Biogeosciences Discuss.,
doi:10.5194/bg-2017-10-RC1, 2017

Biogeosciences Discussions

Interactive comment on "Insignificant effects of elevated CO_2 on bacterioplankton community in a eutrophic coastal mesocosm experiment" *by* Xin Lin et al.

Anonymous Referee #1

Received and published: 21 February 2017

The manuscript addresses the research question if bacterial communities in eutrophic coastal areas will be affected by elevated CO2 concentration. The topic is highly relevant given the possible effects of changes in oceanic carbon chemistry on bacterioplankton communities and subsequent biogeochemical nutrient cycles. The authors state that they found "insignificant effects of elevated CO2 on bacterioplankton communit(ies)", however their methodology and experimental setup is poor and insufficient to test the hypothesis.

The major criticisms of the manuscript is that the bacterial community composition (BCC) resulted from contamination of tubing and material used, as well as non-axenic phytoplankton cultures and hardly represents a natural bacterioplankton community.

Even if the bacteria found in the mesocosms were of marine origin, the initial community composition is unknown and not shown to be similar among the mesocosms. Therefore the results and study are not reproducible.

In fact, samples of the initial days are missing. The BCC after 4 days looks different between mesocosms, yet 3 replicates are missing in the figures, results section and statistical analysis without mentioning. Generally, it appears bizarre that a study addressing the BCC response to elevated CO2 filters away all seawater bacteria before inoculating the water with non-axenic phytoplankton lab cultures. Phytoplankton culture parameters possibly selected for a fast-growing bacterial community that was adapted to phytoplankton bloom conditions and variation in water pH due to phytoplankton respiration processes. This would mean that the studied BCC was likely preconditioned to fluctuations in CO2 with non-adaptive species outcompeted in semi-batch phytoplankton cultures prior to the experiment. A discussion or mentioning of this is missing. Data about other microbial measurements, such as bacterial activities or cell counts, are missing – questioning if bacterioplankton actually was the initial target of the study. Did the authors develop the network method themselves as references in the method section about networks are missing? In that case the method should have been validated. The flaws of experimental design, setup and continuous samplings are complemented by insufficiently described materials and methods.

Text and style of the manuscript are poor: several references are misplaced, missing or incorrectly cited in the reference list. The text contains word/grammar mistakes, word-autocorrect errors and the style of the text is inconsistent throughout the manuscript.

Specific comments. The title is misleading. The effects of elevated CO2 on BCC were not statistically tested prior to day 6 when CO2 concentration actually differed between treatments and the bacterioplankton community was artificially induced by contamination. I doubt that the authors' results support the statement "Insignificant effects of elevated CO2 on bacterioplankton community in a eutrophic coastal mesocosm experiment"

Methods: page 5, line 18. What was the purpose of filtering the seawater for the mesocosms if the aim of the study was to study the bacterioplankton community? If the majority of the bacteria originated with the phytoplankton cultures, why does the community composition in Fig S.1 look very different from the community composition of the mesocosms at day4? At day4, the class distribution of LC mesocosms shows nearly 50% Epsilonbacteria in D4.1, while no Epsilonbacteria are reported from the coccolithophore or diatom cultures.

page 5, line 20. The insitu seawater pCO2 was 650 μatm. How relevant are control mesocosms where the pCO2 concentration is lowered? Despite it changing the carbon chemistry, seawater with 400 μatm seems not to reflect the eutrophic coastal environment in the Wuyuan Bay during January and is therefor a questionable control to test the hypothesis.

page 6, line3. How did the pH change over time and when were samples taken? During phytoplankton blooms, this has major importance as pH changes with respiration during the day and can shift largely over the course of 24 hours.

page 6, line 8. Mesocosms were bubbled with air containing 1000 ppm and 400 ppm CO2, yet differences in CO2 concentrations could not be maintained throughout the experiment. Why?

page 7, line 3. Can the authors show that the bacterial community composition at the beginning of the experiment was the same in all mesocosm bags? If not, their hypothesis cannot be tested!

page 7, line 14. BCC at day zero or 1 was not sampled.

page 7, line 18. Sequential filtering prior DNA extraction – missing discussion about the majority of bacteria not being included in the results (particle attached and algae associated/attached bacteria were filtered away).

page 7, line 19. Which DNA extraction protocol was used? phenol/chloroform method?

The method description is insufficient.

page 8, line 9. The QIIME pipeline is not sufficiently described. How many raw sequences were obtained? How many samples were sequenced/passed quality control? Which pipeline parameters were used? How was the phylogenetic tree produced? What kind of tree is it?

Section 2.5 is missing references, parameter description or validation of the method, the link to the sequencing center IEG is insufficient here.

Results page 10, line 11. Additional to pCO2 levels, the measured pH should be shown in a graph.

The results sections contain many passages of discussion that should not be included here (for example page 11, line 19 or page 14, line 16).

page 11, line 16. How many sequences were included in the results? How many reads were obtained per sample? Why were some replicates not included in the results?

page 12, line 20. Was the BCC tested for differences prior to day6? If so, results are not described or included in Table2.

On page 12, some bacteria phyla were selected for analysis, does it mean that the rest was ignored in analysis after this point and in the network analysis?

How similar/different are mesocosm replicates? Inter-treatment variability seems to be very high, possibly coupled to initial differences in bacterial communities in the different mesocosms.

page 14, line 12. Naming of OTUs is weird (e.g. OTU 4331023), the high numbers suggest many OTUs, but only 4992 were reported.

Can the authors support the results with bacterial abundance data? If certain bacteria increase/decrease in relative abundance, is this due to a change in community composition or an overall increase/decrease in cell numbers? This would stress the effect of

the phytoplankton bloom on bacterial growth and BCC.

Discussion The discussion is too short, selective and does not truly discuss the results in a broad perspective. For example:

Page 15, line 17. If the BCC resulted from phytoplankton culture inoculum, the bacteria were adapted to growth alongside phytoplankton in cultures and closed containers and resulting pH ranges due to phytoplankton respiration (possibly for several years, depending on when phytoplankton strains were isolated, non-adapted bacteria would have been outcompeted prior to the experiment). Therefore, the results should not be generalized but discussed in this perspective.

page 17, line 22. The authors "speculate that the stimulation of growth of Flavobacteria could have been due to the enhanced activation of proteorhodopsin under the HC treatment at the early stage of diatom bloom". This is pure speculation based only on selective reading of the literature and has no place here in the absence of any evidence of expression of proteorhodopsin.

Figures: Figure 1 is not relevant for the manuscript.

In Figure 2, SE or SD (description missing in Figure legend) should be shown both upwards and downwards.

Figure 3 misses a description of replicate numbers. Why does day 4 only have one replicate? It would aid the reader to have spaces between the different days. Inter-replicate variability is apparent, mesocosm 8 for example has a distinct BCC compared to other LC mesocosms (increase of Phaeobacter over time), however this is not discussed in the paper.

Figure 4, which information does this figure show that are not visual in Figure 3? How many replicates were included?

Figure 5, which data were used for the network? Which day/replicates? How are differences in replicate numbers accounted for? How are "OTUs with importance" eval-

uated?

Fig S1, how representative is the diatom BCC if it comes from two species? Is it the sum/average of cultures? Replicates? When were samples taken? During inoculation or before/after the experiment? BCC likely changes throughout the course of phytoplankton growth (as shown by the authors in the mesocosm experiment) and can affect the BCC of the inoculum.

Fig S2, the Figure text is not sufficient. How was the tree generated? What kind of tree is this? Is it rooted? Which parameters were used when it was generated? Is it relevant?

S5, the figure illustrates that the bacterioplankton diversity is widely spread in the early days of the experiment, and it is obvious that replicates at day 4 are missing. Yet a discussion of these results is missing in the text.

S6, The figure legend is misleading. The PCA legend does not show the different mesocosm replicates and they are not mentioned in the figure text. How similar are replicates (at the same day)?

Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2017-10, 2017.