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Interactive comment on “Arctic microbial community dynamics influenced by elevated CO₂ levels” by C. P. D. Brussaard et al.

Anonymous Referee #3

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This paper examines the effects on microbial community dynamics of elevated CO₂ levels in Arctic mesocosms. In general, the science is done well – the methods are appropriate and I recognize the substantial effort put into these types of mesocosms experiments. However, there are some problems in presentation/interpretation that must be addressed. Many of my minor comments are below, but there are two overriding/general comments to be made first of all.

The first is more minor - 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. Normally, a $t=0$ in an experiment is set to correspond with a certain start of manipula-

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tions/additions/etc. and we hence observe reactions downstream of this point when the change was initiated. Logically, given the ms title/goal, one would interpret your natural $t=0$ to coincide with the start of CO₂ manipulations - this is the independent variable you are manipulating. 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?). 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).

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. It is especially important in these types of microbial ecological experiments when replicates of natural populations are concerned - we all know the variable nature of responses that can be obtained. Also, why do replicates if you are not going to benefit from the fact that they allow you to then perform statistics and strengthen your conclusions? One of the answers one may give is that, even if not doing stats, they allow you to check for variability in the treatment bottles/mesocosms. This point is exactly the concern I have in many of the interpretations made of “differences” between CO₂ treatments. 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. 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. 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. It will also naturally simplify the rather complex Results/Discussion by removing some of the “fluff” that is not statistically relevant.

Specific comments

- 1) P.11,I.11: Remove “of”.
- 2) P.12,I.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.
- 3) P.12,I.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.
- 4) P.13,I.13: “HP” is not defined.
- 5) P.13,I.16: I believe you meant to say “. . .maintained (grazing assays) and processed (counting). . .”.
- 6) P.15,I.26: Do these “highest average growth rates”, and other highlighted differences, equate to statistically significant differences? No mention of stats is made anywhere in the paper.
- 7) P.16,I.3: Pico II showed max values of 4000-5000, not 1500-1700 – those are the values of the Nano I.

8) P.16,I.9: Again, with the spread of the triplicates and lack of stats here, I would say they are not different from any other treatment.

9) P.16,I.15-17: Ibid.

10) P.17,I.9: Ibid.

11) P.19,I.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 - be more precise.

12) P.21,I.24: There are two “De”s.

13) P.22,I.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 to prove this difference if you are going to highlight it/state it as a “difference”.

14) P.22,I.28: Again, all these three growth rates overlap and are not different.

15) P.24,I.15: Italicize *Bathycoccus*.

16) P.25,I.9: Prove that this is true - the difference at the end of phase 3 is 4.5×10^6 vs 5.5×10^6 and looking at the spread of the replicates, I doubt they will be significantly different.

17) P.25,I.15: Haptophyceae.

18) P.26,I.2: I would temper this statement, as “profound” is a bit too strong since most of your differences do not seem statistically significant.

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

Figure 1: Your “0” label for phase 0 is off-center in panel F.

Figure 2: There is a mismatch between the panels and legend over pico vs nano.

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Figure 3-5 and 7-8: p-values should accompany your regression values.

Figure 6: I would put both panels at the same scale ($\times 10^6$) - this way it would be quite obvious that V4 ($0.1-0.9 \times 10^6$) was well under V5 ($1-25 \times 10^6$). It would also match all the subsequent figures which are (or should be) at the $\times 10^6$ scale. Lastly, fix population” in the y-axis legend of panel B.

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

Figure 8: Change the scale of panel A to $\times 10^6$ to match all the previous figures.

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