Dear Prof. Achterberg,

Thank-you for reviewing this manuscript, the previous reviews indeed helped us to strengthen the manuscript and we are very pleased that it is now acceptable for publication. We have made the minor amendments suggested, as can be seen in the marked up version attached, and addressed the one comment (in blue italic).

Associate Editor Decision: Publish subject to minor revisions (Editor review) (21 Jun 2017) by E.P. Achterberg

Comments to the Author:

The resubmitted manuscript has substantially improved following two rounds of reviews. In particular the substantial and thorough amendments to the manuscript following the latest reviews have greatly improved the manuscript. The text flows well, and the scientific reasoning is well laid out and expressed.

The use of statistical techniques to show the different behaviours with time of the various phytoplankton communities that were subjected to different CO2 treatments has strongly improved the manuscript.

The authors have followed the recommendations by the reviewers and thereby greatly strengthened the manuscript.

I suggest a number of minor amendments to the manuscript before it can be published.

Line 29: replace dissolution with uptake ***amended***

Line 29: after carbon dioxide write (CO2) ***amended***

Line 29: replace in with by ***amended***

Line 30: replace atmosphere with ocean ***amended***

Line 32: replace highly with strongly ***amended***

Line 37: replace CO2 with fugacity of CO2 (fCO2) ***amended***

Line 39: their abundances with its abundance ***amended***

Line 429: between “shift towards” place with increasing fCO2 ***amended***

Line 50: replace activities with emissions ***amended***

Line 50: write: primarily caused by the burning…… ***amended***

Line 56: replace productivity with production ***amended***

Line 81: replace nutrients with nutrient ***amended***

Line 81: replace concentration with concentration ***amended***

Line 84: write: carbon sequestration in deep waters and sediments ***amended***

Line 101: replace under with with ***amended***

Line 101: write: temperature conditions ***amended***

Line 117: replace hung with reached ***amended***

Line 127: replace fCO2 with fugacity of CO2 (fCO2) ***amended***

Line 134: write: threshold fCO2 level, if present…… ***amended***

Line 136: write: Initial fCO2 was 240…… ***amended***

Line 166: replace concentrations with levels ***amended***

Line 300 and 302: replace of with in ***amended***

Line 306: write: phytoplankton abundance ***amended***

Line 337: write: low fCO2 compared to high fCO2 ***amended***

Line 448: replace on with for ***amended***

Line 499: is there evidence from other mesocosm experiments of stimulation of SYN due to bubbling or enclosing? ***Yes, this has been seen in a few other mesocosm experiments, we have added this into the text: "Alternatively, effects of enclosure and the techniques (bubbling) used to ensure a homogenous water column may have stimulated SYN within the mesocosms, which has been found to occur in several mesocosm experiments (Paulino et al., 2008; Gazeau et al., 2017)."(Line 498-501).***

Line 504: replace occur with upwell ***amended***

Line 660: replace predicted with projected ***amended***

Alterations in microbial community composition with increasing fCO_2: a mesocosm study in the eastern Baltic Sea

Katharine J. Crawfurd[1], Santiago Alvarez-Fernandez[2], Kristina D. A. Mojica[3], Ulf Riebesell[4], Corina P. D. Brussaard[1,5]

[1]{NIOZ Royal Netherlands Institute for Sea Research, Department of Marine Microbiology and Biogeochemistry and Utrecht University, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands}

[2]{Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung, Biologische Anstalt Helgoland, 27498, Helgoland, Germany}

[3] {Department of Botany and Plant Pathology, Cordley Hall 2082, Oregon State University, Corvallis, Oregon 97331-29052, USA}

[4]{GEOMAR Helmholtz Centre for Ocean Research Kiel, Biological Oceanography, Düsternbrooker Weg 20, 24105, Kiel, Germany}

[5]{Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94248, 1090 GE Amsterdam, The Netherlands}

Correspondence to: K. Crawfurd (kate.crawfurd@gmail.com) and C. P. D. Brussaard (corina.brussaard@nioz.nl)

Abstract

Ocean acidification, resulting from the ~~dissolution~~uptake of anthropogenic carbon dioxide ~~in~~(CO_2) by the ~~atmosphere~~ocean, is considered a major threat to marine ecosystems. Here we examined effects of ocean acidification on microbial community dynamics in the eastern Baltic Sea, during the summer of 2012 when inorganic nitrogen and phosphorus were ~~highly~~strongly depleted. Large volume in situ mesocosms were employed to mimic present ~~to~~, future and far future CO_2 scenarios. All six groups of phytoplankton enumerated by flow cytometry (<20 µm cell diameter) showed distinct trends in net growth and abundance with CO_2 enrichment. The picoeukaryotic phytoplankton groups Pico-I and II displayed enhanced abundances, whilst Pico-III, *Synechococcus* and the nanoeukaryotic phytoplankton groups were negatively affected by elevated fugacity of CO_2 (fCO_2~~.~~). Specifically, the numerically dominant eukaryote, Pico-I, demonstrated increases in gross growth rate with increasing fCO_2 sufficient to double ~~their abundances.~~its abundance. Dynamics of the prokaryote community closely followed trends in total algal biomass despite differential effects of fCO_2 on algal groups. Similarly, viral abundances corresponded to prokaryotic host population dynamics. Viral lysis and grazing were both important in controlling microbial abundances. Overall our results point to a shift, with increasing fCO_2, towards a more regenerative system with production dominated by small picoeukaryotic phytoplankton.

1 Introduction

Marine phytoplankton are responsible for approximately half of global primary production (Field et al., 1998), with shelf sea communities contributing an average 15-30 % (Kulinski and Pempkowiak, 2011). Since the industrial revolution atmospheric carbon dioxide (CO_2) concentrations have increased by nearly 40 % due to anthropogenic ~~activities~~emissions, primarily caused by the burning of fossil fuels and deforestation (Doney et al., 2009). Atmospheric CO_2 dissolves in the oceans where

it forms carbonic acid which reduces seawater pH, a process commonly termed, ocean acidification (OA). Currently, along with warming sea surface temperatures and changing light and nutrient conditions, marine ecosystems face unprecedented decreases in ocean pH (Doney et al., 2009; Gruber, 2011). Ocean acidification is considered one of the greatest current threats to marine ecosystems (Turley and Boot, 2010) and has been shown to alter phytoplankton primary ~~productivity~~production with the direction and magnitude of the responses dependent on community composition (eg. Hein and Sand-Jensen, 1997; Tortell et al., 2002; Leonardos and Geider, 2005; Engel et al., 2007; Feng et al., 2009; Eberlein et al., 2017). Certain cyanobacteria, including diazotrophs, demonstrate stimulated growth under conditions of elevated CO_2 (Qiu and Gao, 2002; Barcelos e Ramos et al., 2007; Hutchins, 2007; Dutkiewicz et al., 2015). However, no consistent trends have been found for *Synechococcus* (Schulz et al., 2017 and references therein). The responses of diatoms and coccolithophores also appear more variable (Dutkiewicz et al., 2015 and references therein), although coccolithophore calcification seems generally negatively impacted (Meyer and Riebesell, 2015; Riebesell et al., 2017). OA has also been reported to increase the abundances of small-sized photoautotrophic eukaryotes in mesocosm experiments (Engel et al., 2007; Meakin and Wyman, 2011; Brussaard et al., 2013; Schulz et al., 2017).

Recently, data regarding the effects of OA on taxa-specific phytoplankton growth rates were incorporated into a global ecosystem model. The results emphasized that elevated CO_2 concentrations can cause changes in community structure by altering the competitive fitness, and thus competition between phytoplankton groups (Dutkiewicz et al., 2015). Moreover, OA was found to have a greater impact on phytoplankton community size structure, function and biomass than either warming or reduced nutrient supply (Dutkiewicz et al., 2015). Many OA studies have been conducted using single-species under controlled laboratory conditions and therefore cannot account for intrinsic community interactions that occur under natural conditions. Alternatively, larger-volume mesocosm experiments allow for OA manipulation of natural communities and as such, are more likely to capture and quantify the overall response of the natural ecosystems. To date, the majority of

these experiments started under replete nutrient conditions or received nutrient additions (Paul et al., 2015 and references therein). Thus, little data is available for oligotrophic conditions, which are present in ~75% of the world's oceans (Corno et al., 2007).

Whilst environmental factors such as temperature, light, ~~nutrients~~nutrient and CO_2 ~~concentration~~concentrations regulate gross primary production, loss factors determine the fate of this photosynthetically fixed carbon. Grazing, sinking and viral lysis affect the cycling of elements in different manners, i.e. transferred to higher trophic levels through grazing, carbon ~~storage~~sequestration in deep waters and sediments ~~via sinking~~, and cellular content release by viral lysis (Wilhelm and Suttle, 1999; Brussaard et al., 2005). Released detrital and dissolved organic matter (DOM) is quickly utilized by heterotrophic bacteria, thereby stimulating activity within the microbial loop (Brussaard et al., 2008; Lønborg et al., 2013; Sheik et al., 2014; Middelboe and Lyck, 2002). Consequently, bacteria may be affected indirectly by OA through changes in the quality and/or quantity of DOM (Weinbauer et al., 2011). Viral lysis has been found to be as important as microzooplankton grazing to the mortality of natural bacterio- and phytoplankton (Weinbauer, 2004; Baudoux et al., 2006; Evans and Brussaard, 2012; Mojica et al., 2016). Thus far, most studies examining the effects of OA on microzooplankton abundance and/or grazing have found little or no direct effect (Suffrian et al., 2008; Rose et al., 2009; Aberle et al., 2013; Brussaard et al., 2013; Niehoff et al., 2013). To our knowledge, no viral lysis rates have been reported for natural phytoplankton communities under conditions of OA. A few studies have inferred rates based on changes in viral abundances under enhanced CO_2, but the results are inconsistent (Larsen et al., 2008; Brussaard et al., 2013). Therefore, the effect of OA on the relative share of these key loss processes is still understudied for most ecosystems.

Here we report on the temporal dynamics of microbes (phytoplankton, prokaryotes and viruses) under the influence of enhanced CO_2 concentrations in the low-salinity (around 5.7) Baltic Sea. Using large mesocosms ~~under~~with in situ light and temperature conditions, the pelagic ecosystem was exposed to a range of increasing CO_2 concentrations from ambient to future and far future

concentrations. The study was performed during the summer in the Baltic Sea near Tvärminne when conditions were oligotrophic. Our data show, that over the 43 day long experiment, enhanced CO_2 concentrations elicited distinct shifts in the microbial community, most notably an increase in the net growth of small picoeukaryotic phytoplankton.

2 Materials and Methods

2.1 Study site and experimental set-up

The present study was conducted in the Tvärminne Storfjärden (59° 51.5' N, 23° 15.5' E) between 14 June and 7 August, 2012. Nine mesocosms, each enclosing ~55 m^3 of water, were moored in a square arrangement at a site with a water depth of approximately 30 m. The mesocosms consisted of open ended polyurethane bags 2 m in diameter and 18.5 m in length mounted onto floating frames covered at each end with a 3 mm mesh. Initially, the mesocosms were kept open for 5 days to allow for rinsing and water exchange while excluding large organisms from entering with the 3 mm mesh. During this time, the bags were positioned such that the tops were submerged 0.5 m below the water surface and the bottoms ~~hung~~reached down to 17 m depth in the water column. Photosynthetically active radiation (PAR) transparent plastic hoods (open on the side) prevented rain and bird droppings from entering the mesocosms, which would affect salinity and nutrients, respectively. Five days before the CO_2 treatment was to begin, the water column of the mesocosms was isolated from the influence of the surrounding water. To do so, the 3 mm mesh was removed and sediment traps (2 m long) were attached to close off the bottom of the mesocosms. The top ends of the bags were raised and secured to the frame 1.5 m above the water surface to prevent water entering via wave action. The mesocosms were then bubbled with compressed air for 3.5 min, to remove salinity gradients and ensure that the water body was fully homogeneous.

The present manuscript includes results from six of the original mesocosms, due to the unfortunate loss of three mesocosms which were compromised by leakage. The mean fugacity of CO_2 (fCO_2) during the experiment, i.e. days 1-43, for the individual mesocosms were as follows: M1, 365 µatm;

M3, 1007 µatm; M5, 368 µatm; M6, 821 µatm; M7, 497 µatm; M8, 1231 µatm (Table 1). The gradient of non-replicated fCO_2 of the present study (as opposed to a smaller number of replicated treatment levels) was selected as a balance between the necessary, but manageable, number of mesocosms and minimizing the impact of the high potential for loss of mesocosms to successfully address the underlying questions of the study (Schulz et al., 2013). Moreover, it maximizes the potential of identifying a threshold fCO_2 level concentration, if present (by allowing for a larger number of treatment levels). Carbon dioxide manipulation was carried out in four steps and took place between days 0 to 4 until the target fCO_2 was reached. Initial ~~fugacity of CO_2 (fCO_2)~~ was 240 µatm. For fCO_2 manipulations, 50 µm filtered natural seawater was saturated with CO_2 and then injected evenly throughout the depth of the mesocosms as described by Riebesell et al. (2013). Two mesocosms functioned as controls and were treated in a similar manner using only filtered seawater. On day 15, a supplementary fCO_2 addition was made to the top 7 m of mesocosms numbered 3, 6, and 8 to replace CO_2 lost due to outgassing (Paul et al., 2015; Spilling et al., 2016). Throughout this study we refer to fCO_2 which accounts for the non-ideal behavior of CO_2 gas and is considered the standard measurement required for gas exchange (Pfeil et al., 2012).

Initial nutrient concentrations were 0.05 µmol L^{-1}, 0.15 µmol L^{-1}, 6.2 µmol L^{-1} and 0.2 µmol L^{-1} for nitrate, phosphate, silicate and ammonium, respectively. Nutrient concentrations remained low for the duration of the experiment (Paul et al., 2015, this issue) and no nutrients were added. Salinity was relatively constant around 5.7. Temperature was more variable; on average temperature within the mesocosms (0-17 m) increased from ~8 °C to a maximum on day 15 of ~15 °C and then decreased again to ~8 °C by day 30. For further details of the experimental set-up, carbonate chemistry dynamics and nutrient concentrations throughout the experiment we refer to the general overview paper by Paul et al. (2015).

Collective sampling was performed every morning using depth integrated water samplers (IWS, HYDRO-BIOS, Kiel). These sampling devices were gently lowered through the water column collecting ~5 L of water gradually between 0-10 m (top) or 0-17 m (whole water column). Water was collected

157 from all mesocosms and the surrounding water. Subsamples were obtained for enumeration of
158 phytoplankton, prokaryotes and viruses. Samples for viral lysis and grazing experiments were taken
159 from 5 m depth using a gentle vacuum-driven pump system. Samples were protected against sunlight
160 and warming by thick black plastic bags containing wet ice. Samples were processed at in situ
161 temperature (representative of 5 m depth) under dim light and handled using nitrile gloves. As viral
162 lysis and grazing rates were determined from samples taken from 5 m depth, samples for microbial
163 abundances reported here were taken from the top 10 m integrated samples.
164 The experimental period has been divided into four phases based on major physical and biological
165 changes (Paul et al., 2015): Phase 0 before CO_2 addition (days -5 to 0), Phase I (days 1-16), Phase II
166 (days 17-30) and Phase III (days 31-43). Throughout this manuscript the data are presented using
167 three colors (blue, grey and red), representing low (mesocosms M1 and M5) intermediate (M6 and
168 M7) and high (M3 and M8) fCO_2 ~~concentrations~~levels (Table 1).
169
170 **2.2 Microbial abundances**
171 Microbes were enumerated using a Becton Dickinson FACSCalibur flow cytometer (FCM) equipped
172 with a 488 nm argon laser. The samples were stored on wet ice and in the dark until counting. The
173 photoautotrophic cells (<20 µm) were counted directly using fresh seawater and were discriminated
174 by their autofluorescent pigments. Six phytoplankton clusters were differentiated based on the
175 bivariant plots of either chlorophyll (red autofluorescence) or phycoerythrin (orange
176 autofluorescence, for *Synechococcus* and Pico-III) against side scatter. The size of the different
177 phytoplankton clusters was determined by gentle filtration through 25 mm diameter polycarbonate
178 filters (Whatman) with a range of pore sizes (12, 10, 8, 5, 3, 2, 1, and 0.8 µm) according to Veldhuis
179 and Kraay (2004). Average cell sizes for the different phytoplankton groups were 1, 1, 3, 2.9, 5.2, and
180 8.8 µm diameter for the prokaryotic cyanobacteria *Synechococcus* spp. (SYN), picoeukaryotic
181 phytoplankton I, II and III (Pico-I-III), and nanoeukaryotic phytoplankton I, and II (Nano-I, II),
182 respectively. Pico-III was discriminated from Pico-II (comparable average cell size) by a higher orange

183 autofluorescence signature, potentially representing small-sized cryptophytes (Klaveness, 1989);
184 alternatively large single cells or microcolonies of *Synechococcus* (Haverkamp et al., 2009). The
185 cyanobacterial species *Prochlorococcus* spp. were not observed during this experiment. Counts were
186 converted to cellular carbon by assuming a spherical shape equivalent to the average cell diameters
187 determined from size fractionations and applying conversion factors of 237 fg C μm^{-3} (Worden et al.,
188 2004) and 196.5 fg C μm^{-3} (Garrison et al., 2000) for pico- and nano-sized plankton, respectively.
189 Microbial net growth and loss rates were derived from exponential regressions of changes in the cell
190 abundances over time.
191 Abundances of prokaryotes and viruses were determined from 0.5 % glutaraldehyde fixed, flash
192 frozen (-80 °C) samples according to Marie et al. (1999) and Brussaard (2004), respectively. The
193 prokaryotes include heterotrophic bacteria, archaea and unicellular cyanobacteria, the latter
194 accounting for maximal 10 % of the total abundance in our samples, as indicated by their
195 autofluorescence. Briefly, thawed samples were diluted with sterile autoclaved Tris-EDTA buffer (10
196 mM Tris-HCl and 1 mM EDTA, pH 8.2; Mojica et al., 2014) and stained with the green fluorescent
197 nucleic acid-specific dye SYBR-Green I (Invitrogen Inc.) to a final concentration of the commercial
198 stock of 1.0×10^{-4} (for prokaryotes) or 0.5×10^{-4} (for viruses). Virus samples were stained at 80 °C for
199 10 min and then allowed to cool for 5 min at room temperature in the dark. Prokaryotes were
200 stained for 15 min at room temperature in the dark (Brussaard, 2004). Prokaryotes and viruses were
201 discriminated in bivariate scatter plots of green fluorescence versus side scatter. Final counts were
202 corrected for blanks prepared and analyzed in a similar manner as the samples. Two groups of
203 prokaryotes were identified by their stained nucleic acid fluorescence, referred here on as low (LNA)
204 and high (HNA) fluorescence prokaryotes.
205
206 **2.3 Viral lysis and grazing**
207 Microzooplankton grazing and viral lysis of phytoplankton was determined using the modified
208 dilution assay, based on reducing grazing and viral lysis mortality pressure in a serial manner allowing

for increased phytoplankton growth (over the incubation period) with dilution (Mojica et al., 2016). Briefly, two dilution series were created in clear 1.2 L polycarbonate bottles by gently mixing 200 µm sieved whole seawater with either 0.45 µm filtered seawater (i.e. microzooplankton grazers removed) or 30 KDa filtered seawater (i.e. grazers and viruses removed) to final dilutions of 20, 40, 70 and 100 %. The 0.45 µm filtrate was produced by gravity filtration of 200 µm mesh sieved seawater through a 0.45 µm Sartopore capsule filter. The 30 KDa ultrafiltrate was produced by tangential flow filtration of 200 µm pre-sieved seawater using a 30 kDa Vivaflow 200 PES membrane tangential flow cartridge (Vivascience). All treatments were performed in triplicate. Bottles were suspended next to the mesocosms in small cages at 5 m depth for 24 hours. Subsamples were taken at 0 and 24 h, and phytoplankton abundances of the grazing series (0.45 µm diluent) were enumerated by flow cytometry. Due to time constraint, the majority of the samples of the 30 kDa series were fixed with 1 % (final concentration) formaldehyde : hexamine solution (18 % v/v : 10 % w/v), for 30 min at 4 °C, flash frozen in liquid nitrogen and stored at –80 °C until flow cytometry analysis in the home laboratory. Fixation had no significant effect (student's t-tests, p-value >0.05) as tested periodically against fresh samples. The modified dilution assay was only run for Mesocosms 1 (low fCO_2) and 3 (high fCO_2) due to the logistics of handling times. Experiments were performed until day 31. Grazing rates and the combined rate of grazing and viral lysis were estimated from the slope of a regression of phytoplankton apparent growth versus dilution of the 0.45 µm and 30 kDa series, respectively. A significant difference between the two regression coefficients (as tested by analysis of covariance) indicated a significant viral lysis rate. Phytoplankton gross growth rate, in the absence of grazing and viral lysis, was derived from the y-intercept of the 30 kDa series regression. Similarly, significant differences between mesocosms M1 and M3 (low and high fCO_2) were determined through analysis of covariance of the dilution series for the two mesocosms. A significance threshold of 0.05 was used and significance is denoted throughout the manuscript by an asterisk (*). Occasionally, the regression of apparent growth rate versus fraction of natural water resulted in a positive slope (thus no reduction in mortality with dilution). In addition, very low phytoplankton

abundances can also prohibit statistical significance of results. Under such conditions dilution experiments were deemed unsuccessful (see for limitations of the modified dilution method, Baudoux et al., 2006; Kimmance and Brussaard, 2010; Stoecker et al., 2015).

Viral lysis of prokaryotes was determined according to the viral production assay (Wilhelm et al., 2002; Winget et al., 2005). After reduction of the natural virus concentration, new virus production by the natural bacterial community is sampled and tracked over time (24 h). Briefly, free viruses were reduced from a 300 ml sample of whole water by re-circulation over a 0.2 µm pore size polyether sulfone membrane (PES) tangential flow filter (Vivaflow 50, Vivascience) at a filtrate expulsion rate of 40 ml min^{-1}. The concentrated sample was then reconstituted to the original volume using virus-free seawater. This process was repeated a total of three times to gradually wash away viruses. After the final reconstitution, 50 ml aliquots were distributed into six polycarbonate tubes. Mitomycin C (Sigma-Aldrich) (final concentration, 1 µg ml^{-1}, maintained at 4 °C), which induces lysogenic bacteria (Weinbauer and Suttle, 1996) was added to a second series of triplicate samples for each mesocosm. A third series of incubations with 0.2 µm filtered samples was used as a control for viral loss (e.g. viruses adhering to the tube walls) and showed no significant loss of free viruses during the incubations. At the start of the experiment, 1 ml subsamples were immediately removed from each tube and fixed as previously described for viral and bacterial abundance. The samples were dark incubated at in situ temperature and 1 ml subsamples were taken at 3 h, 6 h, 9 h, 12 h and 24 h. Virus production was determined from linear regression of viral abundance over time. Viral production due to induction of lysogeny was calculated as the difference between production in the unamended samples and production of samples to which mitomycin C was added. Although mortality experiments were initially planned to be employed for mesocosms 1, 2, and 3 representing low, mid and high fCO_2 conditions, mesocosm 2 was compromised due to leakage. Additionally, due to logistical reasons assays were only performed until day 21.

To determine grazing rates on prokaryotes, fluorescently labeled bacteria (FLBs) were prepared from enriched natural bacterial assemblages (originating from the North Sea) labeled with 5-([4,6-

261 Dichlorotriazin-2-yl]amino) fluorescein (DTAF, 36565 Sigma-Aldrich 40 µg ml^{-1}) according to Sherr et
262 al. (1993). Frozen ampoules of FLB (1-5 % of total bacterial abundance) were added to triplicate 1 L
263 incubation bottles containing whole water gently passed through 200 µm mesh. Twenty ml samples
264 were taken immediately after addition (0 h) and the headspace was removed by gently squeezing air
265 from the bottle. The 1 L bottles were incubated on a slow turning wheel (1 rpm) at in situ light and
266 temperature conditions (representative of 5 m depth) for 24 h. Sampling was repeated after 24 h. All
267 samples were fixed to a 1 % final concentration of gluteraldehyde (0.2 µm filtered; 25 % EM-grade),
268 stained (in the dark for 30 min at 4 °C) with 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI)
269 solution (0.2 µm filtered; Acrodisc ®25 mm Syringe filters, PALL Life Sciences; 2 µg ml^{-1} final
270 concentration; Sherr et al., 1993) and filtered onto 25 mm, 0.2 µm black polycarbonate filters (GE
271 Healthcare life sciences). Filters were then mounted on microscopic slides and stored at -20 °C until
272 analysis. FLBs present on a ~0.75 mm^2 area were counted using a Zeiss Axioplan 2 microscope.
273 Grazing (µ d^{-1}) was measured according to $N_{T24} = N_{T0} * e^{-\mu t}$, where N_{T24} and N_{T0} are the number of FLBs
274 present at 24 h and 0 h, respectively.
275
276 **2.4 Statistics**
277 Non-metric multidimensional scaling (NMDS) was used to follow microbial community development
278 in each mesocosm over the experimental period. NMDS is an ordination technique which represents
279 the dissimilarities obtained from an abundance data matrix in a *2*-dimensional space (Legendre and
280 Legendre, 1998). In this case, the data matrix was comprised of abundance data for each
281 phytoplankton group in each mesocosm for every day of sampling. The treatment effect was
282 assessed by analysis of similarity (ANOSIM; Clarke, 1993) and inspection of the NMDS biplot. ANOSIM
283 compares the mean of ranked dissimilarities of mesocosms between fCO_2 treatments (low: 1, 5, 7;
284 high: 6, 3, 8) to the mean of ranked dissimilarities within treatments per phase. The NMDS plots
285 allowed divergence periods in the development and community composition between treatments to
286 be visually assessed (period 1 from day 3-13 and period 2 from days 16-24). Net growth rates of each

of the different microbial groups were calculated for these identified divergence periods. Relationships between net growth rates and peak cell abundances with fCO_2 were evaluated by linear regression against the average fCO_2 per mesocosm during each period or peak day. A generalized linear model was used to test the relationship between prokaryote abundance and carbon biomass with an ARMA correlation structure of order 3 to account for temporal autocorrelation. The model fulfilled all assumptions such as homoscedasticity and avoiding autocorrelation of the residuals (Zuur et al., 2007). A significance threshold of $p \leq 0.05$ was used and significance is denoted by an asterisk (*). All analyses were performed using the statistical software program R, using packages nlme (Pinheiro et al., 2017) and vegan (Oksanen et al., 2017) (R core Team, 2017). Where average of low and high mesocosm abundance data are reported, values represent the average of mesocosms 1, 5, 7 (mean fCO_2 365-497 µatm) and 6, 3, 8 (821-1231 µatm), respectively.

3 Results

3.1 Total phytoplankton dynamics in response to CO_2 enrichment

During Phase 0, low variability in phytoplankton abundances ~~of~~in the different mesocosms (1.5 ± 0.05 x 10^5 ml^{-1}) indicated good replicability of initial conditions prior to CO_2 manipulation (Fig. 1). This was further supported by the high similarity between microbial communities ~~of~~in the different mesocosms as indicated by the tight clustering of points in the NMDS plot during this period (Fig. 2). During Phase 0, the phytoplankton community (<20 µm) was dominated by pico-sized autotrophs, with the prokaryotic cyanobacteria *Synechococcus* (SYN) and Pico-I accounting for 69 % and 27 % of total phytoplankton abundance, respectively. After CO_2 addition, there were two primary peaks in phytoplankton, which occurred on day 4 in Phase I and day 24 in Phase II (Fig. 1a). The phytoplankton community became significantly different over time in the different treatments (ANOSIM, p=0.01, Fig. 2). Two periods were identified based on their divergence (Fig.2), the first (NMDS-based period 1) followed the initial peak in abundance (days 3-13) with highest abundances occurring in the

elevated CO_2 mesocosms (Fig. 1a). During the second period (NMDS-based period 2, days 16-24), abundances were higher in the low fCO_2 mesocosms (Fig. 1a). In general the NMDS plot shows that throughout the experiment, mesocosm M1 followed the same basic trajectory as mesocosms M5 and M7, whilst mesocosm M3 followed M6 and M8 (Fig. 2). Thus, the two mesocosms (representing high and low fCO_2 treatments) deviated from each other during Phase I and were clearly separated during Phases II and III (Fig. 2).

Phytoplankton abundances in the surrounding water started to differ from the mesocosms during Phase 0 (on average 44 % lower) which was primarily due to lower abundances of SYN. This effect was seen from day -1, prior to CO_2 addition but following bubbling with compressed air (day -5). On day 15, a deep mixing event occurred as a result of storm conditions (with consequent alterations in temperature and salinity) and as a result phytoplankton abundances in the surrounding open water diverged more strongly from the mesocosms but remained similar in their dynamics (Fig. 3). Microbial abundances in the 0-17 m samples were slightly lower but showed very similar dynamics to those in the 0-10 m samples (Fig. S1).

3.1.1 *Synechococcus*

The prokaryotic cyanobacteria *Synechococcus* (SYN) accounted for the majority of total abundance, i.e. 74 % averaged across all mesocosms over the experimental period. Abundances of SYN showed distinct variability between the different CO_2 treatments, starting on day 7, with the low CO_2 mesocosms exhibiting nearly 20 % lower abundances between days 11-15 as compared to high fCO_2 mesocosms (Fig. 3a). SYN net growth rates during days 3-13 (NMDS-based period 1) were positively correlated with CO_2 ($p=0.10$, $R^2=0.53$; Table 2, Fig. S2a). One explanation for higher net growth rates at elevated CO_2 could be the significantly ($p<0.05$) higher grazing rate in the low fCO_2 mesocosm M1 (0.56 d^{-1}) compared to the high fCO_2 M3 (0.27 d^{-1}) as measured on day 10 (Fig. 4a). After day 16, SYN abundances increased in all mesocosms and during this period (days 16-24) net growth rates had a significant negative correlation to fCO_2 ($p=0.05$, $R^2= 0.63$; Figs. 3a, Table 2 and Fig. S3a).

Consequently, the net increase in SYN abundances during this period was on average 20 % higher at low fCO_2 compared to high fCO_2. This corresponded to higher total loss rates in high fCO_2 treatments measured on day 17 (0.33 vs 0.17 d^{-1} for M3 and M1, respectively; Fig. 4a). The higher net growth most likely led to the peak in SYN abundance observed on day 24 (max. 4.7 x 10^5 ml^{-1}), which was negatively correlated with fCO_2 ($p=0.01$, $R^2=0.80$; Table 3, Fig. S4a). After this period (days 24-28), SYN abundances declined at comparable rates in the different mesocosms, irrespective of fCO_2 (Fig. 3a). Abundances in the low fCO_2 mesocosms remained higher into Phase III (Fig. 3a). SYN abundances in the surrounding water were generally lower than in the mesocosms, with the exception of days 17-21.

3.1.2 Picoeukaryotes

In contrast to the prokaryotic photoautotrophs, the eukaryotic phytoplankton community showed a strong positive response to elevated fCO_2 (Fig. 1b). Pico-I was the numerically dominant group of eukaryotic phytoplankton, accounting for an average 21-26 % of total phytoplankton abundances. Net growth rates leading up to the first peak in abundance (from day 1 to 5) had a strong positive correlation with fCO_2 ($p<0.01$, $R^2=0.90$; Fig. 3b, Table 3, Fig. S5a). Accordingly, the peak on day 5 (max. 1.1 x 10^5 ml^{-1}; Fig. 3b) was also correlated positively with fCO_2 ($p=0.01$, $R^2=0.81$; Table 3, Fig. S4b). During Phase I, from days 3-13 (i.e. NMDS-based period 1), net growth rates of Pico-I remained positively correlated to CO_2 concentration ($p=0.01$, $R^2=0.80$; Table 2, Fig. S2b). However, during this period there was also a decline in abundance (days 5-9; $p<0.01$, $R^2=0.89$; Table 3, Fig. S5b) with 23 % more cells lost in the low fCO_2 mesocosms. Accordingly, following this period, gross growth rate was significantly higher in the high fCO_2 mesocosm M3 as compared to the low fCO_2 mesocosm M1 (day 10, $p<0.05$; Fig. 4b). Pico-I abundances in the surrounding open water started to deviate from the mesocosms after day 10, and were on average around half that of the low fCO_2 mesocosms (Fig. 3b). Following a brief increase (occurring between days 11-13) correlated to fCO_2 ($p<0.01$, $R^2=0.94$; Table 3, Fig. S4c), abundances declined sharply between days 13-16 (Fig. 3b), coinciding with a significantly

higher total mortality rate in the high fCO_2 mesocosm M3 (day 13; Fig. 4b). Viral lysis was a substantial loss factor relative to grazing, for this group, comprising an average 45 % and 70 % of total losses in M1 and M3, respectively (Table S1). During NMDS-based period 2, net growth rates of Pico-I were significantly higher at high fCO_2 ($p=0.05$, $R^2=0.64$; Table 2, Fig S3b). By day 21, abundances in the high fCO_2 mesocosms were (on average) ~2-fold higher than at low fCO_2 (maximum abundances 8.7×10^4 ml^{-1} and 5.9×10^4 ml^{-1} for high and low fCO_2 mesocosms; $p=0.01$, $R^2=0.84$; Table 3, Fig. S4d). Standing stock of Pico-I remained high in the elevated fCO_2 mesocosms for the remainder of the experiment (7.9×10^4 vs 4.3×10^4 ml^{-1} on average for high and low fCO_2 mesocosms, respectively; Fig. 3b). Additionally, gross growth rates during this final period were relatively low (0.14 and 0.16 d^{-1} in M1 and M3, respectively) and comparable to total loss rates (averaging 0.13 and 0.10 d^{-1} over days 25-31, for M1 and M3, respectively; Fig. 4b).

Another pico-eukaryote group, Pico-II, slowly increased in abundance until day 13, when it increased more rapidly (Fig. 3c). Gross growth rates measured during Phase I were high (0.69 and 0.72 d^{-1} on average in the low and high fCO_2 mesocosms M1 and M3, respectively; Fig. 4c), and comparable to loss processes (0.46 and 0.58 d^{-1}), indicative of a relatively high turnover rate of production. Overall net growth rates during days 3-13 (NMDS-based period 1) did not correlate to CO_2 ($p=0.52$, $R^2=0.11$; Table 2, Fig. S2c). However, during periods of rapid increases in net growth, abundances were positively correlated to CO_2 concentration (days 12-17; $p=0.01$, $R^2=0.82$; Table 3, Fig. S5c). Accordingly, the peak in abundances of Pico-II on day 17 displayed a distinct positive correlation with fCO_2 ($p<0.01$, $R^2=0.93$; Table 3, Fig. S4e), with maximum abundances of 4.6×10^3 ml^{-1} and 3.4×10^3 ml^{-1} for the high and low fCO_2 mecososms, respectively (Fig. 3c). In M8 (the highest fCO_2 mesocosm), abundances increased for an extra day with the peak occurring on day 18, resulting in an average 23 % higher abundances. During the decline of the Pico-II peak (days 16-24), net growth rates were negatively correlated with fCO_2 ($p=0.10$, $R^2=0.52$; Table 2, Fig S3c). Moreover, the rate of decline was faster for the high fCO_2 mesocosms during days 18-21 ($p<0.01$, $R^2=0.85$). The Pico-II abundances in

390 the surrounding water were comparable to the mesocosms during Phases 0 and I, lower during
391 Phase II and higher during Phase III (Fig. 3c).
392 Pico-III exhibited a short initial increase in abundances in the low fCO_2 treatments, resulting in nearly
393 2-fold higher abundances at low fCO_2 by day 3 compared to the high fCO_2 treatment (Fig. 3d). After
394 this initial period, net growth rates of this group had a significant positive correlation with fCO_2 (days
395 3-13; p=0.04, R^2=0.67; Table 2, Fig. S2d). In general, during Phase I gross growth (p<0.01, days 1, 3,
396 10; Fig. 4d) and total mortality (p<0.05, days 1, 6, 10; Fig. 4d) were significantly higher in the low fCO_2
397 mesocosm M1, as compared to the high fCO_2 mesocosm M3 resulting in low net growth rates. During
398 Phase II (days 16-24, NMDS-based period 2) the opposite occurred; i.e. net growth rates were
399 negatively correlated with fCO_2 (p<0.01, R^2=0.86; Table 2, Fig S.3d). Maximum Pico-III abundances
400 (day 24: 4.2 x 10^3 and 8.3 x 10^3 ml^{-1} for high and low fCO_2) had a strong negative correlation with fCO_2
401 (p<0.01, R^2=0.91; Table 3, Fig. S4f). Pico-III abundances remained noticeably higher in the low fCO_2
402 mesocosms during Phases II and III (on average 80 %; Fig. 3d). Unfortunately, almost half of the
403 mortality assays in this second half of the experiment failed (see Materials and Methods), but the
404 successful assays suggest that losses were minor (<0.15 d^{-1}; Fig. 4d) and primarily due to grazing, as
405 no significant viral lysis was detected (Table S1).
406
407 **3.1.3 Nanoeukaryotes**
408 Nano-I showed maximum abundances (4.3 ± 0.4 x 10^2 ml^{-1}) on day 6 (except M1 which peaked on day
409 5), independent of fCO_2 (p=0.23, R^2=0.33; Fig. 3e). There was, however, a negative correlation of net
410 growth rate with fCO_2 during days 3-13 (NMDS-based period 1; p=0.01, R^2=0.79; Table 2, Fig. S2e). A
411 second major peak in abundance of Nano-I occurred on day 17, with markedly higher numbers in the
412 low fCO_2 mesocosms (4.1 x 10^2 ml^{-1} as compared to 2.4 x 10^2 ml^{-1} in high fCO_2 mesocosms; p=0.04,
413 R^2=0.67; Fig. 3e, Table 3 and Fig. S4g). Total loss rates in the high fCO_2 mesocosm M3 on days 6 and
414 10 were 2.3-fold higher compared to the low fCO_2 mescososm M1 (Fig. 4e), which may help to
415 explain this discrepancy in total abundance between low and high fCO_2 mesocosms. Viral lysis made

up to 98 % of total losses in the high fCO_2 mesocosm M3 during this period, whilst in M1 viral lysis was only detected on day 13 (Table S1). Peak abundances (around 5.0 x 10^2 ml^{-1}) were much lower compared to those in the surrounding waters (max ~2.4 x 10^3 ml^{-1}; Figs. 3e and S6a). During Phase II, Nano-I abundances in the surrounding waters displayed rather erratic dynamics compared to those of the mesocosms, but converged during certain periods (e.g. days 19-22). No significant relationship was found between net loss rates and fCO_2 for the second NMDS-based period ($p=0.26$, $R^2=0.30$; Table 2, Fig S.3e). At the end of Phase II, abundances were similar in all mesocosms but diverged again during Phase III (days 31-39) due primarily to a negative effect of CO_2 on Nano-I abundances, as depicted in the average 36 % reduction in Nano-I.

The temporal dynamics of Nano-II, the least abundant phytoplankton group analysed in our study, displayed the largest variability (Fig. 3f), perhaps due to the spread of this cluster in flow cytographs (which may indicate that this group represents several different phytoplankton species). No significant relationship was found between net growth rate and fCO_2 for this group for the two NMDS-based periods (Table 2, Figs S2f and S3f) nor with the peak in abundances on day 17 ($p=0.13$, $R^2=0.46$; Fig. S4h). Moreover, no consistent trend was detected in mortality rates (Fig. 4f). Similar to Nano-I, abundances in the surrounding water were often higher than in the mesocosms (max 3.5 x 10^2 ml^{-1} vs 1.1 x 10^4 ml^{-1}, respectively; Figs. 3f and S6b).

3.1.4 Algal carbon biomass

The mean combined biomass of Pico-I and Pico-II showed a strong positive correlation with fCO_2 throughout the experiment ($p<0.05$, $R^2=0.95$; Fig. 5a), an effect already noticeable by day 2. Their biomass in the high fCO_2 mesocosms was, on average 11 % higher than in the low fCO_2 mesocosms between days 10-20 and 20 % higher between days 20-39. Conversely, the remaining algal groups showed an average 10 % reduction in carbon biomass at enhanced fCO_2 (days 3-39, the sum of SYN, Pico-III, Nano-I and II ; $p<0.01$; Fig. 5b). The most notable response was found for the biomass of Pico-III, which showed an immediate negative response to CO_2 addition (Fig. S7a) and remained, on

442 average, 29 % lower throughout the study period (days 2-39). For Nano-I and II the lower carbon
443 biomass only became apparent during the end of Phase I and beginning of Phase II (days 14-20; Fig.
444 S7b). Due to its small cell size, the numerically dominant SYN accounted for an average of 40 % of
445 total carbon biomass.
446
447 **3.2 Prokaryote and virus population dynamics**
448 Prokaryote abundance in the mesocosms was positively related to total algal biomass independent of
449 treatment ($p<0.05$, $R^2=0.33$; Fig. 8) and generally followed total algal biomass (Fig. S7c). The initial
450 increase in total prokaryote abundances occurred during the first few days following the closure of
451 the mesocosms (Fig. 6a). This was primarily due to increases in the HNA-prokaryote group (Fig. 6b)
452 which displayed higher net growth rates (0.22 d^{-1}) compared to the LNA-prokaryotes (0.14 d^{-1} ~~for~~on
453 days -3 to 3; Fig. 6c). A similar, albeit somewhat lower, increase was also recorded in the surrounding
454 waters (Fig. 6a). The decline of the first peak in prokaryote abundances coincided with the decay in
455 phytoplankton abundance/biomass (Figs. 1a and S7c). Concurrently the share of viral lysis increased,
456 representing 37-39 % of total mortality on day 11 (Fig. 7b). No measurable rates of lysogeny were
457 found for the prokaryotic community during the experimental period (all phases). From days 10 to 15
458 prokaryote dynamics (total, HNA and LNA) became noticeably affected by CO_2 concentration with a
459 significant positive correlation between net growth and fCO_2 during Phase I (days 3-13 NMDS-based
460 period 1; Table 2, Fig. S2 g and h). In the higher fCO_2 mesocosms, the decline in prokaryote
461 abundance occurring between days 13 and 16 (Fig. 6a) was largely (70 %) due to decreasing HNA-
462 prokaryote numbers (Fig. 6b). The grazing was 1.6-fold higher in the high fCO_2 mesocosm M3
463 compared to M1 (0.36 ± 0.13 and 0.14 ± 0.08 d^{-1} on day 14; Fig. 7a). At the same time, virus
464 abundance increased in the high fCO_2 mesocosms (Fig. 6d).
465 During Phase II, prokaryote abundances increased steadily until day 24 (for both HNA and LNA),
466 corresponding to increased algal biomass (Figs. 6 and S7c) and lowered grazing rates (Fig. 7a).
467 Specifically, during days 16-24 (NMDS-based period 2), the HNA-prokaryotes showed an average 10

% higher abundances in the low, as compared to the high fCO_2 mesocosms (Fig. 6b). However, a significant negative correlation of net growth rates and fCO_2 was only found for LNA (Table 2, Fig S3g and h). No significant differences in loss rates between M1 and M3 were found during Phase II (p=0.22, 0.46 days 18 and 21 respectively; Fig. 7). Halfway through Phase II (day 24), the prokaryote abundance in the surrounding water leveled off (Fig. 6a). Prokaryote abundance ultimately declined during days 28-35 (Fig. 6a), whereby the net growth of LNA was again negatively correlated with enhanced CO_2 (p=0.02, R^2=0.76; Table 2, Fig S3g). Unfortunately, no experimental data on grazing and lysis of prokaryotes is present after day 25. However, viral abundances increased steadily at 2.2 x 10^6 d^{-1}, concomitant with a decline in prokaryote abundance (Fig. 6a and d). There was no significant correlation between viral abundances and fCO_2 during Phases II and III (p=0.36, R^2=0.21).

4 Discussion

In most experimental mesocosm studies, nutrients have been added to stimulate phytoplankton growth (Schulz et al., 2017) therefore little data exists for oligotrophic phytoplankton communities. In this study, we describe the impact of increased fCO_2 on the brackish Baltic Sea microbial community during summer (nutrient depleted; Paul et al., 2015). Small-sized phytoplankton numerically dominated the autotrophic community, in particular SYN and Pico-I (both about 1 µm cell diameter). Our results demonstrate variable effects of fCO_2 manipulation on temporal phytoplankton dynamics, dependent on phytoplankton group. In particular, Pico-I and Pico-II showed significant positive responses, whilst the abundances of Pico-III, SYN and Nano-I were negatively influenced by elevated fCO_2. The impact of OA on the different groups was, at times, a direct consequence of alterations in gross growth rate, whilst overall phytoplankton population dynamics could be explained by the combination of growth and losses. OA effects on community composition in these systems may have consequences on both the food web and biogeochemical cycling.

Comparison with surrounding waters

During Phase 0, the microbial assemblage showed good replicability between all mesocosms, however they had already began to deviate from the community in the surrounding waters. This was most likely a consequence of water movement altering the physical conditions and biological composition of the surrounding water body. The dynamic nature of water movement in this region has been shown to alter the entire phytoplankton community several times over within a few months, due to fluctuations in nutrient supply, advection, replacement/mixing of water masses and water temperature (Lips and Lips, 2010). Alternatively, effects of enclosure and the techniques (bubbling) used to ensure a homogenous water column may have stimulated SYN within the mesocosms~~.~~, which has been found to occur in several mesocosm experiments (Paulino et al., 2008; Gazeau et al., 2017). By Phases II and III, the microbial abundances within the mesocosms were distinctly different from the surrounding waters, with generally fewer SYN and Pico-I, and more Nano-I and Nano-II. Our statistical analysis shows that during this time, there was little similarity between the surrounding waters and mesocosms regardless of the CO_2 treatment level. Thus, the deviations during this time were most likely due to an upwelling event in the archipelago (days 17-30; Paul et al., 2015). Cold, nutrient-rich deep water has been shown to ~~occur~~upwell during summer, with profound positive influence on ecosystem productivity (Nômmann et al., 1991; Lehman and Myrberg, 2008). A relaxation from nutrient limitation in vertically stratified waters disproportionately favours larger-sized phytoplankton, due to their higher nutrient requirements and lower capacity to compete at low concentrations dictated by their lower surface to volume ratio (Raven, 1998; Veldhuis et al., 2005). Inside the mesocosms, which were isolated from upwelled nutrients, picoeukaryotes dominated similar to a stratified water column. Following this upwelling event, the pH of the surrounding waters dropped from 8.3 to 7.8, a level comparable to the highest CO_2 treatment (M8) on day 32 (Paul et al., 2015). Suggesting that other factors contributed to the observed differences between mesocosms and surrounding water, than can be accounted for by CO_2 concentration alone e.g. nutrients. Alternatively, the magnitude and source of mortality occurring in the surrounding water may have been altered, compared to within the mesocosms, after such an

upwelling event. Although the grazer community in the surrounding waters was not studied during this campaign, it is likely that the grazing community was completely restructured during the upwelling event (Uitto et al., 1997). It is nonetheless noteworthy that the phytoplankton groups with distinct responses to CO_2 enrichment (either positive or negative) in the low (ambient) fCO_2 mesocosms diverged from those in the surrounding water before the upwelling event occurred.

Phytoplankton dynamics

Synechococcus showed significantly lower net growth rates and peak abundances at higher fCO_2. Both in laboratory and mesoscosm experiments, *Synechococcus* has been reported to have diverse responses to CO_2, with approximately equal accounts of positive (Lu et al., 2006; Schulz et al., 2017), negative (Paulino et al., ~~2007~~2008; Hopkins et al., 2010; Traving et al., 2014,) and insignificant changes (Fu et al., 2007; Lu et al., 2006) in net growth rate with fCO_2. This variable response is probably due, at least in part, to the broad physiological and genetic diversity of this species. In the Gulf of Finland alone, 46 different strains of *Synechococcus* were isolated in July 2004 (Haverkamp et al. 2009). Direct effects on physiology have been implied from laboratory studies. One isolate, a phycoerythrin rich strain of *Synechococcus* WH7803 (Traving et al., 2014) elicited a negative physiological effect on the growth rate from increased CO_2. This was most likely a consequence of higher sensitivity to the lower pH (Traving et al., 2014), and the cellular cost of maintaining pH homeostasis or conversely a direct effect on protein export. Additionally, Lu et al. (2006) reported increased growth rates in a cultured phycocyanin rich but not a phycoerythrin rich strain of *Synechococcus*, suggesting that pigments may play some part in defining the direct physiological response within *Synechococcus*. In addition, within natural communities (Paulino et al., ~~2007~~2008; Hopkins et al., 2010; Schulz et al., 2017) variability can also arise from indirect effects such a altering competition with other picoplankton (Paulino et al., ~~2007~~2008). The delay and dampened effect of fCO_2 on SYN abundances within our study was more likely due to indirect effects arising from alterations in food web dynamics than to direct impacts on the physiology of this species.

546 Specifically, significant differences in grazing rates of SYN between M1 and M3 (days 10 and 17, no
547 significant lysis detected) could be responsible for the differing dynamics between the mesocosms at
548 the end of Phase I and beginning of Phase II.
549 The gross growth rates of Pico-I were significantly higher ($p<0.05$) at high fCO_2 compared to the low
550 CO_2 concentrations during the first 10 days of Phase I. Moreover, no differences were detected in the
551 measured loss rates, demonstrating that increases in Pico-I were the due to increases in growth
552 alone. The stimulation of Pico-I by elevated fCO_2 may be due to a stronger reliance on diffusive CO_2
553 entry compared to larger cells. Model simulations reveal that whilst near-cell CO_2/pH conditions are
554 close to those of the bulk water for cells <5 µm in diameter, they diverge as cell diameters increase
555 (Flynn et al., 2012). This is due to the size-dependent thickness of the diffusive boundary layer, which
556 determines the diffusional transport across the boundary layer and to the cell surface (Wolf-Gladrow
557 and Riebesell, 1997; Flynn et al., 2012). It is suggested that larger cells may be more able to cope
558 with fCO_2 variability as their carbon acquisition is more geared towards handling low CO_2
559 concentrations in their diffusive boundary layer, e.g. by means of active carbon acquisition and
560 bicarbonate utilization (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). Moreover, as the Baltic
561 Sea experiences particularly large seasonal fluctuations in pH and fCO_2 (Jansson et al., 2013) due to
562 the low buffering capacity of the waters, phytoplankton here are expected to have a higher degree of
563 physiological plasticity. Our results agree with previous mesocosm studies, which reported enhanced
564 abundances of picoeukaryotic phytoplankton (Brussaard et al., 2013; Davidson et al, 2016; Schulz et
565 al., 2017), and particularly the prasinophyte *Micromonas pusilla* at higher fCO_2 (Engel et al., 2007;
566 Meakin and Wyman, 2011). Furthermore, Schaum et al. (2012) found that 16 ecotypes of
567 *Ostreococcus tauri* (another prasinophyte similar in size to Pico-I) increased in growth rate by 1.4-1.7
568 fold at 1,000 compared to 400 µatm fCO_2. All ecotypes increased their photosynthetic rates and
569 those with most plasticity (those most able to vary their photosynthetic rate in response to changes
570 in fCO_2) were more likely to increase in frequency within the community. It is possible that Pico-I cells
571 are adapted to a highly variable carbonate system regime and are able to increase their

572 photosynthetic rate when additional CO_2 is available. This ability would allow them to out-compete
573 other phytoplankton (e.g. nanoeukaryotes in this study) in an environment when nutrients are
574 scarce.
575 The net growth rates and peak abundances of Pico-II were also positively affected by fCO_2. Gross
576 growth rates were significantly higher at high fCO_2 on only two occasions (days 10 and 20) and were
577 accompanied by high total mortality rates. Pigment analysis suggests that both Pico-I and Pico-II are
578 chlorophytes (Paul et al., 2015) and as such may share a common evolutionary history (Schulz et al.,
579 2017); thus Pico-II may be stimulated by fCO_2 in a similar manner to Pico I. Chlorophytes are found in
580 high numbers at this site throughout the year (Kuosa, 1991), suggesting the ecological relevance of
581 Pico-I and Pico-II in this ecosystem. In addition, Pico-II bloomed exactly when Pico-I declined which
582 may suggest potential competitive exclusion.
583 Pico-III showed the most distinct and immediate response to CO_2 addition. The significant reduction
584 in gross growth rates observed during Phase I suggests a direct negative effect of CO_2 on the
585 physiology of these cells. For this group, the lower gross growth rates were matched by lower total
586 mortality rates with increased fCO_2. Although the mean cell size of Pico-III and Pico-II were
587 comparable (2.9 and 2.5 µm, respectively), they showed opposing responses to fCO_2 enrichment
588 (lower Pico-III abundances at high fCO_2). These differences may arise from taxonomic differences
589 between the two groups. Pico-III displayed relatively high phycoerythrin orange autofluorescence,
590 likely representing small-sized cryptophytes (Klaveness, 1989), although rod-shaped *Synechococcus*
591 up to 2.9 µm in length (isolated from this region; Haverkamp et al., 2009) or *Synechococcus*
592 microcolonies (often only two cells in the Baltic; Motwani and Gorokhove, 2013) cannot be excluded.
593 In agreement with Pico-III response to CO_2 enrichment, Hopkins et al. (2010) reported reduced
594 abundances of small cryptophytes under increased CO_2 in a mesocosm study in a Norwegian fjord
595 near Bergen.
596 Lastly, the two nanoeukaryotic phytoplankton groups also displayed a negative response to fCO_2
597 enrichment, whereby Nano-II was the least defined, most likely due to a high taxonomic diversity in

598 this group. Nano-I started to display lower abundances at high fCO_2 during Phase I (after day 10),
599 which was likely the result of greater differences between gross growth and total mortality
600 (compared to low fCO_2). Alternatively, enhanced nutrient competition due to increased abundances
601 of SYN and Pico-I (and later on also Pico-II) at elevated fCO_2 may also have contributed to the
602 dampened response of Nano-I in the high fCO_2 mesocosms. The overall decline in Nano-I, during
603 Phase II, and sustained low abundances during Phase III may well have been the result of grazing by
604 the increased mesozooplankton abundances during Phase II (Lischka et al., 2017).
605
606 **Microbial loop**
607 The strong association of prokaryote abundance with algal biomass, present throughout the
608 experiment, suggests that the effect of CO_2 was an indirect consequence of alterations in the
609 availability of phytoplankton carbon. Others have reported a tight coupling of autotrophic and
610 heterotrophic communities at this location, with an estimated 35 % of the total net primary
611 production being utilized directly by bacteria or heterotrophic flagellates (Kuosa and Kivi, 1989),
612 suggesting a highly efficient microbial loop in this ecosystem. In addition to phytoplankton exudation,
613 viral lysis may also contribute to the dissolved organic carbon pool (Wilhelm and Suttle, 1999;
614 Brussaard et al., 2005; Lønborg et al., 2013). We calculated that viral lysis of phytoplankton between
615 days 9 and 13 resulted in the release of 1.3 and 13.1 ng C ml^{-1} for M1 and M3, respectively. Assuming
616 a bacterial growth efficiency of 30 % and cellular carbon conversion of 7 fg C $cell^{-1}$ (Hornick et al.,
617 2017), we estimate that the organic carbon required to support bacterial dynamics during this period
618 (taking into account the net growth and loss rates) was 2.9 and 11.5 ng C ml^{-1} in low and high fCO_2
619 mesocosms M1 and M3, respectively. These results suggest that viral lysis of phytoplankton was an
620 important source of organic carbon for the bacterial community. Our results are consistent with
621 bacterial-phytoplankton coupling during this eastern Baltic Sea mesocosm study (Hornick et al.,
622 2017), and agree with earlier work on summer carbon flow in the northern Baltic Sea showing that
623 prokaryotic growth was largely supported by recycled carbon (Uitto et al., 1997). The average net

624 growth rates of the prokaryotes during the first period of increase in Phases 0 and I (0.2 d^{-1}) were
625 comparable to rates reported for this region (Kuosa, 1991). In order to sustain the concomitant daily
626 mortality (between 0.3-0.5 d^{-1}) measured during our study, prokaryotic gross growth rates must have
627 been close to one doubling a day (0.5-0.7 d^{-1}). During Phase I, grazing was the dominant loss factor of
628 the prokaryotic community although there was also evidence that viral lysis was occurring.
629 Bermúdez et al. (2016) reported the highest biomass of protozoans around day 15. This was
630 predominantly the heterotrophic choanoflagellate *Calliacantha natans,* which selectively feeds on
631 particles <1 µm in diameter (Marchant and Scott, 1993; Hornick et al., 2017). Indeed, an earlier study
632 in this area showed that heterotrophic nanoflagellates were the dominant grazers of bacteria,
633 responsible for ingestion of approximately 53 % of bacterial production compared to only 11 % being
634 grazing by ciliates (Uitto et al., 1997). During the first half of Phase II, grazing was reduced and likely
635 contributed to the steady increase in prokaryote abundances. Specifically, a negative relationship
636 between the abundances of HNA-prokaryotes and fCO_2 was detected and corresponded to reduced
637 bacterial production and respiration at higher fCO_2 (Hornick et al., 2017; Spilling et al., 2016).
638 Although CO_2 enrichment may not directly affect bacterial growth, co-occurring global rise in
639 temperature can increase enzyme activities, affecting bacterial production and respiration rates
640 (Piontek et al., 2009; Wohlers et al., 2009; Wohlers-Zöllner et al., 2011). Enhanced bacterial re-
641 mineralization of organic matter may stimulate autotrophic production by the small-sized
642 phytoplankton (Riebesell et al., 2009; Riebesell and Tortell, 2011; Engel et al., 2013), intensifying the
643 selection of small cell size.
644 Mean viral abundances were higher under CO_2 enrichment towards the end of Phase I and into Phase
645 II which is expected under conditions of increased phytoplankton and prokaryote biomass. The
646 estimated average viral burst size, obtained from this increase in total viral abundance and
647 concurrent decline in bacterial abundances, was about 30 which is comparable to published values
648 (Parada et al, 2006; Wommack and Colwell, 2000). Viral lysis rates of prokaryotes were measured
649 until day 25 and indicated that during days 18-25 an average 10-15 % of the total prokaryote

650 population was lysed per day. Moreover, the concurrent steady increase in viral abundances during
651 Phase III indicates that viral lysis of the prokaryotes remained important. Thus, the combined impact
652 of increased viral mortality together with reduced production (Hornick et al., 2017) ultimately led to
653 the decline in prokaryote abundance (this study). Lysogeny did not appear to be an important life
654 strategy of viruses during our campaign. Direct effects of higher fCO_2 on viruses are not expected, as
655 marine virus isolates are quite stable (both in terms of particle decay and loss of infectivity) over the
656 range of pH of the present study (Danovaro et al., 2011; Mojica and Brussaard, 2014). The few
657 studies which have inferred viral lysis rates based on changes in viral abundances show reduced
658 abundances of algal viruses (e.g. *Emiliania huxleyi* virus) under enhanced CO_2 (Larsen et al., 2008)
659 while mesocosm results by Brussaard et al. (2013) indicated a stronger impact of viruses on bacterial
660 abundance dynamics with CO_2 enrichment.
661
662 **5 Conclusions**
663 Due to the low buffering capacity of the Baltic Sea and the paucity of data regarding OA impact in
664 nutrient-limited waters, the results presented here are pertinent to increasing our understanding of
665 how ~~predicted~~projected rises in fCO_2 will affect the microbial communities in this region. Our study
666 provides evidence that cell size, taxonomy and sensitivity to loss can all play a role in the outcome of
667 CO_2 enrichment. Physiological constraints of cell size favour nutrient uptake by small cells under
668 conditions of reduced nutrients and our results show that these effects can be further exacerbated
669 by OA. Gross growth rates along with the complementary mortality rates allowed for a more
670 comprehensive understanding of the phytoplankton population dynamics and thus perception of
671 how microbial food web dynamics can influence the response of the autotrophic and heterotrophic
672 components of the community. Our results further suggest that alterations in CO_2 concentrations are
673 expected to affect prokaryote communities (mainly) indirectly through alterations in phytoplankton
674 biomass, productivity and viral lysis. Overall, the combination of growth and losses (grazing and viral
675 lysis) could explain microbial population dynamics observed in this study. It is noteworthy to

mention, a recent study in the oligotrophic northeast Atlantic Ocean reported a shift from grazing-dominated to viral lysis-dominated phytoplankton community with strengthening of vertical stratification (shoaling the mixed layer depth and enhancing nutrient limitation) (Mojica et al., 2016). Thus, we highly recommend that future research on OA combine mesocosm studies focusing on changes in microbial community composition and activity with experiments aimed at understanding the effects of OA on food web dynamics, i.e. partitioning mortality between grazing and viral lysis (Brussaard et al., 2008).

Author Contribution

Design and overall coordination of research by CB. Organization and performance of analyses in the field by KC. Data analysis by KC, CB, and SA-F. Design and coordination of the overall KOSMOS mesocosm project by UR. All authors contributed to the writing of the paper (KC, KM and CB are lead authors).

Acknowledgements

This project was funded through grants to C.B. by the Darwin project, the Netherlands Institute for Sea Research (NIOZ), and the EU project MESOAQUA (grant agreement number 228224). We thank the KOSMOS project organisers and team, in particular Andrea Ludwig, the staff of the Tvärminne Zoological Station and the diving team. We give special thanks to Anna Noordeloos, Kirsten Kooijman and Richard Doggen for their technical assistance during this campaign. We also gratefully acknowledge the captain and crew of R/V ALKOR for their work transporting, deploying and recovering the mesocosms. The collaborative mesocosm campaign was funded by BMBF projects BIOACID II (FKZ 03F06550) and SOPRAN Phase II (FKZ 03F0611).