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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.

Anonymous Referee #2

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The article describes a brute force deep-sequencing approach to study changes in microbial community structure over a 30-day experimental period inside Arctic marine mesocosms with an increasing gradient of pCO2 without replication. Mesocosms were amended with mineral nutrients after the start of the experiment, in order to induce phytoplankton blooms. Consequently, microbial community development is temporally described as pre- or post nutrient addition. Microbial communities were collected by size fractionations of 3 μm > x < 20 μm and 0.2 μm > x < 3 μm prior to nucleic acid extraction, 16S rDNA amplification and paired-end Illumina amplicon sequencing. The authors found that artificial acidification in mesocosms by pCO2 manipulation did not have a significant impact on structure of microbial communities or on dominant micro-

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bial taxa, with the exception of 15 rare taxa that were positively correlated with pCO2. All statistical analyses demonstrated unequivocally that mesocosm effects, i.e. the physical and chemical perturbation of enclosing seawater in a mesocosm, resulted in the greatest differences in microbial communities (fjord vs mesocosm).

General comments

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?

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

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.

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?

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.

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

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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.

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?

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?

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?

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.

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

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.

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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.

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

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?

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

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.

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)?

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.

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