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Interactive comment on "Increasing P-stress and viral infection impact lipid remodeling of the picophytoplankter *Micromonas pusilla*" *by* D. S. Maat et al.

Anonymous Referee #2

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This is an interesting study focusing on the intact polar lipid (IPL) composition of the picophytoplankton Micromonas and how it is affected by phosphate (P) stress and viral infection under chemostat and batch culture-based experiments. This research follows a nice article published by the same main authors in 2014 in AEM that was focusing on the growth rate and viral infection cycle under the same conditions for the same species. In this present study, the authors found that the IPL cellular composition is impacted by the different nutrient stresses and under viral infection. Moreover, it is hypothesized that there is minimal PG quota required under P-stress as long as cells maintain growth. Finally, they precisely described the IPL-Fatty Acid (FA) cellular composition of Micromonas Mp-LAC38 and one of its virus (MpV-08T), showing similarities

and differences. The results described here significantly contribute to the understanding of the physiological responses of an important marine phytoplanktonic species to nutrient stress and viral infection. These results are a demonstration that within the phytoplanktonic community (besides diatoms, cyanobacteria and haptophytes), the picoplanktonic green algae also modify their lipid composition under P limitation and starvation.

General comments: There are many different factors which are studied in this growth experiment: two different carbon dioxide concentrations, different P stress levels and a virus infection. This is the strength of this study and why I found it original compared to other studies, but in the current form of the manuscript, this is also a weakness. Indeed, I often found myself lost trying to understand what kind of samples we were talking about, especially when dealing with the cultures that grew under P-limited conditions prior to starvation. I was sometimes mixing together the samples taken during limitation and starvation (post-limitation). I invite the authors to re-work their discussion parts when these treatments are mentioned and make the different treatments clear. I really appreciated though that the authors made the distinction between limitation and starvation as these two states are clearly different and generally overlooked in culture-based studies while this is ecologically highly relevant. Even if the CO2 enrichment does not seem to show any significant results, I think that some information about this side of the experiment is missing (e.g. the pH of the cultures, is there any suspected C limitation?...). Since Slapeta et al. 2006, Micromonas pusilla has been considered as a cryptic species (i.e. high genetic variability hidden behind an identical morphology). However, genomic data and global distribution of Micromonas tend to show that strains from different genetic clades are highly divergent and might not be considered as the same species. This is not the subject of this paper, but I highly recommend the authors to specify from which genetic clade the strain Mp-LAC38 is grouping with (sensu Slapeta et al. 2006, or Worden et al. 2009 or else) in order to avoid any confusion in the future. The careful analysis of the lipid composition of Micromonas using HPLC-MS, the comparison of host and viral lipids and the physiological effects of different levels

of P-stress provide valuable information. However, looking at the discussion about ratios of lipids under viral infection, I wondered if there was replication of sampling, or at least, technical replication of lipidomics analysis to give an idea of variability (especially when you see the low proportions of PGs in starved cultures). Besides my concerns (see specific comments below) and critics, I think that this complex experiment and analysis deserve to be seriously considered for publication in Biogeosciences.

Specific comments: Pg 15585, l. 6-20: in the Introduction, it should be specified that phytoplankton are subjected to a number of limitation, and P is only one of them. Sometimes in co-limitation with other, sometimes alone, especially in some oceanic area.

Pg 15586, l. 25: see general comments and specify here the Micromonas clade you worked with.

Pg 15588, l. 17: please specify the duration of a cycle of lysis for the virus MpV-08T as I find it unusual for a one-step growth experiment with a 100% infectivity rate to take so long (i.e. 30 hours) before seeing cell lysis ("minimal" though). I understand that under P stress the viral cycle takes longer; maybe to give numbers for a P-repleted culture infected by the same virus could give a better sense of how the cycle of lysis is delayed. Explain why data are not shown in the article about infected Micromonas under P-replete.

Pg 15591, l. 13-14: could you quickly explain why the FA combinations could not be determined for PGs, it might help the general reader to better understand the method as metabolomics approach is sometimes cryptic to define.

Pg 15591, l.21: could you be more specific about the rationale behind the analysis of these specific ratios. Is it because those are the only ratios for which you detected linear correlations? Or is there a more logical reason for skipping the other ratios?

Pg 15593, l. 17-18: this comment is related to the previous one (see above). Should this assertion be supported by an analysis of the ratio of MGDGs to DGDGs, and how

it evolves with increasing P stress?

Pg 15596, l.8-9: Is the viral genome available? A genomic analysis looking for genes involved in lipid biosynthesis pathways would give some clues about the de novo production if the viruses possess the required genetic information.

Figure 3. The figure has to be better labelled because this probably describes the strongest message of your story but it is complicated to get a simple and quick understanding of it. For example, you should state (on the figure) which symbols are from the limited growth cultures and which ones are from the starved cultures.

Technical error: Pg 15592, l.22: parenthesis missing.

Interactive comment on Biogeosciences Discuss., 12, 15583, 2015.