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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 #1

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The authors present a complex culture-based study to tease apart the multivariate effects of phosphate (P) stress and viral infection on the intact polar lipid composition of the picophytoplankton Micromonas. They found that growth history prior to nutrient starvation impacted the extent of lipid remodeling in both infected and uninfected Micromonas. Additionally, they found that the lipid composition differs between Micromonas and its virus, MpV-08T, and that Micromonas has a minimum quota for PG-lipids to maintain growth. These results contribute to our understanding of the physiological implications of nutrient stress and viral infection in an ecologically-relevant phytoplankton, and illustrate a potential mechanism (i.e., lipid remodeling) for coping with these stressors.

General comments:

This study has several strengths that distinguish it from much of the literature cited. The authors chose an experimental design that examined the impact of both nutrient stress and CO2-enrichment simultaneously, with two levels of intermediate P stress, thus allowing evaluation of a gradient of P limitation, rather than just P replete and P starved growth conditions. Likewise, comparison of host and viral lipids as well as combined analyses of IPL and FA provide new information on the interplay between phytoplankton hosts and viruses, as well as lipid dynamics.

I have several major concerns about this study in its current form. This is clearly a very complex study with many interrelated variables, and one of my concerns is whether or not the appropriate tests/controls have been included to tease apart the effects of the multiple factors. Contributing to my confusion on this point is the inconsistency in how the various treatments are referred to in the manuscript. For example, it is not always clear to me which "P-starved" treatment is being discussed (with the exception of Table 2, where the breakdown of treatments is clear). It may be helpful to develop an alternative naming scheme for the treatments, but regardless, please carefully revisit the manuscript to clarify this so the reader can better follow your conclusions about which treatment showed what trends. Related to this, I would like to see some comments about how effects of the various culturing techniques were accounted for, or if not accounted for, how they may have impacted the results. Finally, I find myself wondering whether there is any lipid remodeling occurring during viral infection under P-replete conditions, and also whether the enzyme activity is affected by viral infection. With so much evidence mounting in the literature regarding viral manipulation of host metabolism, I feel this would serve as a good baseline for comparison with the P-starved/infected treatments and allow better resolution of the specific factors driving each trend.

Additionally, the organization of the paper needs improvement, both for the text (particularly the introduction) and the figures. The statements of rationale for the study

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vastly undersell its significance, and important conclusions are often buried in complex wording in the paragraphs of the discussion. Likewise, I feel that the figures are not arranged in a way that showcases the most important findings. Re-framing the introduction and results/discussion of the manuscript will help readers quickly grasp the significance and value of the data presented.

Specific comments:

Page 15585, line 9: I believe you mean “heterogeneous” here, not “heterogenic” (which is related to genetics).

Page 15586, lines 3-6: It seems a bit odd to me to emphasize nitrogen so much in this paragraph, since this study is focused on phosphorus and not on evaluating the dependence of Micromonas on nitrogen. Consider revising.

Page 15588, lines 4-6: I appreciate the rationale stated here.

Page 15588, line 7: Can you please provide more details about the starvation hereâĂŤhow quickly did starvation set in? How did you determine when the cells were starved?

Page 15588, lines 15: If this viral strain has been used in previous publications, please cite them here.

Page 15588, lines 17: Can you please include an indication of the variance from the MPN assays? My understanding is that the estimate from an MPN assay includes a lower and upper limit, which could be helpful here to judge the potential range in infective virus:host ratio.

Page 15589, lines 25+: Please include more details about the enzymes assays. These assays are very tricky to run correctly, and many variables can influence the outcome. This is especially important since one of the primary conclusions is that the degree of lipid remodeling is related to P-stress and this is the only metric of P-stress included in the paper (if you have another metric such as measured growth rate or doubling time,

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that would also be helpful to include).

Page 15591, line 20: Were the tests performed regressions or correlations? Here the term regression is used, but elsewhere (Page 15592, line 21) it says correlation. I would like to see information here about whether the data conform to assumptions of the test or if a non-parametric test would be more appropriate. It would also be very helpful to have a clearer understanding of what data were included in the analysis (were the virally-infected treatments also included with the uninfected?), and why you might have expected a linear correlation (my opinion is that a non-linear relationship would be more probable, especially if you neared the physiological limits of the organism).

Page 15591, line 23-25: I think it would be valuable to have this data represented in a table similar to Table 2, and there should also be a reference here to the table and/or to Figure 3 since the data is shown there.

Page 15592, line 7: Consider restructuring this paragraph to lead with the interesting phosphate-related results rather than opening with a statement about the lack of effects from CO2, which emphasizes the less compelling result.

Page 15594, lines 6-8: I may be misinterpreting the intention of this sentence, but I don't see why P-supply rate, which certainly influences resource allocation to various cellular structures in addition to growth rate (check out any literature on cellular/ecological stoichiometry), wouldn't also play a role in lipid composition? Don't the results of this paper, which show directly that P-supply influences lipid composition, contradict this statement? Please clarify.

Page 15595, lines 18-20: It's not clear to me what is meant the statement, "a process that does not seem to be relevant for the production of viruses as such." Please elaborate on how this does not seem to be relevant.

Table 1: My feeling is that this table could be moved to the supplemental material, since it is not the major focus of the paper. But I want to be clear that this is still important

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data to publish.

Figure 3: I found this figure to be very complex and difficult to understand since it aggregates data from different treatments, which are generally discussed separately in the text. It would help to have some brackets or different symbols to identify which points are derived from the P-replete, P-limited, and P-stressed treatments. It's misleading to me to not distinguish between these on the plot.

General comment about figures/data: I would like to see some representation of the error associated with the various measurements and calculations represented in the paper to help get a sense for how different the values really are.

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

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