Dear Editor,

We are grateful for the numerous constructive comments on our manuscript from the two referees. Please find below our point by point responses to each referee comment and suggestion, as well as a revised version of our manuscript with and without track changes.

We revised our statistical analyses specifically by applying different multivariate approaches (e.g, Permutational multivariate analysis of variance (PERMANOVA); Distance-based linear modeling (DistLM); Distance-based redundancy analysis (dbRDA); principal component analysis (PCA); cluster analyses). Thereby we were able to account for potential interactions of several variables as proposed by the reviewers. All analyses were performed on entire data sets of physicochemical, metabolic or community variables. Phase-divisions were removed and the method section reworked according the reviewer´s suggestions. Thus, large parts of the manuscript were substantially revised. Thereby, we focus more specifically on bacterial variables and the coupling of bacteria to phytoplankton.

We are currently formatting the data files to be uploaded to the PANGAEA data base.

We thank you for the opportunity to submit a revised manuscript for consideration in Biogeosciences and look forward to hearing a response on the manuscript soon.

Yours Sincerely,

Thomas Hornick, on behalf of all authors

Response to Reviewer #1

We thank reviewer #1 for the constructive comments on our manuscript. Our responses to reviewer comments, including modifications to the manuscript, are detailed in the following:

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 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 CO_2. 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 CO_2 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 CO_2 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 CO_2 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 CO_2 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 CO_2 on planktonic communities. However, we realized that some reported effects might be overemphasized in our old discussion and thus reconsidered their relevance. In the revised version of the manuscript we focus better on bacterial aspects and try to link them more specifically to particular processes, supported by very thoroughly reanalysed statistics (see also comments by and our reply to reviewer #2). Consequently, large parts of the manuscript have been revised according to the suggestions of both reviewers. Further detailed descriptions on changes, which were amended to the manuscript, will be answered in the following responses on the comments raised by the reviewers.

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

Author`s response: We realize that the title was not clear in that respect. Based on our reanalyzed statistics and addressing specifically the coupling of bacteria to phytoplankton, the title has been changed: "Ocean acidification impacts bacteria-phytoplankton coupling 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. In the old version, 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 methods section and increase the word flow. However, we realized that it might be important to include brief descriptions on the measurement of physical and chemical parameters (Lines 134-175), metabolic parameters (Lines 233-245) as well as the mesocosm set-up (Lines 97-111) 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 better described the methods and tried 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. 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 specified this in the revised manuscript (Lines 192-194) and mentioned observations in the ratio between LDNA and HDNA prokaryotes in the results section (Lines 302-305).

REVIEWER COMMENT 5: It is unclear how statistics were used to show the relationship between e.g. bacterial variables and CO_2 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 realized (see rebuttal to reviewer #2), that this might be problematic for interpreting multivariate relationships. We revised the statistics specifically using multivariate approaches. Thereby we used permutational multivariate analysis of variance (PERMANOVA) to test for an effect of the fCO_2-treatment on chemical, metabolic and community data comprising entire datasets throughout the experiment. All phase-separations and applied statistics only comprising particular time-points were removed and data reanalyzed. Additionally we used distance-based redundancy

analysis (dbRDA) (Legendre and Anderson, 1999) for relating/modeling physicochemical variables (including temperature and PAR) to metabolic variable and microbial communities. To elucidate possible effects of the fCO_2-treatment on the co-occurence of different functional groups of the microbial communities, we performed cluster analyses on multiple spearman´s rank correlation coefficients. Thereby *p*-values were corrected for multiple comparisons. By applying multivariate approaches, we accounted for the temporal effect (i.e. two-factoral PERMANOVA with factors time and fCO_2-treatment).

(see section 2.5. Statistical analysis in the revised manuscript)

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 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 understood that a general division in temporal phases is difficult. Hence, we reanalyzed out statistics with multivariate approaches. All phase divisions were removed. We reworked the manuscript to focus clearly on bacteria and the trophic coupling of bacteria to phytoplankton at low nutrient conditions.

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: The whole results section was substantially reworked, based on reanalyzed statistics. Most rather descriptive aspects were removed.

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 whole statistical analyses have been revised (see Reviewer COMMENT 5).

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 (Paul et al., 2015). 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 had to clarify this relationship more detailed and addressed this relationship of picophytoplankton and bacterial biovolumes in section 4.1 (Lines 407-427).

REVIEWER COMMENT 10: Since bacteria are the focus of this manuscript (as I understand 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 realized that changing this order would help to better focus the manuscript on bacteria. We revised the results section accordingly, first describing statistical result and observations in the univariate data sets of bacterial variables and afterwards phytoplankton variables and then focusing on multivariate statistical approaches.

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 CO_2 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 CO_2 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 CO_2-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. Especially, when direct effects of CO_2 on bacterial variables are not expected, direct effects of CO_2 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. However, since Paul et al. (2016) did not report on changes

in carbon export across the study we reconsidered the importance of such observations during this particular study. We reanalyzed statistics, while focusing on consistent effects of CO_2 and the co-occurrence of functional groups of the microbial community.

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

Author`s response: In the revised manuscript we report on all figures also in the results section. Further we improved the quality off all figures.

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 fCO_2, 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 recognised that statements on those topics, which are not supported by measurements will certainly remain speculative. However, distance-based linear modeling (DistLM) and distance-based redundancy analysis (dbRDA) allowed to covering aspects like grazing, etc., by unexplained variance. Most speculative assumptions have been removed and the discussion has been substantially revised.

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Brussaard, C. P. D.: Optimization of procedures for counting viruses by flow cytometry, Appl. Environ. Microb., 70, 1506–1513, doi:10.1128/AEM.70.3.1506-1513.2004, 2004.

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Responses to Reviewer #2 (Linda Rhodes)

We thank the reviewer for the numerous constructive comments on this manuscript and modified the manuscript accorgingly.

REVIEWER COMMENT 1: One major concern is the confounding of fCO_2 levels and microorganisms added with the CO_2-saturated seawater to adjust fCO_2 levels. According to Paul et al (2015), different volumes of 50 µM-filtered seawater were infused in the mesocosms to achieve a gradient of fCO_2. 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 fCO_2 levels, it is not possible to separate the abiotic CO_2 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 CO_2-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 CO_2-saturated water with the "spider" to rapidly and equally distribute the CO_2-saturated water within each mesocosm according to Paul et al. (2015). Moreover, the addition of CO_2 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 and really important issue associated with the addition of CO_2-saturated water. As described in Paul et al. (2015), different amounts of 50 µM prefiltered CO_2-saturated water were added to each mesocosm to reveal different fugacities of CO_2. However, also the control mesocosms were manipulated with the "spider" and were manipulated with prefiltered but not CO_2-saturated water (0.04 % of total volume) so that a similar water treatment occurred. Further, the added amounts of CO_2-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 CO_2-saturated water consequently made up at maximum 0.35% of the total community, when considering an equivalent abundance of organisms compared with the enclosed water in the mesocosms. However, most of the organisms will die during the preparation of CO_2-saturated water. A pH<4 and constant bubbling with CO_2 during night will kill most of the organisms, which remained after pre-filtration (own observations). However, probably the most important issue to consider is that, although a possible introduction of a seed community still could have been occurred, this community has already been present in the mesocosms. Water, used for the CO_2-treatment was taken from the same water masses enclosed in the mesocosms. Thus, there was no addition of a different microbial community. Taking all this into account, the differences in the volume of added CO_2-saturated water and thus the abundance of added microbes are to our understanding negligible and will not substantially influence the interpretation of the results. We amended the text accordingly (Lines 115-119).

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 has highlighted an important issue, especially when making conclusions on bacterial activity parameters. We included temperature and also PAR in our revised statistical multivariate analyses on metabolic and community variables.

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). (see COMMENT 5 by reviewer #1)

REVIEWER COMMENT 4: Throughout the manuscript, there are references to significant differences in values. However, there was only 1 mesocosm per fCO_2 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 CO_2 to apply regression analysis or having the opportunity to analyse tipping points of a response to CO_2 as well as analysing non-linear responses. We agree that we do not know a within-group variation of a single CO_2-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 (i.e. see section 2.4). 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 reanalyzed the data and

applied more appropriate statistical approaches and models like dbRDA (see COMMENT 5 by reviewer #1).

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 fCO_2, 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.

Author´s response: As reviewer 2 addresses right, final supporting data for any evidence of a grazing claim is missing. Therefore, we removed speculative assumptions and incorporated the section 4.2 into other sections of the discussion. The discussion has been reworked substantially.

REVIEWER COMMENT 6: Related to the decline in bacterial biovolume at higher fCO_2 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. As pointed out before, we reworked our statistical analyses and removed such solely graphical interpretations.

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 have been corrected accordingly.

References

Crawfurd, K. J., Brussaard, C. P. D., and Riebesell, U.: Shifts in the microbial community in the Baltic Sea with increasing CO_2, Biogeosciences Discuss., doi:10.5194/bg-2015-606, in review, 2016.

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Ocean acidification impacts~~indirectly alters trophic interaction of heterotrophic~~ bacteria-phytoplankton coupling at low nutrient-conditions

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Abstract

Annually, The oceans absorb about aone fourtha quarter of the yearlyanthropogenicallyyearly produced anthropogenic atmospheric carbon dioxide (CO_2),)), resulting in a decreasedropdecrease in surface water pH, a process termed ocean acidification (OA). Surprisingly little is known about how OA affects the physiology as well as food web interactions of heterotrophic bacteria or the coupling of heterotrophic bacteria to phytoplankton when essential nutrients are limited., since most . Previous experiments were carried out, for the most part, undertaken during productive phases or even after following nutrient additions designed to stimulate algal blooms. Therefore, we undertookconductedundertook an *in situ* large-volume mesocosm (~55 m^3) experiment in the Baltic Sea by simulating different fugacities of CO_2 (fCO_2) extending from present to future conditions. The study was conductedcarried outconducted in July-August after the nominal spring-bloom, in July-August order to maintain low-nutrient conditions throughout the experiment, which. This resulted in a small-sized phytoplankton communitiescommunitycommunities dominated by small-sized functional groups (picophytoplankton). Several positive as well as negative effects on free-living (FL) and particle-associated (PA)). There was no consistent fCO_2-induced effect on Bacterial Protein Production (BPP) and biovolume (BV) could be related to fCO_2-induced differences in phytoplankton composition and subsequent the availability of phytoplankton-derived organic matter. However, dynamics of BV and), cell-specific BPP (csBPP) orof FL heterotrophic bacteria could not be explained exclusively by the availability of phytoplankton-derived organic carbon. The dynamics were also related to enhanced grazing on DNA-rich (HDNA) bacterial cells at higher fCO_2 as revealed by flow cytometry. Additionally, a decoupling of autotrophic production and heterotrophic consumption during the last third of the experiment resulted in low, but significantly higher accumulation of DOC at enhanced fCO_2. Interestingly we could not detect any consistent and direct fCO_2-induced effect on BPP, csBPP nor BVor biovolumes (BVs) of either FL or PA heterotrophic bacteria. In contrast, our results reveal several indirect fCO_2-induced effects on BPP and bacterial BV with potential, when considered as individual components (univariate analyses). Permutational Multivariate Analysis of Variance (PERMANOVA) revealed a significant effect of the fCO_2-treatment on entire assemblages of dissolved and particulate nutrients, metabolic parameters and the bacteria-phytoplankton community. However, distance-based linear modelling only identified

fCO_2 as a factor explaining the variability observed amongst the microbial community composition, but not for explaining variability within the metabolic parameters. This suggests that fCO_2 impacts on microbial metabolic parameters occurred indirectly through varying physiochemical parameters and microbial species composition. Cluster analyses examining the co-occurrence of different functional groups of bacteria and phytoplankton further revealed a separation of the four fCO_2-treated mesocosms from both control mesocosms, indicating that complex trophic interactions might be altered in a future acidified ocean. Possible consequences for ~~oceanic carbon~~ nutrient cycling and carbon export are still largely unknown, in particular in a ~~low~~ nutrient limited~~and high fCO_2 future~~limited ocean.

Key words

Ocean acidification, CO_2 enrichment, trophic interaction, Baltic Sea, KOSMOS mesocosm experiment, bacterial production, phytoplankton~~, DOC accumulation~~

1 Introduction

Since the industrial revolution the oceans have absorbed ca. one half of the anthropogenic carbon dioxide (CO_2).~~), thereby shifting~~). This has resulted in a shift in carbonate ~~chemistry~~ equilibria and pH (e.g. Caldeira and Wickett, 2003~~; Raven et al., 2005; Sabine et al., 2004). During the last decade, the Baltic Sea, experienced a pronounced decrease in pH (~0.1 pH units between 1993 and 2012, International Council for the Exploration of the Sea, 2014). This corresponds to a 30% increase in the concentration of H^+ during this period (IPCC, 2007)~~), with potential consequences for organismal~~organism~~organismal physiology (~~Fabry et al., 2008,~~ Taylor et al., 2012). In~~At the same time, autotrophic organisms can~~In principal, autotrophs should be fertilized by an enhanced CO_2 availability, increasing the production of particulate (POM) and dissolved organic matter (DOM) (~~Egge, et al., 2009;~~ Hein and Sand-Jensen, 1997; Egge, et al., 2009~~; Losh et al., 2012;~~ Riebesell et al., 2007). However, most CO_2 enrichment experiments studying natural plankton assemblages under variable nutrient conditions do not reveal a consistent response of primary production to elevated CO_2 (e.g. Engel, et al., 2005; Riebesell et al., 2007; Bach~~Hopkinson~~ et al., 2016).~~2010; Riebesell et al., 2007). Nevertheless, not only~~). Both ~~the~~ amount~~, but also~~ and ~~the~~ stoichiometric composition of algal DOM and POM can be affected by changes in fCO_2. For example, Riebesell et al. (2007) and~~or~~and Maat et al. (2014) reported an increased stoichiometric drawdown of carbon

(C) to nitrogen (N) at higher levels of fCO_2, most likely as a result from C-overconsumption (ToggweilerToggweillerToggweiler, 1993).

Since Heterotrophic bacteria greatly depend, in oligotrophic systems, are largely dependent on phytoplankton derived organic carbon (e.g. Azam, 1998), andthey will most likelyand as such respond to alterations in both the quantity and quality of phyotplankton derived DOM and POM (e.g. Allgaier et al., 2008; Grossart et al., 2006a).), de Kljijver et al., 2010). Availability and competition for nutrients, however, can substantially impactalterimpact fCO_2-induced changes in activity and biomass of phytoplankton and subsequently of heterotrophic bacteria. In nutrient-depleted or nutrient-limited systems, bacteria arecan becomeare restricted in their utilization of phytoplankton derived organic carbonmatter, depending on the relative availability of inorganic nutrientscarbon (Hoikkala et al., 2009; Lignell et al., 2008).; Thingstad and Lignell, 1997). Consequently, a fCO_2 dependent increasesincreaseincreases in inorganic C-availability for autotrophs may not stimulate heterotrophic activity. This , causing a decoupling of heterotrophic from and autotrophic processes has been termed as a "counterintuitive carbon to nutrient coupling" (Thingstad et al., 2008). Consequently,The accumulation of bioavailable dissolved organic carbon (DOC) and particulate organic carbon (POC) could accumulate), as a consequence of this decoupling in nutrient limited oceanic surface waters with, may have profound consequences for nutrient cycling and the nature of the oceanic carbon pump (e.g.Cauwet et al., 2002; Mauriac et al., 2011; Søndergaard et al., 2000; Thingstad et al., 1997). Given that various studies have reported on limitation of bacterial growth by inorganic nutrients in several parts of the Baltic Sea (e.g. Hoikkala et al., 2009; Kivi et al., 1993; Kuparinen and Heinänen, 1993).; Zweifel et al. 1993). Based on these results,), we soughtintendedsought to evaluate the effects of enhanced fCO_2 on activity and biomass of free-living (FL) as well as particle associated (PA) bacteria during a relatively low productive period characterisedof the year withcharacterised by low levels of nutrients. and low productivity.

2 Methods

2.1 Experimental setup, CO_2 manipulation and sampling

Nine floating, pelagic KOSMOS (Kiel Off-Shore Mesocosms for future Ocean Simulations~~;~~ ~~Riebesell et al., 2013~~2013a, Riebesell, et al., 2013b) mesocosms (cylindrical, 2 m diameter, 17 m long with conical sediment trap extending to 19 m depth) were moored on 12th June 2012 (day -10 = t-10; 10 days before CO_2 manipulation) at 59°51.5´N, 23°15.5'E in the Baltic Sea at Tvärminne Storfjärden on the south-west coast of Finland. Exposed~~Afterwards, the open~~Exposed mesocosm bags were rinsed ~~and water fully exchanged with the surrounding water masses~~ for a period of five days~~. Mesocosms were~~, covered on the top and bottom with a 3 mm net to exclude larger organisms. ~~At~~ Thereby, the containing water was fully exchanged with the surrounding water masses. Five days prior the start of the experiment (t-5)~~,~~), sediment traps were attached to the bottom of each mesocosm at 17 m depth. ~~Further, the~~ In addition, submerged mesocosm bags were drawn~~pulled up~~drawn 1.5 m above the water surface, enclosing and separating ~55 m^3 of water from the surrounding Baltic Sea and meshes were removed. Mesocosms were covered by a photosynthetic active radiation (PAR) transparent roof to prevent nutrient addition from birds and freshwater input from rain. Additionally, existing haloclines were removed in each mesocosm as described in Paul et al. (2015), thereby creating a fully homogeneous water body.

The experiment was conducted between 17th June (t-5) and 4th August (t43) 2012. To minimize environmental stress on enclosed organisms CO_2 addition was performed stepwise over~~on day t0 after sampling and the following~~over three days ~~to minimize environmental stress on organisms until reaching the initial fugacity levels of CO_2 (fCO_2).~~commencing on day t0. CO_2 addition was repeated at t15 in the upper mixed 7 m to compensate for outgassing. Different fCO_2 treatments were achieved by equally distributing filtered (50 µm), CO_2-saturated seawater into the treated mesocosms with a water distributor as described by Paul et al. (2015). Control mesocosms were also manipulated with the water distributor and 50 µM pre-filtered water without CO_2. CO_2 amendments resulted in ca. 0.04-0.35 % increases in the total water volume across mesocosms (Paul et al. 2015). Integrated water samples ~~throughout the whole water column (0-17m~~(0-17 m) were collected from each mesocosm and the surrounding seawater using depth-integrated water samplers (IWS, HYDRO-BIOS, Kiel).

Samples for activity measurements were directly subsampled from the IWS on the sampling boat without headspace to maintain in-situ fCO_2 concentrations during incubation.

Unfortunately, three mesocosms failed~~were lost~~failed during the experiment~~ due to~~, as a consequence of welding faults~~ and thus~~, resulting in unquantifiable water exchanges with the surrounding waters. Therefore, with~~we only refer~~with reference to the six remaining mesocosms~~ during this report, using~~, CO_2 concentrations defining each treatment are reported as the mean~~average~~mean fCO_2 concentration~~from t1 to t43 to characterize~~concentration determined over the initial~~different treatments~~initial 43 days (t1-t43) as described in Paul et al. (2015~~): ~~). The control mesocosms (two replicates) had 365 µatm and 368 µatm ~~(controls);~~fCO_2, respectively. The four treatment mesocosms each had 497 µatm, 821 µatm, 1007 µatm and 1231 µatm fCO_2, respectively. Detailed descriptions on the study site, mesocosm deployment and system, performance of the mesocosm facility throughout the experiment, CO_2 addition, carbonate chemistry, cleaning of the mesocosm bags as well as sampling frequencies of single parameters are~~can be obtained from the experimental overview by~~are given in Paul et al. (2015).

2.2 Physical and chemical parameters

Physical measurements (i.e. temperature and salinity) were performed using a CTC60M memory probe (Sea and Sun Technology, Trappenkamp, Germany~~). For these parameters, the depth integrated mean values are presented. Full descriptions of sampling and analyses of Chl *a*, particulate matter (particulate carbon (TPC), particulate organic nitrogen (PON), total particulate phosphorus (TPP), biogenic silica (BSi)), dissolved organic matter (DOM including dissolved organic carbon (DOC), dissolved orgnaic nitrogen (DON), dissolved organic phosphorous (DOP) as well as dissolved inorganic nutrients (phosphate (PO_4^{3-}), nitrate (NO_3^-)) can be obtained from Paul et al. (2015) and in case of DOP measurements from Nausch et al. ~~) and are calculated as the mean, integrated over the total depth. Photosythetic active radiation (PAR) was measured with a PAR sensor (LI-COR LI-192) at the roof of Tvärminne Zoological Station. ~~(2015).~~

Samples for dissolved inorganic carbon concentrations (DIC) and total pH were gently pressure-filtered (Sarstedt Filtropur PES, 0.2 µm pore size) using a membrane pump (Stepdos). Total pH was determined as described in Dickson et al. (2007) on a Cary 100

(Varian) spectrophotometer in a temperature-controlled 10 cm cuvette using a *m*-cresol indicator dye. (Mosley et al., 2004). DIC concentrations were determined by infrared absorption using a LI-COR LI-7000 on an AIRICA system (MARIANDA, Kiel). Total pH and DIC were used to calculate carbonate chemistry speciation using the stoichiometric equilibrium constants for carbonic acid of Mehrbach et al. (1973) as refitted by Lueker et al. (2000).

Samples for dissolved organic carbon (DOC), total dissolved nitrogen (TDN) as well as dissolved silica (DSi) and dissolved inorganic phosphate (DIP) were filtered through pre-combusted (450 °C, 6h) GF/F filters (Whatman, nominal pore size of 0.7 µm). Concentrations of DOC and TDN were determined using a high-temperature catalytic combustion technique with a Shimadzu TOC-TN V analyser following Badr et al. (2003). DSi concentrations were determined using standard colorimetric techniques (Grasshoff et al. 1983) at the micromolar level with a nutrient autoanalyser (Seal Analytical, Quattro). DIP concentrations were determined with a colorimetric method using a 2 m liquid waveguide capillary cell (Patey et al., 2008, Zhang and Chi, 2002) with a miniaturised detector (Ocean Optics Ltd).

Total particulate carbon (TPC), particulate organic nitrogen (PON) and total particulate phosphorus (TPP) samples were collected onto pre-combusted (450 °C, 6h) GF/F filters (Whatman, nominal pore size of 0.7 µm) using gentle vacuum filtration and stored in glass Petri dishes at -20 °C. Biogenic silica (BSi) samples were collected on cellulose acetate filters (0.65 µm, Whatman) using gentle vacuum filtration (< 200 mbar) and stored in glass Petri dishes at -20 °C. Filters for TPC/PON analyses were dried at 60 °C, packed into tin capsules and measured on an elemental analyser (EuroEA) according to Sharp (1974), coupled by either a Conflo II to a Finnigan Delta^{Plus} isotope ratio mass spectrometer or a Conflo III to a Thermo Finnigan Delta^{Plus} XP isotope ratio mass spectrometer. Filters for TPP were treated with oxidizing decomposition reagent (MERCK, catalogue no. 112936) to oxidise organic phosphorus to orthophosphate. Particulate silica was leached from filtered material. Concentrations of dissolved inorganic phosphate as well as dissolved silica were determined spectrophotometrically according to Hansen and Koroleff (1999).

Samples for chlorophyll *a* (Chl *a*) were filtered on GF/F filters (Whatman, nominal pore size of 0.7 µm) and stored at -20 °C. Chl *a* was extracted in acetone (90 %) and samples

homogenized. After centrifugation (10 min, 800 x g, 4 °C) the supernatand was analysed on a fluorometer (TURNER 10-AU) to determine concentrations of Chl *a* (Welschmeyer, 1994).

Further details on the determination of physical parameters, concentration of Chl *a* as well as dissolved and particulate nutrients can be obtained from Paul et al. (2015).

2.3 Microbial standing stock

Abundance of ~~photoautotrophic cells (<20 µm) and~~ free-living (FL) heterotrophic prokaryotes (HP) and photoautotrophic prokaryotic (*Synechococcus* spp.) as well as eukaryotic cells (<20 µm) were determined by flow cytometry (Crawfurd et al. 2016). Briefly~~In short~~Briefly, phytoplankton were discriminated based on their chlorophyll red autofluorescence and/or phycoerythrin orange autofluorescence (Marie et al., 1999). In combination with their side scatter signal and size fractionation~~,~~ the phytoplankton community could be divided into 6 clusters~~, (Crawfurd et al. 2016),~~, varying in size from 1 to 8.8 µm average cell diameter~~.~~ (Crawfurd et al., 2016). Three groups of picoeukaryotic phytoplankton (Pico I-III), 1 picoprokaryotic photoautotroph (*Synechococcus* spp.) and 2 nanoeukaryotic phytoplankton groups were detected. Biovolume (BV) estimations were based on cell abundance and average cell diameters by assuming a spherical cell shape. The BV sum of Synechococcus and Pico I-III is expressed as BV_{Pico}. The BV sum of Nano I and II will be referred as BV_{Nano}. ~~Abundances of FL HP were determined from 0.5 % glutaraldehyde fixed samples after staining with a nucleic acid specific dye (Crawfurd et al. 2016). Unicellular cyanobacteria (*Synechococcus* spp.) contributed at max 10% of the total counts and, therefore, we use the term heterotrophic prokaryotes (HP). Two groups were identified based on their low (LDNA) and high (HDNA) fluorescence.~~

Abundances of FL prokaryotes were determined from 0.5 % glutaraldehyde fixed samples after staining with the nucleic acid-specific dye SYBR green I (Crawfurd et al. 2016). Unicellular cyanobacteria (*Synechococcus* spp.) contributed~~Particle-associated (PA) HP~~contributed maximally 10% of the total counts. Two additional groups were identified based on their low (LDNA) and high (HDNA) fluorescence. This identification was based on gating of SYBR green I fluorescence against the side scatter signal (CrawfurdBrussaard, 2004 with adaptation according to Mojica et al., 20162014). Particle-associated (PA) prokaryotes were enumerated by epifluorescence-microscopy on a Leica Leitz DMRB fluorescence

239 microscope with UV- and blue light excitation filters (Leica Microsystems, Wetzlar,
240 Germany). Fresh samples were gently mixed to prevent particle settling and a 15 mL
241 subsample ~~of 15 mL~~ was filtered on a 0.1-% Irgalan Black coloured 5.0 µm polycarbonate-
242 filter (Whatman, Maidstone, UK) (Hobbie et al., 1977). ~~Thereafter,~~ Filters were fixed with
243 glutaraldehyde (Carl Roth, Karlsruhe, Germany, final conc. 2 %) and stained for 15 min with
244 4´6-diamidino-2-phenylindole (DAPI, final conc. 1 µg mL^{-1}) (Porter and Feig, 1980) directly
245 on the filtration device and rinsed twice with sterile filtered habitat water before air-drying
246 and embedding in Citifluor AF1 (Citifluor Ltd, London, UK) on a microscopic slide (Rieck et
247 al.~~,~~., 2015). Counts~~Due to mainly small, equally distributed particles~~Counts were made based
248 on ~~the filters throughout the experiment,~~ 15 random unique squares as~~were counted with~~as
249 observed at a magnification of 1000x. The total number of heterotrophic PA
250 prokaryotes~~HP~~prokaryotes was enumerated by subtracting Chl *a* autofluorescent cells from
251 DAPI-stained cells (Rieck et al., 2015).~~.~~

252 BV of~~was~~of FL and PA prokaryotes were calculated separately. ~~for FL and PA HP.~~. For FL
253 ~~HP,~~prokaryotes we ~~used~~ estimated BVs on the basis of an average cell volume of 0.06 μm^3
254 ~~reported by~~ (Hagström et al.~~,~~ ~~(~~., 1979). BV of PA prokaryotes~~HP~~prokaryotes were calculated
255 from measurements of 1600 cells across~~from~~across 3 different mesocosms (346 µatm, 868
256 µatm, 1333 µatm) and~~as well as different~~and three time points (t0, t20, t39) throughout the
257 experiment (~~t0, t20, t39) according to~~ Massana et al.~~,~~ ~~(~~., 1997). A~~The~~A resulting average BV
258 of 0.16 μm^3 per cell was ~~further~~ used to calculate BV of PA prokaryotes~~HP~~prokaryotes
259 derived from cell abundances. We~~The BV sum of both size fractions is expressed as total BV~~
260 ~~of HP (BV_{HP}). Thereby, cell numbers of PA HP were interpolated with R (R Core Team,~~
261 ~~2014), using splines, to calculate daily abundances. Further, we use~~We subsequently adopted
262 the term "~~HP" and "~~heterotrophic bacteria"~~,~~" ~~synonymously,~~", since ~~heterotrophic~~ bacteria
263 account for the majority of non~~heterotrophic~~non- photosynthetic prokaryotes in surface waters
264 (~~Karner et al., 2001;~~ Kirchman et al. 2007).

265 ~~Changes in Chl *a* and BV of heterotrophic bacteria are dependent on various factors, which~~
266 ~~are not necessarily related to each other. Therefore, we have standardized BV_{HP} to total Chl *a*~~
267 ~~known as a measurement for phytoplankton biomass (Falkowski and Kiefer, 1985). Thereby,~~
268 ~~we express a ratio (BV_{HP} : Chl*a*), describing the distribution of heterotrophic bacterial BV and~~
269 ~~phytoplankton biomass in relation to *f*CO_2.~~

~~2.4 Bacterial production and community respiration~~

2.4 Metabolic parameters

Rates of bacterial protein production (BPP) were determined by incorporation of ^{14}[C]-leucine (^{14}C-Leu, Simon and Azam, 1989) according to Grossart et al. (2006a). Triplicates and a formalin-killed control were incubated with ^{14}C-Leu (213 mCi mmol^{-1}; Hartmann Analytic GmbH, Germany) at a final concentration of 165 nM, which ensured saturation of the uptake systems of both FL and PA bacteria. Incubation was performed in the dark at *in situ* temperature (between 7.8 °C and 15.8 °C) for 1.5 h. After fixation with 2% formalin, samples were filtered onto 5.0 µm (PA bacteria) nitrocellulose filters (Sartorius, Germany) and extracted with ice-cold 5% trichloroacetic acid (TCA) for 5 min. Thereafter, filters were rinsed twice with ice-cold 5% TCA, once with ethanol (50% v/v), and dissolved in ethylacetate for measurement by liquid scintillation counting (Wallac 1414, Perkin Elmer). Afterwards, the collected filtrate was filtered on 0.2 µm (FL bacteria) nitrocellulose filters (Sartorius, Germany) and processed in the same way as the 5.0 µm filters. Standard deviation of triplicate measurements was usually <15%. The amount of incorporated ^{14}C-Leu was converted into BPP by using an intracellular isotope dilution factor of 2. A conversion factor of 0.86 was used to convert the produced protein into carbon (Simon and Azam, 1989). Cell-specific BPP rates (csBPP) were calculated by dividing BPP-rates by abundances of FL~~PA~~FL prokaryotes and PA~~FL~~PA HP.

Community respiration (CR) rates were calculated from oxygen consumption during an incubation period of 48 hours at *in situ* temperature in the dark by assuming a respiratory quotient of 1 (Berggren et al., 2012). Thereby oxygen concentrations were measured in triplicate in 120 mL O_2 bottles without headspace, using a fiber optical dipping probe (PreSens, Fibox 3), which was calibrated against anoxic and air saturated water. ~~Further descriptions are given by Spilling et al. (2015).~~

Primary production (PP) was measured using radio-labeled $NaH^{14}CO_3$ (Steeman-Nielsen, 1952) from 0-10 m depth integrated samples. After incubation of duplicate samples with 10 µL of ^{14}C bicarbonate solution (DHI Lab, 20 µCi mL^{-1}) in 8 mL vials at 2,4,6, 8 and 10 m for 24 h, samples were acidified with 1 M HCl to remove remaining inorganic ^{14}C. Radioactivity was determined by using a scintillation counter (Wallac 1414, Perkin Elmer).

PP was calculated knowing the dark-control corrected ^{14}C incorporation and the fraction of the ^{14}C addition to the total inorganic carbon pool according to Gargas (1975). Further detailsdescriptions on the measurement of CR and PP are given by Spilling et al. (2016a).

2.5 Statistical analyses

~~We used the nonparametric Spearman´s rank correlation coefficient to measure statistical dependence between variables. Significance is determined as $p < 0.05$. Statistical analyses and visualisation were performed using R 3.1.2. (R Core Team, 2014) and R package "ggplot2" (Wickham, 2009).~~

Permutational multivariate analysis of variance – PERMANOVA (Anderson, 2001, McArdle and Anderson, 2001) was used to determine associations between physical/chemical variables and biotic variables. PERMANOVA (perm=9999) was performed to test for significant differences in variance over time and between fCO_2-treated mesocosms (Anderson et al., 2008). Environmental data were normalized according Clarke and Gorley (20062001). Biotic abundance data were log(x+1) transformed (Clarke and Gorley, 2006Green, 1988). PERMANOVA partitions the total sum of squares based on the experimental design and calculates a distance based pseudo-*F* statistic for each term in the model. Distance-based linear modeling (DistLM) was implemented to relate physical/chemical predictor variables and the multivariate assemblage of biotic variables (Supplementary Table S1) (Legendre and Anderson, 1999; McArdle and Anderson, 2001; Anderson et al., 2008). The DistLM routine was based on the AIC model selection criterion (seeAkaike, 1973, Akaike, 1974, Burnham and Anderson et al., 2008, 2004) using a step-wise selection procedure. In case of equally AIC-ranked models (difference <1), a model with fewer parameters was preferred. Unconstrained ordination methods were used to visualize and determine effects of fCO_2 on biotic and physical/chemical variables in multivariate space, thereby maximizing the total overall variation (Anderson et al., 2008). A Principal Component Analysis (PCA) was performed on normalized chemical data to identify chemical gradients and patterns between the differently fCO_2-treated mesocosms over time (ClarkeMardia et al., 1979; Venerables and Gorley, 2006Ripley, 2002). Distance based redundancy analysis (dbRDA) was used for visual interpretation of the DistLM in multi-dimensional space (Anderson et al., 2008). Multivariate analyses of physicochemical, metabolic and community data were performed on a reduced data set comprising 10 time points (t5-t29, every 3rd day, t31), containing all measured

activity variables (BPP, areal PP and CR). Missing values of nutrient data or abundance data (based on every other day measurements) were estimated as means of the preceding and following measurement day. No activity data waswere interpolated or data extrapolated in general.

Cluster analyses were performed based on Spearman´s rank correlation coefficients calculated for each mesocosm between all possible combinations of LDNA, HDNA, pico- and nanophytoplankton abundances as well as total Chl *a*. Thereafter, *p*-values were corrected for multiple testing according Benjamini and Hochberg (1995). The R-package pvclust was used to assess the uncertainty in hierarchical cluster analysis (Suzuki and Shimodeira, 2015). For each cluster, AU (approximately unbiased) *p*-values (between 0 and 1) were calculated via multiscale bootstrap resampling (Suzuki and Shimodaira, 2015).

PERMANOVA, distLM and dbRDA were carried out using Primer 6.0 and PERMANOVA + for PRIMER software (Clarke and Gorley, 2006, Anderson et al., 2008). All other analysis, including PCA and the visualisation of result was performed with R 3.2.5 (R Core Team, 2016) using packages Hmisc (Harrell et al., 2016), vegan (Oksanen et al., 2016), pvclust (Suzuki and Shimodeira, 2015), gplots (Warnes et al., 2016) and ggplot2 (Wickham, 2009).

3 Results

~~Paul et al. (2015) defined general phases of the experiment by physical characteristics of the water column (temperature) as well as the first fCO_2 manipulation at t0 (Phase 0 = t-5 to t0, Phase I = t1 to t16, Phase II = t17 to t30, Phase III = t31 to t43). These phases characterize also changes in Chl *a* concentration and chemical bulk parameters. However, heterotrophic bacteria differed in their response with a variable time delay. Consequently, we divided the experiment into new phases based on changes in activity and BV of heterotrophic bacteria. To provide clarity with respect to other publications of the same study, we termed the following phases: **P1 = t0 to t8, P2 = t8 to t26 and P3 = t26 to t43**. The time between closing of the mesocosms and the first fCO_2 manipulation was defined as Phase P0 = t-5 to t0. P1 describes an initial phase without observed fCO_2 related responses in BPP, csBPP or BV. During P2 several positive as well as negative fCO_2 mediated effects on BPP, csBPP and BV were observed, which could be related to the availability of phytoplankton derived organic carbon~~

and effects of bacterial mortality. The end of P2 is defined by reaching the BV maximum of FL heterotrophic bacteria at t26.

3.1 Phytoplankton dynamics

Concentration of Chl *a* increased after closing of the mesocosms until t5, followed by a decline until the end of P1 (t8) (Figure 1). During P0 and P1 no significant fCO_2 related differences in total concentration of Chl *a* could be observed. During P2, concentrations of Chl *a* increased again, driven by increasing BV of nanophotoautotrophs (BV_{Nano}) until reaching the respective BV maximum of nanophotoautotrophs as well as Chl *a* at t16-t17 (Figure 1). Thereby, nanophotoautotrophs yielded significantly lower BV with increasing fCO_2 between t13-17 (r_s=0.68, p<<0.01, n=30), which was reflected in lower concentrations of Chl *a* in the 3 highest fCO_2 treated mesososms at the Chl *a* maximum at t16. Thereafter, both concentrations of Chl *a* and BV_{Nano} declined until t22-t28, respectively. During the whole P2, Chl *a* was highly positively correlated to BV_{Nano} (r_s=0.87, p<<0.01, n=123). From t22 until the end of the experiment, Chl *a* yielded overall low, but higher concentrations in the 3 highest fCO_2 treated mesocosms (r_s=0.71, p<<0.01, n=76).

BV of picophotoautotrophs (BV_{Pico}) was positively correlated to overall Chl *a* development during the initial phases P0 and P1 (r_s=0.64, p<<0.1, n=66), but showed a strong negative correlation to Chl *a* during P2 and P3 (r_s=-0.81, p<<0.1, n=162). Especially after the breakdown of Chl *a* at t16/t17, BV_{Pico} increased strongly towards the BV maximum at t24 and remained constant until the end of the experiment (Figure 1). The increase was mainly driven by BV of *Synechococcus* spp., which accounted for a generally high proportion of BV_{Pico} (31 ± 2 % to 59 ± 2 %) during this study (Figure S1). All four groups of picophotoautotrophs distinguished by flow cytometry, however, revealed positive or negative fCO_2 related effects on BV (Figure 2). During different periods the smallest sized photoautotroph Pico I (~1 µm) as well as Pico II showed strong fertilization effects of fCO_2, whereas *Synechococcus* spp. and Pico III were not and/or negatively affected by fCO_2.

3.23.1 Bacterial dynamicsproduction (BPP) and biovolume (BV)

Heterotrophic bacterial BV was mainly made up by comprised predominantly of FL bacteria, as. PA bacteria contributed maximallyto onlymaximally 2 ± 0.7 – 10 ± 0.7 % (mean

4.8 ± 0.6 %) of total bacterial BV. PA bacteria, however, accounted for a substantial fraction of overall BPP (27 ± 1 – 59 ± 7 %, mean 39 ± 4 %). ~~Both bacterial size-fractions showed distinct dynamics in BV, BPP and csBPP during the course of the experiment. Interestingly, we could not reveal any consistent and direct fCO_2 effect~~ There was no significant effect of fCO_2 on BPP, csBPP or BV of neither FL ~~or~~ nor PA heterotrophic bacteria~~. Nonetheless, we observed several fCO_2 related differences between the mesocosms in BPP of PA bacteria between t16 and t23 as well as BV, BPP and csBPP of FL bacteria within P2.~~

~~During the initial phases P0 and P1 changes in BPP and BV of both bacterial size-fractions paralleled changes in Chl *a* and BV_{Pico}. Thereby, no significant differences or only weak correlations in FL and PA bacterial BV as well as BPP and csBPP were observed with changes in fCO_2 (Table 1). At t8,~~ (p_{perm} > 0.05), however~~, FL bacterial BPP and csBPP yielded 4-5 times higher rates in the fCO_2 treated mesocosms compared to both controls (Figure 3). These higher FL BPP rates were well reflected in significantly higher BV of FL bacteria with increasing fCO_2 from t10 to t13 (r_s=0.72; p<<0.01; n=24). Between t8-t13, FL bacterial BV was positively correlated to BV_{Pico} (r_s=0.52, p<<0.01, n=36), but particularly to BV_{PicoI} (r_s=0.77, p<<0.01, n=36). Surprisingly, after t13/t14, FL bacterial BV declined only in the three highest fCO_2 treated mesocosms until t18 (Figure 3). In parallel, BPP of both bacterial size-fractions increased after the breakdown of Chl *a* at t16 and yielded significantly~~ a significant temporal effect was observed (p_{perm} < 0.05). Both bacterial size-fractions had distinct dynamics in abundance, BPP and csBPP during the course of the experiment. BPP and bacterial abundances were closely related to Chl *a* and BV of nano- and picophytoplankton, trending along with Chl *a* until t10 and then continuing to increase with BVs of nanophotoautotrophs and Chl *a*. The period between t16 and t26, following a sharp decrease in Chl *a* at t16 revealed highest BPP rates across the experiment with lower rates at higher fCO_2 for PA ~~bacteria (r_s=-0.52, p<0.01, n=24)~~ as well as FL bacteria~~. (r_s=-0.51, p=0.01, n=24) between t16 and t26. Standardizing BPP~~. CsBPP-rates ~~to cell abundance, however, revealed only significantly~~ were lower ~~csBPP rates~~ at elevated~~higher~~elevated fCO_2 for only the FL bacteria during this period~~. (r_s= 0.61, p<0.01, n=24). Although we measured similar responses in BPP for PA and FL bacteria between t16 and t26, BV of both size-fractions~~. Additionally, BVs of FL and PA bacterial revealed contrasting dynamics (~~Figure 3, Figure~~ Fig. 1, Fig. S1).~~S2).~~ PA bacterial BVs~~BV~~BVs declined with the decay of Chl *a*, whereas FL BVs~~bacteria~~BVs increased strongly ~~in BV,~~

~~which was positively correlated to~~ associated with an increase in BV of picophotoautotrophs ~~until~~during this period. The ratio of HDNA:LDNA prokaryotes, which both making up FL bacteria, showed also differences between the experimental~~end of P2. P3~~experimental treatments. Between t14-t25 the ratio of HDNA:LDNA was lower at higher fCO_2.

~~3.3~~3.2 Phytoplankton dynamics

Chl~~characterized by declining BPP rates~~Chl *a* concentration exhibited distinct maxima at two time periods (t5 and t16~~BV of heterotrophic bacteria. FL or PA BPP, csBPP or BV~~t16). The second maximum was associated with an increase in the BV of nanophotoautotrophs (BV_{Nano}) (Fig. 2). This increase was reduced in mesocosms containing higher concentrations of fCO_2 between t13-t17. The differences in BV_{Nano} between the treatments were reflected~~not or negatively correlated to Chl *a*~~reflected in lower concentrations of Chl *a* in the 3 highest fCO_2-treated mesososms at t16. Chl *a* and BV_{Nano} concentrations declined after t16. In contrast, BV of picophotoautotrophs (BV_{Pico}) increased after t11, associated with an increase in BV of *Synechococcus* spp., which accounted for 31 ± 2 % to 59 ± 2 % of BV_{Pico} across the period of this study (Fig. S2).S2).~~or DOC during this period (Table 1).~~ All four groups of picoautotrophs distinguished by flow cytometry, exhibited time-dependent positive or negative relationships with fCO_2 (Fig. 3, Fig. S2, Fig. S3). The Pico I (~1 µm) and Pico II taxa infrequently exhibited strong fertilization effects in response to the fCO_2-treatment. In contrast, *Synechococcus* spp. and Pico III were infrequently negatively affected by the fCO_2-treatment.

3.3 Relation between functional heterotrophic and autotrophic groups

A cluster analysis of pairwise Spearman correlations between functional bacterial and phytoplankton groups revealed a separation based on fCO_2-treatment. Specifically the four CO_2 amended mesocosms were readily distinguishable from the control treatments. Multiple bootstrap resampling (Suzuki and Shimodaira, 2015) supported this, but only significantly for the threetwo highest fCO_2-treated mesocosms. The two highest fCO_2-treatments revealed a positive correlation of LDNA bacteria and Pico I, which could not be observed in any other experimental treatment. In all CO_2-treated mesocosm we observed positive correlations between *Synechococcus* spp. and Pico III as well as *Synechococcus* spp. and Pico I, which were not present in both control mesocosms. In contrast positive correlations between LDNA

and HDNA were not detected in any fCO_2-treatement. Additionally positive correlations between Pico and Nano II as well as HDNA and Cyanobacteria were only present in both controls and the lowest fCO_2-treatment (Fig. 4).

After t10, the ratio between heterotrophic prokaryotic BV and Chl *a* varied between the fCO_2-treatments, but did not show a consistent pattern. After t17, however, the control mesocosms revealed a higher ratio compared to all fCO_2-treated mesocosms (Fig. 5).

3.4 Multivariate physicochemical characterisation

Integrated water temperature and PAR ranged between 8.0 - 15.9 °C and 11.2 - 66.8 mol m^{-2} day^{-1} during the experiment, respectively. Integrated water temperature reached the maximum at t15 and dropped again to 8.2 °C at t31.

PERMANOVA results (Table 1) on a multivariate assemblage of dissolved (DOC, TDN, Phosphate, Bsi) and particulate (TPC, PON, POP, BSiPBsi) nutrients showed significant temporal (Time-$F_{9,10}$=11.1, p=0.0001) and spatial variations along the fCO_2-gradient (fCO_2-$F_{4,10}$=2.6, p=0.02). PCA ordination of the same chemical dataset strongly reflects the temporal pattern, separating the initial time points before t11 from other time points of the experiments along the first PCA axis (Fig. 6). Thereby, Eigenvectors of TPC and PON loaded highest on PCA axis 1 (Table 2). PCA axis two was mainly characterized by high eigenvectors of dissolved phosphate as well as dissolved and particulate silica. The first two PCA axes explained 69 % of variation and cumulatively 80% with including axis three (Table 2).

3.5 Multivariate characterisation of metabolic parameters

PERMANOVA on the resemblance matrix of normalized metabolic variables (BPP, areal PP, CR) revealed significant temporal (Time-$F_{9,10}$=6.7, p=0.0002) and spatial variations along the fCO_2-gradient (fCO_2-$F_{4,10}$=2.64, p<0.03) (Table 3). DistLM identified significant effects of Temperature (p<0.03), Phosphate (p<0.02), DOC (p<0.05) and BSiPBsi (p<0.02) on the multivariate assemblage of metabolic variables (Table 4). The step-wise procedure selects PAR, temperature, DOC and phosphate as determining factors (AIC=59.6; R^2=0.26; number of variables=4). The dbRDA ordination separates the temporal development. Thereby, 92 % of the variability in the fitted model and 24 % of the total variation is explained by the first two dbRDA axes (Fig. 6).

3.6 Multivariate characterisation of the bacterioplankton and phytoplankton community

PERMANOVA on the resemblance matrix of a multivariate assemblage comprising variables of bacterial and phytoplankton communities (abundances of Pico I-III, Nano I-II, FL bacteria (HDNA, LDNA), PA bacteria, *Synechococcus* spp.Cyanobacteria and Chl *a*) revealed significant temporal (Time-$F_{9,10}$=56.8, p=0.0001) and spatial variations along the fCO_2-gradient (fCO_2-$F_{4,10}$=14.9, p=0.0001) (Table 5). DistLM identified significant effects of fCO_2 (p<0.02), Temperature (p<0.001), Phosphate (p<0.003), TPC (p<0.001), BSiPBsi (p<0.001) and POP (p<0.001) on the multivariate assemblage of bacterial and phytoplankton communitiescommunity (Table 6). The step-wise procedure selects fCO_2, temperature, TPC and phosphate as determining factors (AIC=67.2; R^2=0.44; number of variables=4). The dbRDA reveals a separation along the gradient of fCO_2 on the second dbRDA axis. The first dbRDA axis represents the overall temporal development. Thereby the first two dbRDA axesaxis capture 74 % of the variability in the fitted model and 32 % of the total variation.

4 Discussion

Although OA and its ecological consequences have received growing recognition during the last decade (Riebesell and Gattuso, 2015), surprisingly little is known about the ecological effects on heterotrophic bacterial biomass, production or themicrobial foodweb

~~interactions~~the coupling of bacterio- and phytoplankton at nutrient ~~depleted or nutrient~~ limited conditions~~., since most of the~~. Previous experiments were~~ carried out~~, for the most part, conducted during~~ the~~ productive phases of the year (e.g. phytoplankton blooms), under eutrophic conditions (e.g. coastal areas~~)),~~) or ~~even~~ with nutrient additions (e.g. Grossart et al., 2006a; Allgaier et al., 2008; Brussaard et al., 2013; Bach~~Grossart et al., 2006a; Lindh et al., 2013; Riebesell, 2013~~Bach et al, 2016). However, large parts of the oceans are nutrient-limited or experience extended nutrient-limited periods during the year (Moore et al., 2013). Thus, we conducted our experiment in July-August, when nutrients and phytoplankton production were relatively low in the northeastern Baltic Sea (Hoikkala et al., 2009; Lignell et al., 2008) and exposed a natural plankton community to different levels of CO_2.

4.1 Phytoplankton-bacterioplankton coupling at low nutrient conditions

Heterotrophic bacteria are important recyclers of autochtonous DOM in aquatic systems and play an important role in nutrient remineralisation in natural plankton assemblages (Kirchman 1994)., Brett et al., 1999). BV and production of heterotrophic bacteria are highly dependent on quantity and quality of phytoplankton-derived organic carbon and usually are tightly related to phytoplankton development (e.g.Grossart et al., 2003; Grossart et al., 2006b; Allgaier et al., 2008).Rösel and Grossart, 2012; Attermeyer et al., 2014; Attermeyer et al., 2015~~). During the~~). During this study, low nitrogen availability limited overall autotrophic production (Paul et al., 2015~~;~~, Nausch et al., 2016~~2015~~2016). This resulted in a post spring bloom phytoplankton community, dominated by picophytoplankton~~, which~~ (Paul et al., 2015). This is consistent~~known to account~~consistent with previous reports of picophytoplankton accounting for a large fraction of total phytoplankton biomass in oligotrophic, nutrient poor systems (e.g. Platt et al., 1983; Agawin et al., 2000). ~~Nevertheless,~~ Chl *a* dynamics indicated~~of Chl *a* revealed~~indicated two minor blooms of larger phytoplankton during the first half of the experiment~~. One developed directly after the closing of the mesocosms, followed by a second one driven by nanophytoplankton (Paul et al., 2015). Albeit, picophytoplankton~~, although picophytoplankton still accounted for mostly >50 % of the total Chl *a* during this period (Paul et al., 2015;, Spilling et al., 2016b). The phytoplankton development was also reflected in the PCA ordination of dissolved and particulate nutrients, clearly separating the preceding~~entire~~preceding period before t11, including the first peak of Chl *a*, from the other observations during the experiment on principal component 1 (Fig. 6).

The separation was primarily driven by concentrations of particulate matter (Table 2), which decreased until t11 and subsequently sank out of the water column (Paul et al., 2015). ~~One reason might be, that picoplanktonic cells are generally favoured compared to larger cells in terms of resource acquisition and subsequent usage at low nutrient conditions~~

Bacterial BV and BPP paralleled phytoplankton development during this period. With the decay of the initial phytoplankton bloom, a second bloom event resulted, comprised primarily of nanophytoplankton and picophytoplankton resulted (Crawfurd et al., 2016). A decrease in nanophytoplankton BV and Chl *a* concentrations after t16/t17, benefitted both FL heterotrophic bacteria and picophotoautotrophs. The increased availability of DOM, resulting from cell lysis and remineralisation of POM was associated with increases in the BV of both groups and bacterial production levels (Fig. 1, Fi. S1). We attributed these increases to the cells of Picoplankton which, due to their high volume to surface ratio as well as a small boundary layer surrounding these cells, ~~(~~, are generally favoured compared to larger cells in terms of resource acquisition at low nutrient conditions (Raven, 1998; Moore et al., 2013~~; Raven, 1998). However, when~~). If cell size is the major factor determining the access to dissolved nitrogen and phosphorous, bacteria should be able to compete equally or better with picophytoplankton at low concentrations (Suttle et al., 1990; Drakare et al., 2003).~~Drakare et al., 2003; Suttle et al., 1990). On the other hand, BV and production of heterotrophic bacteria are highly dependent on quantity and quality of phytoplankton-derived organic carbon and usually are tightly related to phytoplankton development (~~Suttle et al., 1990; Drakare et al., 2003). However, when phytoplankton is restricted in growth due to the lack of mineral nutrients, a strong comensalisticcomensalistic~~Attermeyer et al., 2014; Attermeyer et al., 2015~~; Grossart et al., 2003; Grossart et al., 2006b; Rösel and Grossart, 2012~~). Consequently, observed fCO_2 induced effects on phytoplankton abundance, phytoplankton losses due to grazing and viral lysis as well as fCO_2-related differences in phytoplankton composition altered the availability of phytoplankton-derived organic matter for FL and PA heterotrophic bacteria (Crawfurd et al., 2016; Paul et al., 2015). Subsequent, changes in BV and production of both size fractions in relation to differences in fCO_2 were observed. However, we could not reveal any consistent pattern of fCO_2-induced effects on the coupling of phytoplankton and bacteria. Changes in BV and production of heterotrophic bacteria were rather indirectly related to different positive as well as negative fCO_2-correlated effects on the phytoplankton during relatively short periods. These periods, however, comprised phases with high organic

~~matter turnover (e.g. breakdown of Chl *a* maximum). This notion emphasizes the importance to the oceanic carbon cycle, especially during long periods of general low productivity. The last phase of the experiment (P3), however, revealed also a decoupling of autotrophic production and heterotrophic consumption, leading to relatively low, but still significantly higher accumulation of DOC at enhanced fCO_2. Nonetheless, we observed additionally fCO_2-mediated differences in FL bacterial BV and cell-specific BPP rates, which could be related to effects of enhanced bacterial grazing at higher fCO_2 (Crawfurd et al., 2016). Predicting effects on heterotrophic bacteria in a future, acidified ocean might consequently depend on several complex trophic interactions of heterotrophic bacteria within the pelagic food web.~~

~~4.1 Bacteria-phytoplankton coupling at low nutrient concentrations~~

~~Heterotrophic bacteria are important recyclers of autochtonously produced DOM in aquatic systems and play an important role in nutrient regeneration in natural plankton assemblages (Kirchman 1994, Brett et al., 1999). When phytoplankton is restricted in growth due to the lack of mineral nutrients, often a strong commensalistic~~ relationship between phytoplanktonic DOM production and bacterioplanktonic DOM utilization may~~has been observed~~may evolve (Azam et al., 1983; Bratbak and Thingstad, 1985~~;~~). ~~Alterations in either growth conditions of phytoplankton or DOM availability for~~, Joint et al., 2002). Although heterotrophic ~~bacterioplankton, but also losses of phyto- and bacterioplankton due to grazing or viral lyses can influence the competition for~~ microbes may indirectly limit primary production by depriving phytoplankton of nutrients ~~and DOM remineralization (Azam et al., 1983; ~~, they would not be able to outcompete autotrophs completely since this would remove their source of substrates for carbon and energy substrate (Bratbak and Thingstad, 1985~~; Caron~~, Joint et al., 2002).~~1988; Sheik et al., 2014). The availability of DOM for heterotrophic bacteria may also change, when they attach to living algae and organic particles. As~~2002). Such a ~~consequence, ~~relationship might explain the paralleled increase in FL bacterial and picophytoplankton BV.

PA bacteria are typically~~often less affected~~typically impacted to a lesser extent by nutrient limitation due to consistently~~the generally~~consistently higher nutrient availability at particle surfaces (e.g. Grossart and Simon, 1993). ~~In our study, ~~This was reflected in this study by the ~~relatively ~~maintenance of high csBPP rates associated~~of~~associated with PA heterotrophic bacteria throughout the whole~~entire ~~experiment. Overall~~However~~Overall, PA ~~heterotrophic~~

bacteria contributed only a minor fraction (maximal 10 ± 0.7 %) to the overall ~~heterotrophic~~ bacterial BV, which is typical~~usually reported~~typical for oligotrophic or mesotrophic ecosystems (Lapoussière et al., 2010). Nevertheless, their ~~the~~ substantial contribution ~~of PA heterotrophic bacteria~~ to overall BPP emphasizes their importance, especially during such low productive periods (e.g. ~~Simon et al., 2002,;~~ Grossart, 2010). ~~Generally,~~ PA heterotrophic bacteria are essential for the remineralization of nutrients from autotrophic biomass, which would otherwise sink out from surface waters (Grossart, 2010).~~Cho and Azam, 1988; Turley and Mackie, 1994).~~ Leakage of hydrolysis products and~~as well as~~and the attachment and detachment of bacteria to and from particles stimulate production ~~of the FL bacterial size fraction~~ amongst free-living bacteria (~~Cho and Azam, 1988;~~ Smith et al., 1992; Grossart 2010~~et al., 2003, Smith et al., 1992) as well as equally sized~~) and picophytoplankton.~~, which would be able to compete with bacteria in terms of nutrient uptake. During the breakdown of Chl *a* after t16/t17, both FL heterotrophic bacteria and picophotoautotrophs benefitted from fresh, remineralized POM and their BV and production greatly increased (Figure 3, Figure S2). The contrasting dynamics of PA heterotrophic bacteria might be a result of particle losses via sinking (Turley and Mackie, 1994).~~.

4.2 Effects of ~~*f*~~CO_2 ~~-related effects~~ /pH on ~~bacterial coupling to~~ phytoplankton-~~derived organic matter~~bacterioplankton coupling at low nutrient conditions

The ~~Several previous studies demonstrated that responses~~The response of heterotrophic bacteria ~~due~~ to changes in fCO_2 has~~were~~have been previously shown to be related to phytoplankton rather than being a direct effect of pH or CO_2 (e.g. Allgaier et al., 2008, Grossart et al., 2006a).~~2006). Also during~~2006a). Here, neither BPP nor BV of neither FL nor PA bacteria suggested a direct effect of CO_2 (PERMANOVA). Differences in FL bacterial BV, BPP, and the ratio of HDNA/LDNA, occurred along the gradient of fCO_2, but were limited to short time periods. Furthermore, these changes were not consistent with fCO_2 resulting in both increases and decreases of a particular variable at specific times (Fig. 1). Periods where fCO_2-related effects were apparent comprised periods with high organic matter turnover (e.g. breakdown of Chl *a* maximum). However, Paul et al. (2015) could not reveal any effect of fCO_2 on the export of carbon, neither across the study period nor at individual time points. Thus it is reasonable to assumespeculate that these small fCO_2-related differences

in bacterial variables arewere a consequence of other altered components of the aquatic food web, and dothereby did not necessarily manifest as changes in carbon export.

Given the inability to relate individual aspects of microbial metabolism or community composition to fCO_2 concentrations, we sought to determine whether an impact was evident using a multivariate approach. Chemical, metabolic and community matrices exhibitedwere shown to exhibit large variations in relation to a strong temporal effect throughout the whole sampling period ($p<<0.01$, Table 1, Table 3, Table 5). In addition, an effect of the fCO_2-treatment was also evident in all three multivariate assemblages, albeit explaining far less of the observed variability in chemical and metabolic variables ($p<0.03$, Table 1, Table 3, Table 5). However, when relating physiochemical to metabolic variables (DistLM, Table 4), neither fCO_2 nor pH were suitable to explaining the observed variability. In contrast, fCO_2 contributed to explaining the variability amongst the bacterioplankton-phytoplankton community dynamics (DistLM, Table 6). Taken together, this ~~study, BPP and BV of both~~ suggests that effects of fCO_2-treatments manifest indirectly, through either altering physiochemical parameters or more likely the composition of the microbial community with possible but so far hidden consequences for, as an impact on microbial metabolism.

4.3 fCO_2/pH effects on phytoplankton alter indirectly phytoplankton-bacterioplankton coupling at low nutrient conditions

Autotrophic organisms can be fertilized by an enhanced CO_2 availability, altering growth conditions of phytoplankton and increasing the production of particulate (POM) and dissolved organic matter (DOM) (Hein and Sand-Jensen, 1997; Egge, et al., 2009; Riebesell et al., 2007; Losh et al., 2012). As a consequence of this increased photosynthetic fixation rate, both quantity and quality of dissolved organic matter (DOM) available for heterotrophic ~~bacterial size fractions were strongly linked to phytoplankton dynamics and revealed~~ bacteria are impacted, with potential implications for the nature of coupling between phytoplankton and bacterioplankton at low nutrient conditions (Azam et al., 1983; Bratbak and Thingstad, 1985). So far, CO_2 enrichment experiments examining natural plankton assemblages (e.g. Engel, et al., 2005; Hopkinson et al., 2010; Riebesell et al., 2007; Bach et al., 2016) did not reveal a consistent pattern of species response or primary production to elevated CO_2. Spilling et al. (2016a) could not detect any effect of increased CO_2 on total primary production, even though Crawfurd et al. (2016) reported effects of CO_2 on several ~~indirect responses to fCO_2, resulting~~

~~from alterations in phytoplankton community composition and biomass. One small~~ groups of picophytoplankton. During our study, although one larger picoeukaryote (Pico III) was negatively impacted by fCO_2, two small picoeukaryotes (Pico I~~) with cell diameters of ~1 µm,~~ Pico II) benefitted from the ~~stepwise~~ CO_2 addition, yielding significantly higher growth rates and BVs~~BV~~BVs at higher fCO_2 ~~after t3~~ (Crawfurd et al., 2016~~) (Figure 2~~). This is consistent~~in line~~consistent with ~~a few~~ recent evidence~~studies, indicating~~evidence suggesting a positive impact~~effect~~impact of enhanced fCO_2 on the abundance of small picoeukaryotic phytoplankton (Brussaard et al., 2013; Newbold et al., 2012; ~~Endo et al., 2013;~~ Sala et al., ~~2015). After t5~~2015, Bach et al., 2016). Both picoeukaryotic groups were identified as variables explaining the separation along the ~~fCO_2~~-gradient of fCO_2 on the second and third dbRDA-axes~~axis~~ in the DistLM ordination of the bacteria-phytoplankton community. Specifically, Pico I was ~~controlled by~~ highly negatively correlated (r_S=-0.67) to dbRDA axis two. However, dbRDA indicated also opposing effects of fCO_2 on Pico II (r_S=0.54) and HDNA prokaryotes (r_S=-0.31), being positively or negatively correlated with axis three. Indeed, sharp increases in $BV_{Pico\ II}$ at high fCO_2 between t14-17 were associated with decreases in BV_{HDNA}.

Although we are not able to draw solid conclusions on the interaction of these two particular groups of organisms, a cluster analysis of pairwise Spearman correlations between functional groups of bacteria and phytoplankton revealed a distinct clustering with mesocosms based on fCO_2 concentration (Fig. 4). We also detected a change in the ratio of heterotrophic bacterial BV to Chl *a* between the different fCO_2-treatments, though this change was not visible for the entire study duration and not consistent with fCO_2. These results strongly suggest that trophic interactions between functional groups of bacteria and phytoplankton might be changing in a future acidified ocean.

In nutrient poor systems, variable growth rates of phytoplankton, DOM quality and quantity, but also losses of phyto- and bacterioplankton due to grazing or viral~~and~~ or ~~viral lysis with highest reported viral lysis and loss rates at t10 and t13, respectively (Crawfurd et al., 2016). Interestingly, viral lysis could only be observed under high CO_2 conditions, but not at ambient CO_2 levels, which might be related to higher Pico I productivity at increased fCO_2 (Crawfurd et al., 2016). Consequently, at high fCO_2 biomass production of FL heterotrophic bacteria was fuelled by bioavailable organic matter from viral lysis and grazing of algal cells (Brussaard et al., 1995; Brussaard et al. 2005;~~ lyses may potentially contribute to this observed decoupling

of phytoplankton and bacterioplankton at high fCO_2 (Azam et al., 1983; Bratbak and Thingstad, 1985; Sheik et al., 2014). The viral shunt orCaron et al., 1988; ~~Sheik et al., 2014). Thus, fertilization effects in photoautotrophic picoplankton during CO_2-addition and subsequent losses (Crawfurd et al., 2016) resulted indirectly in fCO_2-related differences in FL bacterial BV between t8 and t14 due to larger availability of picophytoplankton-derived DOC.~~

~~In parallel a second phytoplankton bloom developed, mainly driven by nanophytoplankton, which yielded significantly lower BV at higher fCO_2 (Crawfurd et al., 2016). This was also reflected in lower Chl *a* concentrations at highest fCO_2 (Paul et al., 2015). During breakdown of Chl *a* after t16/t17, both BPP of FL and PA bacteria yielded significantly lower rates at higher fCO_2, possibly due to the result of lower amounts of nanophytoplankton-derived organic carbon. Nonetheless, differences in BV and csBPP dynamics of FL heterotrophic bacteria between t14 and t26 could not be explained exclusively by the availability of phytoplankton-derived organic carbon, but were rather caused by higher bacterial losses mainly due to grazing at enhanced fCO_2 as reported by Crawfurd et al. (2016).~~

~~4.1 Consequences of fCO_2-related differences in bacterial mortality for trophic relationships~~

~~Not only heterotrophic bacterial activity but also mortality plays an important role in nutrient regeneration in natural plankton assemblages (e.g. Caron 1994). Two major factors determining bacterial mortality are viral lysis and grazing (e.g. Liu et al., 2010). The viral shunt generates mainly bioavailable DOM and stimulates autotrophic and heterotrophic microbes simultaneously. Advantages in competition for dissolved organic nutrients will primarily benefit heterotrophic bacteria (e.g. Joint et al., 2002). In contrast, the consumption of bacterial biomass by~~or bacterivory may release phytoplankton from competition with bacteria for limiting nutrients (e.g. Bratbak and Thingstad, 1985~~;,;~~ Caron ~~et al.,~~ and Goldman, 1990). ~~Additionally, carbon is directly transferred to higher trophic levels (Atkinson, 1996; Sherr et al., 1986; Schnetzer and Caron, 2005). Both will certainly impact the tight phytoplankton-bacteria coupling at low nutrient concentrations. However, possible effects of~~ How increased fCO_2 will ~~on the impact of~~will affect these processes (e.g. viral lysis and bacterial grazing ~~for trophic interactions are~~) under nutrient limited conditions remains so far uncertain.~~largely unknown. Only a few studies have reported on~~uncertain. Bacterial grazing by mixotrophs, which would also directly benefit from increased CO_2 availability (Rose et al.,

2009), may provide a mechanism for recycling of inorganic nutrients, otherwise bound in ~~ocean acidification research~~ bacterial biomass, as a means for supporting phytoplankton growth (e.g.Sanders, 1991; Hartmann et al., 2012; Calbet et al., 2012; Mitra et al. 2014). However, other studies examining bacterial grazing under different nutrient conditions reported conflicting positive and negative~~indicated both no effects as well as effects~~negative results of increased fCO_2 (e.g. Brussaard et al., 2013; Rose et al., 2009).~~; Suffrian et al., 2008).~~

~~During our study FL heterotrophic bacterial BV surprisingly dropped only in the highest fCO_2 treated mesocosms after t13/t14 and stayed low until t22. In particular, the delay of FL bacterial BV increase after the Chl *a* break down at t16/t17 was rather long, since heterotrophic bacteria usually react on much shorter time scales to alterations in phytoplankton derived organic matter (e.g. Azam et al., 1993). Crawfurd et al. (2016), however, reported significantly higher bacterial grazing at enhanced fCO_2 from grazing assays at t15. Consequently, higher availability of DOM after the decay of the phytoplankton bloom did stimulate BPP, but this biomass production was directly channelled to a larger proportion by grazing to higher trophic levels at enhanced fCO_2 (Atkinson, 1996; Schnetzer and Caron, 2005; Sherr et al., 1986). Nevertheless, we also may add viral lysis here as a possibility for a higher bacterial mortality. Indeed, viral abundance was higher at enhanced fCO_2 but increased already after t8 and remained on a constant level until t22 (Crawfurd et al., 2016). Although we~~it is unlikely that viral lysis caused the observed fCO_2 related differences in bacterial BV dynamics between t13/t14 and t26, it still might have added to some of the fCO_2 related effects during this period.

~~In addition, Crawfurd et al. (2016) reported following flow cytometry analysis an accompanying drop of HDNA, but not LDNA bacteria between t13/t14 and t19, which altered finally the proportion of HDNA:LDNA bacteria in relation to fCO_2 between t14 and t26. Differentiation of LDNA and HDNA bacteria according to the cell's nucleic acid content can indicate differences in cell size (Gasol and del Giorgio, 2000), but is more likely a measure for the cell's activity (Gasol and del Giorgio, 2000; Lebaron et al., 2001; Schapira et al., 2009). Although we cannot draw any conclusion, if cell size or cell activity was finally the determining factor, preferential grazing on HDNA heterotrophic bacteria seems~~ are unable to draw defined conclusions on how this myriad of complex biological processes are impacted by fCO_2, it is very likely that there is an impact on trophic interactions which may account for

the portion of unexplained variance we observed in our mulitvariate analyses. likely (Gasol et al., 1999, Hahn and Höfle, 2001; Vaqué, 2001). This resulted, however, in a higher contribution of LDNA and possibly smaller as well as less active cells to the heterotrophic bacterial population. At higher fCO_2 subsequent FL cell-specific BPP rates were reduced and BPP maxima more delayed in time between t16 and t26.

Unfortunately, we are not able to relate that an impact of these processes is likely and may thus account for a portion of the unexplained variance we observed in our results to any possible group of grazing organisms. Nevertheless, results from Flow Cytometry and counting of protozoa as well as mesozooplankton indicated possible grazers (Bermúdez et al., 2016, Crawfurd et al., 2016, Lischka et al., 2015). Bermúdez et al. (2016) reported highest biomass of protozoans around t15. Biomass was thereby substantially made up by the heterotrophic choanoflagellate *Calliacantha natans* (Bermúdez, pers. comm.). *Calliacantha natans* was demonstrated to feed in a size-selective mode only on particles < 1 µm in diameter (Marchant and Scott, 1993) and thus could be a possible predator on heterotrophic bacteria. Additionally, Crawfurd et al. (2016) distinguished one group of phototrophic picoeukaryotes by flow cytometry (Pico II), which only increased in BV and thereby yielded significantly higher BV at higher fCO_2 during the period, when abundance of HDNA bacteria was reduced due to grazing. Although we do not have any evidence for grazing of both particular groups of organisms, the type of nutrition would have implications for trophic interactions. If the dominant grazers consisted of mixotrophic organisms and would be able to fix carbon, they may have directly benefited from increased CO_2 availability (Rose et al., 2009). Consequently, grazing on bacteria by mixotrophs might have acted as a direct conduit for primary productivity supported by the use of inorganic nutrients, which would otherwise be unavailable and bound in bacterial biomass (Hartmann et al., 2012; Mitra et al. 2014; Sanders, 1991).

4.2 Decoupling of fCO_2-related effects on autotrophic production from bacterial consumption during P3

Exudation of carbon rich substances by phytoplankton is one of the major sources of labile DOM for heterotrophic bacteria (Larsson and Hagström, 1979). Exudation is highest under nutrient-poor conditions, when nutrient limitation impedes phytoplankton growth, but not photosynthetic carbon fixation (Fogg, 1983). Reported fCO_2 related increases in primary

~~production or in the consumption of inorganic carbon relative to nitrogen (e.g. Riebesell et al., 1993, Riebesell et al., 2007) may potentially enhance exudation and subsequently alter phytoplankton-bacteria interactions at higher fCO_2 (de Kluijver et al., 2010). During the last phase of the experiment (P3) we indeed observed relatively low, but still significantly higher DOC accumulation at enhanced fCO_2 (Figure 4). Although Spilling et al. (2016) could not reveal any significant differences in primary production due to fCO_2, also pools of Chl *a* and TPC as well as C:N_{POM} showed positive effects related to fCO_2~~mulitvariate analyses. ~~(Paul et al., 2015). However, BPP and heterotrophic bacterial BV of both size-fractions did not reveal any similar fCO_2-related differences to DOC concentration or phytoplankton dynamics. This could lead to the assumption, that heterotrophic bacteria were restricted in growth during P3. Similar findings have been previously described by other studies, which reported on DOC-accumulation caused by a limitation of DOM in surface waters (Cauwet et al., 2002; Larsen et al., 2015; Mauriac et al., 2011; Thingstad et al., 1997, Thingstad et al., 2008). However, generally strong increase in viral abundance and higher reported viral lysis of several phytoplankton groups at higher fCO_2 would have also generated fresh bioavailable DOM during this period (Crawfurd et al., 2016). Additionally, larger zooplankton increased strong in BV (Lischka et al., 2015). Therefore an accumulation of DOC by escaping bacterial utilization seems likely, since heterotrophic bacteria were possibly controlled by viral lysis and grazing. Nevertheless, remineralized nutrients and carbon from the breakdown of the earlier phytoplankton blooms were bound to a higher extend in autotrophic biomass at higher fCO_2 (Paul et al., 2015). This is also reflected in a lower ratio of BV_{HP} : Chl*a* with increasing fCO_2 (Figure 5). However, during P3 fCO_2-related differences did not impact sinking flux (Paul et al., 2015). This was probably related to the domination of small-sized unicellular phytoplankton, which only contributed indirectly via secondary processing of sinking material to the carbon export (Richardson and Jackson, 2007, Paul et al., 2015). On the other hand, total CR rates were significantly reduced at higher fCO_2 (Spilling et al., 2015) during P3. Interestingly, this finding would suggest lower CR at higher DOC concentrations. However, CR was strongly correlated to heterotrophic bacterial BV and thus reflected in the proportion of BV_{HP} : Chl *a*. Consequently, the counterintuitive difference in CR during P3 is most likely a result of the "heterotrophy" of the system, which was lower at higher fCO_2 (Figure 5).~~

5 Conclusion

The use of large-volume mesocosms~~Microbial processes can be affected either directly or indirectly via a cascade~~The use ~~of effects through the response of non-microbial groups or changes in water chemistry (Liu et al., 2010). Our large-volume mesocosm approach~~mesocosms allowed us to test for multiple *f*CO_2-related effects on dynamics of heterotrophic bacterial activity and their biovolume ~~dynamics on~~ in a near-realistic ecosystem ~~level~~ by including trophic interactions from microorganisms up to zooplankton. Complex~~Thereby, we~~Complex interactions between various trophic levels, which can only be properly addressed at~~specifically~~at the scale of whole ecosystems, are important for understanding and predicting *f*CO_2-induced effects on aquatic food webs and biogeochemistry in a future, acidified ocean. We examined these impacts in a nutrient-depleted system, which is representative for large parts of the oceans ~~in terms of low nutrient concentrations and productivity~~ (Moore et al., 2013). ~~During most time of the experiment,~~ Heterotrophic bacterial productivity was, for the most part, tightly coupled to the availability of phytoplankton-derived organic matter. ~~and thus responded to *f*CO_2 related alterations in pico- and nanophytoplankton biovolume, albeit with contrasting results. So far, this is the first ecosystem~~. When accounting for temporal development and taking into account trophic interactions using multivariate statistics, changes in nutrient composition, metabolic parameters and bacteria-phytoplankton communities revealed a significant effect of the *f*CO_2-treatment. Although not consistent throughout the experiment, differences in the ratio of heterotrophic bacterial BV to Chl *a* during the last half of the experiment suggest that a future ocean will become more autotrophic during low productive periods as a result of altered trophic interactions between functional groups of bacteria and phytoplankton. There is additional support for this conclusion from examining the atmospheric exchange of CO_2 (Spilling et al., 2016b). During the limited time-scale of this study, the~~which cannot only report on positive, but also on significantly negative~~the observed effects of *f*CO_2 did not manifest as altered carbon export (Paul et al., 2015). However~~higher *f*CO_2 on bacterial~~However, over several years, maintained changes in nutrient cycling, as a consequence of a permanent decoupling between bacteria and phytoplankton, are likely tomay arise and impact the nature of the carbon pump.

6 Data availability

Data of primary production and respiration can be obtained from Spilling et al. (2016b; doi: 10.1594/PANGAEA.863933). Other variables from the experiment (e.g. total particulate and dissolved nutrients) can be found in Paul et al. (2016; doi:10.1594/PANGAEA.863032). Flow Cytometry data can be obtained from Crawfurd et al. (2016). The primary ~~production. During the experiment, bacterial mortality from grazing and viral lysis had a strong impact on bacterial biovolume. In particular, fCO_2 induced effects on bacterial grazing and its impact on higher trophic levels are still poorly understood and have been greatly neglected in ocean acidification research. In our study, however, there was a period when autotrophic production was decoupled~~ and respiration data can be found in Spilling et al. (2016b; doi: 10.1594/PANGAEA.863933). Other variables ~~from heterotrophic consumption, which resulted in a low, but significantly higher accumulation of DOC, with potential consequences for carbon cycling in the upper ocean. Reasons and consequences of these findings can unfortunately not be generalized, since we did not perform specific bioassays to test for limiting~~the experiment (e.g. total particulate and dissolved ~~nutrients. Thus, we highly encourage implementing such bioassays during further experiments at low nutrient conditions. Our study reveals a number of fCO_2 induced effects, which led to responses in biovolume and productivity of heterotrophic bacteria. Consequently, complex trophic interactions of heterotrophic bacteria in the pelagic food web, which can only be successfully addressed in whole ecosystem studies, seem to be the key for understanding and predicting fCO_2 induced effects on aquatic food webs and biogeochemistry in a future, acidified ocean.~~) can be found in Paul et al. (2016; doi:10.1594/PANGAEA.863032).

Data of Bacterial Protein Production and bacterial abundances will be available with final publication. A PANGEA data repository will be created.

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Table 1: Spearman´s rank correlation (Spearman´s rank correlation coefficient r_s; p-value; n) of heterotrophic prokaryotic biovolume (BV_{HP}), bacterial protein production (BPP) and cell-specific BPP of size fractions I) 0.2-5.0 µm (free living; FL) and II) >5.0 µm (particle-associated; PA) with fCO_2, dissolved organic carbon (DOC), community respiration (CR), chlorophyll *a* (Chl *a*) and total as well as group-specific biovolumes of pico- and nanophotoautotrophs (*Synechococcus* spp, Pico I-III, Nano I-II) during the different phases of the experiment. (n.s. = not significant)

	FL size			PA size		
	BV_{HP}	BPP	csBPP	BV_{HP}	BPP	csBPP
fCO_2	P0: -	P0: -	P0: -	P0: -	P0: -	P0: -
DOC	P0: -	P0: -	P0: -	P0: -	P0: -	P0: -
CR	P0: -0.71; <0.01; 12 P1: 0.58; <<0.01; 42 P2: 0.64; <<0.01; 106 P3: 0.59; <<0.01; 36	P0: n.s. P1: n.s. P2: 0.72; <<0.01; 36 P3: n.s.	P0: n.s. P1: n.s. P2: 0.51; <0.01; 36 P3: n.s.	P0: -0.62; 0.03; 12 P1: 0.5; 0.03; 18 P2: 0.5; <0.01; 36 P3: n.s.	P0: n.s. P1: n.s. P2: 0.71, <<0.01; 36 P3: n.s.	P0: n.s. P1: n.s. P2: n.s. P3: n.s.
Chl *a*	P0: n.s. P1: 0.77; <<0.001; 48 P2: -0.77;<<0.001; 112 P3: n.s.	P0: -0.59; 0.04; 12 P1: 0.48; 0.02; 24 P2: -0.41; <0.01; 41 P3: n.s.	P0: -0.89; 0.02; 6 P1: n.s. P2: n.s. P3: n.s.	P0: -0.65; 0.02; 12 P1: 0.39; 0.05; 24 P2: n.s. P3: n.s.	P0: n.s. P1: 0.51; 0.01; 24 P2: -0.49, <0.01; 41 P3: n.s.	P0: n.s. P1: n.s. P2: -0.41; 0.01; 41 P3: -0.31; 0.05; 41
BV_{Nano}	P0: n.s. P1: n.s. P2: -0.75; <<0.01; 112 P3: -0.46; <<0.01; 51	P0: n.s. P1: n.s. P2: -0.35; 0.02; 42 n.s.	P0: n.s. P1: n.s. P2: n.s. P3: 0.35; 0.05; 33	P0: n.s. P1: n.s. P2: n.s. P3: -0.32; 0.05; 39	P0: 0.83; 0.04; 6 P1: n.s. P2: -0.44, <0.01; 42 P3: n.s.	P0: n.s. P1: n.s. P2: 0.34; 0.03; 42 P3: n.s.
BV_{Pico}	P0: 0.74; <0.01; 12 P1: 0.79; <<0.01; 48 P2: 0.91; <<0.01; 112	P0: n.s. P1: 0.52; <0.01; 24	P0: n.s. P1: n.s. P2: n.s.	P0: n.s. P1: 0.71; <<0.01; 24	P0: n.s. P1: 0.58; <0.01; 24 P2: 0.73, <<0.01; 42	P0: n.s. P1: n.s. P2:

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	P3: n.s.	P2: 0.65; <<0.01; 42 P3: n.s.	P3: n.s.	P2: 0.31; 0.04; 42 P3: n.s.	P3: n.s.	0.37; 0.01; 42 P3: n.s.
BV_{Syn}	P0: 0.87; <<0.01; 12 P1: 0.86; <<0.01; 48 P2: 0.89; <<0.01; 112 P3: n.s.	P0: n.s. P1: 0.5; 0.01; 24 P2: 0.56; <<0.01; 42 P3: -0.44; <0.01; 38	P0: n.s. P1: n.s. P2: n.s. P3: -0.47; <0.01; 33	P0: n.s. P1: 0.64; <<0.01; 24 P2: n.s. P3: n.s.	P0: 0.83; 0.04; 6 P1: 0.55; <0.01; 24 P2: 0.55, <<0.01; 42 P3: -0.5; <0.01; 38	P0: n.s. P1: n.s. P2: 0.37; 0.01; 42 P3: n.s.
BV_{PicoI}	P0: 0.9; <<0.01; 12 P1: 0.82; <<0.01; 48 P2:0.36;<<0.01;110 P3: -0.28; 0.05; 51	P0: n.s. P1: 0.64; <<0.01; 24 P2: n.s.; P3: n.s.	P0: n.s. P1: 0.53; <0.01; 24 P2: n.s. P3: -0.34; 0.05; 33	P0: n.s. P1: 0.6; <0.01; 24 P2: n.s. P3: n.s.	P0: n.s. P1: 0.65; <<0.01; 24 P2: n.s. P3: n.s.	P0: 0.83; 0.04; 6 P1: n.s. P2: n.s. P3: n.s.
BV_{PicoII}	P0: -0.76; <0.01; 12 P1: 0.6; <<0.01; 48 P2: n.s.; P3: 0.36; 0.01; 51	P0: n.s. P1: 0.54; <0.01; 24 P2: n.s. P3: 0.46; <0.01; 38	P0: n.s. P1: 0.4; 0.05; 24 P2: n.s. P3: n.s.	P0: n.s. P1: 0.58; <0.01; 24 P2: 0.54; <<0.01; 42 P3: n.s.	P0: 1; <<0.01; 6 P1: 0.63; <0.01; 24 P2: n.s. P3: n.s.	P0: 0.94; <0.01; 6 P1: n.s. P2: n.s. P3: n.s.
$BV_{PicoIII}$Res BV_{NanoI}Total	P0: n.s. P0: n.s.	P0: n.s. P0: n.s.	P0: n.s. P0: n.s.		P0: n.s. P0: n.s.	P0: n.s. P0: 1; P0:
BV_{NanoII}	P0: n.s. P1: n.s. P2: -0.76; <<0.01; 112 P3: n.s.	P0: n.s. P1: n.s. P2: -0.37; 0.02; 42 P3: n.s.	P0: n.s. P1: n.s. P2: n.s. P3: n.s.	P0: n.s. P1: n.s. P2: n.s. P3: n.s.	P0: 0.81; 0.05; 6 P1: n.s. P2: -0.46; <0.01; 42 P3: n.s.	P0: n.s. P1: n.s. P2: -0.34; 0.03; 42 P3: n.s.

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Table 1: Results of two-factor permutational multivariate analysis of variance (PERMANOVA)[*] on a resemblance matrix (Euclidian distance) of normalized chemical varables (Phosphate, DOC, TDN, DSi, TPC, PON, POP, BSiPBsi). Time (Ti); fCO_2-treatment (fCO_2); Residuals (Res).

Source of variation	***df***	**SS**	**MS**	**Pseudo-*F***	***p (perm)***	**Unique perms**
Time	9	309.93	34.436	11.118	**0.0001**	9920
fCO_2 [**]	4	31.974	7.9936	2.5808	**0.0246**	9936
Time x fCO_2	36	80.177	2.2271	0.71906	0.8794	9904
Res	10	30.973	3.0973			
Total	59	472				

[*] Permutation was performed with unrestricted permutation of raw data.

[**] Pair-wise test could only be performed for control-mesocosms (n=2) with each fCO_2-treatment (n=1), due to missing replication for each fCO_2-treatment. Pair-wise comparison was only significant between control and the highest fCO_2-treatment (p_{perm}=0.029).

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Table 2: Eigenvectors and -values of the first four axes of a PCA on normalized variables of dissolved and particulate nutrients. Ordination of the PCA is visualized in Fig. 6.

Variable	PC1	PC2	PC3	PC4
DOC	-0.4	-0.23	0.04	0.68
TDN	0.39	0.21	0.21	0.47
Phosphate	-0.1	0.48	-0.74	0.35
DSi	0.3	0.52	-0.03	-0.24
TPC	0.48	-0.06	0.03	0.13
PON	0.46	-0.05	-0.05	0.16
POP	0.36	-0.39	-0.04	0.21
BSiPBsi	0.17	-0.51	-0.63	-0.22
% variation	49.2	19.7	11.4	7.2
cum. % variation	49.2	68.9	80.4	87.6

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Table 3: Results of two-factor permutational multivariate analysis of variance (PERMANOVA)(*) on a resemblance matrix (Euclidian distance) based on normalized metabolic variables (bacterial protein production (BPP), areal primary production (PP) and community respiration (CR)). Time (Ti); fCO_2-treatment (fCO_2); Residuals (Res).

Source of variation	*df*	SS	MS	Pseudo-*F*	*p (perm)*	Unique perms
Time	9	92.128	10.236	6.73	**0.001**	9931
fCO_2 (**)	4	16.044	4.011	2.637	**0.023**	9944
Time x fCO_2	36	42.721	1.1867	0.78018	0.792	9904
Res	10	15.21	1.521			
Total	59	182.46				

(*) Permutation was performed with unrestricted permutation of raw data.

(**) Pair-wise test could only be performed for control-mesocosms (n=2) with each fCO_2-treatment (n=1), due to missing replication for each fCO_2-treatment. Pair-wise comparisons were significant between control and all fCO_2-treatments (p_{perm}<0.04).

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Table 4: Summary of a DistLM procedure for modelling the relationship between physicochemical variables and a resemblance matrix based on a multivariate assemblage comprising normalized data of bacterial protein production (BPP), areal primary production (PP) and community respiration (CR). Non-redundant physicochemical variables were removed prior analysis. Therefore PON and pH were excluded from the subsequent analysis due to high correlations (r_s>0.9) to TPC and fCO_2, respectively.

Variable	SS (trace)	Pseudo-*F*	*p*	Prop.
fCO_2	5.0551	1.6527	0.1759	0.03
Temp(*)	10.209	3.4376	**0.0229**	0.055
PAR(*)	6.2466	2.056	0.1067	0.034
DOC(*)	8.6228	2.8769	**0.0474**	0.047
TDN	4.7628	1.5545	0.1984	0.026
Phosphate(*)	12.319	4.1994	**0.0111**	0.068
DSi	0.26167	0.083	0.9648	0.001
TPC	7.7827	2.5842	0.0613	0.004
POP	5.0171	1.6399	0.1818	0.027
BSiPBsi	11.688	3.9696	**0.0111**	0.064

(*) variables selected in step-wise procedure based on AIC.

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Table 5: Results of two-factor permutational multivariate analysis of variance (PERMANOVA)(*) on a resemblance matrix (Bray Curtis similarity) based on log(X+1) transformed abundances of Pico I-III, Nano I-II, FL bacteria (HDNA, LDNA), PA bacteria, Cyanobacteria and Chl *a*. Time (Ti); fCO_2-treatment (fCO_2); Residuals (Res).

Source of variation	*df*	SS	MS	Pseudo-*F*	*p (perm)*	Unique perms
Time	9	201.83	22.426	56.754	**0.0001**	9923
fCO_2 (**)	4	23.631	5.9077	14.951	**0.0001**	9940
Time x fCO_2	36	19.859	0.55164	1.396	0.151	9915
Res	10	3.9515	0.39515			
Total	59	271.01				

(*) Permutation was performed with unrestricted permutation of raw data.

(**) Pair-wise test could only be performed for control-mesocosms (n=2) with each fCO_2-treatment (n=1), due to missing replication for each fCO_2-treatment. Pair-wise comparisons were significant between control and all fCO_2-treatments (p_{perm}<0.01).

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Table 6: Summary of a DistLM procedure for modelling the relationship between physicochemical variables and a multivariate assemblage comprising variables of the bacterial and phytoplankton community. The resemblance matrix (Bray Curtis similarity) was based on log(X+1) transformed abundances of Pico I-III, Nano I-II, FL bacteria (HDNA, LDNA), PA bacteria, *Synechococcus* spp. and Chl *a*. Non-redundant physicochemical variables were removed prior analysis. Therefore PON and pH were excluded from the subsequent analysis due to high correlations (r_s>0.9) to TPC and fCO_2, respectively.

Variable	SS (trace)	Pseudo-*F*	*p*	Prop.
fCO_2(*)	20.469	4.7386	**0.0119**	0.075
Temp(*)	51.838	13.718	**0.0001**	0.191
PAR	10.791	2.4051	0.0813	0.039
DOC	11.14	2.4864	**0.0769**	0.041
TDN	9.4456	2.0945	0.1078	0.034
Phosphate(*)	25.649	6.063	**0.0029**	0.095
DSi	9.5766	2.1246	0.103	0.035
TPC(*)	36.038	8.8955	**0.0002**	0.133
POP	52.171	13.827	**0.0001**	0.193
BSiPBsi	36.439	9.01	**0.0005**	0.134

(*) variables selected in step-wise procedure based on AIC.

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