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Biogeosciences Discussions

Interactive comment on "Response of bacterioplankton community structure to an artificial gradient of pCO_2in the Arctic Ocean" *by* R. Zhang et al.

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Response to general comments of referee 2: We appreciated the detailed and constructive comments from referee 2. As a part of team work in EPOCA, our study used DNA fingerprinting technique (T-RFLP) to analyze and compare large amount of samples (e.g. 159 samples of 19 sampling points from all 9 mesocosms). In two companion papers, Sperling et al. and Roy et al. presented size fraction (particle-attached and free-living) bacterial community dynamics. These three papers provided the most detailed and systematic description about bacterial community changes under artificial pCO2 gradient in marine ecosystem so far. For the concern of water used in our

study, we did use sterilized ultrapure water, produced by Milli-Q academic A10 system. In addition, we included negative controls for all PCR amplification and did not find contaminations. Furthermore, as suggested by referee, we included more references, improved our description of statistics and provided more information about bacterial activity in the revised manuscript.

Response to specific comments of referee 2: 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 and community structure varied with time: : :but not the degree of ocean acidification." Response: We have rewritten this part to avoid confusion in the revised manuscript.

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. Response: We agree with the reviewer that T-RFLP does not have resolution at species level and the usage of "species richness" is misleading. We use "taxonomic richness" in the revised manuscript.

p. 10646 line 12-13: Any hypothesis why/how total alkalinity could influence community composition? Response: This sentence was replaced with a more appropriate description of environmental control on bacterial community composition.

p. 10646 line 23-24: Amann et al. is probably not the best reference for "microbial loop" Response: We have corrected the error as suggested.

p. 10647 line 12: are the values concerning the global average? Please state Response: Revised as suggested.

p. 10647 line 13-14: consider to revise grammar/language Response: We have revised

the language of this sentence.

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 mesocosms 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 Response: We have corrected the error, cited and discussed these two studies as suggested.

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) Response: Revised.

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 - 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 Response: We agreed with the comments. A brief introduction of experimental setup, with all necessary information, are presented in first two paragraphs of "Materials and methods" in the revised manuscript. We also included some background information in the abstract and introduction as suggested.

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 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. 10651 line 7: please provide sequence of primers Response: We have provided all necessary information of DNA extraction, PCR and T-RFLP procedure in the revised manuscript.

p. 10649 line 16: see concerns about use of "simple" double-distilled water above Response: Please see our response to the general comments.

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.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 kind of similarity-index was used for BIOENV? What about the most obvious factors temperature and chl? Response: The description of statistical analysis is extended and is placed in a separate paragraph in the revised manuscript.

p. 10652 line 12: State here what you mean by ": : :uncompleted: : :". The reader finds it only in the next paragraph. Response: We have revised the language of this observation.

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. 10655 line 8: ": : :environmental changes, which commonly occurred in all mesocosms: : :" Which ones can this be? Temperature, chl,: : :? 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? Response: In the new version, we concluded that the bacterial community structure

was controlled by multiple biological and chemical factors. Even the best correlation in BIOENV analysis did not show high coefficient value. So we removed the discussion of the contribution of individual parameter on bacterial community composition in the revised manuscript.

p.10653 line 28: briefly explain “Libshuff analysis” in Methods section. Response: Revised.

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) Response: In the revised manuscript, we removed the discussion on nutrient since it is too speculative.

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. Response: Revised.

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? Response: We revised this discussion and only focused on the coupling of bacteria and phytoplankton, which was shown by our observation and supported by other studies.

p. 10656 line 24-26: “This evidence proved: : :” Consider to revise grammar/language. Response: Revised.

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). Response: References are cited.

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? Response: Revised and the comparison to accompanying studies is discussed. Bacterial activity measured in this experiment is presented by Piontek et al., 2012 and is discussed in our manuscript.

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. Response: We agreed with the comments and this discussion was removed in the revised manuscript, as suggested by reviewer 1.

Fig. 1: Clarify meaning of colours in the caption. 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 Response: The figure legends have been revised as suggested.

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