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Biogeosciences Discussions

Interactive comment on "Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms" *by* A.-S. Roy et al.

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General comments:

1) Title: "Ocean acidification" is a rather bombastic for interpretation of results from a temporally- and physically-limited mesocosm experiment. Perhaps a milder statement such as "Acidification shows negligible impacts on high-latitude marine bacterial communities in coastal pelagic mesocosms" would be more accurate?

We disagree on removing "Ocean acidification" because it is the standard expression for CO2-induced seawater acidification. Furthermore, this experiment was supported by the European Project Ocean Acidification and therefore justifies the use of such

term in the derived article tiles. A further proof of the wide use of this term is the title of the Biogeoscience special issue in which this article is aimed to be published. This special issue is indeed entitled "Arctic Ocean acidification: pelagic ecosystem and biogeochemical responses during a mesocosm study". In this context, the present title is well suited for this experiment.

2) Do the authors have a hypothesis or set of hypotheses that they set out to test?

We hypothesized that increased acidification would significantly alter microbial community structure. The null-hypothesis was that acidification would have no effect on arctic mesocosm microbial communities.

3) Why mention all pCO2 treatments in the mesocosm experiment if only six were subjected to microbial community analysis? I agree with Reviewer #1 that the mesocosms not analyzed in the present study should be removed from the manuscript to avoid confusion.

See comment 1, above

4) 250 sequencing samples are described in the manuscript, however I counted only 7 mesocosms (6 mesocosms + fjord) x 9 time points = 63 samples (I do not consider replicate amplification reactions to be separate sequencing samples as the replicates were pooled prior to sequencing). From where do the 250 sequencing samples arise?

Previously, we inappropriately reported the collection time points and omitted to include t18; this was corrected throughout the manuscript. The total of samples sequenced come from 7 mesocosms * 10 time points = 70 samples * 2 (for each size fraction) = 140 * 2 (for 2 different filtered volumes at collection time) = 280 – 24 samples which didn't have enough material = a total of 256 independent samples sequenced. We previously rounded off the number of sequenced samples to 250 but in order to clarify the reviewer's concern, this number was changed to the exact number of sequenced samples of 256.

5) RNA and DNA were isolated together, however the authors have not clarified whether DNA used for amplicon generation, or whether reverse-transcribed rRNA was used. If RNA was not used at all, then a short sentence might be included in the M&M that RNA was isolated but not utilized in the present study. Ribosomal RNA gene/transcript terminology should be checked.

Following the comments of the reviewer, the material and method section was screened for correct terminology and a short sentence about the RNA was included as suggested in the method's section 2.3.

6) The authors report 2 510 000 sequences per treatment (20 000 000 sequences total) >this calculation disagrees with the six treatments shown in the paper (fjord, control, 4 x pCO2 manipulations). In the abstract the authors mention seven treamtents, however this number assumes that the duplicate control mesocosms are counted as separate treatments. As the control mesocosms were lumped together for statistical analyses (ANOVA in particular), then only six treatments were analyzed: fjord + control + 4 with elevated pCO2.

The number of sequences within a treatment cannot be lightly estimated. However, the OTU table produced by the analysis contained 256 samples, each rarefied to a depth of 81,181 sequences; giving a grand total of 20 782 336 sequences. The total number of sequences was rounded up to 20 000 000 to facilitate reading of the manuscript. However, the manuscript was screened in respect to the reviewer's comment and all number stated were proofed.

7) If really wanted to look at short time scale changes in microbial communities, why examine rDNA and not rRNA, especially since the RNA was available?

The rationale for studying rDNA was to determine if the composition of the microbial community was changing in response to the changes in the environmental factors. While the rRNA would also have been useful, it is by itself not as easy to deconvolute the influencing factors on the changing abundance of rRNA transcripts. Even if it can

be effective in a controlled experiment with a simple community, rRNA analysis was not feasible in regards to the financial and logistical problems of this study; hence justifying the rDNA analysis.

8) Could the authors please comment on their choice of 20 μm pre-filtration? Does this small pore size not greatly increase the possibility that many particle-associated bacteria are missed? Why not 50 μm or 100 μm pre-filtering to remove debris?

The decision to use a 20 μm pre-filter was taken by the EPOCA community and was done in order to establish a certain comparability in between all studies from this large mesocosms project. This pre filtration was further approved to assure the entire photoautotrophic cells present in the mesocosms will be sampled for the bacterial community dynamic study of Brussaard et al., 2012.

9) Statistics The overall approach to statistical analyses is very sound and thorough. I do have some questions, however: 1. It was not explicitly stated in the methods section whether amplicons were chimera-checked prior to statistical analysis. I assume that these steps were taken in addition to the QIIME quality filtering steps? 2. Why were ANOVA, RDA *and* PCoA analyses performed, when the significance of particular structuring variables could have been obtained from only the RDA? 3. Why does Figure S1 show the indirect gradient analysis PCoA ordination *overlaid* with the forward selection results from the RDA, when the direct gradient analysis RDA shows this without overlay? 4. Why were FDR-corrected p-values also subjected to Bonferroni correction for the g-test done in QIIME?

1. Chimera checking is implicit in the "closed-reference" OTU picking strategy that was applied here: by searching reads against a reference and discarding those that fail to hit, we will only retain a chimeric sequence if that chimera is represented in the reference database. 2. PCoA was not involved in determining structuring variables, and was simply a method of visualizing the variance in the data in lower-dimensional space. The reviewer is correct that ANOVA and RDA analyses are rather redundant, and the

RDA would suffice. We were attempting to be rigorous by using two separate statistics. 3. The reason we plotted the PCoA, with environmental variables “overlaid”, was to obtain the widest spread across variance space. If the RDA would have been plotted, the axes would have been constrained to only include variance associated with our environmental parameters, therefore removing any variation due to unknown factors. The authors think it is more honest to plot the PCoA (unconstrained), and to use the RDA to test for the significance and importance of environmental parameters. 4. The FDR-corrected p-values were not subjected to an additional Bonferroni correction. These corrections were done independently. However, in regards to the reviewer comment, this specification was removed from the manuscript to avoid confusing the reader.

10) It would be nice to see the authors comment on how their choice of experiment duration (30 days) may or may not have influenced the results that they obtained.

It is quite impossible to speculate how the duration of the experiment would have affected the sequence number or the OTUs identified in this study. However, the evaluation of the effect of changing pCO2 on microbial community during a longer period of time is suggested as future work in the conclusion (last sentence) using the results on adaptative evolution of Emiliania huxleyi of Lohbeck et al., (2012).

11) Specific comments p 13326, line 17-18: within each abundant phylum line 19: axis lengths.

These errors have been amended.

12) p 13333, line 13: line ending “which did show a response”. I think this should read “which did show an indirect response”, since there is no way to distinguish whether their slight increase was due directly to pCO2 or simply to increased algal carbon availability.

The sentence was revised and corrected for “which show a response to elevated CO2” to avoid confusion. However, the direction of the response (direct or indirect) was not established here and further analyses are needed in order to further characterise the

response.

13) p 13334, line 17: “methanogenenic” is misspelled. I think this sentence can be removed as it is purely speculative. As this is an exploratory study of pCO2 impacts on microbial diversity, it is not necessary to speculate about metabolic links between interesting taxa.

The manuscript was revised but the sentence was kept as metabolic functions are one of the authors interest.

14) Table 3: (a) and (b) not marked, table division not clear.

The table was revised to improve division and letters were added.

15) Table 4: Should read: "significantly correlated with pCO2". Instead of "general response to elevated pCO2", wouldn’t it simply be easier to report the correlation coefficients for each taxon, with a positive or negative sign to indicate direction of response? Table description should be re-written to improve clarity. Also, if only 6821 OTUs were found after clustering at 97%, from where does OTU 114612 come? Assuming that only unique sequences were used for alignment/clustering/taxonomy, why not use *unique* OTU numbers for this table?

The terms “Significantly correlated with pCO2” could be mistaken as increasing with the increase of pCO2 which is not accurate for the present table. Indeed, in some taxa, the response is decreasing or else. Therefore, the term “general response to elevated pCO2” was kept to diminish possible misunderstanding.

The use of signs was considered however for some response no appropriate sign could be found and therefore text was kept. The table legend was improved to avoid confusion. The OTU number is actually the Greegenes individual number attributed when sequencing is done by the sequencing program (see comment 5, above). It does not correspond to a rank number within the total sequence number; number 114612 correspond to a taxon. We agree that it could lead to confusion and corrected the column

table header to Greengenes OTU identifier.

16) Figure 1: Is it possible to include a second plot that shows changes in bacterial counts over time?

The authors do not have access to a bacterial count over time. However, this subject is covered by other authors (e.g Brussaard et al., 2012) who participated to this large mesocosms experiment.

17) Figure 2: the legend should consist of one column to help clearly identify stacked bar colors/taxon identities. However, doesn't Figure 3, which summarizes the data in Figure 2, obviate the need for Figure 2, which is quite a large figure to digest? One suggestion might be to move Figure 2 into the Supplementary Material, since it is essentially the same as Figure S2.

We decided to leave the legend of figure 2 under the figure as it facilitates the colour association and gives a better visualisation as a column on the right side. The position of figure 2 within the manuscript is explained in the response to comment #8, see above.

18) Figure 3: what are the units for the y-axis? cells per ml-1? Irrelevant treatments should be removed from the figure. Phase0/Phase 1/Phase2/Phase3 demarcations can be removed, as they are only briefly described in section 3.1 but never again referred to as such in the text. Where is the fjord treatment in this plot (145 μatm)?

The units for the y-axis, mean number of reads per sample, were added to the figure. No treatment was considered irrelevant as they all help to draw the whole bacterial community reaction in between the treatments. The fjord treatment is represented by the black bars (see key on right-hand side of the plot).

19) Figures 4, S3, S4: The responses of these taxa to pCO2 have already been summarized nicely in the text and in Table 4, therefore I do not believe that these figures make a significant contribution to the paper and can be removed.

We understand the reviewer's concern; however, these figures were included to improve the understanding of the pCO2 effect by providing the readers with a more accessible way to the information. Furthermore, since the Editor did not raise concerns about the manuscript length, we decided to keep these figures to facilitate visualisation of the pCO2 effect.

References:

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M., Schulz, K. G., Bellerby, R., Ludwig, A., Czerny, J., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated CO2 levels, Biogeosciences, 9, 12309–12341, 2012.

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