation to define and average many permutations of

an out of bag score in prediction performance. This provides an effective procedure for high-dimensional data with small sample sizes (Biau and Scornet, 2016) and is popular within a number of related disciplines in the water sciences (Tyralis, 2019) ecological/species distribution models (Luan et al., 2020) and bioinformatics/high throughput genomics (Chen and Ishwaran 2012; Boulesteix et al., 2012). This additional information and the references cited will be included in the methods section.

RC1-6: Authors would be well advised to standardise the format of journals for references, mostly abbreviations but also full names, e.g. Nature Communications. Please check the format of references in the manuscript.

Response RC1 – 5: We note that the export of references did not function as intended and will correct them in our revised version of the manuscript.

RC1-7: Figure 2C: This figure lacks the axis title of the right Y-axis, which is the total chl-a concentrations in μg/L.

Response RC1 – 7: Thank you for bringing this to our attention. This axis title will be added to the figure.

Minor comments

RC1-8: Page 3, Line 91: Please check this sentence.

RC1-9: Page 6, Line 78-84: Dichloromethane:Methanol:Phosphate buffer, Dichloromethane / Methanol / Trichloroacetic acid buffer, it is better to unify the two forms. N2 required subscript.

RC1-10: Page 7, Line 11: n = 34in total, lack of space.

RC1-11: Page 22, Line 88: This sentence lacks a full stop. Line 13: 2m and 17m, lack of space.

RC1-12: Figure 8A: Check R^2 in the diagram.

Response RC1 – 8-12: These corrections will be implemented.