Biogeosciences Discuss., 9, C6656–C6661, 2012
www.biogeosciences-discuss.net/9/C6656/2012/

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.

A.-S. Roy et al.

sroy@geomar.de

Received and published: 19 December 2012

Major concerns:

1) 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 $\sim$1420 μatm when no taxonomic analyses have been performed on this mesocosm? Hence, the analyzed sample in this work with the highest pCO2 is the $\sim$1050 μatm mesocosm.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

The experiments described in this article were part of a large mesocosm experiment framework that provides the basis for the manuscripts presented in a special issue of Biogeosciences. Therefore it was necessary to situate our experiments in the context of this framework. However, to eliminate any confusion, the complete manuscript was screened to clarify the mesocosms description. Furthermore, it is specified on line 6 of the abstract that only six of the nine treatments were analysed and the raison for analysing only six of nine mesocosms is stated in the section 2.2 of the methods.

2) 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 CO2 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 addition, justification and description of nutrients addition are covered in the manuscript from Schultz et al., 2012 and we refer to this article in the method 2.1 section p. 13324, line 2. The last sentence of the introduction was amended to remove the aim to observe the effect of nutrients addition on the phytoplankton bloom as it was not a pre-defined aim of the experiment. .Nutrients were added during the experiment following the decline of the natural phytoplanktonic bloom which used all nutrients present in the mesocosms. After this bloom, nutrients were depleted and phytoplankton and bacteria declined dramatically. Therefore, it was decided by the experimentalists, that to characterise the effect of elevated pCO2 it would be essential to continue the experiment and thus nutrients were added to create an upwelling-like supply and stimulate another bloom (Schultz et al., 2012; personal comments Schultz).

BGD

9, C6656–C6661, 2012

3) 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 reviewer is correct. We have amended the text throughout the manuscript to clarify that the size fraction variable is actually a sampling strategy, and not an experimental treatment.

4) 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.

Sequences were clustered against the Greengenes reference database, and taxonomy was determined using RDP. The Red algae are diverse group of both unicellular and multicellular eukaryotes. Red algae are broadly distributed in polar oceans (Wulff et al., 2009).

Cyanobacteria were identified to a different resolution during the sequencing process and in regard to the referee's comment, the manuscript was scanned to ensure no misused of the terms and thus remove confusion. Cyanobacteria were sometimes classified as including chloroplast and sometimes as true Cyanobacteria. To avoid classification error, we created a group called "Cyanobacteria and eukaryotic chloroplast" including all cyanobacterial species identified to chloroplast. This group was abundant enough to be included in the most abundant phylum figure (Fig. 2). However, the taxa identified as free-living cyanobacteria were not abundant enough and were therefore included in the "others" group detailed in figure S2; therefore the use of the term Cyanobacteria represents the Cyanobacteria presented in the "others" figure.

Other concerns:

BGD

9, C6656–C6661, 2012

5) 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.

Only 106 singleton OTU's were not included in the present analysis and the inclusion of such singleton would have not seriously bias the results (see method section 2.4).

The OTU # is the Greengenes prokMSA identifier and can be identified in the Greengenes database at http://greengenes.lbl.gov/cgi-bin/nph-search.cgi. As the OTU's identification is explained in method section 2.4, no further explanatory material was provided. The OTU numbers were kept in Table 4 but the column's header was changed to Greengenes OTU identifier

6) 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.

"Free-living" and "particle-associated" have been changed to "small size fraction" and "large size fraction", respectively, throughout the manuscript.

7) 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.

The chl. a distribution patterns in the various mesocosm was used to select samples and showing only the samples selected for this high throughput sequencing would prevent the reader from obtaining a global view of the mesocosm development with time and we therefore Figure 1 was retained in the main manuscript.

8) Figure 2 & 3: it's difficult to link both figures. Figure 2 summarizes the bacterial

BGD

9, C6656–C6661, 2012

diversity (OTU) and figure 3 the relative abundance (Illumina reads). Because the authors merged the treated mesocoms 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.

Both figures are essential to convey the whole picture as figure 2 represents the most abundant phyla in % in each analysed mesocosms and figure 3 represent the mean absolute abundance of the most abundant phyla between the fjord, the combined control mesocosms and the combined manipulated mesocosm. These figures present independent results and are complementary.

We were interested in understanding the mean taxa abundances for the overall mesocosm communities, and whether or not they were significantly different across treatments. This is why we reported SE (estimation of how close the true population mean is to the sample mean) in figure 3, rather than the SD (degree to which replicate measurements differ from the sample mean). However, we agree with the reviewer that presenting the SD can be informative in this case (for an estimate of the variation between replicates), so this information has been included in a table (Table S1) in the supplement.

9) 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.

Heat maps changed for contour plots and the concern about using extrapolation and contour plots is understood; however this presentation is the best visual representation to show variation in abundance with respect to varying pCO2. The terms "continuous interpolation" was added to the legend of figure 4 to clarify this explapolation.

Tables

10) The legend of table 4 is poorly written and confusing.

BGD

9, C6656–C6661, 2012

The legend for Table 4 has been re-written for clarity.

Minor corrections

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

The text was corrected as suggested.

12) 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 and TRFLP. These authors documented taxonomic shifts between treatments.

Due to the vast amount of literature concerning ocean acidification, we have restrained ourselves to reference only to mesocosm projects as results from completely different experimental design (flow-through versus mesocosm) could lead to misinterpretation of our results. Indeed, Witt et al., 2011 worked on biofilms in an outdoor flow-through system and the present experiment worked on phytoplankton in mesocosms.

Interactive comment on Biogeosciences Discuss., 9, 13319, 2012.