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Interactive comment on "Ocean acidification indirectly alters trophic interaction of heterotrophic bacteria at low nutrient conditions" *by* Thomas Hornick et al.

Thomas Hornick et al.

hornick@igb-berlin.de

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We kindly thank Reviewer #1 for the review and taking the time to provide numerous constructive comments on our manuscript. We will consider almost all comments and suggestions when revising the manuscript and have responded below with our comments and description of changes we will make to the manuscript. Wherever possible we will incorporate the suggestions. In case, we are not able to follow the suggestion, we hope that we have clearly explained our reasoning.

REVIEWER COMMENT 1: This manuscript addresses an interesting, relevant and timely issue - how bacteria and their C processing may be affected by ocean acidification. As is also pointed out, there are no reasons to expect strong direct effects, while

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there may be indirect effects channelled through other parts of the food web. This topic is addressed in large scale mesocosms with differing levels of CO2. Unfortunately, I don't find that the manuscript is very clear or efficient in addressing the issue. It is a difficult approach to study a large suite of variables that are to a large extent interdependent and try to understand what has actually happened. In my view, this study shows very minor (if any) effects of CO2 on the bacterial variables measured, and it is hard to clearly link those minor effects to any particular process. Linguistically, I think the manuscript is clear, but I think results are overstated and relationships over-interpreted, and that the paper lacks a clear focus and structure.

Author's response: We acknowledge that reviewer 1 raised these critical points. In contrast to most other studies dealing with effects of ocean acidification, we did not add nutrients to study the effects of changing CO2 on nutrient cycling in a plankton community at naturally low nutrient conditions. The purpose of the experiment was to especially test effects of changes in CO2 on a nutrient limited phytoplankton community and if possible effects on this phytoplankton community can feed back on bacterial activity and abundance. No pronounced direct effects of CO2 on bacterial variables were observed throughout the experiment. Although only minor effects could be observed in this study, the obtained results will be crucial to better understand the role of nutrients on both direct and indirect effects of CO2 on planktonic communities. However, we realize that some reported effects might be overemphasized in our old discussion and thus will reconsider their relevance. In the revised version of the manuscript we will focus better on bacterial aspects and try to link them more specifically to particular processes, supported by a very thoroughly reanalysed statistics (see comments by and our reply to reviewer #2). Consequently, large parts of the manuscript will be revised according to the suggestions of reviewer 1. Further detailed descriptions on changes, which will be amended to the manuscript and will be answered in the following responses on the comments raised by reviewer 1.

REVIEWER COMMENT 2: It is unclear in the title what "trophic interaction" refers to.

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Author's response: We realize that the title was not clear in that respect. We will change the title of the revised manuscript and address it as a question to avoid any overstatement of the results mentioned by reviewer 1: "Does ocean acidification alter nutrient- prokaryote-phytoplankton relationships at low nutrient conditions?

REVIEWER COMMENT 3: There is too little information given to be able to evaluate the methods applied by reading this paper alone. There is a lot of self-referencing to papers covering the same experiment in all parts of the manuscript and this is problematic. Important information that is missing in the methods is for example the dimensions of the mesocosms and the principles behind measuring physical and chemical parameters.

Author's response: Thanks for highlighting this important issue. We reduced on purpose as much information as possible, which is given in the core paper by Paul et al. (2015) (i.e. measurements of dissolved and particulate nutrients) to condense our results section and increase the word flow. However, we realize that it might be important to include brief descriptions of measurements of physical and chemical parameters as well as the mesocosm set-up for providing a better background on the experiment, although this was already done in the core paper by Paul et al. (2015). In the revised manuscript we will better specify the methods and try to reduce self-referencing to papers covering the same experiment wherever possible.

REVIEWER COMMENT 4: No information is given on the methods behind the estimation of low and high DNA bacteria. Results are included in the figures on low vs. high DNA bacteria, but not mentioned in the results text.

Author's response: Two groups of heterotrophic prokaryotes were identified based on their low (LDNA) and high (HDNA) fluorescence (Lines 150-151). This identification was based on gating of SYBR green I fluorescence (nucleic-acid specific dye) against the side scatter signal determined by flow cytometry (Brussaard, 2004 with adaptation according to Mojica et al., 2014) as discussed in Crawfurd et al. (2015). We will specify

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this in the revised manuscript and will also include data of the ratio between LDNA and HDNA bacteria in the results section.

REVIEWER COMMENT 5: It is unclear how statistics were used to show the relationship between e.g. bacterial variables and CO2 within a given time period - how did you account for time within each period?

Author's response: So far, statistics were solely based on spearman rank correlation. Thereby, we assigned a spearman rank correlation between two variables using all measurements within a given time period. We realize (see rebuttal to reviewer #2), that this might be problematic for interpreting multivariate relationships. We revised the statistics using multivariate approaches, i.e. distance-based redundancy analysis (dbRDA) (Legendre and Anderson, 1999) for testing multispecies responses of bacterial activity (bacterial protein production, respiration) and the microbial and phytoplankton community (abundance data) on chemical (dissolved and particulate nutrients) and physical (i.e. temperature) parameters. Our dbRDA results suggest that activity and community was significantly driven by fCO2, pH, temperature and concentrations of total particulate and dissolved organic carbon. In addition, we applied generalized linear and additive modelling to account for temporal correlations (time).

REVIEWER COMMENT 6: There is referencing in the results part. Lines 211-218 should be deleted. This manuscript should be able to stand on its own and not make the assumption that we have or will read the other papers from the same experiment. The motivation for dividing into P1 - P3 should be more explicit.

Author's response: The revised manuscript will be part of a special issue comprising several manuscripts with a focus on different aspects of the described experiment. Since most of the experiments are based on a division of the experiment in phases as described by Paul et al. (2015), we decided to give a short description of these phases to avoid confusions with all other manuscripts. This phase division by Paul et al. (2015) was solely based on Chl a and temperature, which does not always match

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bacterial parameters or changes in particulate and dissolved nutrient pools. Therefore, we intended to use a different phase division based on major changes in bacterial biovolume. However, we understand that a general division in temporal phases might be difficult. Hence, we decided to avoid a phase division for all statistical analyses. This paragraph will be reworked to focus clearly on bacteria and the heterotrophic processes of the experiment.

REVIEWER COMMENT 7: Lines 228-229 "During P2, concentrations of Chl a increased again". I don't think this concurs with the graph.

Author's response: We agree that the text was not clear and thus it will be improved in the revised manuscript.

REVIEWER COMMENT 8: Lines 236-237 A Spearman rank correlation does not allow to make an interpretation that distinguishes some treatments from others.

Author's response: We agree on that. The description in lines 236-237 is only based on a graphical evaluation. The text will be revised accordingly.

REVIEWER COMMENT 9: Lines 238-240 This negative relationship between BV of picos and Chl a is puzzling, especially since BV makes out the majority of phytoplankton biomass during the second half of the experiment.

Author's response: The relationship between BV of picophytoplankton and total Chl a does not reflect the total amount of Chl a or the contribution of picophytoplankton on total Chl a. At t13-t17 picophytoplankton contributed to ca. 50% of the total Chl a, but it's contribution increased from t17-t22 up to ca. 80% and stayed between ca. 80-100% upon the end of the experiment. In parallel, Chl a decreased after t17-t22 and stayed low until the end of the experiment. Therefore, BV of picophytoplankton and Chl a are negatively correlated during this period. However, we realized that we have to clarify this relationship more detailed and will amend the test and statistics accordingly.

REVIEWER COMMENT 10: Since bacteria are the focus of this manuscript (as I un-

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derstand the introduction), the results regarding bacteria should be placed first, not phytoplankton.

Author's response: Since heterotrophic processes, mediated by bacteria are dependent on nutrient conditions as well as autotrophic processes mediated by phytoplankton, we intended to describe nutrients and phytoplankton first. However, we realize that changing this order would help to better focus the manuscript on bacteria. We will revise the manuscript accordingly.

REVIEWER COMMENT 11: The effects of the treatments on the bacterial variables throughout the experiment are very small. The only statistical effects reported are for P1 and by looking at the graphs (Fig. 3), the relationships with CO2 are hard to discern. Then a few time points are selected and emphasized in the results and discussion because they show differences in relation to CO2 treatments, but they make out a short period of the experiment.

Author's response: Although effects of the treatment on bacterial variables are small and only present for short time periods, they might have a huge impact on oceanic carbon cycling. Largest differences between the CO2-treatments on bacterial protein production (BPP) were measured after the breakdown of the Chl a maximum at t17, when BPP reached highest values throughout the experiment. During such periods, which are usually short in time, a relatively high turnover of organic matter occurs in natural systems. Therefore, these periods are of large importance for remineralisation processes and the carbon export. We thanks reviewer 1 for highlighting this issue and will give a better reasoning for choosing such periods in time. Especially, when direct effects of CO2 on bacterial variables are not expected, direct effects of CO2 on phytoplankton and nutrient pools might then indirectly feedback on bacterial variables during such periods of high organic matter turnover, when bacteria are most likely favoured and the bacterial metabolism is stimulated. We realized that this might have been not stated clear enough and will amend the revised version of the manuscript accordingly.

REVIEWER COMMENT 12: Figure 4 is not commented on in the results text?

Author's response: We will relate data presented in figure 4 to phytoplankton and bacterial biomass in the results section.

REVIEWER COMMENT 13: The discussion overall is a little tough to follow, since is not very closely aligned to or focused on the main issue. The discussion shows the difficulties in knowing what a statistical relationship means in this kind of study - the relative role of resource abundance, grazing and viral infections can only be speculated around. Still there are plenty of statements like "...revealed several indirect responses to fCO2, resulting from alterations in phytoplankton community composition and biomass". I am not convinced that the data support such statements.

Author's response: Unfortunately, we did not perform additionally experiments to justify the role of resource limitation (C/N/P), mixotrophy, or viral infections after day 25. We recognise that statements on those topics, which are not supported by measurements will certainly remain speculative. Therefore, we will focus in the revised version of the manuscript on aspects, which are well supported by data and try to remove any speculative statements.

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