Response to referees of bg-2012-370

We thank the referees for their constructive reviews which will improve the scientific quality of our manuscript entitled 'Arctic microbial community dynamics influenced by elevated CO_2 levels'. Below please find our comments to the specific issues raised by the referees.

Specific comments of referee #1

3. 'Page 12316, line 15 and 16, and page 1231711-13. I am not convinced that the concentration of Nanophytoplankton III group was highest in the high pCO2 mesocosms. In fig 1e, the abundance of Nanophytoplankton III in the high pCO2 is similar as found in the intermediate pCO2 mesocosms.'

The statement made was tested statistically using ANOVA with the square root transformed abundances as the dependent variable, mesocosm as Factor, and the sampling day as covariate; and testing specified hypothesis contrasts in the Post-hoc hypothesis tests were used (all within the software packages of SYSTAT version 13.00.05 (SYSTAT Software, Inc. 2009, San Jose, CA). Statistical testing showed that Nanoeukaryoties III were significantly higher for the high pCO2 mesocosms during phase 2 and 3 ($p<0.000$). We will add this to the text.

'Page 12318, line 2. Too strong statement. I do agree that these groups are most probably algal viruses, based on the dynamics in these two viral groups and the nano- I and pico- phytoplankton group. However, the authors can't totally rule out the chance that these two groups consist of viruses infecting other hosts. Please de-emphasize the statement.'

The referee is correct and we will rephrase the sentence.

'Page 12320, line 23. Too speculative. It is not possible to give a measurement on the grazing of HP as these results were not presented in the paper. Is the pers com based on measurements from the same experiment? If so, would it be possible to include these data in the present manuscript?'

We will rephrase this sentence and add the grazing rate. The personal communication is indeed based on measurements from the same experiment. The authors of those data like to publish the data in a separate paper.

'Page 12321, line 12. Why is only Nanophytoplankton mentioned? In Fig 1b, there is also a high dominance of Phytoplankton II in Phase 1. The abundance of the Nanoplankton group is actually much lower compared to the Phytoplankton II group in this phase. In addition, figure 2 is confusing as the labeling of figure 2a reads Nanophytoplankton whilst the figure legend tells that this is Picophytoplankton. Are the labels of the y-axis in the two figures reversed? Please correct.'

We discus algal particulate organic carbon that is based on a conversion factor of organic carbon per unit algal cellular volume, and therefore it is possible that the algal cellular carbon is higher for Nanophytoplankton I with lower cell abundances than Picophytoplankton II. We apologize for the typo in the figure legend and we will correct this (fig 2a is Nano-algal POC and fig 2b is Pico-algal POC).

'P12325, line 8-13. It is a bit speculative to assume that grazing was a major regulating factor on the HP community when it was not measured.'

We will take out the part on the grazing.

5. ‘Page 12326, line 9-10. Why was grazing pressure measured only on the Phytoplankton groups and not on the heterotrophic prokaryotes? Such data would have provided a more comprehensive picture of the top-down effects in the microbial food web. ‘

Grazing was measured on the heterotrophic prokaryotes by colleagues that were part of the multi-group nature of this mesocosms experiment under the EPOCA project. We agree that this would have provided an even more comprehensive picture, however, the analysis of those samples/data is not complete yet and the it be published in a separate paper at a later stage.

‘Page 12326, line 16-19. The conclusion on Carbon transport due to viral lysis should be more balanced as grazing was also mentioned to be an important loss factor of these populations at e.g. page 12318.’

We did refer to grazing but we agree that improvement of this section is needed. We will change this section accordingly.

9. ‘…However, the last conclusion (in the abstract) regarding viral lysis and effect of carbon cycling should be modified as grazing also was reported to be a major top-down predator on the phytoplankton community.’

We will change accordingly, adding the relevance of grazing.

10. ‘How was virus to prokaryote ratios calculated? Was it calculated based on the total viral count or only the smaller viral groups (V1, V2,V3) ?’

VBR was based on total virus count. We will add this to the P12318, L24-25.

‘Page 12311, line 11-27: unclear, please rewrite.’

We are not sure what the referee means. We believe by changing the second sentence somewhat it may become clearer, which we will do.

‘Page 12318, line 19. The heading for part 3.4 does not cover the content of the section as HP dynamics is discussed together with vira dynamics and VBR.’

We will change it to “HP and viral dynamics”.

11. ‘Page 12311, line 7-10, unclear.’

We will change this into “Only few studies have reported on OA-induced changes of phytoplankton community composition (Tortell et al., 2002, Engel et al., 2008; Meakin and Wyman, 2011; Feng et al., 2009) and the ecological consequences of OA on natural phytoplankton dynamics are still understudied.”

‘Page 12312, line 15; include “and” before screened through a 3mm mesh.’

Will be done.

‘Page 12314, line 4 and 5: Is it correct to have the cell size group 3-5 um as the last? Why is this size group not following 2-3um in line 4?’

Yes, the cellular size range for Nanophytoplankton IV is correct. It was named cluster nanophytoplankton IV because it was the last cluster showing up in the flow cytometric analysis

(see fig. 1e). To prevent confusion when presenting the different clusters during the different phases of the experiment we did not rename it based on cellular size but on time of appearing.

‘ Change virus to bacterium ratio (VBR) to virus to prokaryote ratio (VPR)’
Will be done.

‘Page 12313, line 13; write heterotrophic prokaryotes (HP) as this is the first time HP is mentioned.’
Will be done.

‘Page12319, line 22, “some viral lysis” imprecise, please rewrite.’
We agree and will rewrite this sentence.

13. ‘Fig 1 might be reduced by combining the groups Nanophytoplankton III and IV as the abundance of these groups are very low and with similar dynamics.’
We do not agree as they formed distinct flow cytometric clusters and therefore prefer to keep them as separate figures.

Specific comments of referee #2

‘Page 12312, line 11. Is hard to understand that Day t -7, means that were 7 days before time 0. Authors could explain better this terminology from the beginning.’
Will be done.

‘Page12314, line24. Taking into account that authors have measured grazing by microzooplankton, why authors have not measured grazers abundance as ciliates, and/or heterotrophic dinoflagellates in the dilution experiments (at least)?’
The natural zooplankton abundances have been measured by others in this joint experiment and we will refer to their paper in the Discussion of the revised manuscript. We performed the dilution experiments under the well acknowledged assumptions of the routine Landry & Hassett dilution method for microzooplankton grazing. Besides, it went beyond practical feasibility to analyze for all dilutions bottles at T=0 and T=24h also the zooplankton community.

‘Authors gave data of viral lysis on phototrophs, how did they estimate this? Please explain. Also for viral lysis on HP, presumably Weinbauer et al. (pers comm).’
Viral lysis on phototrophs was estimated from the decline in specific phytoplankton abundance (net loss rate) during the period of increase in specific virus abundance, under the assumption that the burst sizes are reasonable for the specific phytoplankton host (based on published studies) and taking into account the losses due to grazing. We will explain this better. Indeed, viral lysis on HP was obtained by Weinbauer (pers. communication) as indicated in the manuscript.

‘Results Page 121315, line 19, after nanophytoplankton you should indicate Fig. 1b, 1c).’
Will be done.

‘Page 12318. Grazing rates were measured in triplicate samples for each type of CO2 conditions. However, in table 1, the SD is not shown. This is because some of the replicates have failed?’

Replicates did not fail but we mistakenly used averages. We will change this and will add the standard error and significance level (p-value).

‘Authors said that for the low CO2 treatment, during phase 2 grazing on nanophytoplankton I, was higher than in phase 1 and phase 3. Were these differences significant? It could be that the low success of grazing in mid and high addition of CO2, was due to a negative effect of CO2 on grazers?’

We will indicate the statistical significance in the revised manuscript. There was no negative effect of CO2 on grazers observed (by colleagues Aberle et al. 2012 in this joint experiment), which we will mention in the Discussion of the revised manuscript.

‘Authors assumed that the depletion of nano and picophytoplankton is due to viruses (V4 and V5):
a) Why authors do not follow the same organization for Fig. 6 as for Fig. 1? (first picophytoplankton virus and after nanophytoplanktonic viruses).
b) It is not clear that the decline of algae and the appearance of viruses is more important in acidified samples than in less ones, right?.
c) it is not clear whether grazers followed a similar dynamic as viruses. But, no counts of grazers.’

We presented the nanophytoplankton virus (V4) before the picophytoplankton virus (V5) because of its dynamics, showing the increase in V4 earlier than that of V5. We present our data per time period and thus found it logical to present V5 after V4.

It is indeed not clear that the decline of Nanophytoplankton I is more important in the high CO2 mesocosms than in the mid and low pCO2, however, for picophytoplankton I this is clear (and discussed).

Aberle et al. (2012) did not find that grazers increase in abundance (or biomass). However, grazing rates are of more importance as potential factor for decline of algal abundance and this was measured and discussed.

‘Page 12319, line 21. Authors said the sum of the net growth rates of 0.5 – 0.55 d-1 with the loss rates by grazing and some viral lysis (what does “some viral lysis” means?)’

See also our comment to referee 1. We agree this is not clear and we will rewrite this sentence.

‘Page 12320, line 25. I think that the low burst size obtained for HP is not uncommon for these Arctic waters. For instance, Boras et al. (2010) reported a burst size of 1-59 viruses per bacterium in North Svalbard during summer.’

We thank the referee for pointing out this paper and will make reference to it.

‘Page 12321, line 29. How did you calculate viral burst size of nanophytoplankton I?’

The net increase in V4 was divided by the concurrent decline in net nanophytoplankton abundance (assuming only half of the net decline was due to viral lysis). We will add an explanation in the revised manuscript.

'Page 12324, line 29 same for viral burst size of picoeukaryotes I.'

The net increase in V5 was divided by the concurrent decline in net picophytoplankton abundance. We will add an explanation in the revised manuscript.

'All figures are referred with capital letters while in the text are in lowercase.'

Originally both text and figures were having capital letters. The editorial office has changed the text to lowercase letters, but not the figures. We will correct the figures accordingly.

'Figure 7 c, does not have any letter in the graph.'

We thank the referee for noting this and we will correct this.

Specific comments of referee #3

'The design/layout of the experiments/phases/results are not entirely clear as they are presented. Part of this issue, I imagine, stems from the multidisciplinary/multi-group nature of the mesocosms with which the authors are probably trying to synchronize, but the time labeling could be clarified in this paper.'

The referee is correct stating that the multidisciplinary and multi-group nature of the experiment has led to the design and layout of the experiment, the phases and the time labeling. We will clarify more clearly the t0 (as requested by the referee).

'A minor point - the "low CO2 treatments" are not treatments - they are the controls since you did not manipulate them (at least two of the three?).'

These mesocosms were treated the same as the other mesocosms but not with pCO2 enrichments. Therefore, we believe it is still a treatment and like to maintain this as such in the manuscript.

'Related to the general presentation issue, I find it may be useful to have some sort of table with vertical columns as your four "phases" and rows composed of the different aspects (growth rates, loss rates, abundances, etc.) for each group (Pico I, Nano I, HP, etc.) you want to highlight as a way of summarizing what is going on (and what changes/differences are significant - see below).'

We fully understand what the referee is aiming for and have tried this at an earlier stage of the manuscript preparation but due to the fact that abundances can increase and decline (and thus consequently also growth and loss rates can vary) within the different phases, it is difficult to average or present ranges and in the end it did not become clearer. Therefore, we choose not to include such table.

'The second point is one of more major concern - there is a substantial lack of statistical rigor throughout the manuscript which must be corrected. Statements of "differences" or "increases/decreases" vs one treatment or another must be backed up with stats

and limited to those actually showing verifiable differences…. Unfortunately, your replicates are not true replicates as they seem to exist on a continuum of pCO2 levels (not three samples with the same level), but I believe that all of the line graphs should be converted from 9 individual lines to three lines (one per treatment of low/mid/high) with error bars showing ranges of the triplicates (not SDs) since their pCO2 levels can be roughly grouped (and are not so spread) into those three "levels" (and you can address this reality in the text). This would much simplify the visuals, making them much easier to read and you would more clearly see which lines no longer statistically overlap between one treatment or another.'

We will add the statistics (see also our comment to referee 1 on the topic): The hypothesis that there was no difference in growth rates of microbes in diverse time frames during the mesocosm experiment One-way ANOVA was used. Testing overall differences in standing stock of diverse flow cytometric clusters during different time periods was performed using ANOVA with the square root transformed abundances as the dependent variable, mesocosm as factor, and the sampling day as covariate. To test the specified hypothesis contrasts in the Post-hoc hypothesis tests were used. The grazing rates were calculated in Excel, including the standard error of the grazing rates (i.e. standard error of the estimated slope of the regression line). P-values indicate if the grazing rates differ significantly from zero (all within the software packages of SYSTAT version 13.00.05).

Concerning the replicates comment: With the relatively low amount of possible replicates statistical power of linear regressions are the same, if not superior, than compared to ANOVA based analyses. Also, a gradient approach is not so vulnerable to the loss of one or two mesocosm units in comparison to a replicated design. There are more advantages, nicely summarized in Havenhand et al. (2010 - Designing ocean acidifi cation experiments to maximise inference, in the Guide for Best Practices. The initial pCO2 were chosen to cover with seven out of nine mesocosms levels projected until the end of this century. As primary production and air/sea gas exchange at pCO_2 levels higher than those in the atmosphere shift carbonate chemistry speciation towards lower pCO_2 and higher saturation states with respect to aragonite, the two highest pCO_2 levels were chosen to keep two treatments at the end of the experiment still under-saturated with respect to aragonite.

'It is OK to leave the regressions as individual points (as they are) since you are correlating the exact pCO2 values which vary between replicates. Honestly, when I look at Figs 2/6/7/8, I find it hard to believe there are many real differences between "levels" given the spread of the individual replicates.'

We have statistically checked our statements and all are statistically significant. We will add the p-values in the figures.

'Fig 1 shows a few more potential differences that will need to be verified by stats: peak of PicoI/NanoI in Phase 2 for high CO2, peak of PicoII in Phase 1 for low/mid CO2, etc. Addressing these shortcomings will strengthen the conclusions and not give the impression of too much being made of small differences by focusing on real statistical changes.'

Also all statements related to Figure 1 are statistically tested and found to be significant. We will add this in the revised manuscript.

1) ‘P.11,l.11: Remove “of”.’
We will do this.

2) P.12,l.11: ‘Here, and throughout the methods (and paper in general), be careful to attach the minus sign (-) to the number and then both to the “t” when you want to convey “day minus 7” = t-7, since an added space seems like you are indicating a range.’
We will use the “to” to indicate all time periods in order to avoid confusion.

3) ‘P.12,l.20: Enriched with CO2 – some explanation is required here as it is stated the mesocosms were open to the atmosphere and therefore should degas if they have a pressure/concentration high than the air.’
The addition of CO2 was gradual between day *t*–1 and day *t*4 (compare Fig. 2) by pumping varying amounts of the CO2-enriched seawater (compare Table 1) through a dispersal device which was lowered to about 13m depth in the mesocosms and pulled up again for several times, resulting in an even distribution throughout the water column. We will add this to the Material & Methods section and refer for more detail to the paper by Schulz et al. (2012) in the special issue of Biogeosciences.

4) ‘P.13,l.13: “HP” is not defined.’
We thank the referee for noting and will define HP.

5) ‘P.13,l.16: I believe you meant to say “: : :maintained (grazing assays) and processed (counting): : :”.’
We will change this accordingly.

6) ‘P.15,l.26: Do these “highest average growth rates”, and other highlighted differences, equate to statistically significant differences?’
Statistics on the average growth rates did not show significant differences, however the abundances did. We will add the statistical test results in the revised manuscript.

7) ‘P.16,l.3: Pico II showed max values of 4000-5000, not 1500-1700 – those are the values of the Nano I.’
We apologize for this mistake, thank the referee for noting this, and will correct it.

8) ‘P.16,l.9: Again, with the spread of the triplicates and lack of stats here, I would say they are not different from any other treatment..’
Statistically tested and significant. We will add statistics results in the revised manuscript.

9) ‘P.16,l.15-17: Ibid.’
Statistically tested and significant. We will add statistics results in the revised manuscript.

10) ‘P.17,l.9: Ibid’
Statistically tested and not significant. We will correct this in the revised manuscript.

11) ‘P.19,l.22: What exactly does “some viral lysis” mean? Was all the measured grazing not enough when added to the net growth rate and hence X viral lysis was required’

See also reply to other referees on this topic. Indeed it is not necessary to explain and viral lysis is estimated to be only minor and thus can be ignored. We will rephrase this sentence.

12) 'P.21,l.24: There are two "De"s.'
Will be deleted.

13) 'P.22,l.15: A two-fold change in environmental microbial ecology is often not a significant change at all, considering the stochasticity of the systems and methodological concerns - this is why stats should be used throughout t prove this difference if you are going to highlight it/state it as a "difference".'
Statistically tested and the high CO2 levels were found to be significantly different from the mid and low pCO2. We will add this to the revised manuscript.

14) 'P.22,l.28: Again, all these three growth rates overlap and are not different.'
Yes, statistically not significantly different from each other. We did not state they were different from each other but will add the statistics in the revised manuscript for clarity. As we also mention the growth rates in the Results we will only present average with standard deviation in the Discussion section (and that statistically not significantly different from each other).

15) 'P.24,l.15: Italicize Bathycoccus.'
Will do.

16) 'P.25,l.9: Prove that this is true - the difference at the end of phase 3 is 4.5x10^6 vs 5.5x10^6 and looking at the spread of the replicates, I doubt they will be significantly different.

17) 'P.25,l.15: Haptophyceae.'
Will change.

18) 'P.26,l.2: I would temper this statement, as "profound" is a bit too strong since most of your differences do not seem statistically significant.'
The differences were statistically significant, despite we will leave out "profound" to satisfy the referee.

'Table 1: Deviations are needed here to assess whether anything is significant.'
Will do.

'Figure 1: Your "0" label for phase 0 is off-center in panel F.'
We will change this.

'Figure 2: There is a mismatch between the panels and legend over pico vs nano.'
Thanks for noting, we will change this.

'Figure 3-5 and 7-8: p-values should accompany your regression values.'
We will add those.

‘Figure 6: I would put both panels at the same scale (x10ˆ6) - this way it would be quite obvious that V4 (0.1-0.9x10ˆ6) was well under V5 (1-25x10ˆ6). It would also match all the subsequent figures which are (or should be) at the x10ˆ6 scale. Lastly, fix population” in the y-axis legend of panel B.’

Then one does not see any dynamics. Instead we will change the scale to a max of 25x10^5 (i.e. 2.5x10^6) and add a note in the legend that the scales differ.

‘Figure 7: The “C” is missing in panel C.’

We thank the referee for noting, we will correct this.

‘Figure 8: Change the scale of panel A to x10ˆ6 to match all the previous figures.’

We will do so.