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

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The goal of this study was to assess the effect of ocean acidification (OA) on the bacterial community during an "induced phytoplankton bloom" in a coastal area. The coastal water was filtered onto 0.1 um (but some bacteria were present at the start of the experiment) then three xenic phytoplankton cultures were added to the mesocosms. Despite the massive sequencing work, there are important points that have not been addressed by the authors in the experimental design as well as in the sampling and analysis steps thus weakening the paper. The authors do not show the community structure of the "contaminated water" at the beginning of the experiment (prior phyto-

plankton amendment) and this is a critical point in order to be able to state whether there is an effect or not of OA on bacterial community structure. It would be important to discuss how different the contaminated water community was in comparison to the bacterial community associated with the phytoplankton strains.

Response: We appreciate the comments from reviewer #2. The description of the experimental design, sampling and analysis have been strengthened in the revised manuscript. Our experiment was designed as an intermediary step between laboratory and natural community field experiments, with isolates of non-axenic phytoplankton being added to filtered natural waters. In this way, we were able to investigate the effect of OA on phytoplankton and bacterioplankton in eutrophic waters while minimizing the complexity of shifting compositions of natural phytoplankton communities. In other words, we aimed to study the effects of ocean acidification on some model phytoplankton species and phytoplankton culture-originated bacterioplankton in a larger scale experiment compared to the lab experiment. Therefore, this experiment could not truly reflect the effects of ocean acidification on field natural phytoplankton and bacterioplankton communities. The outdoor mesocosm system was not sterile, and it was impossible to avoid the bacteria from outside through sampling and air-sea exchange during the experiment. Our data showed that the local bacterioplankton communities were very different from bacterioplankton originated from phytoplankton culture by day 4 based, on the comparison of the bacterioplankton community at day 4 and the original bacterioplankton community. And some bacterioplankton that were not detected in the original phytoplankton culture appeared in samples collected at day 4. Therefore, we conclude that the environmental bacterioplankton outcompete the phytoplankton-originated bacterioplankton from day 0 to day 4. Since the day 2 data were lacking, it seems likely that the environmental bacterioplankton became dominant even before day 4. This suggests the bacterioplankton studied in this paper were mainly natural bacterioplankton. The points mentioned above have been added to the results and discussion section. We agree that it is important to discuss the contaminated water community in comparison to the bacterial community associated with the phytoplank-

ton by showing the bacterial community structure at day 2 and day 4. We tried to do sampling at day 2 and day 4. But eventually we could successfully extract enough DNA only from bag 1, bag 6 and bag 7 at day 4 for sequencing, probably due to high concentration of TEP (Transparent Exopolymer Particles) (Sugimoto et al., 2007, Ramaiah et al., 2000). Bacteria were not detectable by flow cytometry in the filtered seawater prior to inoculation. Three species of non-axenic phytoplankton with bacterioplankton were mixed and then inoculated into each mesocosm bag. Because the mixture added was the same, we considered the initial bacterioplankton community was similar in each mesocosm bag. We described the experimental design in a more detailed way to clarify why we used this approach in the revised manuscript. The limitations of our experimental design and approaches were also pointed out in the manuscript (Page 7 Line 16-22, Page 8 Line 1-5).

I would encourage the authors to present also the bacterial abundance data (the authors say that bacteria were present in the "contaminated water and I assume that they have counted them) that will be very useful to understand the bacterial dynamic and response to OA. Furthermore, the DOC and POC data should be included here since the authors state that data those have been packaged in another paper.

Response: We agree that it is better to discuss the correlation between bacterioplankton abundance and community structure in the manuscript as well as DOC and POC data in this paper. The bacteria abundances were shown in Yibin Huang et al entitled “responses of phytoplankton and bacterial metabolism to CO2 enrichment during a coastal mesocosm experiment” (in the second round revision at Limnology and Oceanography). DOC and POC data were shown in Nana Liu et al (in press at Marine Environmental Research) . The section Environmental parameters and experimental timeline is confusing. The authors could consider to include a table that summarizes the nutrient trends and if possible other important data (bacteria count, viral count, phytoplankton count, DOC and POC) RE: Sorry for the confusion. We agree that the nutrient trends, bacteria abundance data, phytoplankton abundance data, DOC and

POC data are important for supporting our main results (Viral counting was not done in this mesocosm experiment). However, these data were packaged in other papers either published or under revision as mentioned above. We think it is not appropriate to use these data directly in this paper. We have cited these papers containing bacteria counts, phytoplankton counts, and DOC and POC data.

Some graphs in the main text and in the SI are not very informative such as phylum distribution and genus distribution graphs and confuse the message of the paper. The SI material needs more explanation and for instance the PCA graphs do not show very clearly the findings.

Response: We improved the legends of the supplementary figures and the text to make them more informative. For example, the software used to construct the phylogenetic tree and the type of phylogenetic tree has been added into the legend of Fig.S2. The explanation of different replicates of the HC and LC treatment has been clarified in the legend Fig. S6 (PCA graph).

It would be useful that the authors would comment the use of their primers in the light of the Environ Microbiol. 2016 May;18(5):1403-14. doi:10.1111/1462-2920.13023. Epub 2015 Oct 14: Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples by Parada et al.

Response: The choice of primers amplifying 16S genes is crucial. Sequencing depth, high coverage of the taxa of interest, the ability to compare results with prior studies, accuracy in relative abundances and the phylogenetic resolution of the sequenced PCR products should be considered when choosing suitable primers (Parada et al., 2016) We used primers 341F (5’-CCTACGGGNGGCWGCAG-3’) and 805R (5’-GACTACHVGGGTATCTAATCC-3’) targeting the 16S V3-V4 region, which has successfully been applied in previous studies (Hugerth et al., 2014). Thus we used 341F/805R primers that were well accepted for bacteria diversity studies. For our

study, using 341F/805R was appropriate considering the ability to compare results with prior studies, accuracy in relative abundances and the phylogenetic resolution of the sequenced PCR products. The paper mentioned above mainly discussed about the primers 515F-Y/926R and 515F-C/806R targeting the 16S V4-V5 region. The advantage of these two pairs of primers is that it should match bacteria as well as archaea. Therefore, the archaea were missing in our data set based on the primers 341F/805R we used in this study. We think primers 515F-Y/926R are better candidates because of their better coverage and their sequences have been validated in Parada et al. Thus we think 515F-Y/926R will be useful for future bacteria diversity studies. The limitations of the primers used in this study has been added to the discussion section (Page 21 Line 13-14).

The English and the structure of the paper should be revised.

Response: The text and the structure has been revised carefully.

References: Hugerth, L. W., Wefer, H. A., Lundin, S., Jakobsson, H. E., Lindberg, M., Rodin, S., Engstrand, L., et al. 2014. DegePrime, a program for degenerate primer design for broad-taxonomic-range PCR in microbial ecology studies. Applied and Environmental Microbiology, 80: 5116–5123.

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