We thank the reviewers for taking the time to review this manuscript and we have carefully considered and addressed their comments (in blue italic).

Reviewer 1:

As I also reviewed the original submission, I am omitting a summary of the manuscript here. Although the authors changed the title of their manuscript, the interpretation of the presented data hasn't changed much (or at least it doesn't come across to the reader). At the moment it is relatively hard to judge the presented manuscript/data as there are still many cases where the statements/conclusions/claims by the authors are not supported by the results. Unfortunately, the authors do not present any further analyses or statistics to back up their statements (I have given a few examples below; however, this list is not comprehensive!).

We have added new statistical analyses including a non-metric multidimensional scaling (NMDS) plot of autotrophic eukaryote and prokaryote abundances which shows the development of the community in time. This shows that there is a "divergence" between the community development of the low and high fCO_2 mesocosms. An analysis of dissimilarities (ANOSIM) shows that this is significant ($p=0.01$)(Lines 307-308 of revised manuscript and Fig.2). The NMDS also allowed us to identify the periods during which divergence occurred. Linear regressions of net growth rates against fCO_2 (averaged over the period) for the individual groups allowed us to further examine the differences (Lines 308-312; Table 2, Figs. S2 and S3). Mesocosms M1 and M3 are clearly in two separate clusters. (Lines 314-315; Fig. 2). We have also carefully examined all claims made and tried to ensure that all are adequately statistically tested and that these test results are presented in the text.

An overall suggestion to the authors would be to look for someone who would be able and willing to aid with the manuscript re-structuring and re-writing, but who is himself or herself not involved in these experiments (someone in their departments maybe? I think it would help to get an outside-the-box opinion here for the interpretation of the data). At the current state, there seem to be too many misinterpretations in the manuscript in order to recommend publication (even with some revisions). I have made some more specific comments below.

We asked two colleagues to contribute (statistical analysis and interpretation and writing); both are now included as co-authors.

In general, the authors try to make (big) claims about the effect of CO2 on the microbial community; however, basically provide neither analyses/statistics that support these claims nor is it visible in the figures.

We have added further statistical analyses as described above. We have also taken care not to overstate the differences we see.

Further, the authors have not given any type of error on their measurements making it hard to see actual differences.

A series of mesocosms with incremental steps of fCO_2 addition was chosen as the experimental design which minimises the risk of failure due to losing mesocosms. This was favoured over a replicated approach (Lines 129-133). Statistical analyses are therefore regressions and do not have errors attached. Unfortunately we were unable to perform grazing and lysis experiments on replicate mesocosms due to the logistics of sample preparation and analysis on site. We have added these considerations into the text (Lines 221-222; 253-256 of revised manuscript).

Overall, in many places it seems that the authors see what they want to see (i.e. an effect of CO2 concentrations on the microbial community).

We have added further statistical analyses to support all claims made on fCO_2-induced differences.

Again, I would recommend to add an overview of the experimental setup to the methods section. I understand that the experimental setup is described in a different manuscript for the special issue. However, as a reader of this manuscript, I actually do not want to have to read another manuscript first in order to find out what the authors did here; at least a summary would be needed here.

We added a section describing the experimental set-up (Lines 110-125 of revised manuscript).

Many places in the manuscript are still not written to the extent that they can be easily followed, for example: "The higher loss rates (days 5 to 9; Fig. 3e) resulted in a decrease in abundance, which was stronger for the low fCO2 mesocosms (as illustrated by M1) due to the significantly higher gross growth rates for the high fCO2 mesocosm (represented by M3; Fig. 3b). The positive correlation of Pico I peak abundance with fCO2 on day 13 (Fig. 3h, R2=0.94) was lost upon another decline in abundance. Significantly higher losses at high fCO2, a combination of grazing and lysis, resulted in a more dramatic crash at high fCO2 and abundances becoming similar again around day 17 (Fig. 3a)." This paragraph is simply not understandable for someone not working on these experiments without having to read the sentences multiple times (which disrupts the flow reading dramatically).
We have improved the readability of the entire manuscript and it has been reviewed and edited by two additional co-authors.

The figures were not labeled at the end of the manuscript (no matter whether this is because of forgetting to label them or whether the submission software omitted to do so); so, for the purpose of the review I assumed that the figures were 1-11 in the way they came out of the printer and I am referring to them in that way.
We apologise for the inconvenience this may have caused and made certain that all are clearly labelled in the revised manuscript.

In general, I think that the authors should try to improve the readability of their manuscript by increasing the quality of their figures, e.g., not having figures that span nearly two full pages (see Figure 3) or omitting repetitive data.
We have completely re-organised the figures to improve their appearance and readability. We have taken note of the reviewer's comments and tried to ensure that they are well presented and have omitted repetitive data.

Showing the ambient data together with the mesocosm is essential to judge whether changes in the mesocosms were, for example, induced by enclosement or were naturally happening outside as well. This does not mean that, in case changes were induced by enclosement, that differences between mesocosms are not useful; they are valuable data. Not presenting the ambient microbial community leaves the reader with guessing what happened and potentially suspecting that the data was omitted because they would alter the conclusions of the paper. If the authors would like to increase the understandability of their manuscript, I highly recommend adding the ambient data to the current main manuscript figures. For example, Suppl. Figure 1 shows that changes in the Synechococcus population are pretty much the result of 'bottle effects' (I agree that a mesocosm is a large bottle, but it is an enclosement). Is this possibly related to cleaning procedures or stirring of the mesocosms as outlined in Paul et al.? While other groups are coherent with the ambient water.
We acknowledge the reviewer's concerns and have added these data into the main figures and Results sections generally (Lines 316-323) and of each group (Figs. 1,3,6 and Figs. S1, 6,7). They are shown as black lines in accordance with the other manuscripts in the special issue. We originally excluded microbial data from the outside water because of the dynamic water movement (including periods of upwelling) in the surrounding waters of this region, which complicates comparisons between them. We now also discuss microbial dynamics in relation to outside water (Lines 490-520 of revised manuscript).

In response to the example given by the reviewer: the mesocosms were kept open for 5 days (t-10 to t-5) for rinsing and free exchange of <3mm plankton. The bags were then closed and bubbled for 3.5 min with compressed air (t-5) to ensure a homogenous water column. Five more days were then allowed before CO_2 manipulation (t-5-t0). This time line suggests that the bubbling could have stimulated Synechococcus growth. Alternatively the surrounding waters, which are very dynamic, may have altered resulting in lower abundances outside the mesocosms. We have acknowledged this in the Results: "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)." (Lines 316-318) and Discussion:" 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." (Lines 491-499).

After looking into Paul et al. for the experimental setup, it seemed that the CO2 concentrations were actually measured during the mesocosms, why aren't the actual measured concentrations used for any of the plots instead of the targeted concentrations (which were only achieved in the first few days but weren't maintained)? This seems such an obvious thing to do and a fact that is completely ignored by the authors.
Actually, we did work with the measured values. Table 1 lists the average fCO_2 for each mesocosm over the duration of the experiment. Linear regressions of abundance or growth rate are plotted against actual fCO_2 for the day or period analysed in the specific mesocosms. We have better clarified this in the text and Figure captions.

Abstract: "Of these groups 2 picoeukaryotic groups increased in abundance whilst the other groups, including prokaryotic Synechococcus spp., decreased with increasing fCO2." Looking at both Figure 2a and Suppl. Figure 1a, I seriously do not see where this statement comes from! There is a short period of time (~ 7-16 days) where Syn. are lower in the low fCO2 than in the higher ones but for most of the experiment it is the other way around. Unfortunately, the authors also do not supply any other analysis or statistics that could possibly back up their claim. Unfortunately, these kind of statements and interpretation is occurring throughout the manuscript, which leads me to the suggestion that the current manuscript is far from being acceptable for publication at this time.
We believe that the reviewer has misread the sentence. We were indeed stating, as does the reviewer, that Synechococcus abundance generally decreased at higher fCO_2. Peak abundance regression against fCO_2 was provided to support this. We now also provide significant linear regression of net growth rates against fCO_2 (Table 2 Fig. S3a) to support this and have added further analyses in the Results section "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 compared to high fCO_2."(Lines 333-337 of revised manuscript). (We have added similar data to the results of each of the phytoplankton groups to support our claims).

Figure 1: The authors claim that the decline in total phytoplankton around the end of phase 2 and throughout phase 3 is due to a decrease in Synechococcus. This is supported by Figure 2a. However, Suppl Figure 1a shows Synechococcus populations staying up rather than declining to concentrations in Figure 1. After doing some research through this manuscript, I found that the difference between the figures is the depth reference here, which is 0.3-10 m for Figure 1 and 0-17 m for Suppl. Figure 1. This difference actually suggests that either the distribution of Synechococcus is not equal throughout the water column or that there is a sinking out of Synechococcus? I don't know why this is, maybe the authors should think about this deviation which is currently not acknowledged anywhere.
Indeed in the top 10m samples Synechococcus accounts for >90% of the loss in total phytoplankton abundance for days 24-28. In the 0-17 m samples they account for 60%. The distribution of Synechococcus is not equal throughout the water column; as a result of vertical stratification phytoplankton abundances were higher in the surface waters. There is no noticeable sinking of Synechococcus, otherwise they would be higher in the 0-17 m samples.

Figure 1: The authors say that the total phytoplankton abundance at the end of phase 1 is significantly higher in the high fCO2 treatment than the low fCO2 treatments. Again, I am really sorry, but I cannot

see this in the plots and the authors again don't provide any analysis or statistics for this. (see p 10 lines 18-20).
By this we meant the second half of Phase I. We have re-written this section to clarify and now show additional statistical analyses to support our claims (Lines 308-311 of revised manuscript).

Panel d: What are the errors on these measurements?
They are the specific growth rates for individual mesocosms for a specific time period so there is no error.

I wonder whether these differences are actual changes or just variation ?
During re-writing we decided to omit this panel. All the linear regressions of net growth against fCO_2, as averaged for the specific time period for each mesocosm, now have p-values to test for significance.

Further, this is comparing growth rates from time point 10 days to growth and loss rates of time points 4-7. In my opinion, this is totally misleading as day 10 is obviously different than 4-7 as can be seen in panel a. (see p 11, l 10-13).
This section has been re-written such that we state more clearly that we are examining differences between the mesocosms from days 3-13 "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). "(Lines 327-333 of revised manuscript). The significantly higher loss rates on day 10 may therefore serve to explain the lower abundances at lower fCO_2 during that time.

Figure 2: Panel c is absolutely unnecessary here (and in following figures); it is the same data as in panel a and its addition here suggests that it is indeed different data and is making the figure unnecessarily large.
We have completely reorganised the figures to improve readability and presentation, this panel has been removed.

Figure 2a legend: It is not necessary to present 4 (!) different significance levels here. For most claims in the manuscript no analyses or statistics are provided while here it is over the top. Either s.th. is significantly different or not.
Agreed, we now only present <0.05 significance.

p 10, l 21 and following: If CO2 has a strong positive effect on Pico 1 and 2, how can they then be comparable in abundances to the surrounding water? Maybe I don't understand this sentence.
Pico I and II are clearly different in abundance from the surrounding waters, we have removed this during re-writing and apologise if this was not clear.

Suppl. Figure 1: This is (probably) the most informative figure of the entire manuscript.
We have added the surrounding water abundances to the main figures to make them more informative.

p 11, l3-5: This claim is not supported by any analysis. Any time series statistics, PCA, similarity analysis, community composition comparisons ?
We have removed this particular sentence, have added new statistical analyses and carefully checked that all claims are fully backed up.

p 17, l 17-18: this is an odd comparison in size fractions and the results are not exclusive of each other.

We removed this sentence as the statement is also made in the sentence before.

p 18, l 20-22: There is consistency among the mesocosms maybe, but there is already a deviation to the surrounding water indicating an effect of enclosement, at least on some groups.
We have added comparisons between mesocosms and surrounding waters to the Results and Discussion sections (Lines 316-321 and 490-520, respectively).

Figure S3 is missing! –a) pico iii poc b)Nano I and II POC c) total POC
We apologise for the inconvenience of this omission; it has been rectified.

Reviewer 3
This study investigates the effect of different fCO2 levels on different microbial groups as determined by flow cytometry in mesococm experiments in the Gulf of Finland. Clear and differential effects of fCO2 levels were found for the various microbial groups. In two mesocosms (low and high fCO2), viral infection and grazing were assessed, parameters that have not been very often measured in ocean acidification experiments.
The experimental approach and the methods are appropriate and appropriately described. Useful information on the potential effect of ocean acidification on different microbial groups was obtained.

The differences between M1 and M3 (growth rates, viral infection and grazing) are a bit overemphasized. Differences between two mesocosms can certainly be calculated and this might be ok in some experimental approaches. But in this case samples have to be taken repeatedly within one mesocosm to estimate the entire variability of the approach. This was certainly not done for the viral infection and grazing measurements. Also, the authors use this analysis to make a comparison between two treatments, but this can also be done when the experimental treatments are replicated. I appreciate the effort that the authors made, but they put to much weight on this analysis. They are right not to mention the effect in the abstract and should reduce the reference to these findings in the discussion. It is ok to discuss that briefly as potential effect, however, in the absence of statistical prove, this part short be less prominent.
We appreciate reviewer's appreciation of the research. We have further reduced emphasis on the loss assay results. It is indeed unfortunate that we were not able to replicate the experiments on more mesocosms but it was logistically / practically not feasible. The experiments were carried out in triplicate within mesocosms and the results are tested statistically but we understand the reviewers concerns that there may be differences between two mesocosms rather than treatments. The new non-metric multidimensional scaling (NMDS) plot does show that M1 and M3 do diverge from each other and cluster well with the other low and high fCO_2 mesocosms respectively.

Despite the comments of one reviewer, the presentation -especially in the discussion- remains poor. Many sentences are awkward or hard to understand.
We have rewritten and improved readability of the manuscript and it has been read and edited by two additional co-authors.

Also, the arguments are sometimes presented in a sloppy way. Although I think I understand what the authors want to say and have no serious objection, the argumentation has to be improved and presented with more rigor.
We have added and improved our statistical analyses which has allowed us to be more specific and has strengthened our argumentation. We have added new statistical analyses including a non-metric multidimensional scaling (NMDS) plot of autotrophic eukaryote and prokaryote abundances which shows the development of the community in time. This shows that there is a "divergence" between the community development of the low and high fCO_2 mesocosms. An analysis of dissimilarities (ANOSIM) shows that this is significant ($p=0.01$)(Lines 307-308 of revised manuscript and Fig.2). The NMDS also allowed us to identify the periods during which divergence occurred. Linear regressions of net growth rates against fCO_2 (averaged over the period) for the individual groups allowed us to further examine the differences (Lines 308-312; Table 2,Figs. S2 and 3). Mesocosms

M1 and M3 are clearly in two separate clusters. (Lines 314-315; Fig. 2). We have also carefully examined all claims made and tried to ensure that all are adequately statistically tested and that these test results are presented in the text.

Minor comments:

Page 2, Line 5/6: Does this mean that TOTAL algal biomass is related to prokaryotic biomass, despote of differential effects of fCO2 on algal groups? Please specify.

We have added two new Figures, one showing total algal biomass which closely resembles the Figure showing total prokaryote abundances (Fig. S7c) and another which shows a positive correlation between total algal biomass and prokaryote abundance (Fig. 8). We have also altered this sentence to:" Dynamics of the prokaryote community closely followed trends in total algal biomass despite differential effects of fCO_2 on algal groups." (Lines 39-40 of revised manuscript).

Page 3, Lines 21-23: specify the direction of the responses.

Sentence changed to: "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." (Lines 104-106 of revised manuscript).

Page 10, Results: I think there should be a short part on phase 0.

We have added this at the beginning of the Results section (Lines 300-306 of revised manuscript).

Page 11, Line 10: 'most so'. generally?

This section has been rewritten more specifically (Lines 327-330).

Line 19: Are low and high fCO2 mesocosms nowhere defined.

Initially as high, intermediate and low in Table 1. and in the Materials & Methods section. We now also added an extra comment at the end of the statistics section where we refer to only high and low mesocosms (Lines 294-296).

Line 22: What does 'not so clear' mean? Please use a more rigorous (scientific language). This comment holds also for many other occasions; not to all will be referred to.

We have extensively rewritten the manuscript and added statistical analyses to overcome this issue.

Page 12, Line 2-4: I.e., 26% of total phytoplankton abundances were found on average in the high high fCO2 mescosms?

Sentence rewritten: "Pico-I was the numerically dominant group of eukaryotic phytoplankton, accounting for an average 21-26 % of total phytoplankton abundances."(Lines 348-349)

Please use more verbs in your sentences, this would increase the readability of the text. This holds for the entire text.

The manuscript has been rewritten and edited by two additional co-authors.

Line 22: What exactly do you mean by 'matched'.

We have rewritten this as 'comparable to total loss rates' (Line 371-372 of revised manuscript).

Page 13, Lines 6-7: These? Please explain better what you mean.

We have removed the sentence

Line 8: explain better why the word seems is appropriate here.

We have rewritten this section to make it more rigorous (Lines 364-379).

Line 17 remove just
Line 22: remove indeed
Line 24: remove perfectly
Line 25: remove indeed

We have made the changes accordingly.

Page 14
Line 2: largely - be more precise
This sentence has been removed

Line 3 after 'reasons', add: see materials and methods
This has been added (Lines 399-400 of revised manuscript)..

Lines 4-7: Awkward sentence
Sentence removed during rewriting

Line 23: Dynamics is I think a word that requires the singular.
Corrected.

Line 23-25: No idea what the authors mean.
Sentence rewritten as: "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)." (Lines 422-424).

Page 15
Line 7: change into to to
Sentence has been altered to "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." (Lines 432-433)

Line 2-3: A correlation could be calculated
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. This has been included as Fig. 8 and in the Results section "Prokaryote abundance in the mesocosms was positively related to total algal biomass independent of treatment ($p<0.05$, $R^2=0.33$; Fig. 8) and generally followed total algal biomass (Fig. S7c)" (Lines 445-446).

Page 16, Lines 2-3: give test and significance level
This sentence was removed however all results are now supported by significance levels.

Page 16/17; There was only one mesocosm for low fCO2 level, right?
Yes, we have now clarified this (Lines 459 of revised manuscript).

Discussion
1st paragraph: There is no real explanation given. Only net growth and loss rates are observed. What has been actually 'examined'?
The Discussion has been completely re-written to improve readability.

2nd paragraph: Why would only nano-sized and not other phytoplankton profit from the nutrient upwelling? Also, the argumentation is not very clear.
We have added the following text: "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)" (Lines 505-508)

Page 19
1st paragraph: 'difficult' is sufficient. In addition, the authors could just refer to the pigment analysis. The molecular analyses were not done, so there is no need to refer to them.

We have re-written this section and refer only to the pigment analyses (Lines 572-573 of revised manuscript).

Again, no explanation is given, just rates are listed. The reader has to try to figure out what the authors have calculated. This has to be explained better.
We have added explanations and tried to improve the clarity and rigor of the arguments throughout the Discussion.

2nd paragraph: what are 'relatively' high loss? How can, based on net changes of abundances and some loss rates estimates, turnover of organic matter be calculateds.
This has been removed during re-writing.

Following pages
Same problems as with other parts of the discussion. Please spend more time on a better and especially more rigorous explanation and argumentation! It is necessary that one can follow your arguments in the text!
Again, we have improved and thoroughly re-written the Discussion.

Figures: The legend starts should start with a caption that specifies the intention of the graph.
We have improved the Figure legends

~~Shifts in the size structure of the~~ Alterations in microbial community ~~in the Baltic Sea~~ composition with increasing fCO_2: a mesocosm study in the eastern Baltic Sea

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Abstract~~:~~

Ocean acidification, ~~due to~~ resulting from the dissolution of ~~anthropogenically produced~~anthropogenic carbon dioxide in the atmosphere, is considered a major threat to marine ecosystems. ~~We~~Here we examined ~~the~~ effects of ocean acidification on ~~the~~ microbial community ~~structure~~dynamics in the ~~Gulf of Finland,~~eastern Baltic Sea, during the~~,~~ summer of 2012 when inorganic nitrogen and phosphorus were highly depleted~~, summer. Using large~~. Large volume in situ mesocosms were employed to ~~simulate~~mimic present to future and far future CO_2 scenarios~~, we observed distinct trends with increasing fCO_2 in each of the 6~~. All six groups of phytoplankton enumerated by flow cytometry (<20 µm cell diameter~~). Of these groups 2~~) showed distinct trends in net growth and abundance with CO_2 enrichment. The picoeukaryotic ~~groups increased in abundance~~ phytoplankton groups Pico-I and II displayed enhanced abundances, whilst ~~the other groups, including prokaryotic~~Pico-III, *Synechococcus* ~~spp., decreased with increasing~~and the nanoeukaryotic phytoplankton groups were negatively affected by elevated fCO_2. ~~Gross growth rates increased~~Specifically, the numerically dominant eukaryote, Pico-I, demonstrated increases in gross growth rate with increasing fCO_2 ~~in the dominant picoeukaryote group~~ sufficient to double their abundances~~ whilst reduced losses allowed the other picoeukaryotes to flourish at higher fCO_2. Converting abundances to particulate organic carbon we saw a large shift in the partitioning of carbon between the size fractions which lasted throughout the experiment. The prokaryotes largely~~. Dynamics of the prokaryote community closely followed ~~the~~trends in total algal biomass ~~with responses to increasing fCO_2 reflecting the altered phytoplankton community dynamics.~~despite differential effects of fCO_2 on algal groups. Similarly, ~~higher~~ viral abundances ~~at higher fCO_2 seemed related to increased prokaryote biomass.~~corresponded to prokaryotic host population dynamics. Viral lysis and grazing were both important in controlling ~~prokaryotic~~microbial abundances. Overall our results point to a shift towards a more regenerative system with ~~potentially increased~~

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~~productivity but reduced carbon export.~~production dominated by small picoeukaryotic phytoplankton.

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1 Introduction

~~Ocean acidification (OA) caused by anthropogenic carbon dioxide (CO_2) release and its subsequent dissolution in the oceans is considered one of the great threats that marine ecosystems face (Turley and Boot, 2010). Direct and indirect effects are predicted to have a large impact on these ecosystems (IPCC, 2007). Phytoplankton production has been found susceptible to OA, depending on the phytoplankton community composition (eg. Hein and Sand-Jensen, 1997; Tortell et al., 2002; Leonardos and Geider, 2005; Engel et al., 2007; Feng et al., 2009). Calcification of coccolithophores, which influence sedimentation via calcium carbonate ballasting, is generally reduced (Meyer and Riebesell, 2015). Diatoms, important for organic matter burial, have been found to benefit in some cases (Feng et al., 2009) but not in others (Tortell et al., 2002). Certain cyanobacteria, including diazotrophs, have been seen to benefit from elevated CO_2 concentrations (Qiu and Gao, 2002; Barcelos e Ramos et al., 2007; Hutchins, 2007). Direct CO_2 effects are also reported for small sized photoautotrophic eukaryotes (Engel et al., 2007; Meakin and Wyman, 2011; Brussaard et al., 2013).~~

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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, primarily 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

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Boot, 2010) and has been shown to alter phytoplankton primary productivity 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). ~~whereby shelf sea communities contribute 15-30% of this (Kulinski and Pempkowiak, 2011). Whilst environmental factors, such as temperature, light, nutrients and CO_2 concentration, regulate gross primary production bottom-up, loss factors (i.e., grazing, viral lysis and sedimentation) determine the fate of the carbon fixed by phytoplankton. Ingested carbon transfers to higher trophic levels, sinking of phytoplankton and faeces may lead to carbon storage in sediments, and viral lysis is a major driver of carbon release to dissolved and detrital organic matter (DOM; Wilhelm and Suttle, 1999; Brussaard et al., 2005; Lønborg et al., 2013). Through viral lysis the cell content of the host is released into the surrounding water and utilized by heterotrophic bacteria, thereby stimulating the microbial loop (Brussaard et al., 2008; Sheik et al., 2014). Bacteria may also be affected either directly by OA, or indirectly via changes in the quality~~ Moreover, OA was found to have a greater impact on

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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 and CO_2 concentration 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 in 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 ~~at least~~ as important ~~a loss factor~~ as microzooplankton grazing ~~for~~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

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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. ~~The effect of ocean acidification on the relative share of these key loss processes is, however, still understudied for most ecosystems, particularly for brackish coastal systems. Low salinity affects the pH buffering capacity due to low total alkalinity and is as such of interest for OA studies.~~ Here we report on the temporal dynamics of microbes (phytoplankton, prokaryotes and viruses) under the influence of enhanced CO_2 concentrations ~~and~~ in ~~relation to viral lysis and grazing control.~~the low-salinity (around 5.7) Baltic Sea. Using large mesocosms ~~at~~under in situ light and temperature, the ~~Baltic Sea~~ pelagic ecosystem was exposed to a range of increasing CO_2 concentrations from ambient to future and far~~-~~future concentrations. ~~This~~The study was performed during the summer in the ~~Gulf of Finland~~Baltic Sea near Tvärminne~~, with salinity around 5.7 and low dissolved inorganic nitrogen and phosphorus concentrations. During~~ when conditions were oligotrophic. Our data show, that over the 43 day long experiment ~~the smallest~~, enhanced CO_2 concentrations elicited distinct shifts in the microbial community, most notably an increase in the net growth of small picoeukaryotic phytoplankton ~~especially showed distinct responses to the treatment conditions~~.

2 Materials and Methods

2.1 Study site and experimental set-up

~~The 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 with a depth of 17 m were moored in a square arrangement within the archipelago. For details on 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, this issue). After deployment the mesocosms were kept open for 5 days with 3 mm mesh screening over the top and bottom openings before being closed at the bottom and pulled above the sea surface at the top. Photosynthetically active radiation (PAR) transparent plastic hoods (open on the side) prevented rain and bird droppings from entering~~

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Comment [k1]: Add brief summary of experimental set-up

~~the mesocosms. Six mesocosms were sampled for the current study, unfortunately three were lost due to leakage. Initial fugacity of CO_2 (fCO_2) was 240 µatm. The mean fCO_2 during the experiment, i.e. days 1-43, for the individual mesocosms was as follows: M1, 365 µatm; M3, 1007 µatm; M5, 368 µatm; M6, 821 µatm; M7, 497 µatm; M8, 1231 µatm . Throughout this study we refer to fCO_2 which takes into account the non-ideal behavior of CO_2 gas and is the standard measurement required for gas exchange calculations (Pfeil et al., 2013).~~

~~For fCO_2 manipulations,~~ 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 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 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 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 ~~whole~~ depth of the mesocosms as described by Riebesell et al. (2013). Two mesocosms functioned as controls and were treated in ~~four steps between days 0 to 3 until target fCO_2 was reached~~a similar manner using only filtered seawater. On day 15, a ~~further~~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~~. The remaining mesocosms received similar treatment without CO_2.~~ (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~~, i.e. nitrate, phosphate, silicate and ammonium,~~ 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~~, and stayed~~. 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 ~~was initially ≈8°C and rose to ≈15°C~~within the mesocosms (0-17 m) increased from ~8 °C to a maximum on day 15 ~~before falling to ≈8°C~~of ~15 °C and then decreased again~~.~~ to ~8 °C by day 30. For further details of the experimental

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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 ~~daily in the~~every morning~~,~~ using ~~an~~depth integrated water ~~sampler, from the top (0-10 m) and from the whole water column (0-17 m) of~~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 from all mesocosms and the surrounding water.~~—~~ Subsamples were obtained for enumeration of phytoplankton, prokaryotes and viruses. Samples for viral lysis and grazing experiments were taken from 5 m depth using a gentle vacuum-driven pump system. Samples were protected against ~~daylight~~sunlight and warming by thick black plastic bags containing wet ice. ~~In the laboratory the samples~~Samples were processed at in situ temperature ~~and dimmed~~(representative of 5 m depth) under dim light and handled using nitrile gloves. As viral lysis and grazing rates were determined from samples taken from 5 m depth, samples for microbial abundances reported here were taken from the top 10 m integrated samples. ~~For abundances from 0-17 m and the surrounding water see Supplementary data (Table S1 and Fig.S1).~~

The ~~experiment~~experimental period has been divided into ~~4~~four phases based on major physical and biological changes ~~occurring~~ (Paul et al., 2015~~).~~): Phase 0 before CO_2 addition (days -5 to 0), ~~phase~~Phase I (days 1-16), ~~phase~~Phase II (days 17-~~22~~30) and ~~phase~~Phase III (days ~~23~~31-43). Throughout this ~~study~~manuscript the data are presented using ~~3~~three colors (blue, grey and red), representing low (mesocosms M1 and M5) intermediate (M6 and M7) and high (M3 and M8) fCO_2 concentrations (Table 1).

2.2 Microbial abundances

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Microbes were enumerated using a Becton Dickinson FACSCalibur flow cytometer (FCM) equipped with a 488 nm argon laser. ~~The photoautotrophic cells (<20 µm) were counted directly fresh and were discriminated by their autofluorescent pigments (Marie et al., 1999). The samples were held on wet ice in the dark until counting. Based on their chlorophyll red autofluorescence and the presence of phycoerythrin orange autofluorescence in combination with side scatter signal, the phytoplankton community could be divided into 6 clusters. Phytoplankton cell~~The samples were stored on wet ice and in the dark until counting. The photoautotrophic cells (<20 µm) were counted directly using fresh seawater and were discriminated by their autofluorescent pigments. Six phytoplankton clusters were differentiated based on the bivariant plots of either chlorophyll (red autofluorescence) or phycoerythrin (orange autofluorescence, for *Synechococcus* and Pico-III) against side scatter. The size of the different phytoplankton clusters was determined by gentle filtration through 25 mm diameter polycarbonate filters (Whatman) with a range of pore sizes (12, 10, 8, 5, 3, 2, 1, and 0.8 µm) according to Veldhuis and Kraay (2004). Average cell sizes ~~of~~for the different phytoplankton groups were 1, 1, 3, 2.9, 5.2, and 8.8– µm diameter for the prokaryotic cyanobacteria *Synechococcus* spp. (SYN), picoeukaryotic phytoplankton I, II and III (Pico-I-III), and nanoeukaryotic phytoplankton I, and II (Nano-I, II), respectively. Pico-III was discriminated from Pico-II (comparable average cell size) by a higher orange autofluorescence~~. Cyanobacterial~~ signature, potentially representing small-sized cryptophytes (Klaveness, 1989); alternatively large single cells or microcolonies of *Synechococcus* (Haverkamp et al., 2009). The cyanobacterial species *Prochlorococcus* spp. were not observed during this experiment. ~~Assuming the cells~~ Counts were converted to ~~be~~cellular carbon by assuming a spherical shape equivalent to the average cell diameters determined from size fractionations and applying conversion factors of 237 fg C μm^{-3} (Worden et al., 2004) and 196.5 fg C μm^{-3} (Garrison et al., 2000) for pico- and nano-sized plankton, respectively~~, cellular carbon was calculated based on the average cell diameters. Net~~. Microbial net growth and loss rates ~~of phytoplankton and heterotrophic prokaryotes~~ were derived from exponential ~~regression analysis~~regressions of changes in the cell abundances over time.

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Abundances of prokaryotes and viruses were determined from 0.5 % glutaraldehyde fixed, flash frozen (-80 °C) samples according to Marie et al. (1999) and Brussaard (2004), respectively. ~~The prokaryotes include bacteria, archaea and unicellular cyanobacteria, the latter accounting for maximal 10% of the total abundance. In the surface waters of the Baltic Sea most prokaryotes are heterotrophs (Riemann et al., 2008).~~

~~Briefly, thawed samples were diluted with sterile autoclaved Tris EDTA buffer (10mM Tris HCl and 1mM EDTA, pH 8.2)~~The prokaryotes include heterotrophic bacteria, archaea and unicellular cyanobacteria, the latter accounting for maximal 10 % of the total abundance in our samples, as indicated by their autofluorescence. Briefly, thawed samples were diluted with sterile autoclaved Tris-EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.2; Mojica et al., 2014) and stained with the green fluorescent nucleic acid-specific dye SYBR-Green I (Invitrogen Inc.) ~~to a final concentration of the commercial stock of 1×10^{-4} (for prokaryotes) or 0.5×10^{-4} (for viruses). Virus samples were stained at 80°C for 10 min and then allowed to cool for 5 min at room temperature in the dark. Prokaryotes were stained for 15 min at room temperature in the dark (Brussaard, 2004 with adaptation according to Mojica et al., 2014).~~to a final concentration of the commercial stock of 1.0 × 10^{-4} (for prokaryotes) or 0.5 × 10^{-4} (for viruses). Virus samples were stained at 80 °C for 10 min and then allowed to cool for 5 min at room temperature in the dark. Prokaryotes were stained for 15 min at room temperature in the dark (Brussaard, 2004). Prokaryotes and viruses were discriminated in bivariate scatter plots of green fluorescence versus side scatter. Final counts were corrected for blanks prepared and ~~analysed like the samples. Two groups of prokaryotes were identified as low (LDNA) and high DNA (HDNA) fluorescence prokaryotes by their stained nucleic acid fluorescence. Four viral groups (V1–4) were distinguished, whereby V1 V3 showed increasing green nucleic acid fluorescence (with similar side scatter signatures) and cluster V4 had similar green fluorescence to V3 but had higher side scatter similar to a virus infecting nano-eukaryotic algae (Baudoux and Brussaard,~~

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~~2005).~~analyzed in a similar manner as the samples. Two groups of prokaryotes were identified by their stained nucleic acid fluorescence, referred here on as low (LNA) and high (HNA) fluorescence prokaryotes.

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2.3 Viral lysis and grazing

Microzooplankton grazing and viral lysis of phytoplankton was determined using the modified dilution ~~method (Mojica et al., 2016). All seawater handling was performed at *in situ* temperature under dim light conditions using nitrile gloves. Briefly, one of two series of dilutions of 20, 40, 70 and 100% whole seawater (200 µm mesh sieved), was gently mixed with 0.45 µm filtered seawater (i.e. microzooplankton grazers removed) and the second series with 30 KDa filtered seawater (i.e. grazers and viruses removed). The dilution reduced the~~assay, based on reducing grazing and viral lysis mortality pressure in a serial manner ~~and regression analysis allowed loss rates (slope) and gross~~ allowing for increased phytoplankton growth ~~rates, in the absence of grazing and lysis (intercept y axis 30 kDa series), to be determined.~~ (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). ~~Incubations~~All treatments were ~~set up~~performed in triplicate ~~in clear 1.2 L polycarbonate bottles. They~~. Bottles were suspended ~~close~~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 ~~fresh~~ by ~~FCM~~flow cytometry. Due to time constraint, the majority of the samples ~~from~~of the 30 kDa series were fixed ~~to a~~with 1~~%~~ % (final concentration ~~with~~) formaldehyde~~:~~ : hexamine solution (18 % v/v~~:~~ : 10 % w/v), ~~stored~~ for 30

min at 4 °C, flash frozen in liquid nitrogen and stored at –80 °C until flow cytometry analysis. The effects of fixation were in the home laboratory. Fixation had no significant effect (student's t-tests, p-value >0.05) as tested periodically by running duplicate series ofagainst fresh and frozen samples. No differences in analysis between fresh and frozen samples were observed. Incubation experiments were The modified dilution assay was only run with samples from mesocosmfor Mesocosms 1 (low fCO_2) and 3 (high fCO_2);) due to the logistics of handling times it was not possible to do more. . 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 yOccasionally the dilution assays displayed a positive slope rather than a negative slope for 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 not resulting in ano reduction in mortality with dilution). FurthermoreIn addition, very low phytoplankton abundances complicate proper analysis (and consequentlycan also prohibit statistical significance of results) due to the fact that the assay is based on a. Under such conditions dilution series. Such assaysexperiments were deemed failed. Further discussion of potential causes of positive regressions can be found inunsuccessful (see for limitations of the modified dilution method, Baudoux et al., 2006; Kimmance and Brussaard (, 2010) and; Stoecker et al. (., 2015).

Viral lysis of prokaryotes was determined by the method ofaccording to the viral production assay (Wilhelm et al., 2002; Winget et al. (., 2005) adapted from the original method by. After reduction of the natural virus concentration, new virus production by the natural bacterial community is sampled

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and tracked over time (24 h). ~~Wilhelm et al. (2002). Here free viruses are removed from a sample of prokaryotes, samples are then taken every 3 hours for 24 hours for virus enumeration. Any viruses in the samples must come from lysing bacteria and thus the rate of bacterial lysis can be estimated using an appropriate burst size. Briefly, free viruses were removed~~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}. ~~A total of 900 ml of~~ The concentrated sample was then reconstituted to the original volume using virus-free seawater~~, freshly produced by 30 kDa ultrafiltration using a PES membrane (Vivaflow 200, Vivascience) was added in~~ . This process was repeated a total of three ~~steps~~times to gradually wash away ~~free~~ viruses. ~~Finally the sample was diluted back to the original 300 ml volume with virus-free seawater. The samples~~After the final reconstitution, 50 ml aliquots were ~~aliquoted~~distributed into six ~~50 ml~~ polycarbonate tubes. ~~Mytomycin~~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 ~~three~~a second series of ~~the six tubes~~triplicate samples for each mesocosm ~~studied~~. 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 ~~in the dark~~ and 1 ml subsamples were ~~then~~ taken ~~after 3h, 6h, 9h, 12h~~at 3 h, 6 h, 9 h, 12 h and ~~24h. Viruses were later enumerated by the method of Brussaard (2004) to determine their rate of production over time.~~ 24 h. Virus production was determined from linear regression of viral abundance over time ~~(time period used for regression analysis may vary~~. Viral production due to induction of lysogeny was calculated as the difference between ~~sampling days, depending on the temporal virus abundance dynamics).~~production in the unamended samples and production of samples to which mitomycin C was added. Although mortality experiments were ~~performed with~~ initially planned to be employed for mesocosms 1, 2, and 3 ~~as~~representing low, mid and high fCO_2

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conditions, mesocosm 2 was ~~lost~~compromised due to leakage. ~~Due~~Additionally, due to logistical reasons ~~we~~assays were only ~~able to perform these assays~~performed until day 21.

To determine grazing rates on prokaryotes, fluorescently labeled bacteria (~~FLB~~FLBs) were prepared from ~~cultured *Halomonas halodurans*~~enriched natural bacterial assemblages (originating from the North Sea) labeled with ~~594~~5-([4,6-~~Dichlorotriazinyl Aminofluorescein~~Dichlorotriazin-2-yl]amino) fluorescein (DTAF, 36565 Sigma-Aldrich 40 µg ml^{-1}) according to Sherr ~~and Sherr~~et al. (1993). Frozen ampoules ~~containing prey~~of FLB (1-5 % of total ~~bacteria~~bacterial abundance) were added to triplicate 1 L incubation bottles containing whole water gently passed through 200 µm mesh. Twenty ~~milliliter~~ml samples were taken immediately after addition (0 h) and the headspace was removed by gently squeezing air from the bottle ~~so that no air bubble remained. The~~. The 1 L bottles were incubated on a slow turning wheel (1 rpm) at in situ light and temperature conditions (representative of 5 m depth) for 24 h. Sampling was repeated after 24 h. All samples were fixed ~~with~~to a 1 % final concentration ~~0.2 µm filtered~~ of gluteraldehyde (0.2 µm filtered; 25 % EM-grade~~, 25%) and~~), stained ~~with~~ (in the dark for 30 min at 4 °C) with 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) solution (0.2 µm filtered~~(~~; Acrodisc ®~~25mm~~25 mm Syringe filters, PALL Life Sciences~~) DAPI at a~~; 2 µg ml^{-1} final concentration ~~of 2 µg ml^{-1}~~; (Sherr et al., 1993)~~. Samples were incubated for 30 min at 4°C and stored in the dark. The 1 L bottles were incubated on a slow turning wheel (1 rpm) at *in situ* light and temperature conditions for 24 h. 24 h samples were then taken in the same manner as for 0 h. Samples were~~ and filtered onto 25 mm, 0.2 µm black polycarbonate filters (GE Healthcare life sciences~~),~~). Filters were then mounted on microscopic slides and stored at -20 °C until analysis. FLBs present on a ~~≈~~~0.~~75mm^2~~75 mm^2 area were counted using a Zeiss Axioplan 2 microscope. ~~-~~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 present at 24 h and 0 h, respectively.

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385 **2.4 Statistics**

386 Non-metric multidimensional scaling (NMDS) was used to follow microbial community development
387 in each mesocosm over the experimental period. NMDS is an ordination technique which represents
388 the dissimilarities obtained from an abundance data matrix in a *2*-dimensional space (Legendre and
389 Legendre, 1998). In this case, the data matrix was comprised of abundance data for each
390 phytoplankton group in each mesocosm for every day of sampling. The treatment effect was
391 assessed by analysis of similarity (ANOSIM; Clarke, 1993) and inspection of the NMDS biplot. ANOSIM
392 compares the mean of ranked dissimilarities of mesocosms between fCO_2 treatments (low: 1, 5, 7;
393 high: 6, 3, 8) to the mean of ranked dissimilarities within treatments per phase. The NMDS plots
394 allowed divergence periods in the development and community composition between treatments to
395 be visually assessed (period 1 from day 3-13 and period 2 from days 16-24). Net growth rates of each
396 of the different microbial groups were calculated for these identified divergence periods.
397 Relationships between net growth rates and peak cell abundances with fCO_2 were evaluated by
398 linear regression against the average fCO_2 per mesocosm during each period or peak day. A
399 generalized linear model was used to test the relationship between prokaryote abundance and
400 carbon biomass with an ARMA correlation structure of order 3 to account for temporal
401 autocorrelation. The model fulfilled all assumptions such as homoscedasticity and avoiding
402 autocorrelation of the residuals (Zuur et al., 2007). A significance threshold of $p \leq 0.05$ was used and
403 significance is denoted by an asterisk (*). All analyses were performed using the statistical software
404 program R, using packages nlme (Pinheiro et al., 2017) and vegan (Oksanen et al., 2017) (R core
405 Team, 2017). Where average of low and high mesocosm abundance data are reported, values
406 represent the average of mesocosms 1, 5, 7 (mean fCO_2 365-497 µatm) and 6, 3, 8 (821-1231 µatm),
407 respectively.

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409 **3 Results**

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3.1 Total phytoplankton dynamics in response to CO_2 enrichment

During Phase 0, low variability in phytoplankton abundances of 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 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 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). ~~Microzooplankton grazing rates were estimated from the regression coefficient of the apparent growth rate versus fraction of natural seawater for the 0.45 µm series, with the combined rate of viral-induced lysis and microzooplankton grazing being estimated from a similar regression for the 30 kDa series (Baudoux et al., 2006; Kimmance and Brussaard, 2010).~~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).

~~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 were determined by analysis of covariance of regression lines of the dilution series for the two mesocosms. Students T-tests were used to determine significant differences between mesocosms for other parameters.~~

~~**3 Results**~~

~~**3.1 Phytoplankton population dynamics**~~

~~Phytoplankton (total) showed two main peaks in abundance day 4, (phase I) and day 24 (phase II; Fig. 1a). Generally abundances were similar in all mesocosms except during the second half of phase I (days 11-15) when they were greater at higher CO_2 and following this (days 17-22)greater at lower CO_2 concentrations. These trends were largely due to the prokaryotic cyanobacteria *Synechococcus* spp., making up on average 74% of total abundance. In contrast, the total eukaryotic phytoplankton showed a strong positive effect of fCO_2 (Fig. 1b), due to the response of Pico I and II. Abundances in the surrounding waters were more similar to the low fCO_2 than the high fCO_2 mesocosms, demonstrating that the differences between the low and high fCO_2 mesocosms are the effect of the elevated fCO_2. Phytoplankton, prokaryotes and viral abundances in the 0-17m samples were generally lower but showed similar dynamics (Figs. S1 and S2).~~

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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 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.

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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 x 10^4 ml^{-1} and 5.9 x 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 x 10^4 vs 4.3 x 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 the surrounding water were comparable to the mesocosms during Phases 0 and I, lower during Phase II and higher during Phase III (Fig. 3c).

Pico-III exhibited a short initial increase in abundances in the low fCO_2 treatments, resulting in nearly 2-fold higher abundances at low fCO_2 by day 3 compared to the high fCO_2 treatment (Fig. 3d). After this initial period, net growth rates of this group had a significant positive correlation with fCO_2 (days 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, 10; Fig. 4d) and total mortality ($p<0.05$, days 1, 6, 10; Fig. 4d) were significantly higher in the low fCO_2 mesocosm M1, as compared to the high fCO_2 mesocosm M3 resulting in low net growth rates. During Phase II (days 16-24, NMDS-based period 2) the opposite occurred; i.e. net growth rates were negatively correlated with fCO_2 ($p<0.01$, $R^2=0.86$; Table 2, Fig S.3d). Maximum Pico-III abundances (day 24: 4.2×10^3 and 8.3×10^3 ml^{-1} for high and low fCO_2) had a strong negative correlation with fCO_2 ($p<0.01$, $R^2=0.91$; Table 3, Fig. S4f). Pico-III abundances remained noticeably higher in the low fCO_2 mesocosms during Phases II and III (on average 80 %; Fig. 3d). Unfortunately, almost half of the mortality assays in this second half of the experiment failed (see Materials and Methods), but the

successful assays suggest that losses were minor (<0.15 d^{-1}; Fig. 4d) and primarily due to grazing, as no significant viral lysis was detected (Table S1).

3.1.3 Nanoeukaryotes

Nano-I showed maximum abundances (4.3 ± 0.4 x 10^2 ml^{-1}) on day 6 (except M1 which peaked on day 5), independent of fCO_2 (p=0.23, R^2=0.33; Fig. 3e). There was, however, a negative correlation of net 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 second major peak in abundance of Nano-I occurred on day 17, with markedly higher numbers in the 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, 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 10 were 2.3-fold higher compared to the low fCO_2 mescososm M1 (Fig. 4e), which may help to 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 average, 29 % lower throughout the study period (days 2-39). For Nano-I and II the lower carbon biomass only became apparent during the end of Phase I and beginning of Phase II (days 14-20; Fig. S7b). Due to its small cell size, the numerically dominant SYN accounted for an average of 40 % of total carbon biomass.

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~~**3.2 Prokaryote** *Synechococcus* (SYN) showed an initial peak in abundance on day 4 (Fig. 2a), then abundances declined, in all mesocosms until day 7. Between day 7 and 16 high CO_2 mesocosm abundances stabilized but in the lower CO_2 mesocosms continued to drop until t12 before increasing again. This difference may be explained by higher grazing rates (no viral lysis detected), at lower CO_2 as measured inM1 compared to M3 on day 10 (0.56 vs 0.27 d^{-1})(Fig. 2b). Despite deviations in temporal dynamics between the treatments, SYN abundance peaked at day 24 in all mesocosms with around 4.5 x 10^5 cells ml^{-1} (Fig 2a) and was negatively correlated with fCO_2 (R^2=0.77). Total net production during this bloom was greater in the low fCO_2 mesocosms than in the high ones as initial abundances were lower (day 13) and peak abundances higher (day 24; Fig. 2a). The higher losses at~~

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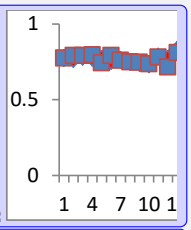
~~higher CO_2 measured on day 17 (M3:0.33 vs M1:0.17) correspond with this. The following decline (days 24-28) seemed largely due to reduced gross growth rates (Fig. 2b). Interestingly in the whole water column (0-17m) only a short decline was seen which may be due to differences in the response of deeper water *Synechococcus* sp. There may be two distinct *Synechococcus* sp. populations, one dominating the top 10m accounting for 70-80% of total until the peak at t23 then around 85% until t26. A smaller population deeper in the water column then shows different dynamics following the bloom crash from t26 onwards. The top population continues to crash but the deeper population stops. From t29 a difference between CO_2 treatments in the % in the top 10m is seen due to first the top population increasing again faster at lower CO_2 and then due to the lower population increasing at high CO_2.~~ **and virus population dynamics**

Prokaryote abundance in the mesocosms was positively related to total algal biomass independent of treatment ($p<0.05$, $R^2=0.33$; Fig. 8) and generally followed total algal biomass (Fig. S7c). The initial increase in total prokaryote abundances occurred during the first few days following the closure of the mesocosms (Fig.

~~**3.1.2 Picoeukaryotes 1**~~

~~Pico I was numerically the second most dominant group of phytoplankton, making up 26% of total phytoplankton abundances on average at highCO_2 and 21% at low CO_2. This amounts to 15% of total POC at high fCO_2 and 10% at low fCO_2 (mean of total POC). The initial peak in abundance at day 5, (Fig. 3a) showed a slight positive trend and strong correlation with fCO_2 for the net growth rate (Fig. 3d, $R^2=0.95$) and abundance (Fig. 3g, $R^2=0.8$). The following crash (days 5-9) may have been The positive correlation of Pico I peak abundance with fCO_2 on day 13 (Fig. 3h, $R^2=0.94$) was lost upon another decline in abundance. Significantly higher losses at high fCO_2, a combination of grazing and lysis, resulted in a more dramatic crash at high fCO_2 and abundances becoming similar again around day 17 (Fig. 3a). Viral lysis was a significant loss factor compared to grazing, i.e. overall on average 45% and 70% of total losses in M1 and M3, respectively (Table S2). An extra addition of CO_2 was given to M3, M6 and M8 because their fCO_2 had approached that of the remaining mesocosms. This~~



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may have stimulated the gross growth in M3 for a longer period in the high fCO_2 mesocosms as compared to M1 (day 19; Fig. 3b). Combined with higher losses at low fCO_2 a positive correlation of net growth rates with fCO_2 was seen (Fig. 3f, R^2=0.71), and almost 2-fold higher abundances at high fCO_2 on day 21 (Fig. 3a, i, R^2=0.84). Pico I was thus greatly stimulated by increased fCO_2, from day 3 throughout the experiment. Standing stock of Pico I remained higher at high fCO_2 for the further duration of the experiment (Fig. 3a), with gross growth matched by total losses (Fig.3b). Surprisingly the higher abundances did not stimulate higher losses during this period, grazing rates were very low in both M1 and M3, and viral lysis was totally responsible for losses on day 31 in both mesocosms (Table S2).

3.1.3 Picoeukaryotes II

A group of larger picoeukaryotes, Pico II (mean diameter of 3 µm) bloomed exactly during the period Pico I was low in standing stock (days 13-21, Fig. 4a) and the peak abundance (day 17) correlated positively with fCO_2 (Fig. 4d). Relatively high total losses of 0.46 and 0.58 d^{-1} in the low and high fCO_2 mesocosms, respectively (average days 6-13) accompanied the high gross growth rates (0.69 and 0.72 d^{-1}) for the same period (Fig. 4b). These indicate high turnover and explain the slow rate of increase in cell abundance until day 13 (Fig. 4a). During the bloom period of Pico II, losses were smaller than the gross growth rate, more so it seems for M3 than M1 (Fig. 4b). Resultant net growth rates correlated with fCO_2 (Fig. 4d, R^2=0.82) with peak abundances 1.4 fold higher at high fCO_2 (Fig. 4a). Higher losses then contributed to the faster decline in abundances at high fCO_2. Phase III was a period of low turnover for Pico II with low gross growth and loss rates resulting in quite stable cell abundances, still higher at high fCO_2, until day 29 after which they declined in all mesocosms (Fig. 4a).

3.1.4 Picoeukaryotes III

Another group with around 2.9 µm cell diameter could be discriminated from Pico II by its higher orange autofluorescence, and as such may represent small-sized cryptophytes. This is just at the lower size range of small cryptophyte (Klaveness, 1989). This group (Pico III) had its highest abundances during phases II and III (days 17-43, Fig. 5a), with a distinct negative correlation to fCO_2 (Fig. 5e, R^2=0.91). Already directly upon the first CO_2 addition (days 0-4) the abundances declined for the high fCO_2 mesocosms (Fig. 5a) with net growth rates negatively correlated to fCO_2 (Fig. 5d, R^2=0.94). Gross growth rates were indeed significantly higher for M1 than M3 at days 1, 4 and 10 (Fig. 5b). Abundances of the Pico III group in the surrounding water followed the low fCO_2 mesocosms perfectly during this first period, indicating that the crash in the high fCO_2 mesocosms was indeed a direct (negative) effect of fCO_2 (Table S1). A similar response of Pico III abundance halting in the high fCO_2 mesocosms and strongly increasing in the low fCO_2 mesocosms occurred directly after the additional fCO_2 purge (day 15). Losses were largely due to microzooplankton grazing. Unfortunately about half of the loss assays in the second half of the experiment failed (for unknown reasons), yet the successful assays suggest that losses were minor (Fig. 5b). There may also be larger cryptophytes present in the community, not counted by the flow cytometer because our data show Pico III most dominant in phase III whilst the specific pigment data shows a decline from phases 0 to III.

3.1.5 Nanoeukaryotes I

The nanoeukaryotes group Nano I consisted of cells with a mean diameter of 5.2 µm and were found with maximum abundances of 5.5 x10^3 ml^{-1} (Fig. 6a). After an initial peak at day 6, the lower fCO_2 mesocosms showed the highest numbers at day 17 (Fig. 6a). This seems initiated by 2.3-fold higher total loss rates for M3 than M1 on days 6 and 10 (Fig. 6b) in combination with 2-fold lower gross growth rates on day 10 (Fig. 6b). Ultimately, this led to net growth rates correlating negatively with

~~fCO_2 for days 10-12 (Fig. 6d, R^2=0.83). Viral lysis occurred predominantly in the high fCO_2 mesocosm throughout the experiment with rates ranging from 0.13 to 0.7 day^{-1} (making up 16 to 98% of total losses; Table S2). A group of viruses which had a flow cytometric signal typical for viruses infecting nanoeukaryotes (V4) were identified but no obvious correlation~~ This was ~~found with any of the phytoplankton groups. Lower total loss rates at days 13 and 17 in both mesocosms allowed a small increase in abundance, peaking on day 17 and negatively correlated to fCO_2 (Fig. 6e, R^2=0.67).~~

~~3.1.6 Nanoeukaryotes II~~

~~The temporal dynamics of Nano II were rather erratic (Fig.~~ primarily ~~7a). Nano II were the largest in size and may have been made up by different phytoplankton species, however~~ due to ~~their low numbers we were unable to discriminate separate groups. The peak in abundance at day 16 showed a negative correlation to fCO_2 (Fig. 7e, R^2=0.61), and was the result of an overall reduced net growth rate with fCO_2 (Fig. 7d, R^2=0.56). The subsequent decline seems the result of reduced gross growth rate (to even zero) and increased loss rate (day 20; Fig.~~ increases in the HNA ~~7b).~~

~~3.1.7 Algal POC~~

~~The calculated mean algal POC shows that fCO_2 had a clear positive effect on the biomass of Pico I and II (Fig. 8a; p<0.0001). The effect became noticeable only a few days into the experiment and the mean Pico I and II POC concentrations in the high fCO_2 mesocosms stayed high for the entire duration of the experiment. At the same time the remaining algal groups showed reduced POC at enhanced fCO_2 (the sum of Pico III, and Nano I and II and *Synechococcus* spp.; Fig. 8b, p<0.01). Particularly Pico III showed a nearly instant and markedly negative response to increased fCO_2 concentration (Fig. S3a). This was a lasting effect as the strongest difference was found in the second half of the experiment. For Nano I and II the higher algal POC concentrations became only apparent~~

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~~from the end of phase I and during phase II (days 14-20; Fig. S3b). Due to its small cell size, the numerically dominant SYN accounted on average for 40% of total POC. Due to the exclusion of 3 mesocosms (see Material and Methods), the number of fCO_2 treatments is reduced to 6, which limits the statistical power of the results. Still, our data show that the responses of the different phytoplankton groups to ocean acidification were evident and consistent.~~

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~~**3.2 Prokaryote population dynamics**~~

~~The prokaryotic temporal dynamics in the mesocosms resembled that in the outside waters (Fig. S2). In general~~ prokaryote ~~abundance in the mesocosms followed the total algal biomass, with an initial increase during the first days following the closure of the mesocosms (Fig. 9a). The increase was mainly due to the HDNA~~group (Fig. 6b) which displayed higher net growth rates (0.22 d^{-1}) compared to the LNA-prokaryotes (~~Fig. 9b). The total prokaryote abundance increased initially at a net growth rate of 0.19 d^{-1}, and more specifically at 0.22 and 0.14~~ 0.14 d^{-1} for days -3 to 3; Fig. 6c). A similar, albeit somewhat lower, increase was also recorded in the surrounding waters (Fig. 6a). ~~the high and low DNA prokaryotes respectively (Fig. 9b and c). There was no significant difference in prokaryote abundance between the treatments at the first peak (day 4). However, grazing was significantly lower (0.3 d^{-1}) in high (M3) than in low (M1; 0.5 d^{-1}) CO_2 treatments, on both days 0 and 4, and viral lysis 3% higher at high CO_2 (Figs. 10b and c). The decline in prokaryote~~ The decline of the first peak in prokaryote abundances ~~from days 5 to 9 seemed due to declining~~coincided with the decay in phytoplankton abundance/biomass (~~Fig~~Figs. 1a~~)~~ and ~~increasing~~S7c). Concurrently the share of viral lysis ~~rates (12-16 % d^{-1}~~increased, representing 37-39 % of total ~~losses in M1 and 37% in M3~~mortality on day 11 (Fig. 7b). ~~, Fig. 10c). Viral lysis assays showed no evidence~~ No measurable rates of lysogeny were found for the prokaryotic community during the ~~experiment~~experimental period (all phases).

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From days 10~~-~~ to 15 prokaryote dynamics (total, HNA and LNA) became ~~clearly~~noticeably affected by ~~fCO_2~~ CO_2 concentration with ~~significantly higher abundances and net growth rates at higher fCO_2 (Fig. 9a). Both the HDNA and the LDNA-prokaryotes (peak abundance on day 13, Fig. 9b and c) showed~~a significant positive correlation ~~with fCO_2 (R^2= 0.92 and 0.79, respectively, total prokaryote R^2= 0.88, Fig. 10d).~~between net growth and fCO_2 during Phase I (days 3-13 NMDS-based period 1; Table 2, Fig. S2 g and h). In the higher fCO_2 mesocosms, the decline in prokaryote abundance ~~following the peak at day~~occurring between days 13 and 16 (Fig. 6a) was largely ~~the result of~~ (70 %) due to decreasing ~~HDNA~~HNA-prokaryote numbers (Fig. 6b). ~~9b). Grazing~~The grazing was ~~indeed significantly~~1.6-fold higher in the high fCO_2 mesocosm M3 ~~but the data for viral lysis were inconclusive due~~compared to ~~a failed assay (for technical reasons) for~~ M1 ~~at~~(0.36 ± 0.13 and 0.14 ± 0.08 d^{-1} on day 14 ~~(~~; Fig. 7a). ~~10b and c). The significantly higher viral abundances, particularly due to~~At the ~~V3 group with highest green fluorescence, for the high fCO_2 mesocosms around that~~same time ~~(Figs. 11a and b) seem to indicate that viral lysis~~, virus abundance increased in the high fCO_2 mesocosms ~~was higher.~~ (Fig. 6d).

During ~~phase~~Phase II, prokaryote abundances increased steadily until day 24 (for both ~~HDNA~~HNA and ~~LDNA~~LNA), corresponding to increased algal biomass (~~Fig. 10e)~~Figs. 6 and ~~low~~S7c) and lowered grazing rates (~~0.1-0.2 d^{-1}; Fig. 10b). Although the overall higher prokaryote standing stock in the low fCO_2 mesocosms was due to enhanced growth around day 16 (Fig. 9a), the net growth rates were comparable after day 17. Moreover, the higher abundances were only found for the HDNA-prokaryotes (Fig. 9b and c). Viral lysis rates were higher for the low fCO_2 mesocosms (Fig. 10c). The higher prokaryote abundances in the low fCO_2 mesocosms appear thus due to the lower grazing prior to the increase, i.e. at the end of phase I (day 14).~~Fig. 7a). 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;

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Fig. 7). Halfway through Phase II (day 24), the prokaryote abundance in the surrounding water leveled off (Fig. 6a). Prokaryote abundance ultimately declined ~~again~~ during days 28-35~~, but less in M1 than in the other mesocosms (Fig. 9a). We unfortunately have no data of the prokaryote loss rates~~ (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~~. However, viral abundances increased steadily at ~~a steady rate of~~ 2.~~2x10^6~~2 x 10^6 d^{-1} ~~(to a maximum of 0.9x10^8 ml^{-1} by day 39; Fig. 11a), implying that viral lysis was at least partly responsible for the~~, concomitant with a decline in prokaryote abundance~~.~~ (Fig. 6a and d). There was no significant ~~difference in~~ correlation between viral abundances ~~between the treatments~~and fCO_2 during ~~this period.~~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.

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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. 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 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; 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; 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). 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. Specifically, significant differences in grazing rates of SYN

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between M1 and M3 (days 10 and 17, no significant lysis detected) could be responsible for the differing dynamics between the mesocosms at the end of Phase I and beginning of Phase II.

The gross growth rates of Pico-I were significantly higher ($p<0.05$) at high fCO_2 compared to the low CO_2 concentrations during the first 10 days of Phase I. Moreover, no differences were detected in the measured loss rates, demonstrating that increases in Pico-I were the due to increases in growth alone. The stimulation of Pico-I by elevated fCO_2 may be due to a stronger reliance on diffusive CO_2 entry compared to larger cells. Model simulations reveal that whilst near-cell CO_2/pH conditions are close to those of the bulk water for cells <5 µm in diameter, they diverge as cell diameters increase (Flynn et al., 2012). This is due to the size-dependent thickness of the diffusive boundary layer, which determines the diffusional transport across the boundary layer and to the cell surface (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). It is suggested that larger cells may be more able to cope with fCO_2 variability as their carbon acquisition is more geared towards handling low CO_2 concentrations in their diffusive boundary layer, e.g. by means of active carbon acquisition and bicarbonate utilization (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). Moreover, as the Baltic Sea experiences particularly large seasonal fluctuations in pH and fCO_2 (Jansson et al., 2013) due to the low buffering capacity of the waters, phytoplankton here are expected to have a higher degree of physiological plasticity. Our results agree with previous mesocosm studies, which reported enhanced abundances of picoeukaryotic phytoplankton (Brussaard et al., 2013; Davidson et al, 2016; Schulz et al., 2017), and particularly the prasinophyte *Micromonas pusilla* at higher fCO_2 (Engel et al., 2007; Meakin and Wyman, 2011). Furthermore, Schaum et al. (2012) found that 16 ecotypes of *Ostreococcus tauri* (another prasinophyte similar in size to Pico-I) increased in growth rate by 1.4-1.7 fold at 1,000 compared to 400 µatm fCO_2. All ecotypes increased their photosynthetic rates and those with most plasticity (those most able to vary their photosynthetic rate in response to changes in fCO_2) were more likely to increase in frequency within the community. It is possible that Pico-I cells are adapted to a highly variable carbonate system regime and are able to increase their photosynthetic rate when additional CO_2 is available. This ability would allow them to out-compete

other phytoplankton (e.g. nanoeukaryotes in this study) in an environment when nutrients are scarce.

The net growth rates and peak abundances of Pico-II were also positively affected by fCO_2. Gross growth rates were significantly higher at high fCO_2 on only two occasions (days 10 and 20) and were accompanied by high total mortality rates. Pigment analysis suggests that both Pico-I and Pico-II are chlorophytes (Paul et al., 2015) and as such may share a common evolutionary history (Schulz et al., 2017); thus Pico-II may be stimulated by fCO_2 in a similar manner to Pico I. Chlorophytes are found in high numbers at this site throughout the year (Kuosa, 1991), suggesting the ecological relevance of Pico-I and Pico-II in this ecosystem. In addition, Pico-II bloomed exactly when Pico-I declined which may suggest potential competitive exclusion.

Pico-III showed the most distinct and immediate response to CO_2 addition. The significant reduction in gross growth rates observed during Phase I suggests a direct negative effect of CO_2 on the physiology of these cells. For this group, the lower gross growth rates were matched by lower total mortality rates with increased fCO_2. Although the mean cell size of Pico-III and Pico-II were comparable (2.9 and 2.5 µm, respectively), they showed opposing responses to fCO_2 enrichment (lower Pico-III abundances at high fCO_2). These differences may arise from taxonomic differences between the two groups. Pico-III displayed relatively high phycoerythrin orange autofluorescence, likely representing small-sized cryptophytes (Klaveness, 1989), although rod-shaped *Synechococcus* up to 2.9 µm in length (isolated from this region; Haverkamp et al., 2009) or *Synechococcus* microcolonies (often only two cells in the Baltic; Motwani and Gorokhove, 2013) cannot be excluded. In agreement with Pico-III response to CO_2 enrichment, Hopkins et al. (2010) reported reduced abundances of small cryptophytes under increased CO_2 in a mesocosm study in a Norwegian fjord near Bergen.

Lastly, the two nanoeukaryotic phytoplankton groups also displayed a negative response to fCO_2 enrichment, whereby Nano-II was the least defined, most likely due to a high taxonomic diversity in this group. Nano-I started to display lower abundances at high fCO_2 during Phase I (after day 10),

which was likely the result of greater differences between gross growth and total mortality (compared to low fCO_2). Alternatively, enhanced nutrient competition due to increased abundances of SYN and Pico-I (and later on also Pico-II) at elevated fCO_2 may also have contributed to the dampened response of Nano-I in the high fCO_2 mesocosms. The overall decline in Nano-I, during Phase II, and sustained low abundances during Phase III may well have been the result of grazing by the increased mesozooplankton abundances during Phase II (Lischka et al., 2017).

Microbial loop

The strong association of prokaryote abundance with algal biomass, present throughout the experiment, suggests that the effect of CO_2 was an indirect consequence of alterations in the availability of phytoplankton carbon. Others have reported a tight coupling of autotrophic and heterotrophic communities at this location, with an estimated 35 % of the total net primary production being utilized directly by bacteria or heterotrophic flagellates (Kuosa and Kivi, 1989), suggesting a highly efficient microbial loop in this ecosystem. In addition to phytoplankton exudation, viral lysis may also contribute to the dissolved organic carbon pool (Wilhelm and Suttle, 1999; Brussaard et al., 2005; Lønborg et al., 2013). We calculated that viral lysis of phytoplankton between days 9 and 13 resulted in the release of 1.3 and 13.1 ng C ml^{-1} for M1 and M3, respectively. Assuming a bacterial growth efficiency of 30 % and cellular carbon conversion of 7 fg C $cell^{-1}$ (Hornick et al., 2017), we estimate that the organic carbon required to support bacterial dynamics during this period (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 mesocosms M1 and M3, respectively. These results suggest that viral lysis of phytoplankton was an important source of organic carbon for the bacterial community. Our results are consistent with bacterial-phytoplankton coupling during this eastern Baltic Sea mesocosm study (Hornick et al., 2017), and agree with earlier work on summer carbon flow in the northern Baltic Sea showing that prokaryotic growth was largely supported by recycled carbon (Uitto et al., 1997). The average net growth rates of the prokaryotes during the first period of increase in Phases 0 and I (0.2 d^{-1}) were

comparable to rates reported for this region (Kuosa, 1991). In order to sustain the concomitant daily mortality (between 0.3-0.5 d^{-1}) measured during our study, prokaryotic gross growth rates must have been close to one doubling a day (0.5-0.7 d^{-1}). During Phase I, grazing was the dominant loss factor of the prokaryotic community although there was also evidence that viral lysis was occurring. Bermúdez et al. (2016) reported the highest biomass of protozoans around day 15. This was predominantly the heterotrophic choanoflagellate *Calliacantha natans*, which selectively feeds on particles <1 µm in diameter (Marchant and Scott, 1993; Hornick et al., 2017). Indeed, an earlier study in this area showed that heterotrophic nanoflagellates were the dominant grazers of bacteria, responsible for ingestion of approximately 53 % of bacterial production compared to only 11 % being grazing by ciliates (Uitto et al., 1997). During the first half of Phase II, grazing was reduced and likely contributed to the steady increase in prokaryote abundances. Specifically, a negative relationship between the abundances of HNA-prokaryotes and fCO_2 was detected and corresponded to reduced bacterial production and respiration at higher fCO_2 (Hornick et al., 2017; Spilling et al., 2016). Although CO_2 enrichment may not directly affect bacterial growth, co-occurring global rise in temperature can increase enzyme activities, affecting bacterial production and respiration rates (Piontek et al., 2009; Wohlers et al., 2009; Wohlers-Zöllner et al., 2011). Enhanced bacterial re-mineralization of organic matter may stimulate autotrophic production by the small-sized phytoplankton (Riebesell et al., 2009; Riebesell and Tortell, 2011; Engel et al., 2013), intensifying the selection of small cell size.

Mean viral abundances were higher under CO_2 enrichment towards the end of Phase I and into Phase II which is expected under conditions of increased phytoplankton and prokaryote biomass. The estimated average viral burst size, obtained from this increase in total viral abundance and concurrent decline in bacterial abundances, was about 30 which is comparable to published values (Parada et al, 2006; Wommack and Colwell, 2000). Viral lysis rates of prokaryotes were measured until day 25 and indicated that during days 18-25 an average 10-15 % of the total prokaryote population was lysed per day. Moreover, the concurrent steady increase in viral abundances during

Phase III indicates that viral lysis of the prokaryotes remained important. Thus, the combined impact of increased viral mortality together with reduced production (Hornick et al., 2017) ultimately led to the decline in prokaryote abundance (this study). Lysogeny did not appear to be an important life strategy of viruses during our campaign. Direct effects of higher fCO_2 on viruses are not expected, as marine virus isolates are quite stable (both in terms of particle decay and loss of infectivity) over the range of pH of the present study (Danovaro et al., 2011; Mojica and Brussaard, 2014). ~~At the start of the experiment the trophic conditions were typical for the Baltic Sea in summer, with depleted nutrient conditions, particularly nitrate (Paul et al., 2015), and a vertically stratified water column following the diatom-dominated spring bloom (Kuosa, 1991). The summer phytoplankton community was dominated by pico- and nano-sized phytoplankton, and these phytoplankton groups were of key importance during the experiment. Already at the start of the experiment more than 95% of the phytoplankton community was smaller than 20 µm cell diameter, and by day 5, 70% was smaller than 2 µm (Paul~~The few studies which have inferred viral lysis rates based on changes in viral abundances show reduced abundances of algal viruses (e.g. *Emiliania huxleyi* virus) under enhanced CO_2 (Larsen et al., 2008) while mesocosm results by Brussaard et al. (2013) indicated a stronger impact of viruses on bacterial abundance dynamics with CO_2 enrichment.

~~et al., 2015). The picoeukaryotic photoautotrophs Pico I and II showed a very strong fertilization effect with enhanced fCO_2, directly following the initial CO_2 additions until the end of the experiment. At the same time, the rest of the phytoplankton (Pico III, Nano I and II, and the prokaryote *Synechococcus* spp.) showed reduced abundances at higher fCO_2. These shifts in the size structure of the community could be explained by examining the gross growth rates in combination with the losses of the individual groups.~~

~~Overall, microbial temporal dynamics in the mesocosms were largely comparable to the surrounding water, with a few exceptions: i.e., phytoplankton Nano I and II occasionally showed much higher~~

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abundances whilst all the picoplankton abundances were lower in the surrounding waters. Higher abundances of nano-sized phytoplankton in the surrounding water were likely due to upwelling of cold, CO_2-rich deep water to the surface, bringing in inorganic nutrients, particularly silicate (Paul et al., 2015). Average temperatures in all the mesocosms and surrounding waters were similar, with the upwelling reducing the temperature from around 15 to 8°C during phase II. Along with reduced PAR (Paul et al., 2015) this generally reduced gross growth of the different phytoplankton groups however no synergistic effects with fCO_2 could be ascertained. The microbial population dynamics in the surrounding water more closely resembled those in the ambient fCO_2 mesocosms, and more importantly the differences were in contrast to the shifts in phytoplankton group dynamics in response to CO_2 enrichment. This implies that enhanced fCO_2 was indeed responsible for the changes seen.

4.1 Phase 0 (days -5 to 0), before CO_2 addition

In most experimental work nutrients have been added to stimulate phytoplankton growth, therefore little data exists for oligotrophic phytoplankton communities (Brussaard et al., 2013) with smaller sized algae typically dominating as they are better competitors for the growth-limiting nutrients (Raven, 1998; Veldhuis et al., 2005). Phase 0 shows the natural state of the ecosystem at the start of the experiment, with indeed a summer community dominated by picophytoplankton. Consistency in phytoplankton abundances across the mesocosms confirmed good replication and baseline data prior to CO_2 manipulation. The flow cytometric phytoplankton community was dominated by cyanobacteria *Synechococcus* spp. (SYN) and the smallest picoeukaryotes (Pico I; both around 1 µm). Picoeukaryotes are found in high numbers at this site throughout the year and *Synechococcus* only in summer when the temperatures are higher (Kuosa, 1991). Microscopic identification of picoeukaryotes is extremely difficult and no species have been described for the region (Kuosa, 1991), however, pigment analyses suggest that Pico I and II are likely to be prasinophytes or other

chlorophytes (Paul et al., 2015). Ideally, performing molecular analyses on the specific algal groups sorted by flow cytometry aids to identify group composition at the species level. Biomass of *Synechococcus* and Pico I increased steadily upon closure of the mesocosms due to high gross growth rates whilst the other groups dropped slightly in abundance. Our grazing rates of *Synechococcus* compare well to the average reported estimate of microzooplankton grazing on cyanobacteria in July in this region of 0.3 d^{-1} (range 0.18-0.53 d^{-1}, Kuosa, 1991). The net growth rates of the total prokaryotic community (0.19 d^{-1}) were also comparable to rates reported for this region (Kuosa, 1991). Because the losses (strongly dominated by grazing) were between 0.3-0.5 d^{-1}, their gross growth rates must have been around 0.5-0.7 d^{-1}.

4.2 Phase I (days 1-16)

According to Paul and coauthors (2015) Phase I was characterised by high productivity and high organic matter turnover. Indeed we saw all phytoplankton groups bloom and we measured relatively high losses by grazing and viral lysis for all groups, responsible for the referred high turnover of organic matter. The prokaryotes responded positively to the increased algal productivity and viral lysis. More specifically, during phase I Pico I benefitted directly and most from enhanced fCO_2 as demonstrated by their significantly ($p<0.05$) higher gross growth rates. Net growth rates of Pico II correlated positively with CO_2 enrichment, but somewhat later into phase I (days 12-17) due to reduced losses.

The stimulation of Pico I by elevated fCO_2 may be due to a stronger reliance on diffusive CO_2 entry compared to larger cells. Model simulations reveal that whilst near-cell CO_2/pH conditions are close to those of the bulk water for cells <5 µm in diameter, they diverge as cell diameters increase (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). This is due to the size-dependent thickness of the diffusive boundary layer, which determines the diffusional transport across the boundary layer and to the cell surface (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). It is suggested that larger

cells may be more able to cope with fCO_2 variability as their carbon acquisition is more geared towards dealing with low CO_2 concentrations in their diffusive boundary, e.g. by means of active carbon acquisition and bicarbonate utilization (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). However, as the Baltic Sea experiences particularly large seasonal fluctuations in pH and fCO_2 (Jansson et al., 2013) due to the low buffering capacity of the waters, phytoplankton here may be expected to have a high degree of physiological plasticity. Previous mesocosm studies reported enhanced abundances of the picoeukaryotic prasinophyte *Micromonas pusilla* at higher fCO_2 (Engel et al., 2007; Meakin and Wyman, 2011). Another summer mesocosm study in the Arctic revealed that even smaller picoeukaryotes, similar to Pico I in our study, showed a positive response to enhanced fCO_2 (Brussaard et al., 2013). Furthermore, Schaum et al. (2012) found that 16 ecotypes of *Ostreococcus tauri* (another prasinophyte similar in size to Pico I) increased in growth rate by 1.4–1.7 fold at 1,000 compared to 400 µatm pCO_2. All ecotypes increased their photosynthetic rates and those with most plasticity, most able to vary their photosynthetic rate in response to changes in fCO_2, were most likely to increase in frequency in the community. It is likely that the picoeukaryotes in our study, which show stimulation by fCO_2 are adapted to a highly variable carbonate system regime and are able to increase their photosynthetic rate when additional CO_2 is available. This ability could allow them to outcompete other phytoplankton (e.g., nanoeukaryotes during phase I) in an environment where nutrients are scarce.

Pico II population dynamics were, despite high gross growth rates, controlled by grazing at the start of the experiment, and only after a reduction in losses during phase II (more so for the high CO_2 mesocosms) could a bloom develop. For Nano I and Nano II the gross growth rates seemed to increase at higher fCO_2, but at the same time the losses also increased. However, differences in growth and loss rates were not statistically significant and thus it stays difficult to underpin why these phytoplankton groups peaked to higher abundances at lower fCO_2 in phase I. Potentially

released competition for nutrients towards the end of phase I (the numerically dominant Pico I and SYN had declined in abundance by then) aided the increase of the nanoeukaryotes.

In general, grazing was a substantial loss factor for all phytoplankton groups during this period and additionally Pico I and II, Nano I and II experienced noteworthy viral mediated mortality. The high grazing rates coincided with high abundances of the ciliate *Myrionecta rubra* at the start of the experiment (Lischka et al., 2015). After day 10 *M. rubra* abundances declined and correspondingly, abundances of most of the phytoplankton groups increased (Lischka et al., 2015). Occasionally grazing rates between the high fCO_2 (M3) and present-day low fCO_2 (M1) mesocosms differed significantly although no general trend could be observed. Very few studies have examined the effects of OA on microzooplankton grazing of phytoplankton (Suffrian et al., 2008; Rose et al., 2009; Brussaard et al., 2013). In neither of 2 mesocosm experiments did Suffrian et al. (2008) nor Brussaard (2013) see significant effects on grazing rates. However, in an on-board continuous culture experiment Rose et al. (2009) found that at elevated CO_2 concentrations higher prey abundances led to higher grazing rates. Similarly, Pico III in the current study during phase I was strongly negatively affected by CO_2 and showed congruently lower grazing rates at higher fCO_2. Nonetheless, this did not seem to hold for the high abundance groups SYN and Pico I, nor for Pico II with comparable abundances to Pico III. Alternatively the significantly reduced gross growth rates at high fCO_2 are the more likely cause for the clear differences in population dynamics between high and low fCO_2 treatments.

In contrast, higher gross growth rates alongside a predominance of viral lysis at high fCO_2 was seen in both Pico II and Nano I during phase I. Metabolically active cells were reported to be infected at higher rates and phytoplankton growing at higher growth rates produced more viral progeny, which could explain this observation (Bratbak et al., 1998; Weinbauer, 2004; Maat et al., 2014). Direct effects of higher fCO_2 on viruses themselves are not expected as marine virus isolates were found to be quite stable (both particle and infectivity) over the range of pH obtained in the present study

(Danovaro et al., 2011; Mojica and Brussaard, 2014). Besides lytic infection, there is the potential for a lysogenic viral life cycle, during which viral DNA is integrated in the host as a prophage (Weinbauer, 2004). We found, however, no evidence that the share of lysogeny compared to the lytic cycle was affected. In fact, the percentage lysogeny was found insignificant during the entire campaign. Mean viral abundances were higher under CO_2 enrichment towards the end of phase I, which is expected to be in response to increased phytoplankton and prokaryote biomass.

During Phase I the high turnover of phytoplankton biomass led to increasing growth of heterotrophic prokaryotes (Hornick et al., 2016). The enhanced net abundances (this study) were heavily grazed and additionally viral lysis became increasingly important (to 60% of total losses at the end of phase I). Bermúdez et al. (2016) reported highest biomass of protozoans around t15. This was predominantly the heterotrophic choanoflagellate *Calliacantha natans* (Hornick et al., 2016). *Calliacantha natans* feeds selectively only on particles <1 µm in diameter (Marchant and Scott, 1993) and therefore may graze on heterotrophic bacteria. During the second half of phase I significantly more prokaryotes were recorded in the high fCO_2 mesocosms, which was likely due to increased availability of dissolved organic carbon at high fCO_2 from higher rates of viral lysis of Pico II and Nano I initially (day 6) and Pico I and Nano II consecutively (day 10).

Assuming a cellular carbon conversion for phytoplankton cells of 237 fg C μm^{-3} (Worden et al., 2004) and 196.5 fg C μm^{-3} (Garrison et al., 2000) for pico- and nano-sized plankton, respectively, we calculated that viral lysis of phytoplankton between days 9 and 13 resulted in the release of 1.1 and 12.4 ng C ml^{-1} for M1 and M3, respectively. Similarly, assuming a bacterial growth efficiency of 30% and cellular carbon conversion of 7 fgC $cell^{-1}$ (Hornick et al., 2016), we estimated that the amount of organic carbon required to support bacterial growth during this period (taking into account the loss of bacterial carbon due to grazing and viral lysis) was 0.7 ngC ml^{-1} in M1 and 11.0 ngC ml^{-1} in M3. Viral

lysis of phytoplankton was thus an important source of organic carbon for the bacterial community and may have led to the observed differences between treatments.

4.3 Phase II (days 17-30)

Phase II displayed a second peak in total phytoplankton abundances related to increased picophytoplankton but reduced nanophytoplankton. Reduced microzooplankton grazing pressure on the picoeukaryotes and *Synechococcus* after day 17 allowed them to increase in abundance during Phase II. Microzooplankton abundances were reduced as compared to the start of the experiment (approximately an order of magnitude lower) and mesozooplankton increased (Lischka et al., 2015). Thus increased grazing of mesozooplankton on microzooplankton may have resulted in reduced grazing of, and proliferation of, picophytoplankton. .

Synechococcus bloomed during phase II, however with significantly lower abundances at higher fCO_2. So although Pico I benefitted from CO_2 enrichment, the similar sized *Synechococcus* did not. *Synechococcus* has shown diverse, strain-specific responses to CO_2 enrichment (Fu et al., 2007; Lu et al., 2006; Traving et al., 2014). As a prokaryote, *Synechococcus* has very different physiology from picoeukaryotes, needing extremely efficient CCMs due to the inefficiency of its Rubisco. Able to concentrate CO_2 to up to 1000-fold higher than the external medium (Badger and Andrews, 1982), they may attain maximal growth rates at the present-day CO_2 concentration (Low-Décarie et al., 2014).

The prokaryote abundance increased steadily during Phase II, again matching total phytoplankton dynamics. Following the initially higher prokaryote abundances at higher fCO_2 in Phase I, we found during phase II decreased abundances of HDNA-prokaryotes at high fCO_2. This fits with the reported reduced bacterial production (Hornick et al., 2015) and respiration measurements (Spilling et al., 2015) in these mesocosms during this time. The differences were due to an indirect effect on the prokaryotes of reduced phytoplankton growth by SYN, Pico III and Nano I leading to lower POC concentrations at higher fCO_2. This was caused by reduced temperature and PAR (Paul et al., 2015).

~~Indeed we saw only low grazing rates for this period and no significant differences in loss by either grazing or lysis, or in DOC (Paul et al., 2015). The steady increase in viral abundances from day 22 onwards indicates that viral lysis of the prokaryotes was substantial, which is confirmed by the halting of prokaryote growth, reduced bacterial production (Hornick et al., 2016) and ultimate decline in prokaryote abundance (this study). The estimated average viral burst size during phase III, obtained from the increase in total viral abundance and concomitant decline in bacterial abundances, was about 30 which is comparable to published values (Parada et al, 2006; Wommack and Colwell, 2000). Viral lysis rates of prokaryotes were measured until day 25 and indicated that on average 10-15% of the total population lysed per day (day 18-25). The final prokaryote abundance at the end of the experiment was in line with a continued lysis in that order of magnitude (corrected for reduced bacterial production; Hornick et al., 2016). Overall, the increased prokaryote activity during the first half of phase II, the relatively low phytoplankton activity during this phase and the (virally induced) mortality of the prokaryote community during the second half of phase II promotes the mineralization and increase in concentration of phosphate (particularly in the low fCO_2 mesocosms; Paul et al, 2015). To what extent elevated CO_2 concentration affects the reduction in P-release from biomass (Nausch et al., 2016), reduced respiration and bacterial production rates as seen in this study (Hornick et al., 2016; Spilling et al., 2016) needs to be explored still.~~

~~4.4 Phase III (days 31-43)~~

~~The positive growth response of the picoeukaryotes to earlier CO_2 enrichment was clearly reflected in the Chlorophyll a concentration, particulate organic carbon and phosphorus, and also in the dissolved organic carbon (DOC) pools in Phase III (Paul et al., 2015). This increase in DOC at high fCO_2 (Paul et al., 2015) may originate from viral lysis of prokaryotes and phytoplankton (Suttle 2005, Lønborg et al., 2013). We measured higher viral lysis rates for SYN, Pico II and Nano I, and similar lysis rates but higher standing stock of Pico I at high fCO_2. Alternatively, increased fCO_2 coupled with~~

low nutrient availability may have stimulated photosynthetic release of DOC and subsequent transparent exopolymer particles (TEP) formation (Engel, 2002; Borchard and Engel, 2012). TEP formation also results from sloppy feeding (Hasegawa et al., 2001; Møller, 2007) and viral lysis, and is thought to promote aggregation and sinking of particulate organic matter (Brussaard et al., 2008; Lønborg et al., 2013). Under the current conditions this would offset the reduced sedimentation associated with smaller cells (Sommer et al., 2002). However, no difference in sedimentation rates was reported between fCO_2 treatments for the current study (Paul et al., 2015). This may have been (partly) obscured by the negative correlation of diatoms, reported to have relatively higher sedimentation rates (Riebesell, 1989; Waite et al., 1997), with fCO_2 during phase III (Paul et al., 2015). At this stage it is hard to draw a final conclusion because at the same time there was a positive correlation with fCO_2 for larger sized diatoms (>20 µm) (Paul et al., 2015). Because of the general urgency to know more about carbon sequestration, we recommend future studies on OA to focus not only on potential shifts in sedimentation due to changes in phytoplankton community composition, but also as a result of changes in phytoplankton size class in combination with the relative share of grazing and viral lysis (Brussaard et al., 2008).

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5 Conclusions

Firstly, our data explain the majority of the phytoplankton dynamics in this mesocosm experiment as more than 90% of the Chl *a* was found in the <20 µm size fraction (Paul et al., 2015). Indeed these data allow us to examine the more detailed changes in community dynamics which are not obvious in the bulk measurements. Distinct shifts between more abundant pico-sized (0.2-3 µm) and nano-sized (3-20 µm) photoautotrophs were seen during the experiment which were also reflected in size-fractionated Chl a concentrations (Paul et al., 2015). Whilst other evident shifts in abundance and net growth rates between different picoeukaryote groups could only be revealed with the current approach of using flow cytometry. Moreover, the complementary grazing and lysis loss rates (along

with the gross growth rates) allowed for a more notable explanation of changes in the phytoplankton and prokaryote community.

Secondly, our study shows that CO_2 enrichment favors the net growth of the very small sized (1 µm) picoeukaryotic phytoplankton. This positive response with fCO_2 is very specific, as neither *Synechococcus* spp., Pico III, nor the nanoeukaryotic phytoplankton groups displayed enhanced growth. Increasing atmosperic CO_2 leads to a number of further global changes, e.g. increasing sea surface temperatures (SST) which in turn strengthens vertical stratification and shoals mixed layer depth (Sarmiento et al., 1998; Toggweiler and Russell, 2008). Such changes in physicochemical conditions have been reported to favor small cells, largely because of reduced nutrient supply to the surface waters (Cermeño et al., 2008; Riebesell et al., 2009; Li et al., 2009; Craig et al., 2013; Mojica et al., 2016). The study by Mojica et al. (2016) shows that under such conditions the share of viral lysis vs grazing for a variety of phytoplankton groups increases, thereby promoting a more regenerative system.

The overall activity of prokaryotes is expected to be affected not only by viral lysis of phytoplankton and prokaryotes themselves, but also by higher SST. This results in increased enzyme activities, production and also respiration rates, polysaccharide release and TEP formation (Piontek et al., 2009; Wohlers et al., 2009; Borchard et al., 2011; Engel et al., 2011; Wohlers-Zöllner et al., 2011). Enhanced bacterial re-mineralization of organic matter could further increase the autotrophic production by the small-sized phytoplankton (Riebesell et al., 2009; Riebesell and Tortell, 2011; Engel et al., 2013). At the same time, through viral lysis and subsequent microbial respiration the biological pump is negatively affected by the production of atmospheric CO_2 (del Giorgio and Duarte, 2002). The evidence presented in the current study indicates that CO_2 enrichment favors small-sized picoeukaryotic phytoplankton, which is further strengthened by increased SST and enhanced vertical stratification. By and large these changes will tend to reduce carbon sequestration.

Due to the low buffering capacity of the Baltic Sea and the paucity of data regarding OA impact in nutrient-limited waters, the results presented here are pertinent to increasing our understanding of how predicted rises in fCO_2 will affect the microbial communities in this region. Our study provides evidence that cell size, taxonomy and sensitivity to loss can all play a role in the outcome of CO_2 enrichment. Physiological constraints of cell size favour nutrient uptake by small cells under conditions of reduced nutrients and our results show that these effects can be further exacerbated by OA. Gross growth rates along with the complementary mortality rates allowed for a more comprehensive understanding of the phytoplankton population dynamics and thus perception of how microbial food web dynamics can influence the response of the autotrophic and heterotrophic components of the community. Our results further suggest that alterations in CO_2 concentrations are expected to affect prokaryote communities (mainly) indirectly through alterations in phytoplankton biomass, productivity and viral lysis. Overall, the combination of growth and losses (grazing and viral 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 ~~CB~~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).

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