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

Dear editor, we thank you and the referees for reviewing our paper for Biogeosciences. Both referees were of the opinion that our work reports interesting data on the effect of P-stress on lipid remodeling and appreciated our approach of distinguishing between P-limited (P-controlled) growth and P-starvation. The main issues which were stated by the referees were 1) the clarity of the manuscript (large amounts of treatments led to confusion) and the need of a clearer naming of the treatments *(referee 1&2)****; 2) the question whether the appropriate controls were used (i.e. whether the different P-treatments, which were induced by different culturing methods, showed only the effects of P and no other effects)*** *(referee 1)****; 3) a need for improvement of Figure 3, showing the relation between P-stress and lipid ratio in a clearer way*** *(referee 1&2)****, and 4) a request for presenting the error of our data and the validity of the test that were used (regression).***

We addressed these issues as follows:

1) ***We propose a new naming scheme for the different P-treatments, whereby P-limitation is used as a general name for the effects of reduced P-availability and whereby the P-limited cells from the chemostats (growing at 0.97 and 0.32 μ_{max}) are now called "P-controlled". Hence, there will be use of the terms P-repletion and P-limitation, whereby P-limitation is further divided into P-controlled exponential growth and P-starvation. We will introduce these names in the Introduction of the revised manuscript, clarify these treatments in a Table and revised and check the manuscript for correct and consistent use of these terms.***
2) ***We explain here in the rebuttal that our experimental set-up is, in our opinion, adequate for answering our research questions, i.e. we believe that we have used the appropriate controls as we think that the different ways of culturing only affected the P-conditions and not any other variables. We will explain this more comprehensively in the revised version of the manuscript. Also we will discuss additional indicators of P-limitation/ stress.***
3) ***We have produced a new Figure 3 that shows lipid remodeling versus photosynthetic efficiency (Fv/Fm) instead of alkaline phosphatase activity, showing a clearer (linear) relation with P-limitation. The clarity of the figure is furthermore improved by clarifying the different culturing methods/ treatments in the figure itself and referring to additional tables that show additional information on the treatments. The 'old' figures can be added as supplement or just supplied as data in a table.***

4) ***To better show the error of our results and thus the differences between the P-treatments, we will supply a supplementary Table to the revised manuscript that shows the averages + standard deviations of the relative IPL abundances of each P-treatment. These are the averages of the two pCO_2 treatments, which did not show any statistical differences in the 2-way ANOVAs. Moreover, we have carried out new regressions, this time with glycolipid:PG ratio versus Fv/Fm (instead of APA), while the ratios of the infected cultures are now left out of these regressions. Regression statistics are shown in the rebuttal as a table and will be added to the manuscript (as text).***

Moreover, further comments are addressed below in more detail. Our rebuttal text is in blue, bold and Italics. Suggested tables are placed in the text. Figures at the end of the document.

Anonymous Referee #1

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 PGlipids 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.

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.

We thank referee 1 for the positive and constructive comments. We also agree that for such a complex study, with many variables, it should be clear, which variables are affecting the outcome. Below, we describe that we can supply additional information that strengthens our conclusions. We will show variability (averages ± standard deviations) of the effects of the P-treatments on the relative IPL abundances and we will add more information on the physiological status of the cells as suggested by referee 1, , i.e. besides alkaline phosphatase activity (APA), also the photosynthetic efficiency (FvFm), growth rate (μ) and doubling time (Td).

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.

We agree that many variables are involved and that therefore it is important to be clear and consistent when it comes down to the naming of the treatments. As both referee 1 and referee 2 commented on this, we realize that this needs improvement in the manuscript. We propose a different naming scheme in which we use 'P-limitation' as a general term for M. pusilla cultures that are affected (in their growth or physiology) by low P-availability. The cells in the P-limited chemostats, which are growing exponentially at 0.97 and 0.32 μ_{max}) will then be named 'P-controlled' as their growth is controlled by the supply rate of P. The P-starved treatments will remain the same, but whereby also the distinction is made between the culture that is pregrown at 0.97 μ_{max} and the one that is pregrown under 0.32 μ_{max} before starvation set in. We will be consistent in the text in mentioning whether we mean (0.97 and 0.32 μ_{max}) P-controlled growth or (0.97 and 0.32 μ_{max}) P-starvation. We will explain this naming clearly in the Introduction. Furthermore we will add a table in the Methods section (Rebuttal Table 1) with an overview of terms and explanation of treatments. We will carefully check the revised manuscript for consistent and unambiguous use of these terms.

Rebuttal Table 1: P-treatments used in the cultivation experiments of M pusilla. Note that all experiments were carried out in the same vessels and conditions (light, nutrients, temperature, etc.).

	Method	***Treatment name***	***Effects on growth***
P-replete	*semi-continuous turbidostat culturing (daily)*	*P-replete*	*Non-constrained exponential growth at 1.0μ_{max}*
P-controlled	*Chemostats: growth rate driven by P-supply rate*	*0.97 μ_{max} P-controlled*	*P-controlled exponential growth at 0.97μ_{max}*
		0.32 μ_{max} P-controlled	*P-controlled exponential growth at 0.32μ_{max}*
P-starved	*Batch culturing; chemostat pumps are stopped (30h)*	*0.97 μ_{max} P-starved*	*Starvation of the 0.97μ_{max} P-controlled cultures; growth nears zero*
		0.32 μ_{max} P-starved	*Starvation of the 0.32 μ_{max} P-controlled cultures; growth nears zero*

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.

Three different culturing techniques were used, whereby each method had its own specific function: 1) semi-continuous culturing was used to keep the P-replete cultures (=controls) growing exponentially at maximum growth rate; 2) chemostat culturing was used for P-controlled exponential growth of M. pusilla at 0.97 and 0.32μ_{max}; and 3) batch culturing was used to induce P-starvation of the cultures that were previously grown under P-controlled (chemostat) conditions. This is also clarified in our new Table 1. Besides the changes that were deliberately induced to create the specific P-treatments (excess, low supply and depletion), there are to our opinion no indications that other factors may have affected these cultures differently. Culturing of all treatments was carried out in the same type of culturing vessels and under the same regime of temperature, light, aeration, etc. We cannot think of other cultivation variables that might have affected M. pusilla IPL composition. We will add a sentence to the M&M section, stressing the fact that all other conditions remained the same: 'The different culturing methods that were used to create the specific P-conditions (P-repletion, P-controlled growth and P-starvation) are not expected to have influenced M. pusilla growth or physiology, as other conditions (e.g. type of vessels, aeration, irradiance, temperature, etc.) were the same between the different culturing treatments."

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.

We are not aware of any IPL remodeling in our P-replete cultures during viral infection. At 30h post-infection (IPL sampling in P-starved, infected cultures) the P-replete cultures were already lysing. Unfortunately no IPL samples were taken at earlier time points for the P-replete, infected cultures. The alkaline phosphatase activity (APA) is indeed affected by viral infection. This can be seen in Figure 3, whereby the infected cultures (grey symbols) show lower APA (x-axis) than the non-infected P-starved cultures. It could be that the infected cells are (due to reduced metabolism) less affected by P-limitation, or viral infection affects APA in some sort of way. Indeed, this implies that APA may not be a good indicator for P-limitation in infected cells and should be left out of the regression in figure 3 (see also comments below). Additionally we will describe the effect of viral infection on APA in the Discussion section of the revised manuscript.

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 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.

We regret that these aspects of the manuscript miss clarity and thank the referee for pointing this out. We will modify the introduction to a hopefully better structured section, whereby we will more comprehensively state the rationale and significance for this study. In the Discussion section of the revised manuscript we will emphasize the most important findings, also in a more comprehensive manner. Also we will supply a new or additional manuscript Figure 3 (figure 1 in this rebuttal), which uses Fv/Fm as indicator of P-limitation and also better shows the impact of the different treatments.

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

We thank the referee for pointing out this mistake. We will change this in the revised version of the manuscript.

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.

For having a clearer focus on the effects of P-availability, it seems indeed better to only mention this in the Discussion section of the manuscript. We will omit this sentence from the introduction of the revised manuscript.

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

We thank the referee for pointing this out. With the new table and information on the culturing methods we hope we to give a better overview of the experimental set-up and clarified the choices we have made.

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

We called this treatment P-starvation because from this point onwards, the cells were not supplied with P anymore, hence starved in P (see also e.g. Macintyre & Cullen, 2005 for this definition). The physiological effects on the cells are directly visible, i.e. growth quickly reduces to (near) zero (Maat et al. 2014) and besides the increases in APA, also the photosynthetic efficiency decreases. For clarification we will make reference (Macintyre & Cullen, 2005) to this definition in the revised manuscript and furthermore supply a new table (see below) which provides the APA, Fv/Fm, growth rate and doubling time of the different culturing treatments.

Rebuttal Table 2: Indicators of M. pusilla P-stress: alkaline phosphatase activity (APA), photosynthetic efficiency (Fv/Fm), growth rate (μ) and doubling time (Td). Note that the growth rates of the P-starved and infected cultures

Treatment	(pre) growth (decimal of μ_{max})	APA (amol cell^{-1} h^{-1})	Fv/Fm (r.u.)	μ (d^{-1})	Td (d)
P-replete	1.0	0.0	0.66	0.72	0.96
P-controlled	0.97	6.1	0.61	0.7	0.99
	0.32	24.3	0.50	0.23	3.01
P-starved, non-infected	0.97	26.8	0.30	0.09	7.6
	0.32	33.3	0.33	0.07	9.4
P-starved, infected	0.97	18.0	0.34	0.0	x
	0.32	28.9	0.28	0.0	x

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

This virus is used in Maat et al. 2014. We will make the correct reference in the revised manuscript.

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.

For the determination of the percentage of infective viruses we compared MPN assays with the virus abundances that were determined by flow cytometry. The calculated MPNs were higher than the number of cells determined by flow cytometry and therefore 100% of the viruses were considered infective. We will provide a supplemental table to the revised manuscript (Table 3 in this rebuttal)

Rebuttal Table 3: MpV abundances calculated by flow cytometry (FCM) and Most Probable Number dilution assay (MPN). Percentage of infective viruses is calculated as MPN/ FCM*100%.

	MpV abundance			
pCO_2 (μatm)	FCM (×10^8 virus ml^{-1})	MPN (×10^8 virus ml^{-1})	MPN 95%C.I. (×10^8 virus ml^{-1})	% infective
370	1.12	4.52	1.5 – 13.6	100
750	2.14	3.28	1.17 – 9.12	100

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, that would also be helpful to include).

APA is considered a thorough indicator of P-limitation or P-stress (Beardal et al. 2001), i.e. the more limited the cells are, the more enzyme they produce to obtain P from other sources than soluble reactive compounds. We do, however, see the referees point that others variables also influence the activity (i.e. viral infection) and that it would be helpful to consider other parameters reflecting P-limited physiology as well. We will add this data in the form of a new table (Table 2 in this rebuttal; see above). Reconsidering the data according to the referees suggestions we see that Fv/Fm as indicator of P-limitation also shows (a possibly better) relation with lipid remodeling. We will therefore modify figure 3 (Figures 1 A&B of this rebuttal), which shows a linear relation between P-limitation/ stress and lipid remodeling and clearer distinction between the different treatments (graphically depicted within figure). We can add the older version (APA vs. lipid remodeling) as supplement or just depict APA values as in Table 2 of this rebuttal and the regression information (slopes, p-values) in the text.

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).

We carried out regressions to test the relation between APA and lipid remodeling. With correlation we actually meant the general synonym for relation and not the statistical test. We understand that this is confusing, so we will change this accordingly in the text. The infected cultures were included in the tests, but (as this might not be appropriate) we will leave them out in the new regression analyses that are now based on Fv/Fm instead of APA. We will now also state clearly in Methods section of the new manuscript on which data the new regressions are based.

Although a non-linear relation might be expected, we also find a clear linear relation of lipid ratio with Fv/Fm as indicator of P-limitation/ stress. We will provide the slopes of the regressions as well as data on the validity of the used tests in the revised manuscript (here now depicted as Table 4). See also Figure 1A&B of this rebuttal for comparison.

Rebuttal Table 4: Regressions of glycolipid to PG ratio versus Fv/Fm as indicator of P-limitation. Of formula y=ax+b; y=glycolipid:PG ratio, x=Fv/Fm.

Regression	***SQDG:PG***	***DGDG:PG***
Constant (b)	*10.11*	*7.10*
Slope (a)	*-11.5*	*-10.2*
n	*6*	*6*
r^2	*0.974*	*0.954*
Analysis of variance		
F	*150.4*	*83.3*
Normality test (Shapiro-Wilk)	*P=0.781*	*P=0.594*
Constant variance test	*P=0.060*	*P=0.040*

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.

We will add this information in a new Table (see Table 2 of this rebuttal) which we will place in the revised manuscript.

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.

We will change this in the revised manuscript, starting with the effects of P-availability and presenting the outcome of pCO_2 after that.

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.

Our message was that the effects of P-starvation on lipid remodeling are more severe than the effects of P-limitation (now P-controlled growth), whereby the growth rate (forced by the P-supply rate) is of minor importance compared to a completely inhibited P-supply. We realize that this was not directly clear from this sentence and will revise this in the new manuscript ("In a natural P-limited system, the extent of P-lipid substitution would be a function of the P-supply rate (e.g. decreased remineralization). However, when the P-availability decreases to 0 and starvation sets in, the substitution rate rapidly increases further, whereby the extent seems to depend on pregrowth conditions").

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.

We meant to say that viruses themselves (the proliferation of viruses) are not expected to be directly favored by lipid remodeling. Later we explain that indirectly viruses might profit from this process, as it is likely that the P from the lipids can be used in metabolic processes that support viral proliferation/ lysis. The referee has a point here in that it is more correct to state that viruses might be favored by this process. We revised this sentence to: "Our findings demonstrate that P-limited, virally infected cells can still decrease the ratio of P-lipids to non-P-lipids under P-starving conditions. This process might even stimulate virus proliferation by redirecting P from lipids to the cell's energy metabolism (photophosphorylation), supplying energy for virus proliferation and resulting in faster lysis and higher burst sizes."

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

We believe that the absence of pCO_2 effects is indeed important information and prefer to show this table as actual results. Above we agreed with changing the order of in the results section, showing the effects of P-limitation before the effects of pCO_2. We therefore suggest to leave both tables in the manuscript, but start with the P-limitation results and after that the pCO_2.

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.

We agree, and will therefore add text to the new figure 3, explaining which data points belong to which treatments (See figure 1A&B of this rebuttal).

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.

All data is retrieved from single treatments (n=1) and therefore we cannot show the error of the measurements. We do however show (with 2-way ANOVAs) that there are no significant differences between the pCO_2 treatments. We will add a supplementary table (see below) showing the averages + standard deviation (of high and low pCO_2) for all P-treatments (hence the averages from the data in the original manuscript Table 1).

Rebuttal table 5: Averages ± standard deviations (s.d.; high and low pCO_2) of relative IPL peak areas under the different P-treatments showing the error of the

	SQDGs (%)	PGs (%)	DGDGs (%)	MGDGs (%)	DGTSs (%)	DGTAs (%)
P-replete	3.0±0.1	10.5±0.7	2.8±0.1	9.5±0.8	2.7±0.6	71.5±0.7
0.97μ_{max} P-controlled	8.8±0.5	6.0±1.9	7.5±0.4	5.7±0.6	4.5±1.4	67.5±2.1
0.32μ_{max} P-controlled	8.9±0.2	4.5±0.0	9.5±0.1	6.5±0.7	2.3±0.2	68.5±0.7

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 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 culturebased studies while this is ecologically highly relevant.

We thank the referee for his/ her positive and helpful comments. We also thank the referee for complimenting us on the use of the different treatments and our differentiation between limitation and starvation. We agree that the terminology and our use of it is confusing and that this needs improvement (see also our comments to referee 1). As discussed above we will add a table (rebuttal Table 1), which describes the names and methods of each treatment and will carefully check the manuscript for the correct use of these terms.

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?...).

The cultures were not limited in pCO_2 as they were constantly aerated. We will add this information to the Methods section of the revised manuscript. The exact CO_2 conditions, including alkalinity, pH, etc. are published in Maat et al. 2014. We will make a clearer reference to these specific parameters in the Methods section of the revised manuscript.

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.

Micromonas pusilla LAC38 is found to cluster with Micromonas pusilla clade A (Martinez Martinez et al. 2015). Unfortunately this was not mentioned in the extensive studies by Slapeta et al. 2006 or Worden et al. 2009. We will add this information to the Methods section of the revised manuscript.

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).

Unfortunately, we only have data from single treatments (n=1), but we can show variability between P-treatments when we combine the pCO_2 treatments (no differences between low and high pCO_2 – see also our comment to referee 1 above). We will add this data as a supplementary table (rebuttal Table 5) showing the averages + error (of high and low pCO_2) for all P-treatments.

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.

We thank the referee for this suggestion

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.

We agree that that is a better introduction of the matter than just focusing on P-limitation, especially because we have a focus on pCO_2 as well. We will start this paragraph accordingly in the revised manuscript.

Pg 15586, l. 25: see general comments and specify here the Micromonas clade you worked with.
We will add the information to the revised manuscript that M. pusilla LAC38 belongs to clade A.

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.
The onset of lysis and the termination of the lytic cycle are indeed delayed under P-limitation. This is indeed relevant information, which we forgot to mention. We will elaborate on this in the Methods section with reference to Maat et al. 2014.

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.
For the PGs no diagnostic (specific) losses of fragments were obtained from which the original molecules could be deduced. We will add a sentence to the Methods section describing this issue.

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?
Besides the differences between the treatments that were described in the results section belonging to Table 2 (which will be table 1 in the revised manuscript), we could only find increasing ratios (with P-stress) for the SQDGs and DGDGs versus the PGs. We will specify this in the results section of the revised manuscript.

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?

We can see a rapidly increasing DGDG:MGDG ratio from P-replete to P-limited followed by slightly decreases under P-starvation. This is indeed interesting data to show and supports the hypothesis that MGDGs are precursors for the DGDGs that increase with increasing P-limitation (P-stress). We will add a supplementary figure showing the relation of the DGDG:MGDG ratio with Fv/Fm (rebuttal figure 2) and discuss this figures in the Discussion section of the new manuscript.

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.

Unfortunately the viral genome is not available. This is a great suggestion and should be checked when it becomes available.

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.

As discussed above we will more clearly label the figure (see rebuttal Figure 1).

Technical error: Pg 15592, l.22: parenthesis missing.3

We thank the referee for pointing this out and corrected accordingly.

Rebuttal References:

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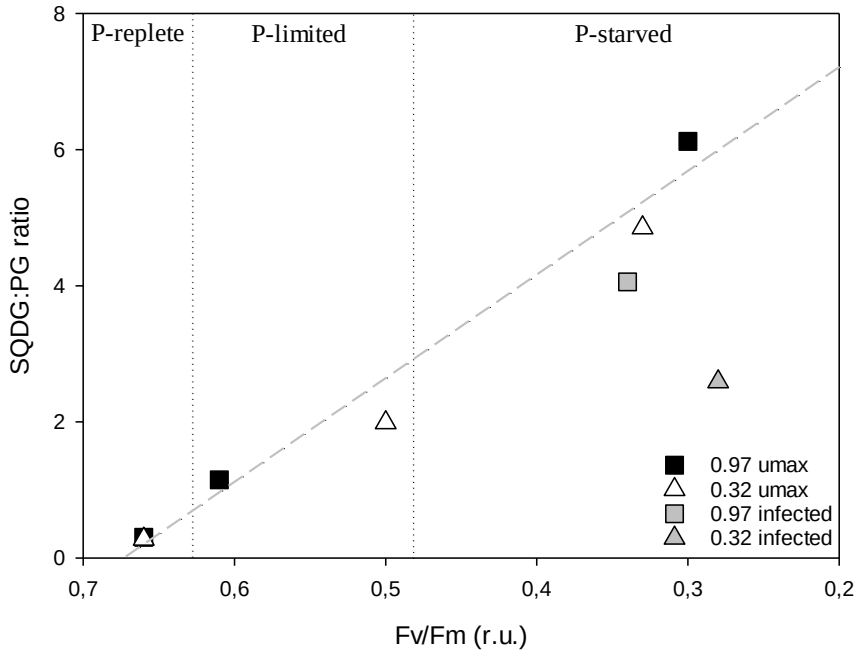

Figure 1A: SQDG:PG ratio versus photosynthetic efficiency (FvFm). Separate regressions for 0.97 and 0.32 cultures. Infected cultures in grey and not included in regression.

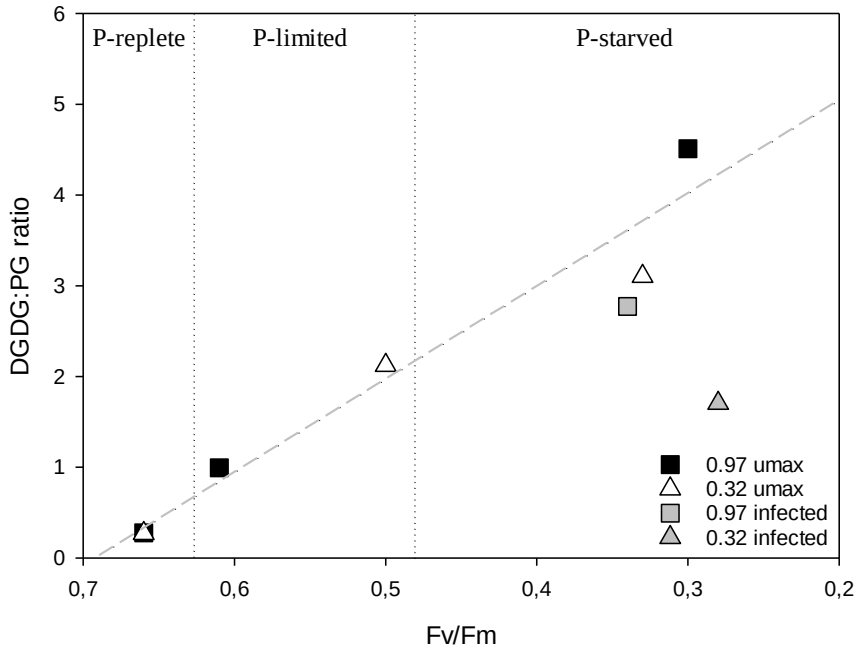

Figure 1B: DGDG:PG ratio versus photosynthetic efficiency (FvFm). Separate regressions for 0.97 and 0.32 cultures. Infected cultures in grey and not included in regression.

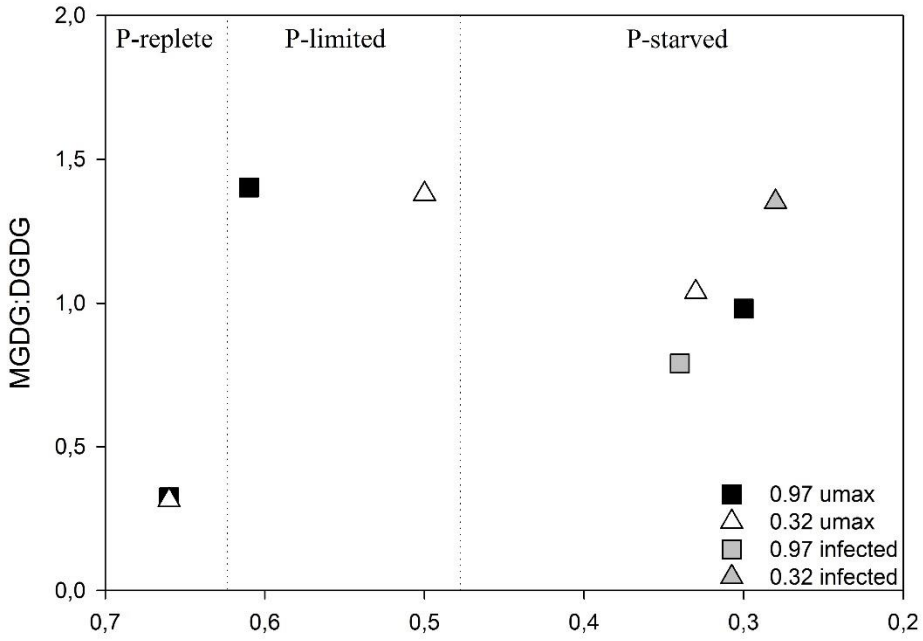

Figure 2: MGDG:DGDG ratio versus photosynthetic efficiency (FvFm). Infected cultures in grey.