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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.

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We kindly thank Linda Rhodes (REVIEWER #2) for her review and taking her time to give constructive comments on our manuscript. We will consider all comments and suggestions when revising the manuscript and have responded below with our comments and description of changes we intend to perform in the revised version of the manuscript. Wherever possible we will incorporate the valuable suggestions. In case, we are not able to follow these suggestions, we hope that we have clearly explained our reasoning.

REVIEWER COMMENT 1: One major concern is the confounding of fCO2 levels and microorganisms added with the CO2-saturated seawater to adjust fCO2 levels. Accord-

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ing to Paul et al (2015), different volumes of 50 μM-filtered seawater were infused in the mesocosms to achieve a gradient of fCO2. This level of filtration will pass viruses, small grazers, and other microorganisms that can influence trophic interactions. Because the volume of added seawater is correlated with fCO2 levels, it is not possible to separate the abiotic CO2 effect from unknown biotic effects. This confounding problem was not addressed in the manuscript and is a serious problem.

Author's response: We are aware of the problem, that a manipulation with CO2-saturated water could impact the planktonic community due to the manipulation itself or the introduced stress by rapid changes in the carbonate system. Therefore, we added CO2-saturated water with the "spider" to rapidly and equally distribute the CO2-saturated water within each mesocosm according to Paul et al. (2015). Moreover, the addition of CO2 was performed in four steps to minimize the stress on the planktonic community by a rapid shift mainly in pH. In addition, reviewer Rhodes pointed out a third issue associated with the addition of CO2-saturated water. As described in Paul et al. (2015), different amounts of 50 μM prefiltered CO2-saturated water were added to each mesocosm to reveal different fugacities of CO2. However, also the control mesocosms were manipulated with the "spider" and were sparged with prefiltered but not CO2-saturated water (0.04 % of total volume) so that a similar water treatment occurred. Further, the added amounts of CO2-saturated water as compared to the total volume of the mesocosm only contributed to 0.08-0.39 %. A possible seed community, which was introduced by the manipulation with CO2-saturated water made up at maximum 0.35% of the total community. Most of the organisms, however, will die during the preparation of CO2-saturated water. A pH<4 and constant bubbling with CO2 during night will kill most of the organisms, which remained after pre-filtration (own observations). Taking all this into account, the differences in the volume of added CO2-saturated water are to our understanding negligible and will not substantially influence the interpretation of the results. We realize that the text was not clear and thus will be improved in the revised manuscript.

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REVIEWER COMMENT 2: Temperature is a major driver of bacterial abundance and production, but it was not included, even as a covariate, for any analysis. Going back to Paul et al (2015), temperature varied nearly 8°C in a non-monotonic fashion over the experimental period. This important variable should not have been ignored.

Author's response: The temperature was similar for all mesocosms and therefore can only potentially have influenced the dynamics of the microbial populations but not the extent of change between the different mesocosms. Nevertheless, the reviewer highlights an important issue, especially when making conclusions on bacterial activity parameters. We included temperature in our revised statistical analysis and will present temperature also in the revised manuscript.

REVIEWER COMMENT 3: Given the number of variables and potential interactions, why wasn't multivariate analysis or similar integrative type of analysis used? Identifying relationships through multiple univariate and bivariate patterns is cumbersome and not necessarily clear to the audience.

Author's response: We agree with reviewer's argument on that and thoroughly 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. Thereby, dbRDA results suggest that activity and community was significantly driven by fCO2, pH, temperature and concentrations of total particulate and dissolved organic carbon. In generalized linear and additive modelling we accounted for the temporal correlation (time). Further, we applied network analysis on significant spearman correlation coefficients between Chl a, temperature, fCO2, pH as well as all groups of nano and picophytoplankton, HDNA and LDNA bacteria revealed by flow cytometry (Crawfurd et al., 2015). Thereby, the 3 highest CO2-treated mesocosms clustered significantly different from both controls and the lowest CO2-treated mesocosm (see Figure 1 below).

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REVIEWER COMMENT 4: Throughout the manuscript, there are references to significant differences in values. However, there was only 1 mesocosm per fCO2 level (except for duplicate controls), and no replicate sampling per mesocosm at each time point. There is no information about variation, and therefore, no statistical basis for making statements about significance. Declared differences are based on subjective assessments, rather than objective data analysis.

Author's response: The reviewer raised an important point about the statistical analyses of the experiment. However, the experiment was designed to catch a gradient of different levels of CO2 to apply regression analysis or having the opportunity to analyse tipping points of a response to CO2 as well as analysing non-linear responses. We agree that we do not know a within-group variation of a single CO2-treatment but this is not mandatory for regression analyses. Statistically, a regression is equally valid compared, i.e. to an analysis of variance (ANOVA) to making statements about significance. Besides, parameters with possible large measurement-variations or small sample volumes (i.e. bacterial protein production (BPP)) were measured in triplicate to account for the variance within the measurement. For these parameters the mean of 3 measurements is presented. However, since these are pseudo-replicates, there is no additional value for any statistical test. We are aware that a spearman rank correlation is based on the rank and only describes the relationship between two variables by using a monotonic function. Therefore, it is probably not appropriate to make conclusions on multivariate interdependent variables. However, we reanalysed the data and applied more appropriate statistical tests and models like dbRDA (see COMMENT 3).

REVIEWER COMMENT 5: The discussion could be more succinct and relevant. Much of section 4.2 can be removed, because it is mostly speculative, and ironically, emphasizes the confounding problem mentioned above. This section also contends that grazing was responsible for the drop in bacterial biovolume at higher fCO2, but there is no supporting evidence from this study to support a grazing claim. This is an important point, because the claim is repeated in both the conclusion and abstract.

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Author′s response: As reviewer 2 addresses right, final supporting data for any evidence of a grazing claim is missing. Therefore, we will remove speculative assumptions and incorporate the section 4.2 into other sections of the discussion. The discussion will be reworked accordingly.

REVIEWER COMMENT 6: Related to the decline in bacterial biovolume at higher fCO2 are the actual results, displayed in Figure 2.I.C. Careful examination of that panel in the figure shows that one of the control mesocosms (368) exhibited a similar decline, for a slightly shorter period of time. In reality, without any information on variation around the data points, it is dangerous to be developing and discussing elaborate explanations of these patterns, if they are even accurate patterns.

Author′s response: We thank the reviewer for pointing out that this was not examined sufficiently previously in the manuscript. We will rework it.

REVIEWER COMMENT 7: Minor points: Discussion: Numbering for the sections need to be corrected. There is no number for the first portion, and two sections labeled “4.1”. Figure 3. y-axis label for Figure 2.I.B should be for cell-specific BPP.

Author‘s response: These 2 points will be corrected accordingly.

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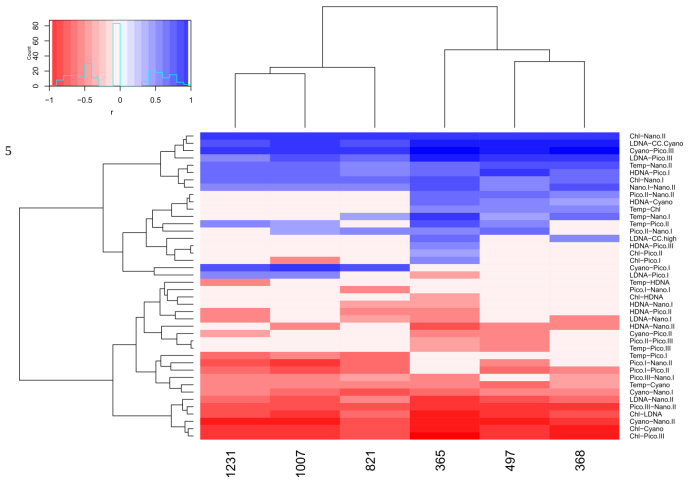

Figure 1: Heatmap and cluster analyses (euclidean distance) on significant spearman correlation coefficients (r) between Chlorophyll *a*, temperature, all groups of nano and picophytoplankton as well as HDNA and LDNA bacteria revealed by flow cytometry (Crawfurd et al., 2015). Thereby the 3 highest CO_2-treated mesocosms clustered significantly different from both controls and the lowest CO_2-treated mesocosm (average levels of fCO_2 t1-t43 are reported for each mesocosm).

Fig. 1.

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