Biogeosciences Discuss., 9, C4502–C4508, 2012
www.biogeosciences-discuss.net/9/C4502/2012/

Biogeosciences Discussions

Interactive comment on "Response of bacterioplankton community structure to an artificial gradient of pCO_2 in the Arctic Ocean" *by* R. Zhang et al.

Anonymous Referee #2

Received and published: 4 October 2012

The study forms part of a large in situ mesocosm experiment to study the effect of ocean acidification on marine planktonic organisms in the Arctic and the related biogeochemistry. The authors used terminal restriction fragment length polymorphism (T-RFLP) analysis in combination with a clone library to follow the bacterial community diversity and phylogenetic composition. The community development over time as well as in relation to environmental parameters and an artificial CO2 gradient was investigated. Bacterial richness and diversity have been shown to be reduced under elevated CO2, as was the relative abundance of Bacteroidetes.

I very much appreciate the high effort that has been undertaken to sample the complete

Full Screen / Esc
Printer-friendly Version
Interactive Discussion
Discussion Paper

CO_2 gradient during the complete experimental period. This effort, together with the sophisticated community fingerprinting methodology, results in one of the most complete data sets achieved on the topic so far. It is unfortunate however that no distinction was made between attached and free-living bacteria, since they seemed to be differently impacted by ocean acidification in a study by Allgaier et al. (2008; also cited in this MS).

A more fundamental concern is the use of bi-dest. water to dissolve the extracted DNA. The current standard in molecular biology is ultra-pure water (or even commercially available products) to avoid contamination of the samples. This is of special importance, when using universal bacterial primers for an amplification procedure, as was done in this study. The authors need to clarify in the MS why this water was appropriate and/or which measures were undertaken to exclude possible contamination of the samples by this source.

Unfortunately, interpretation and discussion of this very interesting data set seems somewhat hastily done. Several important references have been missed (e.g. Arnosti et al., 2011, Newbold et al., 2012; see below for details). In addition, the statistical analysis is somewhat careless or at least poorly described (see below for details). The discussion seems little revised in parts (e.g. doubling of sentences). These points need major revision before publication. Also, the MS would greatly benefit from referring to bacterial activity measures presented by other participants of the experiment in the same issue. It would be very interesting to know, if the community diversity/richness had any influence on bacterial activity and whether this changed with CO_2 level.

Detailed remarks:

p. 10646 line 9-12: "However, the maximum apparent diversity. . ." Sentence is unclear. Did the authors mean that richness and diversity differed in treatments which contained a different phylogenetic structure? How did diversity and richness differ? In addition this sentence reads like a contradiction to the sentence two lines above ". . .richness

BGD

9, C4502–C4508, 2012

and community structure varied with time. . .but not the degree of ocean acidification."

p. 10646 line 10: The term "species" is misleading and should be avoided (also elsewhere in the manuscript), despite the preceding "apparent". T-RFLP cannot be assumed to resolve the community at species level. This should receive one more sentence in the discussion of this MS.

p. 10646 line 12-13: Any hypothesis why/how total alkalinity could influence community composition?

p. 10646 line 23-24: Amann et al. is probably not the best reference for "microbial loop"

p. 10647 line 12: are the values concerning the global average? Please state

p. 10647 line 13-14: consider to revise grammar/language

p. 10648 line 5-6: ". . .the only study of bacterial community structure response to ocean acidification. . ." This is not correct. Two other studies should be cited/discussed:

Arnosti, C., Grossart, H.P., Mühling, M., Joint, I. and Passow, U.: Dynamics of extracellular enzyme activities in seawater under changed atmospheric pCO2: a mesocosm investigation. Aquat. Microb. Ecol., 64, 285-298, 2011.

Newbold, L.K., Oliver, A.E., Booth, T., Tiwari, B., DeSantis, T., Maguire, M., Andersen, G., van der Gast, C.J. and Whiteley, A.S.: The response of picoplankton to ocean acidification. Environ. Microb., published online ahead of print, 2012

p. 10648 line 12-14: ". . .our study provided, for the first time, detailed information on the response of bacterial diversity to ocean acidification." Statement could be controversial (see references above)

p. 10648: Experimental set up and sampling. Despite detailed description elsewhere, some more general details seem to be important to the reader of this paper:

BGD

9, C4502–C4508, 2012

- more exact information on the location of the experiment; this information should also go into abstract and introduction - size of the mesocosms (volume, depth, material); probably also in abstract? - Were there control treatments? M3 and M7? - sampling depth and volume of integrated water sampler

p. 10649 line 10-13: separation step is unclear. Two different mixtures of PCI and CI were used consecutively? How long/which speed was the second centrifugation? Please clarify.

p. 10649 line 16: see concerns about use of "simple" double-distilled water above

p. 10649 line 22: How large is the variance in the amount of template DNA? How was the concentration determined?

p. 10649 line 23: How many cycles in the PCR?

p. 10649 line 25: Generally it is informative to add city and probably even country of every company mentioned. (Also elsewhere in the paper)

p.10650: Presentation of statistical methods has to be extended (see below). This could probably receive a separate paragraph in the Methods section.

p. 10650 line 15: ". . .percentage values. . ." of what?

p. 10651 line 7: please provide sequence of primers

p. 10652 line 12: State here what you mean by ". . .uncompleted. . .". The reader finds it only in the next paragraph.

p.10653 line 1: please clarify: Did the authors pool medium and low treatments for analysis against highest treatments (should also be explained in Methods section)? Or were the 3 levels compared to each other?

p.10653 line 6-16: Explain your statistical analysis strategy in Methods section (see above): Are any of the environmental variables co-varying? Data transformation? What

BGD

9, C4502–C4508, 2012

kind of similarity-index was used for BIOENV? What about the most obvious factors temperature and chl?

p.10653 line 14: The authors state here that salinity is among the parameters correlating best with community. Did salinity change significantly in the mesocosms over the course of the experiment? How could this be explained? Or is the correlation driven by little changes in both, salinity and bacterial community?

p.10653 line 28: briefly explain "Libshuff analysis" in Methods section.

p. 10655 line 8: "...environmental changes, which commonly occurred in all mesocosms..." Which ones can this be? Temperature, chl,...?

p. 10655 line 9-12: "In addition, nutrient manipulation at the middle of the experiment, which induced higher productivity in the mesocosm, could also contribute to the temporal pattern observed for bacterial community structure." Is there a significant difference before and after nutrient addition? Does this influence community response to CO2? (This should be analysed in Results and discussed under point 4.2 in the MS)

p.10655 line 20: "...DMS, which is mainly produced by phytoplankton..." But also by bacteria. To conclude a relation between bacteria and phytoplankton from this seems a bit farfetched.

p. 10655 line 22-25: "Therefore, our study suggested..." Please clarify: Do the authors conclude that phytoplankton or bacteria were related to nutrient stimulation and CO2 manipulation? Or both? How could then direct and indirect correlations be distinguished?

p. 10656 line 15: "Also one parameter..." and line 22: "Furthermore, a pCO2 related chemical parameter..." These are identical sentences, only paraphrased. How could alkalinity influence bacterial community composition?

p. 10656 line 24-26: "This evidence proved..." Consider to revise grammar/language.

BGD

9, C4502–C4508, 2012

p. 10657 line line 16: “Few studies have investigated. . .”. Then they should be cited/discussed. E.g.:

de Kluijver, A., Soetaert, K., Schulz, K.G., Riebesell, U., Bellerby, R.G.J. and Middelburg, J.J.: Phytoplankton-bacteria coupling under elevated CO2 levels: a stable isotope labelling study. Biogeosciences, 7, 3783-3797, 2010

Kim, J. M., Lee, K., Shin, K., Yang, E. J., Engel, A., Karl, D. M. und Kim, H. C. (2011) Shifts in biogenic carbon flow from particulate to dissolved forms under high carbon dioxide and warm ocean conditions Geophysical Research Letters, 38 (8).

p. 10657 line 18-19: “However, if our observation was correct. . .” Sentence unclear. Consider to revise grammar/language. What about bacterial activity measured during this experiment?

p. 10657 line 29 – p. 10658 line 3: “. . .which is contradictory to previous laboratory study. . .” should read “. . .to a previous laboratory study. . .” Besides, this is not surprising, as there have been several contradicting studies of ocean acidification effects on cyanobacteria. E.g.:

Kranz, S.A., Sültemeyer, D., Richter, K.-U. and Rost, B.: Carbon acquisition by Trichodesmium: The effect of pCO2 and diurnal changes. Limnology and Oceanography, 54(2), 548–559, 2009

Czerny, J., Barcelos e Ramos, J. and Riebesell, U.: Influence of elevated CO2 concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium Nodularia spumigena. Biogeosciences, 6, 1865-1875, 2009

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of Nodularia spumigena to pCO2 – Part 1: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973-2988, 2012.

Fig. 1: Clarify meaning of colours in the caption.

BGD

9, C4502–C4508, 2012

Fig. 2: Clarify meaning of colours in the caption. In the lower panel some sampling days were pooled under the same symbol. What is the rationale behind it? State in the caption and maybe in the Methods section. (Also Fig. S1)

Fig. 3: Again colours (also Figs. 4 and 5 as well as S1 and S2)

Fig. 5: State day in caption

Interactive comment on Biogeosciences Discuss., 9, 10645, 2012.