

## ***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 #1**

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Review: “Ocean acidification shows negligible impacts on high-latitude bacterial community structure in coastal pelagic mesocosms” by Roy et al., BioGeoSciences

The aim of this study was to determine the impacts of acidification on the composition of pelagic bacterial communities retrieved from a fjord in Svalbard. To this end, the authors set up several mesocosm experiments with ambient conditions and pCO<sub>2</sub> ranging from ~145 up to ~1420 μatm, from which bacterial assemblages were sampled. To assess and compare bacterial community compositions, originating from two size fractions, environmental 16S rRNA gene amplicons were sequenced using a high-throughput platform (Illumina). Globally, the authors didn't find any significant taxo-

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mic shifts in the composition of bacterial assemblages from different pCO<sub>2</sub>.

Major concerns: Number of mesocosms? The number of mesocosms used in this study is very confusing and requires clarification throughout the text and figures. In the abstract the authors state they analyzed 9 mesocosms. Actually, they studied bacterial community compositions from only 6 mesocosms –as mentioned in material & methods. Why mention in the text the ~1420 μatm when no taxonomic analyses have been performed on this mesocosm? Hence, the analyzed sample in this work with the highest pCO<sub>2</sub> is the ~1050 μatm mesocosm.

Experimental design This is not a bona fide study solely on the effects of acidification on microbial community structure. The authors added nutrients to the mesocosms to trigger phytoplankton blooms. We assume that the authors are trying to put the study in the context of previous literature on the exploitation of enriched CO<sub>2</sub> waters by phytoplankton, but this is not clearly stated in the paper. The context of why the phytoplankton blooms were induced with added nutrient is not mentioned in the introduction. One would think the goal of this study was double: OA and bloom/post-bloom effects. I suggest the authors be clearer about the nutrient addition in the abstract and introduction, and explain why the nutrients were added. As it is it is like an afterthought put into the last sentence.

The conclusion that ‘size fraction’ was the second most important variable in explaining differences in community structure’ ignores the fact that this was a sampling strategy and different organisms would be expected to be in the different size fractions. Enclosing the communities was the main thing that seemed to select for the resulting bacterial community.

The level of taxonomy for the chloroplast sequences needs to be verified, I don't believe they actually had Rhodophytes in the mesocosms. There is also confusion between cyanobacteria and chloroplasts with them being separate sometimes and not other times.

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Other concerns:

How many sequences did not hit the reference collection? Would this seriously bias the results? OTU identifiers The authors refer to OTU by using identifier number, e.g., OTU # 105727 for *Methylotenera*. It's not useful and can be rather confusing, for instance in table 4 (in this table, I suggest removing the OTU identifier column, or provide additional explanatory material

Free-living vs. particle-attached The use of "particle-associated" for the large size fraction is clumsy when a study encompasses phytoplankton detected from plastid 16S genes. The more common small vs. large size fractions would be better terminology. Especially since things can be attached to small particles

Figure concerns Figure 1: authors should remove the sample they did not analyze for bacterial community composition. If the main aim of the study is really OA and its effect on bacterial communities, this figure should be moved to supplementary material.

Figure 2 & 3: it's difficult to link both figures. Figure 2 summarizes the bacterial diversity (OTU) and figure 3 the relative abundance (Illumina reads). Because the authors merged the treated mesocosms together and changed the display it's impossible to scrutinize both figures back-to-back. Also, in figure 3, use of SD instead of SE of the mean would be more informative.

Figure 4: this figure is not a heat map but a contour plot. Although pretty, Contour plots are not really appropriate for a punctuated time series, especially with the use of extrapolation between samples.

Tables The legend of table 4 is poorly written and confusing.

Minor corrections Line 29, p. 13322: remove the comma after "In addition", last sentence of the introduction (line 29.) Line 21, p13330: remove "were not significant"

Discuss? Witt V. et al., *Environ. Microbiol.* 2011: discuss OA effects on biofilm microbial community compositions (from the Great Barrier Reef) using 16S clone libraries

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and TRFLP. These authors documented taxonomic shifts between treatments.

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