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Ocean acidification indirectly alters trophic interaction of heterotrophic bacteria at low nutrient conditions

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Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
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Abstract

Annually, the oceans absorb about one fourth of the anthropogenically produced atmospheric carbon dioxide (CO_2) resulting in a drop in surface water pH, a process termed ocean acidification (OA). Surprisingly little is known about how OA affects physiology as well as food web interactions of heterotrophic bacteria when essential nutrients are limited, since most previous experiments were carried out during productive phases or even after nutrient additions to stimulate algal blooms. Therefore, we conducted 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 carried out after the spring-bloom in July-August to maintain low-nutrient conditions throughout the experiment, which resulted in a small-sized phytoplankton community dominated by picophytoplankton. Several positive as well as negative effects on free-living (FL) and particle-associated (PA) 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) of 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 BV 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 consequences for oceanic carbon cycling, in particular in a low nutrient and high fCO_2 future ocean.

Key words

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

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1 Introduction

Since the industrial revolution the oceans have absorbed ca. one half of the anthropogenic carbon dioxide (CO_2), thereby shifting carbonate chemistry equilibria and pH (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 organism physiology (Fabry et al., 2008, Taylor et al., 2012). At the same time, autotrophic organisms can 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; 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; Hopkinson et al., 2010; Riebesell et al., 2007). Nevertheless, not only the amount, but also the stoichiometric composition of algal DOM and POM can be affected by changes in fCO_2. For example, Riebesell et al. (2007) or 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 (Toggweiller, 1993). Since heterotrophic bacteria greatly depend on phytoplankton derived organic carbon (e.g. Azam, 1998), they will most likely respond to alterations in quantity and quality of phyotplankton derived DOM and POM (e.g. Allgaier et al., 2008; Grossart et al., 2006a). Availability and competition for nutrients, however, can substantially alter fCO_2-induced changes in activity and biomass of phytoplankton and subsequently of heterotrophic bacteria. In nutrient-depleted or nutrient-limited systems, bacteria can become restricted in their utilization of phytoplankton derived organic matter, depending on the relative availability of inorganic nutrients (Hoikkala et al., 2009; Lignell et al., 2008; Thingstad and Lignell, 1997). Consequently, a fCO_2 dependent increase in inorganic C-availability for autotrophs may not stimulate heterotrophic activity. This decoupling of heterotrophic from autotrophic processes has been termed as a "counterintuitive carbon-to-nutrient coupling" (Thingstad et al., 2008). Consequently, bioavailable dissolved organic carbon (DOC) and particulate organic carbon (POC) could accumulate in nutrient limited oceanic surface waters with profound consequences for nutrient cycling and the oceanic carbon pump (Cauwet et al., 2002; Mauriac et al., 2011; Søndergaard

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et al., 2000; Thingstad et al., 1997). Various studies 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 intended to evaluate 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 of the year with low levels of nutrients.

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) 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. Afterwards, the open mesocosm bags were rinsed and water fully exchanged with the surrounding water masses for five days. Mesocosms were covered on the top and bottom with a 3 mm net to exclude larger organisms. At t-5, sediment traps were attached to the bottom at 17 m depth. Further, the submerged mesocosm bags were pulled up 1.5 m above the water surface, enclosing and separating ~55 m^3 of water from the surrounding Baltic Sea and 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. CO_2 addition was performed stepwise on day t0 after sampling and the following three days to minimize environmental stress on organisms until reaching the initial fugacity-levels of CO_2 (fCO_2). 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 as described by Paul et al. (2015). Water samples throughout the whole water column (0-17m) were collected from each mesocosm and the surrounding seawater using depth-integrated water samplers (IWS, HYDRO-BIOS, Kiel).

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
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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 were lost during the experiment due to welding faults and thus unquantifiable water exchanges with the surrounding waters. Therefore, we only refer to the six remaining mesocosms during this report, using the average fCO_2 from t1 to t43 to characterize the different treatments as described in Paul et al. (2015): 365 µatm and 368 µatm (controls); 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 can be obtained from the experimental overview by 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. (2015).

2.3 Microbial standing stock

Abundance of photoautotrophic cells (<20 µm) and free-living (FL) heterotrophic prokaryotes (HP) were determined by flow cytometry (Crawfurd et al. 2016). In short, 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. Three groups of picoeukaryotic phytoplankton (Pico I-III), 1 picoprokaryotic photoautotroph (*Synechococcus*

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Published: 10 March 2016

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.

Particle-associated (PA) HP were enumerated by epifluorescence-microscopy on a Leica Leitz DMRB fluorescence microscope with UV- and blue light excitation filters (Leica Microsystems, Wetzlar, Germany). Fresh samples were gently mixed to prevent particle settling and a subsample of 15 mL was filtered on a 0.1-% Irgalan Black coloured 5.0 µm polycarbonate-filter (Whatman, Maidstone, UK) (Hobbie et al., 1977). Thereafter, filters were fixed with glutaraldehyde (Carl Roth, Karlsruhe, Germany, final conc. 2 %) and stained for 15 min with 4´6-diamidino-2-phenylindole (DAPI, final conc. 1 µg mL^{-1}) (Porter and Feig, 1980) directly on the filtration device and rinsed twice with sterile filtered habitat water before air-drying and embedding in Citifluor AF1 (Citifluor Ltd, London, UK) on a microscopic slide (Rieck et al. 2015). Due to mainly small, equally distributed particles on the filters throughout the experiment, 15 random unique squares were counted with a magnification of 1000x. Total number of PA HP was enumerated by subtracting autofluorescent cells from DAPI-stained cells.

BV was calculated separately for FL and PA HP. For FL HP, we used an average cell volume of 0.06 μm^3 reported by Hagström et al. (1979). BV of PA HP were calculated from measurements of 1600 cells from 3 different mesocosms (346 µatm, 868 µatm, 1333 µatm) as well as different time points throughout the experiment (t0, t20, t39) according to Massana et al. (1997). The resulting average BV of 0.16 μm^3 per cell was further used to calculate BV of PA HP from cell abundances. The BV-sum of both size fractions is expressed as total BV of HP (BV_{HP}). Thereby, cell-numbers of PA HP were interpolated with R (R Core Team, 2014), using splines, to calculate daily abundances. Further, we use the term “HP” and “heterotrophic bacteria” synonymously, since heterotrophic bacteria account for the majority of heterotrophic prokaryotes in surface waters (Karner et al., 2001; Kirchman et al. 2007).

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
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Changes in Chl *a* and BV of heterotrophic bacteria are dependent on various factors, which are not necessarily related to each other. Therefore, we have standardized BV_{HP} to total Chl *a* known as a measurement for phytoplankton biomass (Falkowski and Kiefer, 1985). Thereby, we express a ratio (BV_{HP} : Chl*a*), describing the distribution of heterotrophic bacterial BV and phytoplankton biomass in relation to fCO_2.

2.4 Bacterial production and community respiration

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 PA and FL 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).

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

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).

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,

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

234 both concentrations of Chl *a* and BV_{Nano} declined until t22-t28, respectively. During the
235 whole P2, Chl *a* was highly positively correlated to BV_{Nano} (r_s=0.87, p<<0.01, n=123). From
236 t22 until the end of the experiment, Chl *a* yielded overall low, but higher concentrations in the
237 3 highest fCO_2-treated mesocosms (r_s=0.71, p<<0.01, n=76).

238 BV of picophotoautotrophs (BV_{Pico}) was positively correlated to overall Chl *a* development
239 during the initial phases P0 and P1 (r_s=0.64, p<<0.1, n=66), but showed a strong negative
240 correlation to Chl *a* during P2 and P3 (r_s=-0.81, p<<0.1, n=162). Especially after the
241 breakdown of Chl *a* at t16/t17, BV_{Pico} increased strongly towards the BV maximum at t24 and
242 remained constant until the end of the experiment (Figure 1). The increase was mainly driven
243 by BV of *Synechococcus* spp., which accounted for a generally high proportion of BV_{Pico}
244 (31 ± 2 % to 59 ± 2 %) during this study (Figure S1). All four groups of picophotoautotrophs
245 distinguished by flow cytometry, however, revealed positive or negative fCO_2-related effects
246 on BV (Figure 2). During different periods the smallest sized photoautotroph Pico I (~1 µm)
247 as well as Pico II showed strong fertilization effects of fCO_2, whereas *Synechococcus* spp. and
248 Pico III were not and/or negatively affected by fCO_2.

249 ### 3.2 Bacterial production (BPP) and biovolume (BV)

250 Heterotrophic bacterial BV was mainly made up by FL bacteria, as PA bacteria contributed to
251 only 2 ± 0.7 – 10 ± 0.7 % (mean 4.8 ± 0.6 %) of total bacterial BV. PA bacteria, however,
252 accounted for a substantial fraction of overall BPP (27 ± 1 – 59 ± 7 %, mean 39 ± 4 %). Both
253 bacterial size-fractions showed distinct dynamics in BV, BPP and csBPP during the course of
254 the experiment. Interestingly, we could not reveal any consistent and direct fCO_2 effect on
255 BPP, csBPP or BV of FL or PA heterotrophic bacteria. Nonetheless, we observed several
256 fCO_2-related differences between the mesocosms in BPP of PA bacteria between t16 and t23
257 as well as BV, BPP and csBPP of FL bacteria within P2.

258 During the initial phases P0 and P1 changes in BPP and BV of both bacterial size-fractions
259 paralleled changes in Chl *a* and BV_{Pico}. Thereby, no significant differences or only weak
260 correlations in FL and PA bacterial BV as well as BPP and csBPP were observed with
261 changes in fCO_2 (Table 1). At t8, however, FL bacterial BPP and csBPP yielded 4-5 times
262 higher rates in the fCO_2-treated mesocosms compared to both controls (Figure 3). These
263 higher FL BPP rates were well reflected in significantly higher BV of FL bacteria with

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
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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 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 rates to cell abundance, however, revealed only significantly lower csBPP-rates at higher fCO_2 for 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 revealed contrasting dynamics (Figure 3, Figure S2). PA bacterial BV declined with the decay of Chl *a*, whereas FL bacteria increased strongly in BV, which was positively correlated to BV of picophotoautotrophs until the end of P2. P3 was characterized by declining BPP rates and BV of heterotrophic bacteria. FL or PA BPP, csBPP or BV were not or negatively correlated to Chl *a*, BV of picophotoautotrophs or DOC during this period (Table 1).

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 microbial foodweb interactions at nutrient depleted or nutrient limited conditions, since most of the experiments were carried out during the productive phases of the year (e.g. phytoplankton blooms), under eutrophic conditions (e.g. coastal areas), or even with nutrient additions (Allgaier et al., 2008; Brussaard et al., 2013; Grossart et al., 2006a; Lindh et al., 2013; Riebesell, 2013). 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). During the study, low nitrogen availability limited overall autotrophic production (Paul et al., 2015, Nausch et al., 2015). This resulted in a post spring bloom phytoplankton community, dominated by picophytoplankton, which is known to account for a large fraction of total phytoplankton biomass in oligotrophic, nutrient poor

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Manuscript under review for journal Biogeosciences
Published: 10 March 2016

295 systems (e.g. Agawin et al., 2000). Nevertheless, dynamics of Chl *a* revealed two minor
296 blooms of larger phytoplankton during the first half of the experiment. One developed directly
297 after the closing of the mesocosms, followed by a second one driven by nanophytoplankton
298 (Paul et al., 2015). Albeit, picophytoplankton accounted mostly > 50 % of Chl *a* during the
299 entire experiment (Paul et al., 2015). One reason might be, that picoplanktonic cells are
300 generally favoured compared to larger cells in terms of resource acquisition and subsequent
301 usage at low nutrient conditions due to their high volume to surface ratio as well as a small
302 boundary layer surrounding these cells (Moore et al., 2013; Raven, 1998). However, when
303 cell size is the major factor determining the access to dissolved nitrogen and phosphorous,
304 bacteria should be able to compete equally or better with picophytoplankton at low
305 concentrations (Drakare et al., 2003; Suttle et al., 1990). On the other hand, BV and
306 production of heterotrophic bacteria are highly dependent on quantity and quality of
307 phytoplankton-derived organic carbon and usually are tightly related to phytoplankton
308 development (Attermeyer et al., 2014; Attermeyer et al., 2015; Grossart et al., 2003; Grossart
309 et al., 2006b; Rösel and Grossart, 2012). Consequently, observed fCO_2-induced effects on
310 phytoplankton abundance, phytoplankton losses due to grazing and viral lysis as well as fCO_2-
311 related differences in phytoplankton composition altered the availability of phytoplankton-
312 derived organic matter for FL and PA heterotrophic bacteria (Crawfurd et al., 2016; Paul et
313 al., 2015). Subsequent, changes in BV and production of both size-fractions in relation to
314 differences in fCO_2 were observed. However, we could not reveal any consistent pattern of
315 fCO_2-induced effects on the coupling of phytoplankton and bacteria. Changes in BV and
316 production of heterotrophic bacteria were rather indirectly related to different positive as well
317 as negative fCO_2-correlated effects on the phytoplankton during relatively short periods.
318 These periods, however, comprised phases with high organic matter turnover (e.g. breakdown
319 of Chl *a* maximum). This notion emphasizes the importance to the oceanic carbon cycle,
320 especially during long periods of general low productivity. The last phase of the experiment
321 (P3), however, revealed also a decoupling of autotrophic production and heterotrophic
322 consumption, leading to relatively low, but still significantly higher accumulation of DOC at
323 enhanced fCO_2. Nonetheless, we observed additionally fCO_2-mediated differences in FL
324 bacterial BV and cell-specific BPP rates, which could be related to effects of enhanced
325 bacterial grazing at higher fCO_2 (Crawfurd et al., 2016). Predicting effects on heterotrophic

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Manuscript under review for journal Biogeosciences
Published: 10 March 2016

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 has been observed (Azam et al., 1983; Bratbak and Thingstad, 1985). Alterations in either growth conditions of phytoplankton or DOM availability for heterotrophic bacterioplankton, but also losses of phyto- and bacterioplankton due to grazing or viral lyses can influence the competition for nutrients and DOM remineralization (Azam et al., 1983; Bratbak and Thingstad, 1985; Caron et al., 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 a consequence, PA bacteria are often less affected by nutrient limitation due to the generally higher nutrient availability at particle surfaces (e.g. Grossart and Simon, 1993). In our study, this was reflected in the relatively high csBPP rates of PA heterotrophic bacteria throughout the entire experiment. However, PA heterotrophic bacteria contributed only a minor fraction (maximal 10 ± 0.7 %) to the overall heterotrophic bacterial BV, which is usually reported for oligotrophic or mesotrophic ecosystems (Lapoussière et al., 2010). Nevertheless, 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 (Cho and Azam, 1988; Turley and Mackie, 1994). Leakage of hydrolysis products as well as attachment and detachment of bacteria to and from particles stimulate production of the FL bacterial size fraction (Cho and Azam, 1988; Grossart et al., 2003, Smith et al., 1992) as well as equally-sized 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

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(Figure 3, Figure S2). The contrasting dynamics of PA heterotrophic bacteria might be a result of particle losses via sinking (Turley and Mackie, 1994).

4.1 *f*CO_2-related effects on bacterial coupling to phytoplankton-derived organic matter

Several previous studies demonstrated that responses of heterotrophic bacteria due to changes in *f*CO_2 were related to phytoplankton rather than being a direct effect of pH or CO_2 (e.g. Allgaier et al., 2008, Grossart et al., 2006). Also during this study, BPP and BV of both heterotrophic bacterial size-fractions were strongly linked to phytoplankton dynamics and revealed several indirect responses to *f*CO_2, resulting from alterations in phytoplankton community composition and biomass. One small picoeukaryote (Pico I) with cell-diameters of ~1 µm benefitted from the stepwise CO_2 addition, yielding significantly higher growth rates and BV at higher *f*CO_2 after t3 (Crawfurd et al., 2016) (Figure 2). This is in line with a few recent studies, indicating a positive effect of enhanced *f*CO_2 on the abundance of small picoeukaryotic phytoplankton (Brussaard et al., 2013; Endo et al., 2013; Sala et al., 2015). After t5, Pico I was controlled by grazing and 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 *f*CO_2 (Crawfurd et al., 2016). Consequently, at high *f*CO_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; Sheik et al., 2014). Thus, fertilization effects in photoautotrophic picoplankton during CO_2-addition and subsequent losses (Crawfurd et al., 2016) resulted indirectly in *f*CO_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 *f*CO_2 (Crawfurd et al., 2016). This was also reflected in lower Chl *a* concentrations at highest *f*CO_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 *f*CO_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

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

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).

4.2 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 bacterivory may release phytoplankton from competition with bacteria for limiting nutrients (e.g. Bratbak and Thingstad, 1985, Caron et al., 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 increased fCO_2 on the impact of bacterial grazing for trophic interactions are so far largely unknown. Only a few studies have reported on bacterial grazing in ocean acidification research under different nutrient conditions and indicated both no effects as well as effects of 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 it

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

418 is unlikely that viral lysis caused the observed fCO_2-related differences in bacterial BV
419 dynamics between t13/t14 and t26, it still might have added to some of the fCO_2-related
420 effects during this period.

421 In addition, Crawfurd et al. (2016) reported following flow cytomety analysis an
422 accompanying drop of HDNA, but not LDNA bacteria between t13/t14 and t19, which altered
423 finally the proportion of HDNA:LDNA bacteria in relation to fCO_2 between t14 and t26.
424 Differentiation of LDNA and HDNA bacteria according to the cell's nucleic acid content can
425 indicate differences in cell size (Gasol and del Giorgio, 2000), but is more likely a measure
426 for the cell's activity (Gasol and del Giorgio, 2000; Lebaron et al., 2001; Schapira et al.,
427 2009). Although we cannot draw any conclusion, if cell size or cell-activity was finally the
428 determining factor, preferential grazing on HDNA heterotrophic bacteria seems likely (Gasol
429 et al., 1999, Hahn and Höfle, 2001; Vaqué, 2001). This resulted, however, in a higher
430 contribution of LDNA and possibly smaller as well as less active cells to the heterotrophic
431 bacterial population. At higher fCO_2 subsequent FL cell-specific BPP rates were reduced and
432 BPP maxima more delayed in time between t16 and t26.

433 Unfortunately, we are not able to relate our results to any possible group of grazing
434 organisms. Nevertheless, results from Flow Cytometry and counting of protozoa as well as
435 mesozooplankton indicated possible grazers (Bermúdez et al., 2016, Crawfurd et al., 2016,
436 Lischka et al., 2015). Bermúdez et al. (2016) reported highest biomass of protozoans around
437 t15. Biomass was thereby substantially made up by the heterotrophic choanoflagellate
438 *Calliacantha natans* (Bermúdez, pers. comm.). *Calliacantha natans* was demonstrated to feed
439 in a size-selective mode only on particles < 1 µm in diameter (Marchant and Scott, 1993) and
440 thus could be a possible predator on heterotrophic bacteria. Additionally, Crawfurd et al.
441 (2016) distinguished one group of phototrophic picoeukaryotes by flow cytometry (Pico II),
442 which only increased in BV and thereby yielded significantly higher BV at higher fCO_2
443 during the period, when abundance of HDNA bacteria was reduced due to grazing. Although
444 we do not have any evidence for grazing of both particular groups of organisms, the type of
445 nutrition would have implications for trophic interactions. If the dominant grazers consisted of
446 mixotrophic organisms and would be able to fix carbon, they may have directly benefited
447 from increased CO_2 availability (Rose et al., 2009). Consequently, grazing on bacteria by
448 mixotrophs might have acted as a direct conduit for primary productivity supported by the use

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

of inorganic nutrients, which would otherwise be unavailable and bound in bacterial biomass (Hartmann et al., 2012; Mitra et al. 2014; Sanders, 1991).

4.3 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 (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

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

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

Microbial processes can be affected either directly or indirectly via a cascade of effects through the response of non-microbial groups or changes in water chemistry (Liu et al., 2010). Our large-volume mesocosm approach allowed us to test for multiple fCO_2-related effects on heterotrophic bacterial activity and biovolume dynamics on a near-realistic ecosystem level by including trophic interactions from microorganisms up to zooplankton. Thereby, we addressed specifically 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 tightly coupled to the availability of phytoplankton-derived organic matter and thus responded to fCO_2-related alterations in pico- and nanophytoplankton biovolume, albeit with contrasting results. So far, this is the first ecosystem study, which cannot only report on positive, but also on significantly negative effects of higher fCO_2 on bacterial 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 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 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

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

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.

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Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
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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)

	I) FL size fraction			II) PA size raction		
	BV_{HP}	**BPP**	**csBPP**	**BV_{HP}**	**BPP**	**csBPP**
***f*CO₂**	P0: - **P1: 0.36; 0.01; 48** P2: n.s. P3: n.s.	P0: - **P1: 0.5; 0.01; 24** P2: n.s. P3: n.s.	P0: - **P1: 0.55; <<0.01; 24** P2: n.s. P3: n.s.	P0: - P1: n.s. P2: n.s. P3: n.s.	P0: - P1: n.s. P2: n.s. P3: n.s.	P0: - **P1: 0.41; 0.05; 24** P2: n.s. P3: n.s.
DOC	P0: - P1: n.s. P2: n.s. P3: n.s.	P0: - P1: n.s. **P2: 0.65; <0.01; 17** **P3: -0.35; 0.02; 44**	P0: - P1: n.s. P2: n.s. **P3: -0.35; 0.03; 38**	P0: - P1: n.s. P2: n.s. P3: n.s.	P0: - P1: n.s. **P2: 0.49, 0.05; 17** P3: n.s.	P0: - P1: n.s. P2: n.s. P3: n.s.
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** P3: n.s.	P0: n.s. **P1: 0.52; <0.01; 24** **P2: 0.65; <<0.01; 42** P3: n.s.	P0: n.s. P1: n.s. P2: n.s. P3: n.s.	P0: n.s. **P1: 0.71; <<0.01; 24** **P2: 0.31; 0.04; 42** P3: n.s.	**P0:** n.s. **P1: 0.58; <0.01; 24** **P2: 0.73, <<0.01; 42** P3: n.s.	P0: n.s. P1: n.s. **P2: 0.37; 0.01; 42** P3: n.s.

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

BV_{Syn}	**P0: 0.87; <<0.01; 12**	P0: n.s.	P0: n.s.	P0: n.s.	**P0: 0.83; 0.04; 6**	P0: n.s.
	P1: 0.86; <<0.01; 48	P1: **0.5; 0.01; 24**	P1: n.s.	**P1: 0.64; <<0.01; 24**	**P1: 0.55; <0.01; 24**	P1: n.s.
	P2: 0.89; <<0.01; 112	**P2: 0.56; <<0.01; 42**	P2: n.s.	P2: n.s.	**P2: 0.55, <<0.01; 42**	**P2: 0.37; 0.01; 42**
	P3: n.s.	**P3: -0.44; <0.01; 38**	**P3: -0.47; <0.01; 33**	P3: n.s.	**P3: -0.5; <0.01; 38**	P3: n.s.
BV_{PicoI}	**P0: 0.9; <<0.01; 12**	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.	**P0: 0.83; 0.04; 6**
	P1: 0.82; <<0.01; 48	**P1: 0.64; <<0.01; 24**	**P1: 0.53; <0.01; 24**	**P1: 0.6; <0.01; 24**	**P1: 0.65; <<0.01; 24**	P1: n.s.
	P2:0.36;<<0.01;110	P2: n.s.;	P2: n.s.	P2: n.s.	P2: n.s.	P2: n.s.
	P3: -0.28; 0.05; 51	P3: n.s.	**P3: -0.34; 0.05; 33**	P3: n.s.	P3: n.s.	P3: n.s.
BV_{PicoII}	**P0: -0.76; <0.01; 12**	P0: n.s.	P0: n.s.	P0: n.s.	**P0: 1; <<0.01; 6**	**P0: 0.94; <0.01; 6**
	P1: 0.6; <<0.01; 48	**P1: 0.54; <0.01; 24**	**P1: 0.4; 0.05; 24**	**P1: 0.58; <0.01; 24**	**P1: 0.63; <0.01; 24**	P1: n.s.
	P2: n.s.;	P2: n.s.	P2: n.s.	**P2: 0.54; <<0.01; 42**	P2: n.s.	P2: n.s.
	P3: 0.36; 0.01; 51	**P3: 0.46; <0.01; 38**	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.
$BV_{PicoIII}$	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.
	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.
	P2: 0.6; <<0.01; 112	P2: n.s.	**P2: 0.3; 0.05; 42**	**P2: 0.42; <0.01; 42**	**P2: 0.7; <<0.01; 42**	P2: n.s.
	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.
BV_{NanoI}	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.	**P0: 1; <<0.01; 6**	**P0: 0.94; <0.01; 6**
	P1: 0.45; <<0.01; 48	P1: n.s.	**P1: 0.4; 0.05; 24**	P1: n.s.	**P1: 0.4; 0.05; 24**	P1: n.s.
	P2: -0.53; <<0.01; 112	P2: n.s.	**P2: 0.44; <0.01; 42**	**P2: 0.43; <0.01; 42**	P2: n.s.	**P2: -0.44; <0.01; 42**
	P3: -0.35; 0.03; 51	P3: n.s.	**P3: 0.41; 0.02; 33**	P3: n.s.	P3: n.s.	P3: n.s.
BV_{NanoII}	P0: n.s.	P0: n.s.	P0: n.s.	P0: n.s.	**P0: 0.81; 0.05; 6**	P0: n.s.
	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.	P1: n.s.
	P2: -0.76; <<0.01; 112	**P2: -0.37; 0.02; 42**	P2: n.s.	P2: n.s.	**P2: -0.46; <0.01; 42**	**P2: -0.34; 0.03; 42**
	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.	P3: n.s.

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
Published: 10 March 2016

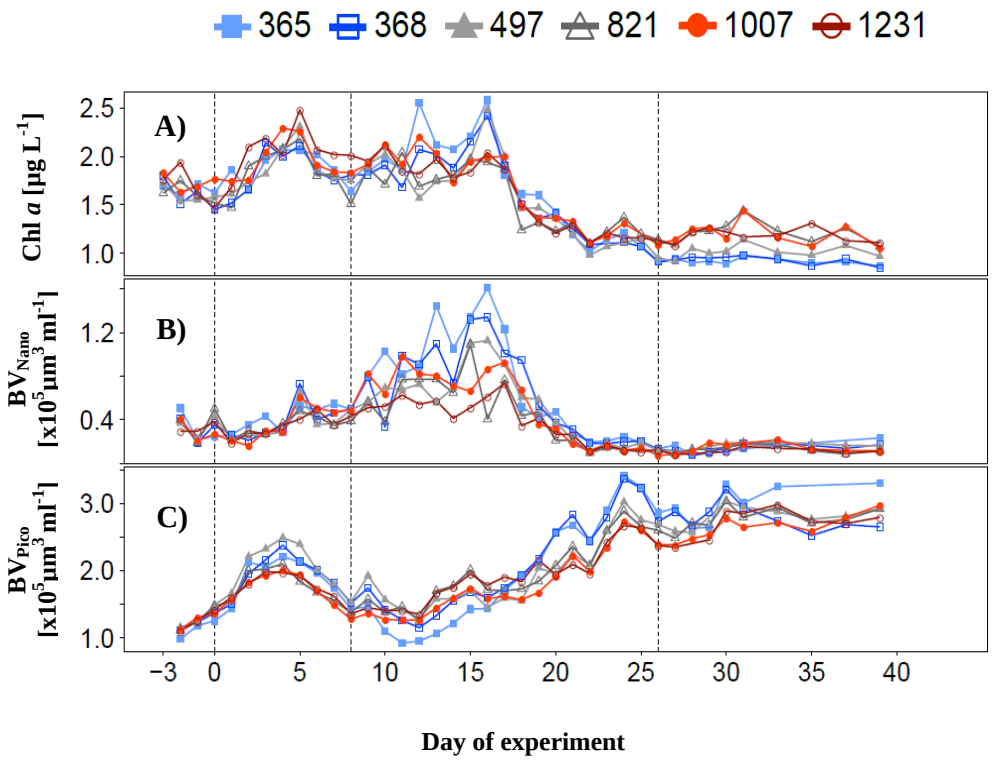

Figure 1. A) Concentration of Chlorophyll *a* [µg L^{-1}], B) biovolume of nanophytoplankton (Nano I and Nano II) [$x10^5$ μm^3 ml^{-1}] and C) biovolume of picophytoplankton (*Synechococcus* spp., Pico I-III) [$x10^5$ μm^3 ml^{-1}] during the course of the experiment. Colours and symbols indicate average fCO_2 [µatm] between t1-t43.

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
Manuscript under review for journal Biogeosciences
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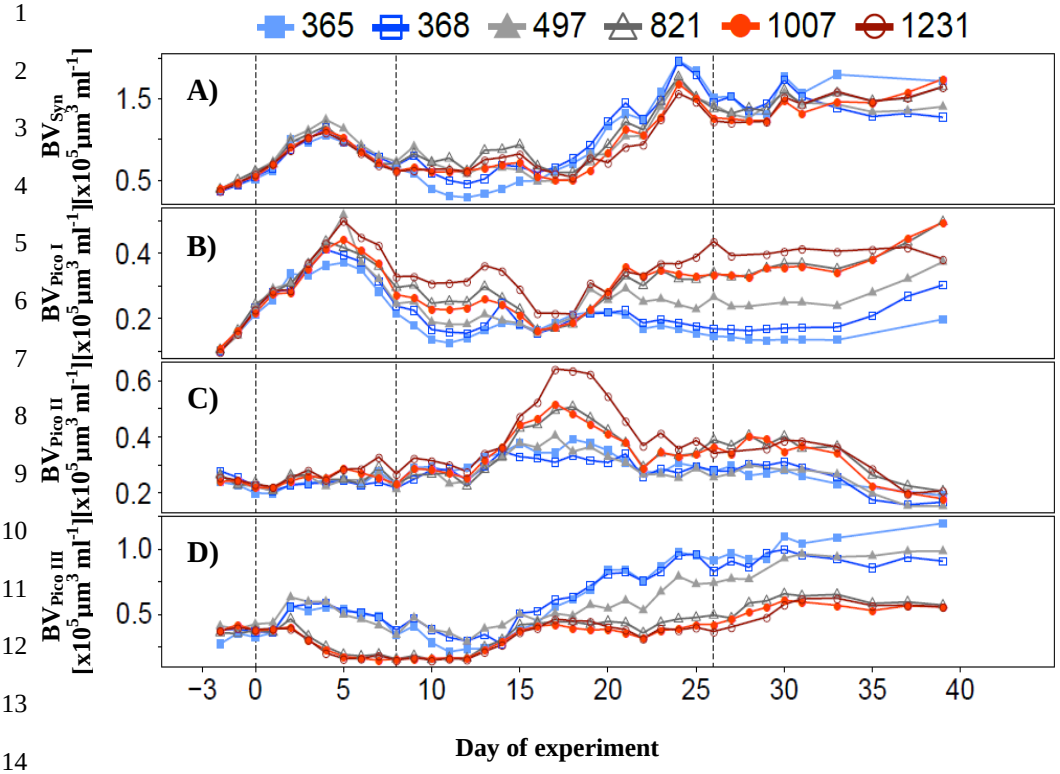

Figure 2. A) biovolume of *Synechococcus* spp. [x10^5 µm^3 ml^{-1}] and B-D) biovolume of picoeukaryote groups I-III (Pico I-III) [x10^5 µm^3 ml^{-1}] during the course of the experiment. Colours and symbols indicate average fCO_2 [µatm] between t1-t43.

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
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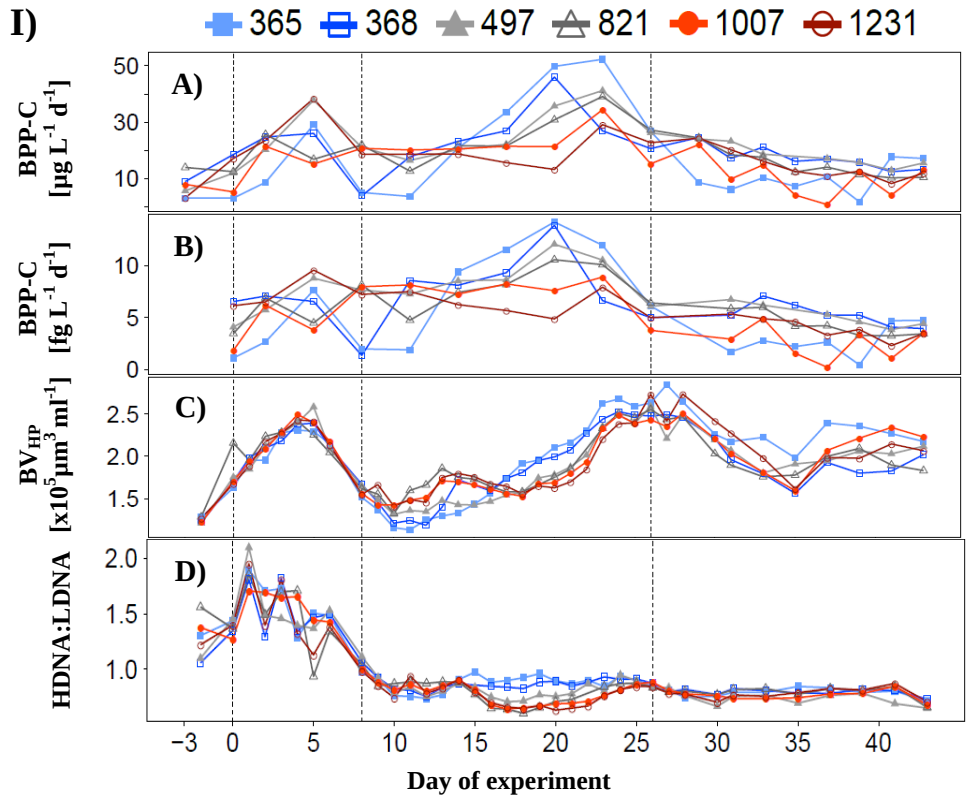

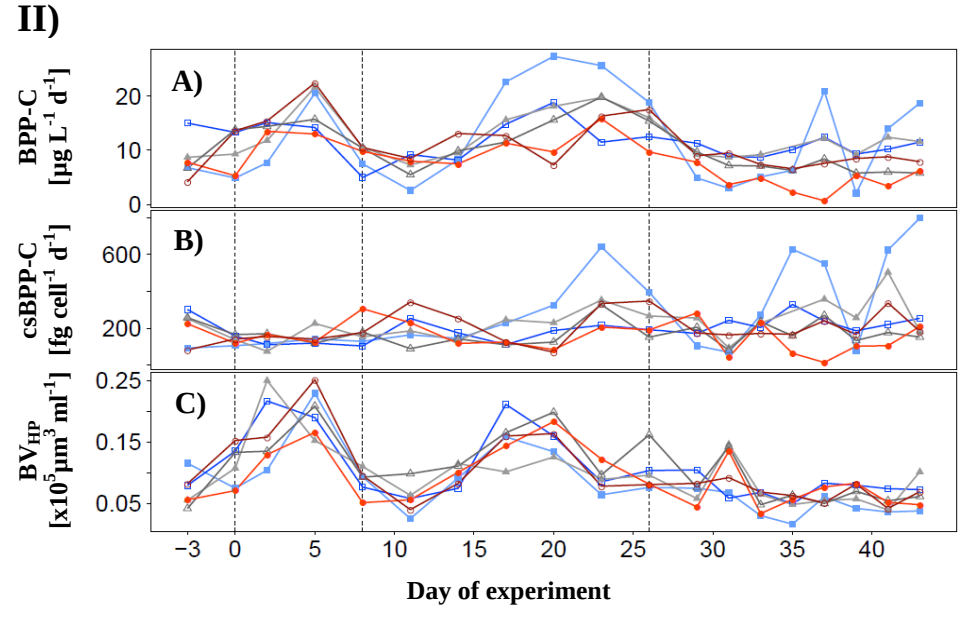

Biogeosciences Discuss., doi:10.5194/bg-2016-61, 2016
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Published: 10 March 2016

Figure 3. A) Bacterial Protein Production (BPP-C) [µg L^{-1} d^{-1}], B) cell-specific Bacterial Protein Production (csBPP-C) [fg $cell^{-1}$ d^{-1}] and C) biovolume of heterotrophic prokaryotes (BV_{HP}) [$x10^5$ μm^3 ml^{-1}] of size fractions I) 0.2-5.0 µm (free-living bacteria) and II) >5.0 µm (particle-associated bacteria) during the course of the experiment. D) Ratio of high versus low nucleic acid stained prokaryotic heterotrophs (HDNA:LDNA), which made up free-living BV_{HP}, revealed from flow cytometry. Colours and symbols indicate average fCO_2 [µatm] between t1-t43.

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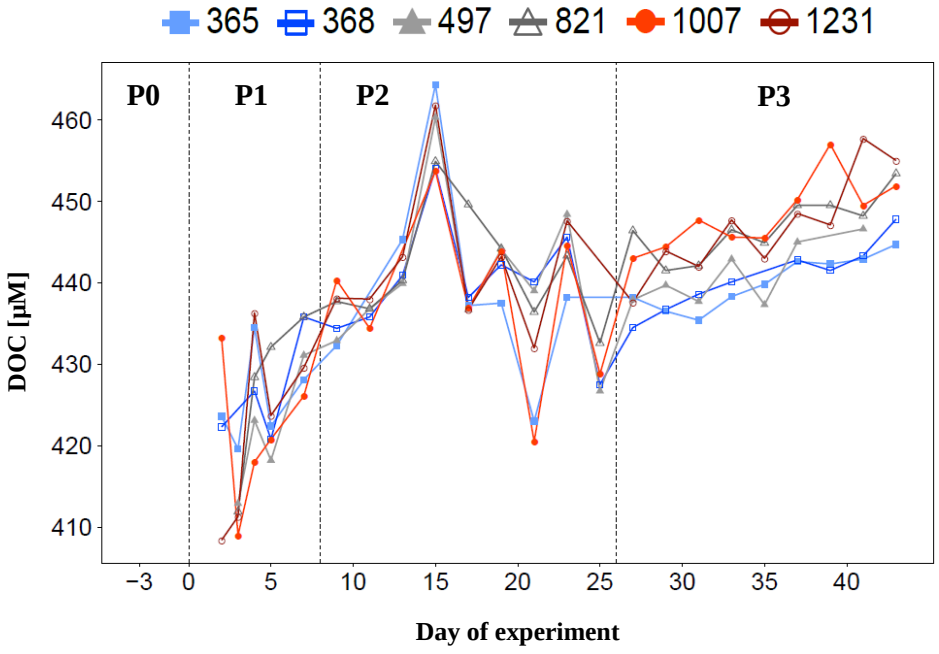

Figure 4. Concentration of dissolved organic carbon (DOC) [µM] during the course of the experiment. During P3, DOC accumulated in the water column, thereby yielding significantly higher concentrations at higher fCO_2 (r_s=0.62; p<<0.01; n=51). Colours and symbols indicate average fCO_2 [µatm] between t1-t43.

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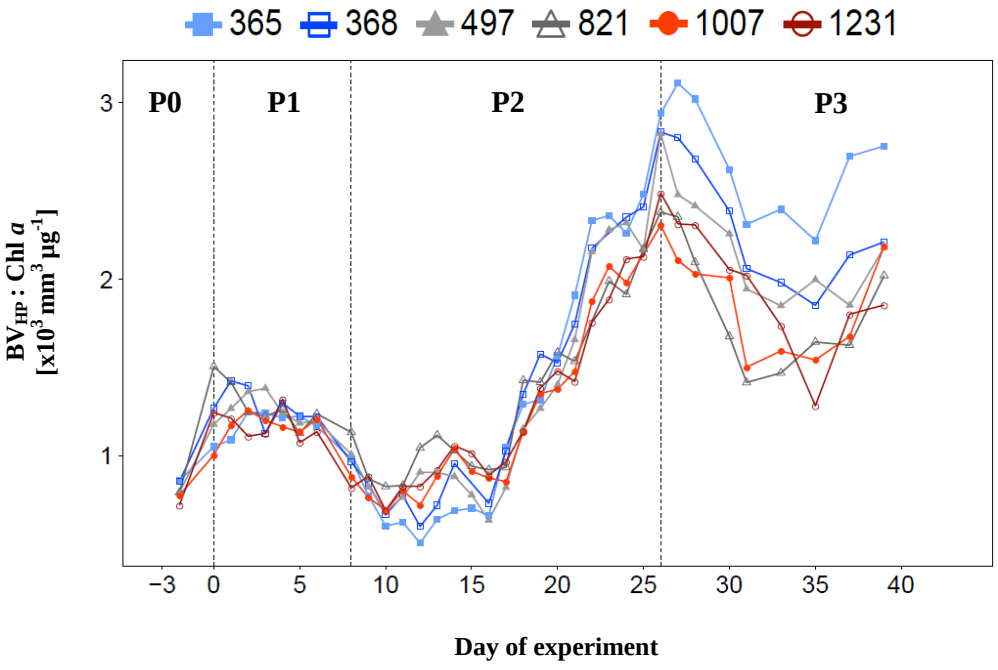

Figure 5. Standardization of heterotrophic prokaryotic biovolume to total Chl *a* (BV_{HP} : Chl *a*) during the course of the experiment. Colours and symbols indicate average fCO_2 [µatm] between t1-t43.