Response to referee comments on 'The Arctic picoeukaryote *Micromonas pusilla* benefits synergistically from warming and ocean acidification' under review for Biogeosciences

Anonymous referee (Referee #1)

The authors assessed the combined impact of ocean acidification (OA) and warming on the growth, photochemical characteristics, and cellular composition of the prasinophyte Micromonas pusilla. M. Pusilla is a common species in Arctic waters, especially in the vertically stratified and nutrient-poor waters found in summer and early fall. Previous observations indicate that the current increase in stratification in the Arctic favors M. Pusilla. Results from this study suggest that, in addition to this trend, OA and warming could also contribute to increase the importance of this key Arctic species in the future. This is a nice, well-crafted physiological laboratory study. The experimental design is straightforward but appropriate. The cells were well acclimated to their new pH and temperature conditions (7 generations), which already makes this data set different from the ones generated during mesocosm studies were the assemblages are exposed to an abrupt change in conditions. Measurement of photochemical and overall growth parameters nicely complement each other, showing how the light and dark metabolisms to OA and warming shape the final response. The results are convincing and well generally discussed.

We thank the reviewer for their kind words on our manuscript.

I am however concerned by the way the authors extrapolate their results to the natural conditions and suggest they be more cautious in this section of the discussion. In the Arctic surface mixed layer, cells do not necessarily achieve their maximum growth rate, which is in sharp contrast to the experimental setup presented in this paper where the cells are nutrient replete and growing at maximum speed. In the surface mixed layer, the biomass and gross growth rate of cells are mostly controlled by the availability of regenerated nutrients such as ammonium. Accordingly, in their introduction, the authors characterise this period of the year as 'nutrient-limited' (Page 2, line 46). Hence, the full and combined positive impacts of OA and warming, as observed during their experiments, could only be achieved in the field if nitrogen supply (regeneration in this case) was not limiting, i.e. if there was a proportional increase between the supply and the demand in nitrogen by M. pusilla. The question is : how would OA and warming affect nitrogen and phosphate regeneration in the upper mixed layer of the water column in the Arctic in summer/fall? For the same reason, I think that the authors should be prudent when comparing M. pusilla and the diatom T.hyalana. To assess which of M. pusilla or T. hyalana will dominate in a warmer and more acidic Arctic, it is important to consider the different life strategies of these two species and ways they utilise the limiting resource. While T. hyalana is a r-strategist rapidly using the resource when available (nitrate), M. pusilla is more of a K-strategist acclimated to a less abundant but regular resource supply (ammonium). The modes of supply of nitrate and ammonium are different as well: mostly upwelling/mixing for nitrate, in-situ regeneration for ammonium. I am sure the authors are well aware of these basic principles. They should consider them in the extrapolation of their results to the field. This will nuance their prediction and open the door to future interesting research.

We agree with the reviewers' comments regarding the applicability of our study for the nutrient limited summer situation, when M. pusilla usually dominates. Nonetheless, picoeukaryotes are also highly abundant in the phytoplankton assemblages in early spring, i.e. before the peak of the spring bloom that is usually dominated by diatoms or Phaeocystis. We thus find it relevant to discuss differences between these functional groups under nutrient-replete conditions. In the revised manuscript, however, we now clarify that our results apply only for nutrient-replete conditions in the abstract (L21), discussion (L401-405 and L420-430) and conclusion sections (L449).

Otherwise, the paper is well written, the data are clearly presented and statistical tests seem appropriate. The non-linearity of the response is interesting and adds a level of complexity and difficulty in our attempt to predict how global change will affect marine systems.

We thank the reviewer for these kind words. In the revised version, we put stronger emphasis on the non-linearity in the observed responses (L343, L352-353).

P2, 46: . . .nutrient limitation. . .As mentioned above, the authors should take into account the fact that nutrient supply is low in these stratified waters and that their experiment was conducted with nutrient-replete cells. Actually, it could be more appropriate to write . . . results in low nutrient concentration. . .instead to infer 'nutrient limitation'.

Following the reviewers' comments, we replaced 'nutrient limitation' by 'low nutrient concentrations' (L46) and address the issue of nutrient-replete experimental conditions as described above.

P3, 62: . . .in relative abundance. . .This means that M. pusilla could actually be less abundant numerically. This is probably not what the authors mean.

We agree with the reviewer that 'higher relative abundances' can mean that a species contributes more to an assemblage even though it is numerically less abundant, and this is exactly what we wanted to describe.

P3, 63: see Hussherr et al. 2017 for an example of the combined impact of OA and light on Arctic pico-phytoplankton.

This relevant study has now been added to the cited references (L64).

P8, 229: . . .Under high temperature, growth was higher at 1000 than at 380. . .Since there is only two points; we don't know if growth 'increases' between these two points. The rest of the observations show that the response is not linear. It could well be the same between these two observations.

We agree with the reviewer that it is not ideal to use the terms 'increasing' and 'decreasing' in these instances, and changed the wording to 'growth was significantly higher under 1000 compared to lower (180 µatm; post-hoc, $t = 5.6$, $p < 0.001$) and higher pCO_2 levels (1400 µatm; post-hoc, $t = 5.9$, $p < 0.001$)' in the revised manuscript (L 232-233).

P9, 252: The authors should add a panel with the changes in N quotas (and N:Chl a ratios) in figure 2. These data are interesting by their own account, and it is difficult to interpret the changes in C:N ratios not knowing how N quotas vary.

Following the reviewers' suggestion, we added a panel showing the N quotas to Figure 2 in the revised manuscript. We have omitted the C:N panel and did not add a N:Chl a one as these do not show any significant responses.

P10, 279: Two 'investigate' in the same sentence.

Following the reviewers' suggestion, one of the instances has been changed to 'studied' in the revised manuscript (L280).

P11, 300: . . .in the summer and autumn when temperature up to 6°C or more can be reached (REF).

Following the reviewers' suggestion the two sentences have been merged into one and now read "M. pusilla is known to dominate Arctic phytoplankton assemblages in the summer and autumn situations (Lovejoy et al., 2007; Marquardt et al., 2016) when surface temperatures of 6°C or more can be reached (Hegseth et al., in press)" (L 301-304).

P 12, 333: . . .Overall, OA had. . . (delete also).

This sentence has been rewritten in response to this and other comments and now reads "Under 6°C and pCO_2 levels expected to be reached by the end of this century, OA had a significantly positive effect on growth and biomass build-up (Figure 1)" (L 335-336).

P12, 333: . . .a significantly positive effect on growth. . .This statement is an oversimplification of the actual results. The positive effect of OA is only clearly observed at 1000 PCO2 and at 6°C. This is not a negative comment. I believe that the most important contribution of this study is to highlight the non-linearity in the response and that this
should not be overlooked.

We agree with the reviewers' comment, and therefore now specify the conditions under which we observed a significantly positive OA effect on growth by writing 'Under 6°C and pCO_2 levels expected to be reached by the end of this century, OA had a significantly positive effect on growth and biomass build-up'. Furthermore, we put more emphasis on the non-linearity of the response, e.g. by writing 'This non-linearity in the observed pCO_2 effects emphasises the importance of experiments with more than two pCO_2 levels in order to properly describe OA-response patterns of organisms'.

P14, 399: . . .may experience growth stimulation under OA. . .Yes, but only if nutrient supply is sufficient to fulfill the nutrient requirements of exponentially growing M. pusilla. What is the main source of nitrogen in the upper part of the water column in the Arctic in summer and fall? Mostly regenerated ammonium. So, the question is if ammonium regeneration will also increase with OA. This is an interesting question. This is somewhat addressed later in the paragraph, but without mentioning the types (nitrate versus ammonium/urea) and sources (mixing or in-situ regeneration) of nitrogen.

Following this as well as the previous comments of the reviewer, we have changed the respective section of the manuscript by clearly referring to nutrient-replete spring situations that we have simulated in our experiment and by mentioning that conditions in the nutrient-limited summer months may differ in the CO_2 and temperature response. Regarding the sources and types of available nitrogen, we now mention that further studies should investigate interactive effects if OA, warming and different "sources and types of nutrients (e.g. mixing-delivered nitrate vs. regenerated ammonium)".

P14, 407: The comparison with the diatom T. hyaline is interesting but should also take into account the types and sources of the limiting nutrient. Are the authors suggesting that M. pusilla would replace T. hyaline as the main blooming species following upwelling/mixing events?

We agree with the reviewer that we need to be more careful about this comparison. We were not trying to suggest that M. pusilla will completely replace blooming species such as T. hyalina. Despite not being the most abundant species, picoeukaryotes still contribute significantly to the large accumulation of biomass during non-limited spring conditions and even slightly larger fractions of small cells may impact the food web dynamics during this important period. These aspects are now specified in the revised manuscript as we specify "The fact that our experiments were conducted under nutrient-replete conditions, which typically favour diatoms over picoeukaryotes, may indicate an even stronger increase in fitness (Collins et al., 2014) and could mean that M. pusilla gains another competitive advantage over phytoplankton like diatoms in the future, in addition to those resulting from changes in stratification (Li et al., 2009). Thus, our findings suggest higher picoplankton contribution to future Arctic phytoplankton assemblages under non-limiting conditions, e.g. early in the growing season when picoeukaryotes can already contribute quite substantially to the phtoplankton standing stocks (Marquardt et al, 2017, Paulsen et al. 2015). How such competition between diatoms and picoeukaryotes would manifest under nutrient-depleted conditions that strongly favour M. pusilla is currently unknown".

D. Campbell (Referee #2)

This is a worthwhile study of an important issue. It is topical and well conducted. I am late with this review, so will offer some quick input on units and figures.

We thank the reviewer for these kind words.

Abstract: Fine

Introduction: Line 51, I think: "In this region, temperatures are rising more than twice as fast as the rest of the globe (Miller et al., 2010)."

Agreed and done (L51-52).

Table 2: There are discrepancies, real or apparent, in the table. Line 1: growth rate d-1, 0.75, implies more than one division per day (0.693 d-1). POC production is 178 fmol cell-1 d-1, but POC quota is 239 fmol cell-1

How can growth rate exceed 1 generation per day, when cells are producing less than a cell quota of carbon per day. At 6C, growth rate constant of 1.06 d-1 implies a generation time of 16 h. But POC production is only 261 fmol cell-1 d-1, while cell quota is 245 fmol cell-1. So cells need a full day to produce a cell worth of carbon, but they are apparently dividing in 16 h. The discrepancies are larger than the quoted error bars on the determinations, so something is going on here with discrepancies among the determinations.

*We thank the reviewer for pointing out these apparent discrepancies. They are due to the fact that POC production rate was calculated by multiplying the POC quota with the growth rate constant μ, which gives the e-folding (2.72) and not the doubling rate of the cell numbers. We agree with the reviewer that this can be confusing. We think that it is more appropriate to use k instead of μ for these calculation, even though μ*POC is commonly used in our scientific community. In the revised manuscript, we now show both μ and k in Table 2, and calculate POC production based on k (L155).*

Table 3: ETRmax does not have to be dimensionless. I x sigmaPSII x phiPSII/(Fv/Fm) or some similar equation can give e- PSII-1 s-1 in absolute units. Likewise for alpha. Figure 1: This figure might be more informative if plotted as cell specific exponential growth rate (panel A, as presented) and C specific exponential growth rate (an arithmetic transform of panel B). This comes back to my concerns about Table 2.

We thank the reviewer for this remark, which we agree with. We have thus changed the units of ETRmax and alpha accordingly throughout the manuscript (e.g. L183-190) and in the tables.

Figure 2: Panel D: why switch to a mass:mass expression, when other panels use molar comparisons. Mole:Mole is more informative, to my mind. Panel A vs. Panel B 200 fmol C cell-1 25 fmol Chl cell-1. But: Each Chl a contains 55 C (not sure if Chl indicates Chl a, or Chl a + c). Either way: 25 fmol Chl cell-1 x 55 C/chl = 1375 fmol C in the chl per cell. So, there is something wrong here with the unit conversions or calibrations. You have more C in the chl per cell, than in the total C per cell. Impossible. Unit conversion error or calibration error somewhere.

Thanks for pointing this out. When reviewing figure 2, we realized that we have accidentally used the wrong units for the Chl a quota, which should be [fg cell^{-1}] instead of [fmol cell^{-1}], as correctly used in Table 2. When converting these to molar units and accounting for the mentioned 55 carbon atoms per Chl a, we find that Chl a accounts for about 1% of the total POC measured in the cell, which seems feasible.

Regarding the suggestion to convert the ratio of C:Chl a to molar units, we prefer to stick to the commonly used weight-based ratios. To clarify that we always refer to Chlorophyll a when using the abbreviation "Chl", we now consistently use "Chl a" throughout the manuscript.

None of this affects the response patterns, but people will use these results for multiple purposes, so reconciling unit issues is worthwhile.

We fully agree with the reviewer and thank him for pointing out these inconsistencies in the units used.