

We thank the reviewers for taking the time to review this manuscript and for the pertinent and constructive comments they have raised. Wherever possible we have incorporated their suggestions and if not I hope that we have clearly explained our reasoning.

Anonymous Referee #1

My main criticisms here are that when I look at the figures, to me it seems as if overall shifts in the microbial community structure (or more precisely changes in the abundances of selected plankton members) do actually change in all the mesocosms but that changes are more pronounced in the ‘high CO₂’ treatments. This means that 1. the title is misleading, and 2. parts of the interpretation and discussion of the data are misleading. However, this fact is not discussed at all in this manuscript.

- 1. We understand Reviewer’s point of view and changed the title into “Shifts in the size structure of the microbial community in the Baltic Sea with increasing CO₂”.***
- 2. We clarify in the Discussion of the revised manuscript that the extent of temporal dynamics differed for the high fCO₂ mesocosms (and not the dynamics itself).***

3. Unfortunately, the authors omit any discussion of factors other than the two that they investigated. For example, what about the changing temperature during the experiments; it ranges from 8-15 deg C, but this isn’t discussed anywhere.

These variables affect the overall dynamics and not specifically the differences between the mesocosms (although we cannot exclude co-stressor effects by other variables on top of CO₂ enrichment for the negative impact particularly – e.g. Pico III and Nano I). We make a statement in the Discussion of revised manuscript.

4. They also omit any discussion about any differences between the mesocosms and the surrounding waters and what the differences could mean (as far as I can see from the Suppl. figures, there are differences).

We chose not to include them in the main figures for 2 reasons. Firstly they are not directly comparable, this location is subject to water movement and during the experiment distinctly different water masses with different physical and biological signatures moved into the surrounding water. Secondly, and due to the previous reasoning, including the phytoplankton abundances in the surrounding waters makes the figures more difficult to read. Occasionally the abundances are much greater than in the mesocosms and it is then harder to discern differences between the mesocosms (see Supplementary Table S1 and Fig S1). Overall, microbial temporal dynamics are largely comparable, with a few exceptions: i.e., phytoplankton Nano I and II show much higher abundances in the outside water whilst all the picoplankton abundances are lower in the surrounding waters. We will add a description on the outside water microbial dynamics in the Results section of the revised manuscript, and add discussion on what may have caused the differences.

5. Further, I find it stunning that the total phytoplankton doesn't vary that much over the different mesocosm treatments (Fig. 1). The authors do not acknowledge or discuss this anywhere.

Total phytoplankton is numerically dominated by Synechococcus, making up to 74% of total (as explained in section 3.1), and Synechococcus especially did not show large variations between the fCO₂ treatments (mesocosms).

6. The authors mention that no nutrients were added to the mesocosms to "resemble the natural bottom-up environmental conditions." Although I can understand why the authors did not add nutrients, however, I doubt that this resembles the natural environmental conditions over a period of six weeks. By enclosing the water masses, the 'natural' nutrient supply, which is either horizontally or vertically, is cut off. But this discussion might also have to be carried out across the different companion manuscripts on these mesocosm experiments submitted to the special issue in BG. But no matter how this discussion turns out, the authors should discuss it in this manuscript. Maybe it is reason, for example, why the start and end abundances of the experiments are sometime quite similar while changes happened in between.

Reviewer is correctly stating that we have not discussed lateral and vertical transport of nutrients, but the summer situation is largely driven by regenerative nutrient supply (Kuosa, 1991). The summer situation is one with vertical stratification and low nutrient concentrations resulting in small-sized phytoplankton dominance that are typically well-controlled by grazing and viral lysis (Kuosa, 1991 and demonstrated by our results). We will specify this more clearly in the Discussion of the revised manuscript. We also measured nutrients outside the mesocosms and found that nitrate, the limiting nutrient, was at similar concentrations inside and outside the mesocosms. Phosphate did increase outside the mesocosms but only after day 25. Silicate was higher and more variable outside the mesocosms.

7. The discussion section could benefit from a bit more 'discussion' rather than just the listing of other articles. How do the results you present actually fit into the literature and what does it mean for your data when other studies have shown certain effects (also see comments below).

We will rework the Discussion accordingly Reviewer's comment.

8. Several sentences and paragraphs are quite lengthy and not easy to understand (all the way to not understandable at all).

We have carefully checked the manuscript and shortened / clarified where we thought necessary (as Reviewer is not specifically mentioning section), and we also ask another native English speaking colleague to read the manuscript for clarity.

9. The introduction is lengthy and repetitive in some places and could be condensed.

We do not agree with Reviewer that the Introduction is lengthy, but we realize it is repetitive at times. We removed redundancies while securing readability.

10. Throughout the manuscript, the numbers of the mesocosms are used, e.g. M1 or M3. This is very confusing especially in the discussion. Could be exchanged for LOW1 or HIGH2 or

something that designates a treatment to that number, especially since M1 and M5 seem to be replicates, as well as M6 and M7, and M3 and M8.

The notation used is consistent across all manuscripts in this special issue and the mesocosms with their mean $f\text{CO}_2$ are presented in Fig. 1. However, we see Reviewer's point and will include a Table, as well as specify better at the start of the Results section as well as in the Discussion which mesocosms are LOW and HIGH.

Table 1. $f\text{CO}_2$ concentrations (μatm) as an average for the duration of the experiment following CO_2 addition and specification of this CO_2 level as low, medium or high. *denotes mesocosms sampled for grazing and viral lysis assays

Mesocosm	M1*	M5	M7	M6	M3*	M8
CO_2 Level	LOW	LOW	INTERMEDIATE	INTERMEDIATE	HIGH	HIGH
Mean $f\text{CO}_2$ (μatm) days 1-43	365	368	497	821	1007	1231

11. what are the 'failed' experiments, are they 'samples lost'? or outliers?

Failed experiments include very low cell abundance samples, complicating proper analysis (and consequently results) of the diluted series, as well as results displaying a positive slope rather than a negative slope for apparent growth rates versus fraction natural water (thus where the dilution does not result in a reduction in mortality). An explanation is now given in the text (M&M section 2.3). We also make reference to paper by Kimmance & Brussaard 2010 describing such issues, as well as Stoecker et al. 2015 which suggest potential causes for positive regressions.

12. - p2, l3: salinity in the Baltic Sea ranges from near-freshwater to near-full seawater, I wouldn't necessarily call it extremely low salinity implying a negative effect, especially since it varies a lot throughout the Baltic Sea.

Reviewer is correct. However, during our study salinity was around 5.7 only. We have deleted this sentence and provided the information about the sampling location in the next sentence (stating also there the actual salinity).

13. - p2, l6: "We examined the effects of ocean acidification in the microbial community during. . ." Do you mean on the community structure or on the carbon export or on primary production rates? Please specify in the abstract.

We specified this in the Abstract of the revised manuscript, making clear we examined effects on microbial community structure.

14. - p2, l25: the threats don't face the marine ecosystems but the marine ecosystems face the threats

Thank you, we corrected this.

15. - p3, l2-9: This paragraph doesn't fit here and disrupts the flow. I would place it to where you describe your experiments.

We have amended the Introduction and moved this information with additional clarification to M&M section, and to paragraph concerning general effect of lower salinity on pH buffer capacity in Discussion.

16. - p3, l10-12: reads awkwardly, split into two sentences

We split into 2 sentences.

17. - p3 l26- p4, l3: this is repetitive

The Introduction is revised and we modified repetitive sections.

18. - p4, l20: which key knowledge, it's not clear from this sentence

Altered.

19. - p4, l24: delete the 'top-down control'

We rephrased this sentence.

20. - p5 and following: the experimental set-up could be made much clearer, maybe use a sketch for this

We follow the general overview paper in this same special issue and make reference to this paper (by Paul et al. 2015), describing the experimental set-up very well (also with figures). We rephrased the sentence making reference to Paul et al. paper in order to clarify this better.

21. - p5, l23: nitrate, phosphate, silicate and ammonium are per definition (dissolved) inorganic nutrients

Agreed and we altered accordingly.

22. - p7, l1-2: Pico III and Pico I do not have comparable cell sizes; one is about 1 micrometer and the one is about 2.9 micrometer in diameter, maybe you meant Pico II and III?

Yes, we thank Reviewer for noting this and have corrected accordingly.

23. - p7, l4: was this conversion factor used for all organisms or just the *Synechococcus*? There are studies that clearly show that the carbon density changes with cell volume with the density being lower at higher volumes (see Verity et al. 1992 L&O or Menden-Deuer and Lessard 2000 L&O) If the same conversion factor was used for all organisms, this would likely bias the results significantly

We have recalculated applying conversion factors of $237 \text{ fg C } \mu\text{m}^{-3}$ (Worden et al. 2004) and $196.5 \text{ fg C } \mu\text{m}^{-3}$ for pico- and nano-sized phytoplankton (Garrison et al. 2000),

respectively according to Mojica et al. 2015 and will use the revised Figures in the manuscript. However the overall dynamics are the same.

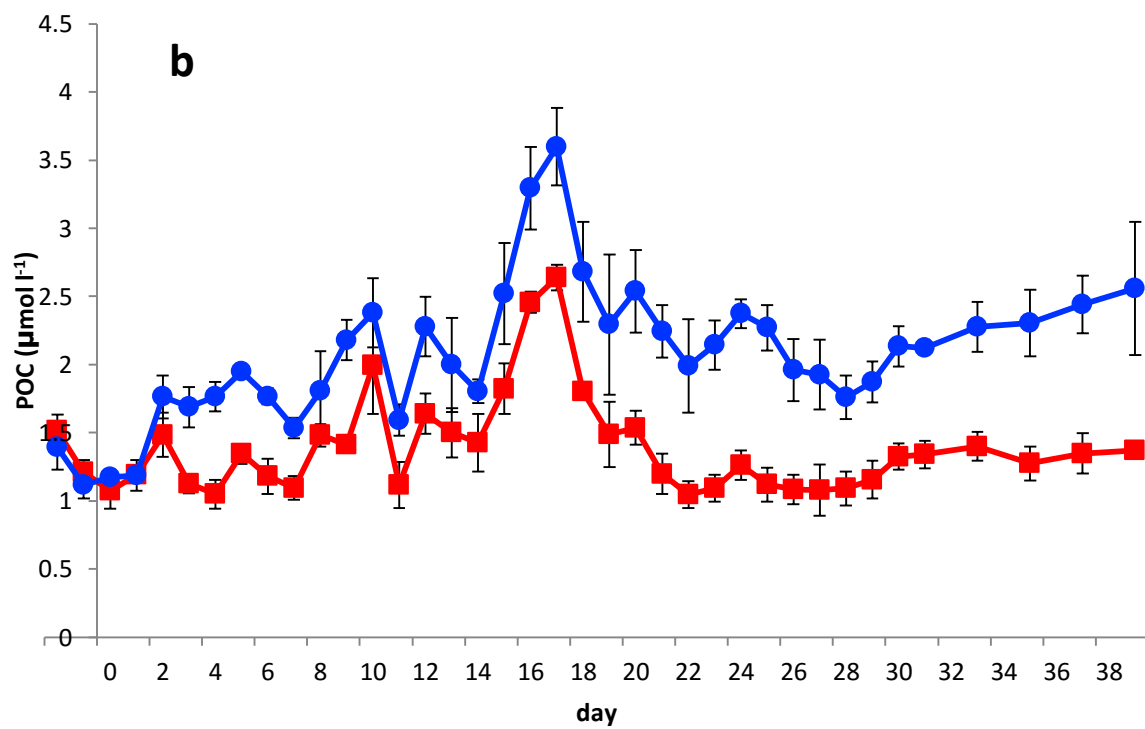
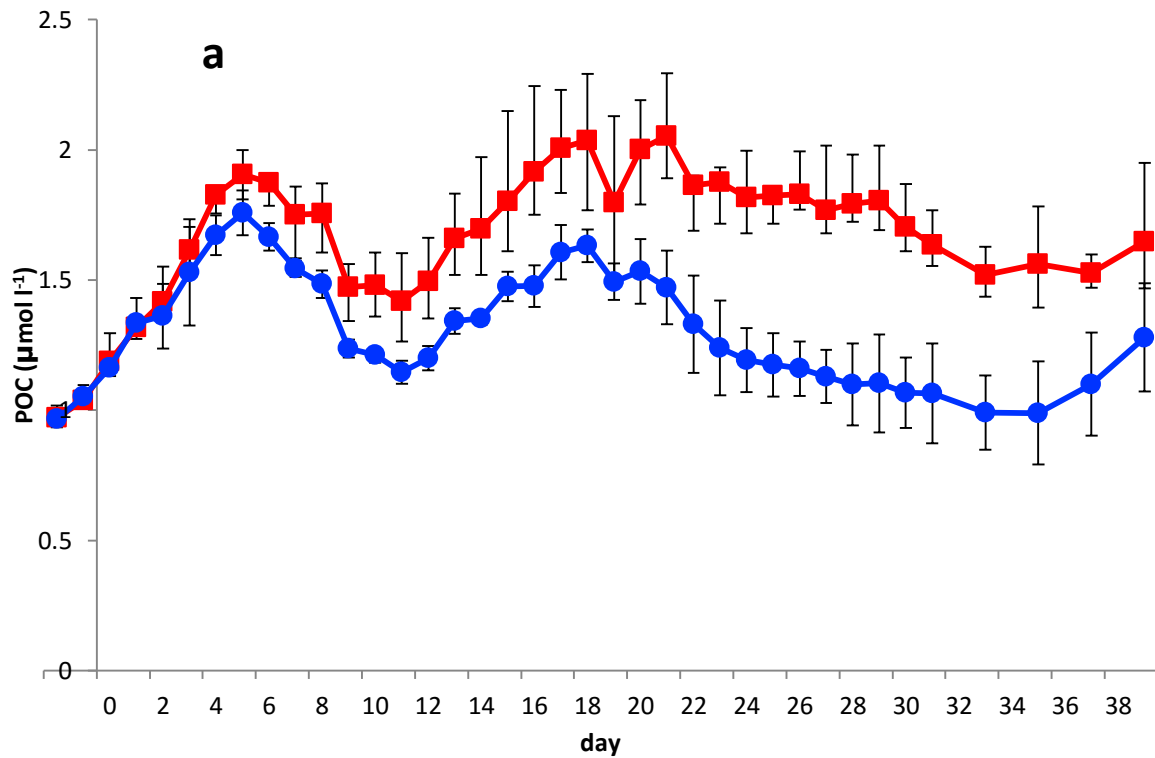


Fig.8. POC calculated from mean cell abundances assuming cells to be spherical and applying conversion factors of $237 \text{ fg C } \mu\text{m}^{-3}$ (Worden et al.2004) and $196.5 \text{ fg C } \mu\text{m}^{-3}$ for pico- and nano-sized plankton (Garrison et al. 2000), respectively according to Mojica et al. (2015). Error bars show one standard deviation. a) Temporal dynamics of Pico I and II b) Temporal dynamics of POC for all other eukaryotes ie. Pico III, Nano I and II.

24. - p7, l11: why not use the term total prokaryotes, because this is what it actually is, and not the heterotrophic prokaryotes which clearly should not include Synechococcus or other photoautotrophic organisms (the 10% argument is not correct here in my opinion).

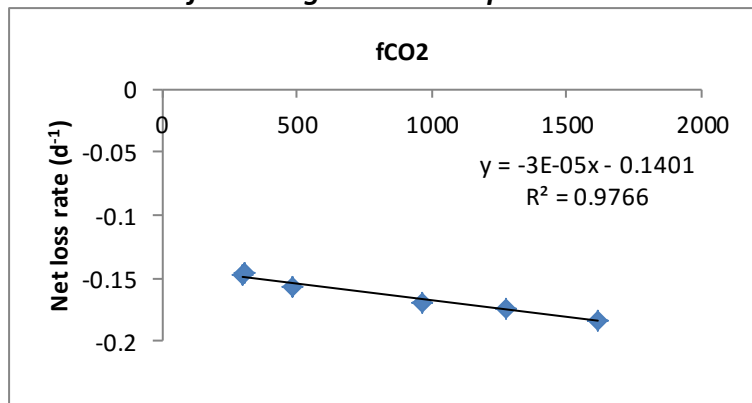
Synechococcus makes up for around 10% of the total prokaryotes in our study, but we understand Reviewer's comment and changed 'heterotrophic prokaryotes' into 'prokaryotes' (and thus also HP into prokaryotes).

25. - p7, l15: final concentrations of what, molar? micromolar? micrograms per kg?

It is a final concentration of the commercial stock, which does not have a specified unit. This is the common way of expressing these final concentrations, but we moved 'commercial stock' directly following 'final concentration of' to improve understanding.

26. - p11, l19: the R^2 is 0.49 in the figure and the regression line doesn't seem like it would be 0.98 either

Actually it is 0.98 and the figure was incorrect; we appologize for the confusion and thank the Reviewer for noting. We have replaced it with the correct one.



27. - p11, l22-25: this part is hard to read. Please rephrase.

We rephrased and split into separate sentences.

28. - p12, l3: wasn't the start of the mesocosm day -5 or day 0 rather than day 13?

We altered the text to make it clearer that we referred to the bloom period.

29. - p12, l5: the decline following what?

Should be the 'following decline', which we now corrected.

30. - p12, l20: there is no such thing as net abundance; you can have net rates but not net abundances (also check throughout the manuscript)

Reviewer is correct and we deleted 'net'.

31. - p12/13: "This may have stimulated the gross growth in M3 as compared to M1 (day 19; Fig. 3b) for a longer period in the high fCO₂ mesocosms, this accompanied by higher losses at low CO₂ resulted in a positive correlation of net growth rates with fCO₂ (Fig. 3e, R² = 0.71) and almost 2-fold higher net abundances at day 21 (Fig. 3a) correlating with fCO₂ (Fig. 3h, R² = 0.84)." I have honestly no idea what this sentence means. It is unnecessarily long and confusing. Further, the 'net abundances', please see comment above, and

We reduced the length of the sentence by splitting it into two and corrected according to Reviewer's comment.

maybe either CO₂ or fCO₂ treatments could be used consistently throughout the manuscript

Noted and we have altered this to fCO₂ throughout (in agreement with the general overview paper by Paul et al. (2015)).

32. - p13, l13-17: unnecessarily long and confusing sentence

We understand Reviewer's concern and have split the sentence into two sentences to improve readability.

33. - p14, l25: what are "CO₂ days"?

Sentence was indeed confusing and we have clarified it now.

34. - p18, l17-19: How do the authors infer a bacterial production rate of about 0.6 d⁻¹ when grazing is about 0.3-0.5 d⁻¹? Is that due to a net positive growth in bacterial abundance? If so, it would be good to mention here. Otherwise the reader might assume steady state as I did here.

Indeed this is due to a positive net growth rate, as stated in the preceding sentence. For clarity we changed the terminology to 'gross growth rates' now. Furthermore, we have moved the actual rate information to the Results to accommodate also Reviewer's comment to include less results and more discussion. We added more discussion on the estimated gross growth rate in comparison to bacterial production rates measured by others (Hornick et al., this issue).

35. - p18, l27: "Also Pico II showed positively correlated net growth rates with CO₂ enrichment, but somewhat later into phase I (days 12-17) due to reduced losses." Awkward start of the sentence, maybe: "Net growth rates of Pico II correlated positively with CO₂ enrichment. . . ."

We thank Reviewer for the improvement and have corrected accordingly.

36. - p20, l25-28: these are mainly results and then one other article is mentioned; but what does this now mean for your data? Do you think that TEP production was a factor regulating the abundances in your study? the actual discussion is missing

We realize the sentence on TEP was a bit of a stand-alone and we chose to delete the sentence as it no longer fits the reworked discussion of these results.

37.- p21, l9-11: This comes out of the blue. How did you examine this?

This is examined using mytomycin C which induces prophage to go into the lytic state. It is explained in the M&M section 2.3. and we have also added this to the Results section in the revised manuscript.

38. - p22, l2: “. . . has a very different physiology,. . .” different from what?

We meant different from picoeukaryotes. We clarified this now in the text.

39. - p22, l17: DOC could have come also from sloppy feeding?

We added this option to the discussion on the topic.

40. - p23, l17: do you mean remineralization of organic matter rather than nutrients?

Yes thanks, we apologize for the mistake and altered the text accordingly.

41. - p23, l22: “multiple other factors”? Please name them here.

We now rephrased the sentence and specifically named SST and stratification.

42. - Fig. 2: Instead of calling it the ‘total prokaryotic phytoplankton’, just call it what it is, the *Synechococcus* population

We have altered the figure accordingly.

43. - Fig. 2: How is the $p < 0.1$ indicated? Is it possibly also the category ‘ $p > 0.05$ ’?

- What are the black dots here and in other plots (and I don’t mean the single asterisks)?

The black dots are the $p < 0.1$ indicators. We have clarified the legend accordingly.

44. - I don’t see any ‘f’ in this figure (and some of the following figures).

True, this was our mistake as not all figures have failed experiments (f). We have deleted this from those figure legends.

45.- Fig. 2: panel b here and in following figures: I understand why the authors want to present the data together, but the plots are really obscured this way and it makes it hard for the reader to discern any data from them. I would suggest to split them into two panels

We have split panel b into two panels for clarity

46.- Fig. 2: Here and following figures, what do you mean by “otherwise no data is a zero”?

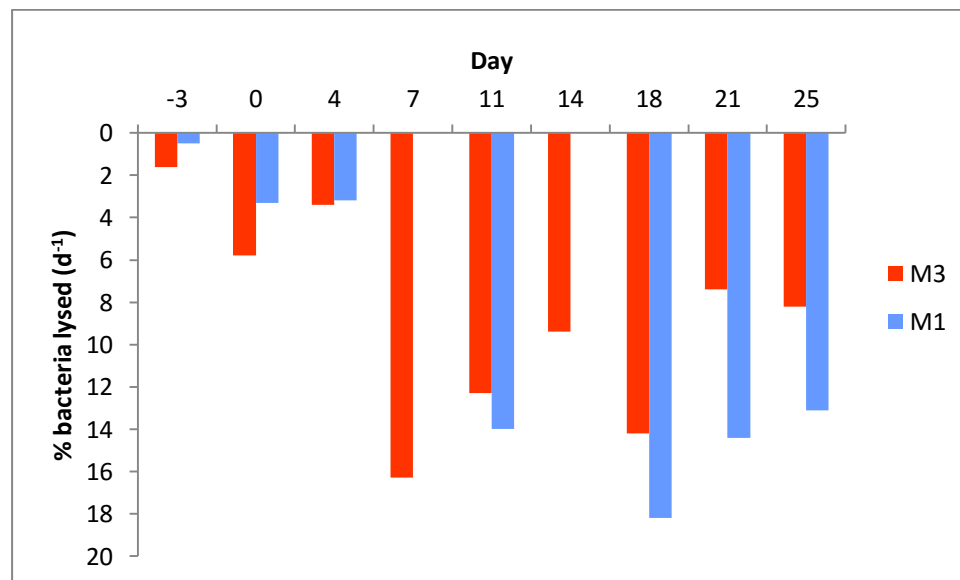
We referred to assays with true zero rates. We understand the misunderstanding and now indicate true zeros (thus not failed assays) with a “0” and made this clear in the figure legends.

47. - Fig. 6: The legend says that linear regression statistics are provided in the plot, however, I couldn’t see a p value.

We apologize as this was a left-over from an earlier version. We show only the r^2 , and made this clear in the legends of the revised manuscript.

48.- Fig. 10: the grazing rate and lysis rate are both loss rates; nevertheless, one of them is presented on a negative scale while the other is presented on a positive scale. I find it confusing.

We agree with Reviewer and have changed the figure now such that all loss rates are presented on a negative scale.



49. - Suppl. Table S1: What are the units here?

They are abundances per milliliter; we have edited this.

50. - Suppl. Table S2: What are the units here?

They are rates per day; we have edited this.

51. - Fig. S1 and S2 legends: the upper layer is mentioned here but the measurements are from the total water mass, i.e. 0.3-17 m rather than 0.3-10 m

We corrected the figure legend.

52. - Fig. S2: panel f is missing

We corrected the figure legend.

53. - Fig. S3 and S4 please use the proper symbol for micromol and not umol

We corrected accordingly.

Garrison, D. L., and others. 2000. Microbial food web structure in the Arabian Sea: A US JGOFS study. Deep-Sea Res. Part II 47: 1387-1422. doi:10.1016/S0967-0645(99)00148-4

Hornick T., Bach, L. T., Crawford, K.J., Spilling, K., Achterberg E.P., Brussaard, C.P.D., Riebesell, U., Grossart, H-P. Effect of ocean acidification on bacterial dynamics during a low productive late summer situation in the Baltic Sea (in prep.) Biogeosciences, 2015.

Kimmance, S. A. and Brussaard, C. P. D.: Estimation of viral-induced phytoplankton mortality using the, in Manual of Aquatic Viral Ecology, pp. 65–73., 2010.

Kuosa, H.: Picoplanktonic algae in the northern Baltic Sea: seasonal dynamics and flagellate grazing, Mar. Ecol. Prog. Ser., 73(2-3), 269–276, doi:10.3354/meps073269, 1991.

Mojica, K. D. A., van de Poll, W. H., Kehoe, M., Huisman, J., Timmermans, K. R., Buma, A. G. J., van der Woerd, H. J., Hahn-Woernle, L., Dijkstra, H. A. and Brussaard, C. P. D.: Phytoplankton community structure in relation to vertical stratification along a north-south gradient in the Northeast Atlantic Ocean, Limnol. Oceanogr., 60(5), 1498–1521, doi:10.1002/lno.10113, 2015.

Paul, A. J., Bach, L. T., Boxhammer, T., Czerny, J., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., Riebesell, U., Road, M., Lismore, E. and Way, E.: Effect of elevated CO₂ on organic matter pools and fluxes in a summer , post spring-bloom Baltic Sea plankton community, Biogeosciences , 1–60, 2015.

Stoecker, D. K., Nejstgaard, J. C., Madhusoodhanan, R., Pohnert, G., Wolfram, S., Jakobsen, H. H., Šulčius, S. and Larsen, A. (2015), Underestimation of microzooplankton grazing in dilution experiments due to inhibition of phytoplankton growth. Limnol. Oceanogr., 60: 1426–1438. doi:10.1002/lno.10106

Worden, A. Z., J. K. Nolan, and B. Palenik. 2004. Assessing the dynamics and ecology of marine picophytoplankton: The importance of the eukaryotic component. Limnol.Oceanogr. 49: 168-179. doi:10.4319/lo.2004.49.1.0168

Biogeosciences Discuss., doi:10.5194/bg-2015-606-RC1, 2016

Interactive comment (in bold, italic) on “Shifts in the microbial community in the Baltic Sea with increasing CO₂” by K. J. Crawford et al.

Also supplied as pdf with Figs embedded in the text (see supplement)

We thank the reviewers for taking the time to review this manuscript and for the pertinent and constructive comments they have raised. Wherever possible we have incorporated their suggestions and if not I hope that we have clearly explained our reasoning.

Anonymous Referee #2

The authors present mesocosms experiment in which they test the effect of ocean acidification on microbial community, by increasing CO₂ levels. Since the ongoing climate change this research is of high importance and data presented in this msc are very valuable. The authors focused on the phytoplankton size fraction <20 µm, as well as to heterotrophic prokaryotes and viruses. The experiment set up is well explained and the msc is in general well written. I do recommend the msc to be published after major revision. Key points that I would recommend to be answered:

1. All though, the target organisms are of key importance for ecosystem functioning, I do not agree that the results are relevant if not being in correlation to the whole phytoplankton community, at least presented as Chl a concentration. If those data exist I highly recommend including them in the msc.

We believe abundance and cell size of the different phytoplankton groups are of key importance as Chlorophyll a consisted mainly of algal groups smaller than 20 µm cell diameter (Paul et al. 2016). Already at the start of the experiment less than 5% was larger than 20 µm diameter. At day 5 even 70% was smaller than 2 µm. Therefore adding Chl a concentration will not add to the current study. We made this clear in the Discussion of the revised manuscript.

2. In Material and Methods it is stated that the samples of surrounding water were taken, but there are not presented in results or at least discussed. It is essential to discuss those data. I would recommend including those data into the graphs reporting about the microbial community changes.

We chose not to include them in the main figures for 2 reasons. Firstly they are not directly comparable, this location is subject to water movement and during the experiment distinctly different water masses with different physical and biological signatures moved into the surrounding water. Secondly, and due to the previous reasoning, including the phytoplankton abundances in the surrounding waters makes the figures more difficult to read. Occasionally the abundances are much greater than in the mesocosms and it is then harder to discern differences between the mesocosms (see Supplementary Table S1 and Fig S1). Overall, microbial temporal dynamics are largely comparable, with a few exceptions: i.e., phytoplankton Nano I and II show much higher abundances in the outside water whilst all the picoplankton abundances are lower in the surrounding waters. We will add a description on the outside water microbial dynamics in the Results section of the revised manuscript, and add discussion on what may have caused the differences.

3. Also, I am not sure if I have understood it correctly – but it seems that CO₂ was added to all mesocosms? In that way you do not have control and any change in microbial community could have been because of some other factor?

We realize that the text was not clear and have improved this in the revised manuscript, i.e., all mesocosms were sparged with water so that a similar water treatment occurred, but no CO₂ was added to the mesocosms that served as present-day controls.

4. What about the temperature?

The temperature was similar for all mesocosms as well as the surrounding water and therefore can only potentially have influenced the dynamics of the microbial populations but not the extent of change between the different mesocosms. We present temperature now briefly at the start of the Results section (of the revised manuscript).

5. What is the usual phytoplankton development dynamics in the Baltic Sea?

We now briefly commented on this at the start of the Discussion.

6. Also, I do not see any shifts in microbial community, but changes abundances during the experiment.

We acknowledge that and clarify in the Discussion of the revised manuscript that the extent of temporal dynamics differed for the high pCO₂ mesocosms (and not the dynamics

itself). Also we changed the title of the manuscript to “Shifts in the size structure of the microbial community in the Baltic Sea with increasing CO₂” to make this clear.

7. Since every experiment need to be repetitive and results comparable with other study site and experiment set up it would be necessary to know the community structure of microbial community. I am aware of the difficulty of taxonomical recognition of size fraction below 20µm, but it is essential. Flow cytometry in an excellent tool, but in an experiment set up of this range I do not believe it is enough. I did like the way how the investigated groups were divided, but the next step in research should be the taxonomical identification.

We agree that taxonomic identification would be a next step but would need flow cytometry sorting and genomics, which goes beyond the scope of the current study. Linking to taxonomic identification by phytoplankton pigment composition analysis is only partly possible as a few large cells may obscure the share of a certain group as compared to total Chl a. Paul et al. (2015) showed that the smaller fraction (<2 µm) was likely to be chlorophytes and prasinophytes. This is mentioned in the Discussion (section 4.1).

8. Not all organisms in the same size fraction have same physiological response to environment drivers.

We recognise this and discussed this in relation to the Pico I and Syn data. We will add a similar line of reasoning for potential differences within a group even as not all are necessarily the same species.

9. Also, the authors in the discussion, do not discuss their data, but cite different authors and their research. If you did not go to the species level – those data cannot be discussed.

We checked the Discussion and amended where needed.

10. The analyzed groups were good explained except the cyanobacteria. The authors distinguish Synechococcus, but no Prochlorococcus. Since the oligotrophy Prochlorococcus could develop in the environment and it can be separated by flow cytometry. Then stated that the prokaryotes include bacteria, archea and unicellular cyanobacteria (together marked as HP) – what cyanobacteria could it be?

Prochlorococcus can indeed be distinguished by flow cytometry, but was not present during this experiment. Therefore cyanobacteria and Synechococcus are used interchangeably during the manuscript. We will make a statement that Prochlorococcus was not observed (Results section).

11. The tittle also does not represent the results – there is not shift in microbial community presented.

We have altered the title to “Shifts in the size structure of the microbial community in the Baltic Sea with increasing fCO₂ ” to more accurately reflect the results.

12. It is not easy to follow the results and discussion with given abbreviations (M1, M5. . .) for mesocosm experiments. It is not clear enough. Maybe a table would be a good way to explain the abbreviations.

The notation used is consistent across all manuscripts in this special issue and the mesocosms with their mean fCO₂ are presented in Fig. 1. However, we see Reviewer’s point of view and will include a Table with the necessary information. See Attachment 1.

Table 1. fCO₂ concentrations (µatm) as an average for the duration of the experiment following CO₂ addition and specification of this CO₂ level as low, medium or high. *denotes mesocosms sampled for grazing and viral lysis assays

Mesocosm	M1*	M5	M7	M6	M3*	M8
CO₂ Level	LOW	LOW	INTERMEDIATE	INTERMEDIATE	HIGH	HIGH
Mean fCO₂ (µatm) days 1-43	365	368	497	821	1007	1231

13. The discussion needs to be rewritten. The results would need to be discussed in more detail. The cited literature and results are maybe not the best choice for the results presented.

It is not clear which examples the reviewer is referring to. However, we have tried to improve the discussion according to Reviewer’s comments.

Paul, A. J., Bach, L. T., Boxhammer, T., Czerny, J., Hellemann, D., Trense, Y., Nausch, M., Sswat, M., Riebesell, U., Road, M., Lismore, E. and Way, E.: Effect of elevated CO₂ on organic matter pools and fluxes in a summer , post spring-bloom Baltic Sea plankton community, Biogeosciences , 1–60, 2015.

Changes made:

1. All comments addressed in text-see track changed version below
2. Addition of Table 1.
3. Addition of Fig 10d
4. All Figs. altered as reviewer requested

Shifts in the size structure of the microbial community in the Baltic Sea with increasing $f\text{CO}_2$

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Abstract:

Ocean acidification, due to dissolution of anthropogenically produced carbon dioxide is considered a major threat to marine ecosystems. ~~The Baltic Sea, The Gulf of Finland, in the Baltic Sea has with extremely low salinity and thus low pH buffering capacity, so is likely to experience stronger variation in pH than the open ocean with increasing atmospheric carbon dioxide.~~ We examined the effects of ocean acidification on the microbial community [structure in the Gulf of Finland, Baltic Sea](#), during ~~the, low salinity (around 5.7), and inorganic nitrogen and phosphorus depleted, summer.~~ Using

large volume *in situ* mesocosms to simulate present to future and far future scenarios. ~~We~~ we ~~observed~~ saw distinct trends with increasing $f\text{CO}_2$ in each of the 6 groups of phytoplankton ~~with~~ diameters below 20 μm that we enumerated by flow cytometry (<20 μm cell diameter). Of these groups ~~two~~ two picoeukaryotic groups increased in abundance whilst the other groups, including prokaryotic *Synechococcus* spp., decreased with increasing $f\text{CO}_2$. Gross growth rates increased with increasing $f\text{CO}_2$ in the dominant picoeukaryote group sufficient to double their abundances whilst reduced grazing losses allowed the other picoeukaryotes to flourish at higher $f\text{CO}_2$. Significant increases in lysis rates were seen at higher $f\text{CO}_2$ in these two picoeukaryote groups. 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 ~~heterotrophic~~ prokaryotes largely followed the algal biomass with responses to increasing $f\text{CO}_2$ reflecting the altered phytoplankton community dynamics. Similarly, higher viral abundances at higher $f\text{CO}_2$ seemed related to increased prokaryote biomass. Viral lysis and grazing were equally both important in controlling prokaryotic abundances. Overall our results point to a shift towards a more regenerative system with potentially increased productivity but reduced carbon export.

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 facing marine ecosystems face (Turley and Boot, 2010). Direct and indirect effects are predicted to have a large impact on ~~marine~~ 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; ~~Hein and Sand-Jensen, 1997; Leonardos and Geider, 2005;~~ Calcification of coccolithophores, which influence sedimentation via calcium carbonate ballasting, is generally reduced (Meyer and Riebesell, 2015).

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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 CO₂ concentrations (Qiu and Gao, 2002; Barcelos e Ramos et al., 2007; Hutchins, 2007; Qiu and Gao, 2002). Even a few studies Direct CO₂ effects are also reported that for small-sized photoautotrophic eukaryotes, can be affected by CO₂ enrichment (Engel et al., 2007; Meakin and Wyman, 2011; Brussaard et al., 2013).

Marine phytoplankton are responsible for approximately half of global primary production (Field et al., 1998), whereby shelf sea communities contribute 15-30% of this (Kulinski and Pempkowiak, 2011). Whilst environmental factors, such as temperature, light, nutrients and CO₂ concentration, regulate gross primary production bottom-up, top-down-controlling-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 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 natural bacterio- and phytoplankton (Weinbauer, 2004; Baudoux et al., 2006; Evans and Brussaard, 2012; Mojica et al., 2015a,2016).

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, heterotrophic prokaryotes and viruses) under the influence of enhanced CO₂ concentrations and in relation to viral lysis and grazing

~~top-down~~ control. Using large mesocosms at *in situ* light and temperature, the Baltic Sea pelagic ecosystem was exposed to a range of increasing $f\text{CO}_2$ concentrations from ambient to future and far-future concentrations. This study was performed during summer in the Gulf of Finland near Tvärminne, with salinity around 5.7 and low dissolved inorganic nitrogen and phosphorus concentrations. During the 43 day long experiment ~~especially~~ the smallest 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 m³ 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 described in detail 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 the mesocosms. Six mesocosms were sampled for the current study, unfortunately three were lost due to leakage. Initial fugacity of CO₂ ($f\text{CO}_2$) was 240 µatm. ~~and raised to 293 (mesocosm M1), 294 (M5), 488 (M7), 1011 (M6) and 1322 µatm (M3) at day 4~~ The mean $f\text{CO}_2$ 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 $f\text{CO}_2$ which takes into account the non-ideal behavior of CO₂ gas and is the standard measurement required for gas exchange calculations (Pfeil et al., 2013).

For $f\text{CO}_2$ manipulations, natural seawater was saturated with CO_2 and then injected evenly throughout the whole depth of the mesocosms in four steps between days 0 to 3 until target $f\text{CO}_2$ was reached. On day 15 a further $f\text{CO}_2$ addition was made to the top 7 m of mesocosms 3, 6 and 8 to replace CO_2 lost due to outgassing. The remaining mesocosms received similar treatment without CO_2 . Initial nutrient concentrations, i.e. ~~dissolved inorganic~~ nitrate, phosphate, silicate and ammonium, were $0.05 \mu\text{mol L}^{-1}$, $0.15 \mu\text{mol L}^{-1}$, $6.2 \mu\text{mol L}^{-1}$ and $0.2 \mu\text{mol L}^{-1}$, respectively, and stayed low ~~during for~~ the duration of the experiment (Paul et al. 2015, this issue). Salinity was around 5.7, temperature was initially $\approx 8^\circ\text{C}$ and rose to $\approx 15^\circ\text{C}$ on day 15 before falling to $\approx 8^\circ\text{C}$ again.

Collective sampling was performed daily in the morning, using an integrated water sampler, from the top (0-10 m) and from the whole water column (0-17 m) of all mesocosms and the surrounding water. Subsamples were obtained for enumeration of phytoplankton, ~~heterotrophic~~ prokaryotes and viruses. Samples for viral lysis and grazing were taken from 5 m depth using a gentle vacuum-driven pump system. Samples were protected against daylight and warming by thick black plastic bags containing wet ice. In the laboratory the samples were processed at *in situ* temperature and dimmed light. As viral lysis and grazing rates were determined from samples taken from 5 m depth, samples for microbial abundances reported 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 has been divided into 4 phases based on major physical and biological changes occurring (Paul et al. 2015). Phase 0 before CO_2 addition (days -5 to 0), phase I (days 1-16), phase II (days 17-22) and phase III (days 23-43). Throughout this study the data are presented using 3 colors (blue, grey and red), representing low (mesocosms M1 and M5) intermediate (M6 and M7) and high (M3 and M8) $f\text{CO}_2$ ~~(Table 1) additions~~.

2.2 Microbial abundances

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 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 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 I (comparable average cell size) by the higher orange autofluorescence. ~~Another common cyanobacterial species, *Prochlorococcus* spp., were as not seen~~observed during this experiment. Assuming the cells to be spherical ~~and containing~~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, ~~c-~~cellular carbon was calculated based on the average cell diameters. Net growth and loss rates of phytoplankton and heterotrophic prokaryotes were derived from exponential regression analysis of the cell abundances.

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. ~~As in~~ the surface waters of the Baltic Sea most prokaryotes are heterotrophs (Riemann et al., 2008). ~~we use the term heterotrophic prokaryotes (HP) in this report.~~

Briefly, thawed samples were diluted with sterile autoclaved Tris-EDTA buffer (10mM Tris-HCl and 1mM EDTA, pH 8.2) and stained with the green fluorescent nucleic acid-specific dye SYBR-Green I (~~Molecular Probes,~~ Invitrogen Inc.) to a final concentration ~~of of the commercial stock of~~ 1×10^{-4} (~~for prokaryote~~ ~~prokaryotes~~) or 0.5×10^{-4} (~~for~~ viruses) ~~of the commercial stock~~. Virus samples were stained at 80°C for 10 min and then allowed to cool for 5 min -at room temperature in the dark. ~~r.~~

~~P~~rokaryotes were stained for 15 min at room temperature in the dark (Brussaard, 2004 with adaptation according to Mojica et al., 2014). ~~P~~rokaryotes 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, 2005).

2.3 Viral lysis and grazing

Microzooplankton grazing and viral lysis of phytoplankton was determined using the modified dilution method (Mojica et al., 2016) ~~dilution method of Landry and Hassett (1982)~~. 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 grazing and lysis pressure in a serial manner and regression analysis allowed loss rates (slope) and

gross phytoplankton growth rates, in the absence of grazing and lysis (intercept y axis 30 kDa series), to be determined. The 0.45 μm filtrate was produced by gravity filtration of $\sim 200 \mu\text{m}$ mesh sieved seawater through a 0.45 μm Sartopore capsule filter. The 30 kDa ultrafiltrate was produced by tangential flow filtration of $\sim 200 \mu\text{m}$ pre-sieved seawater using a 30 kDa Vivaflow 200 PES membrane tangential flow cartridge (Vivascience). Incubations were set up in triplicate in clear 1.2 L polycarbonate bottles. They were suspended close 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. Due to time constraint, samples from the 30 kDa series were fixed to a 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 tested periodically by running duplicate series of fresh and frozen samples. No differences in analysis between fresh and frozen samples were observed. Incubation experiments were run with samples from mesocosm 1 (~~control~~ low $f\text{CO}_2$) and 3 (high $f\text{CO}_2$); due to the logistics of handling times it was not possible to do more. Experiments were performed until day 31. Occasionally the dilution assays displayed a positive slope rather than a negative slope for apparent growth rate versus fraction of natural water (thus not resulting in a reduction in mortality with dilution). Furthermore, very low phytoplankton abundances complicate proper analysis (and consequently results) due to the fact that the assay is based on a dilution series. Such assays were deemed failed. Further discussion of potential causes of positive regressions can be found in Kimmance and Brussaard (2010) and Stoecker et al. (2015).

Viral lysis of prokaryotes was determined by the method of Winget et al. (2005) adapted from the original method by 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 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 virus-free seawater, freshly produced by 30 kDa ultrafiltration using a PES membrane (Vivaflow 200, Vivascience) was added in three steps to wash away free viruses. Finally the sample was diluted back to the original 300 ml volume with virus-free seawater. The samples were aliquoted into six 50 ml polycarbonate tubes. Mytomycin C (Sigma-Aldrich) (final concentration, 1 $\mu\text{g ml}^{-1}$, maintained at 4°C), which induces lysogenic bacteria (Weinbauer and Suttle, 1996) was added to three of the six tubes for each mesocosm studied. A third series of incubations with 0.2 μm filtered samples, ~~the filtrate from the previous step, and thus only free viruses present,~~ 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. ~~Viruses were found not to be lost from the 0.2 μm controls.~~ 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 incubated at *in situ* temperature in the dark and 1 ml subsamples were then taken after 3h, 6h, 9h, 12h and 24h. Viruses were later enumerated by the method of Brussaard (2004) to determine their rate of production over time. Virus production was determined from linear regression of ~~virus-viral~~ abundance over time (time period used for regression analysis may vary between sampling days, depending on the temporal virus abundance dynamics). Although experiments were performed with mesocosms 1, 2, and 3 as low, mid and high $f\text{CO}_2$, mesocosm 2 was lost due to leakage. Due to logistical reasons we were only able to perform these assays until day 21.

To determine grazing rates on prokaryotes, fluorescently labelled bacteria (FLB) were prepared from cultured *Halomonas halodurans* labelled with 594,6-Dichlorotriazinyl Amino fluorescein (DTAF,

40 µg ml⁻¹) according to Sherr [and](#) Sherr (1993). Frozen ampoules containing prey (1% of total bacteria) were added to triplicate 1 L incubation bottles containing whole water gently passed through 200 µm mesh. Twenty milliliter samples were taken immediately (0 h) and the headspace was removed by gently squeezing the bottle so that no air bubble remained. The samples were fixed with 1% final concentration 0.2 µm filtered gluteraldehyde (EM-grade, 25%) and stained with 0.2 µm filtered (Acrodisc® 25mm Syringe filters, PALL Life Sciences) DAPI at a 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 filtered onto 25 mm, 0.2 µm black polycarbonate filters (GE Healthcare life sciences), mounted on microscopic slides and stored at -20°C until analysis. FLBs present on a ≈0.75mm² area were counted using a Zeiss Axioplan 2 microscope. Grazing (µd⁻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.

2.4 Statistics

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; Kimmanse and Brussaard, 2010). 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 showed two main peaks in abundance, at the start of the experiment (day 4, phase I) and day 24 (phase II; Fig. 1a). At the end of phase I the high $f\text{CO}_2$ mesocosms displayed higher phytoplankton abundance than the present day (low) $f\text{CO}_2$, whereas the opposite was found for days 17-22. 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 $f\text{CO}_2$ (Fig. 1b), due to the response of Pico I and II. For all phytoplankton groups, except *Synechococcus* and Pico III, we found ~~that the algal~~ abundances in the surrounding water (Table S1) -largely comparable to the temporal dynamics in the mesocosms, with only occasionally higher abundances for the nanoeukaryotic phytoplankton groups and lower abundances for Pico I and II (Table S1, Fig. S1). The surrounding waters were more similar to the low $f\text{CO}_2$ than the high $f\text{CO}_2$ mesocosms, demonstrating that the differences between the low and high $f\text{CO}_2$ mesocosms are the effect of the elevated $f\text{CO}_2$. Phytoplankton, prokaryotes and viral abundances in the 0-17m samples were generally lower but showed similar dynamics (Figs. S1 and S2).

3.1.1 *Synechococcus*

Synechococcus (SYN) showed an initial peak in abundance on day 4 (Fig. 2a), then abundances declined, most so for the low $f\text{CO}_2$ mesocosms from days 4-7. The net growth rate was being highly strongly negatively correlated with $f\text{CO}_2$ ($R^2=0.98$, Fig. 2d). The loss measurements (only grazing, no viral lysis detected) confirmed that the total loss rate for the low $f\text{CO}_2$ mesocosm M1 was significantly higher than for the high $f\text{CO}_2$ mesocosm M3 on day 10 (0.56 vs 0.27 d^{-1}), whilst the gross growth rate did not differ significantly (Fig. 2b). Net increase in cell abundances regained at increased again from day 12, for in the low $f\text{CO}_2$ mesocosms this continued until the bloom at day 24, whilst the high CO_2 mesocosms peaked at day 15 and then dropped again before increasing from days 19-24. Despite the deviation in temporal dynamics between the treatments, SYN abundance peaked at day 24 in all mesocosms with around 4.5×10^5 cells ml^{-1} (Fig 2a) and was negatively correlated with $f\text{CO}_2$ ($R^2=0.77$). For the low $f\text{CO}_2$ mesocosms total net production was higher during this bloom was greater in the low $f\text{CO}_2$ mesocosms than in the higher $f\text{CO}_2$ mesocosms as initial abundances were lower they started lower (day 13) and ended peak abundances higher (day 24; Fig. 2a). This could be explained by a higher total loss rate for M3 than M1 on day 17 (0.33 vs 0.17). The following decline following (days 24-28) seemed largely due to reduced gross growth rates (Fig. 2b). Thereafter the trend was not so clear until the end of the experiment.

3.1.2 Picoeukaryotes I

Pico I was numerically the second most dominant group of phytoplankton, 21.26% of total phytoplankton abundances on average in the high $f\text{CO}_2$ mesocosms and 26.21% in the low CO_2 mesocosms. This amounts to 15% of total POC at high $f\text{CO}_2$, 10% at low $f\text{CO}_2$ (mean of total POC). The initial increase (peak in abundance at day 5, Fig. 3a) of these small-sized (mean cell diameter $\approx 1 \mu\text{m}$, comparable to SYN) phytoplankton already showed a slight positive trend and strong correlation with $f\text{CO}_2$ for the net growth rate (Fig. 3de, $R^2=0.95$) and abundance (Fig. 3gf, $R^2=0.8$). The higher total net loss rates (days 5 to 9; Fig. 3ed) induced resulted in a decrease in abundance, which was

stronger for the low $f\text{CO}_2$ mesocosms (as illustrated by M1) due to the significantly higher gross growth rates for the high $f\text{CO}_2$ mesocosm (represented by M3; Fig. 3b). The positive correlation of Pico I peak abundance with $f\text{CO}_2$ on day 13 (Fig. 3hg, $R^2=0.94$) was lost upon another decline in abundance. Significantly higher losses at high $f\text{CO}_2$, a combination of grazing and lysis, resulted in a more dramatic crash at high $f\text{CO}_2$ 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 $f\text{CO}_2$ was given to M3, M6 and M8 because their $f\text{CO}_2$ concentration had approached that of the remaining mesocosms. This may have stimulated the gross growth in M3 as compared to M1 (day 19; Fig. 3b) for a longer period in the high $f\text{CO}_2$ mesocosms as compared to M1 (day 19; Fig. 3b); this accompanied by Combined with higher losses at low $f\text{CO}_2$ a resulted in a positive correlation of net growth rates with $f\text{CO}_2$ was seen (Fig. 3fe, $R^2=0.71$), was seen and almost 2-fold higher net abundances at high $f\text{CO}_2$ on day 21 (Fig. 3a) correlating with $f\text{CO}_2$ (Fig. 3hi, $R^2=0.84$). Pico I was thus greatly stimulated by increased $f\text{CO}_2$, from day 3 throughout the experiment. Standing stock of Pico I remained higher at high $f\text{CO}_2$ 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 $f\text{CO}_2$ (Fig. 4d). Relatively high total losses of (0.46 and 0.58 d^{-1} on average days 6-13, in the low and high $f\text{CO}_2$ 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 are indicative of 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 $f\text{CO}_2$ (Fig. 4de, $R^2=0.82$) with peak abundances 1.4 fold higher at high $f\text{CO}_2$ (Fig. 4a). Higher losses at high $f\text{CO}_2$ then contributed to the faster decline in abundances at high $f\text{CO}_2$. 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 $f\text{CO}_2$, 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 mainly, 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 $f\text{CO}_2$ (Fig. 5d5e, $R^2=0.91$). Already directly upon the first $f\text{CO}_2$ addition (days 0-4) the abundances declined for the high $f\text{CO}_2$ mesocosms (Fig. 5a) with net growth rates negatively correlated to $f\text{CO}_2$ (Fig. 5de, $R^2=0.94$). Gross growth rates were indeed significantly higher for M1 than M3 at days 1, 4, 7 and 10 (Fig. 5b). Abundances of the Pico III group in the ambient Fjordsurrounding water followed the low $f\text{CO}_2$ mesocosms perfectly during this first period, indicating that the crash in the high $f\text{CO}_2$ mesocosms was indeed a direct (negative) effect of $f\text{CO}_2$ (Table S1). - A similar response of Pico III abundance halting in the high $f\text{CO}_2$ mesocosms and strongly increasing in the low $f\text{CO}_2$ mesocosms occurred directly after the additional $f\text{CO}_2$ 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 $5.5 \times 10^2 \text{ ml}^{-1}$ (Fig. 6a). After an initial peak at day 6, the lower $f\text{CO}_2$ mesocosms showed the highest numbers at day 17 (Fig. 6a). This seems initiated by 2.3-fold ~~increased-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 leading~~ to net growth rates correlating negatively with $f\text{CO}_2$ ~~for~~ days 10-12 (Fig. 6de, $R^2=0.83$). Viral lysis occurred ~~largely-predominantly~~ in the high $f\text{CO}_2$ 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 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 $f\text{CO}_2$ (Fig. 6ed, $R^2=0.67$).

3.1.6 Nanoeukaryotes II

The temporal dynamics of Nano II were rather erratic (Fig. 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 $f\text{CO}_2$ (Fig. 7ed, $R^2=0.61$), and was the result of an overall reduced net growth rate with $f\text{CO}_2$ (Fig. 7de, $R^2=0.56$). The subsequent decline seems the result of reduced gross growth rate (to even zero) and increased loss rate (day 20; Fig. 7b).

3.1.7 Algal POC

The calculated mean algal POC shows that $f\text{CO}_2$ had a clear positive effect on the biomass of Pico I and II (Fig. 8a; $p < 0.0001$). The effect became noticeable ~~already after only~~ a few days into the experiment and the mean Pico I and II POC concentrations in the high $f\text{CO}_2$ mesocosms stayed high during for the entire duration of the experiment. At the same time the remaining algal groups showed reduced POC at enhanced $f\text{CO}_2$ (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 $f\text{CO}_2$ 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 from the end of phase I and during phase II ~~For Nano I and II the higher algal POC concentrations became only more apparent at the end of phase I~~ (days ~~1014-1620~~ 1014-1620; Fig. S3b). Due to its small cell size, the numerically dominant SYN accounted on average for 40% of total POC. ~~We are aware that d~~Due to the exclusion of 3 mesocosms (see Material ~~and~~ Methods), the number of $f\text{CO}_2$ 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.

3.2 Prokaryote population dynamics

The prokaryotic temporal dynamics in the mesocosms ~~overall~~ 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-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 d^{-1} for 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 at the same time viral lysis was slightly (3% higher at high CO_2 – higher in the high (M1) as compared to the low fCO_2 mesocosm (M3)) (Figs. 10a-10b and bc). The decline in prokaryote abundances from days 5 to 9 seemed due to declining phytoplankton biomass (Fig. 1a) and increasing viral lysis rates (12-16 % d^{-1} representing 39% of total losses in M1 and 37% in M3 on day 11, Fig. 10b-10c). The viral production assays did not show evidence of lysogeny. Viral lysis assays showed no evidence of lysogeny for the prokaryotic community during the experiment (all phases).

From days 10-15 prokaryote dynamics became clearly affected by fCO_2 with significantly higher abundances and net growth rates at higher fCO_2 (Fig. 9a), although we cannot exclude an indirect response due to altered algal dynamics in response to higher fCO_2 . Both the HDNA and the LDNA-prokaryotes (peak abundance on day 13, Fig. 9b and c) showed significant correlation with fCO_2 ($R^2 = 0.92$ and 0.79 , respectively, total prokaryote $R^2 = 0.88$, Fig. 10c-10d). In the higher fCO_2 mesocosms the decline in prokaryote abundance following the peak at day 13 was largely the result of decreasing HDNA-prokaryote numbers (Fig. 9b). Grazing was indeed significantly higher in the high fCO_2 mesocosm M3 but the data for viral lysis were inconclusive due to a failed assay (for technical reasons) for M1 at day 14 (Fig. 10a-10b and bc). The significantly ($p < 0.01$) higher viral abundances, particularly due to the V3 group, with highest green fluorescence, for the high fCO_2 mesocosms around that time (Figs. 11a and b) around that time do seem to indicate that viral lysis in the high fCO_2 mesocosms was higher.

During phase II prokaryote abundances increased steadily until day 24 (for both HDNA and LDNA), corresponding to increased algal biomass (Fig. 110e) and low grazing rates ($0.1\text{-}0.2 \text{ d}^{-1}$; Fig. 10a-10b).

Although the overall higher prokaryote standing stock in the low $f\text{CO}_2$ 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 seen~~ higher for the low $f\text{CO}_2$ mesocosms (Fig. ~~10b~~10c). The higher prokaryote abundances in the low $f\text{CO}_2$ mesocosms ~~appeared~~ thus ~~merely~~ due to the lower grazing prior to the increase, i.e. at the end of phase I (day 14). ~~Again, lysogeny was not observed for the prokaryotic community.~~

Prokaryote abundance ultimately declined again ~~during~~(~~from~~ days 28-35), but ~~less~~ in M1-~~less~~ than in the other mesocosms (Fig. 9a). We unfortunately have no data of the prokaryote loss rates after day 25, however viral abundances increased at a steady rate of $2.2 \times 10^6 \text{ d}^{-1}$ (to a maximum of $0.9 \times 10^8 \text{ ml}^{-1}$ by day 39; Fig. 11a), implying that viral lysis was at least partly responsible for the decline in prokaryote abundance. There was no significant difference in viral abundances between the treatments during this period. ~~Estimating the viral burst size from the increase in viral abundance and concomitant decline in bacterial abundance gives on average 30 viruses per lysed bacterial cell. The addition of mytomycin C to triplicate samples in the prokaryote lysis assays allowed us to determine whether prokaryotes were subject to lysogenic infections. Our results showed no evidence of this and only relatively low levels of lytic infection until the final period.~~

4 Discussion

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 ~~as such~~ these phytoplankton

groups ~~were~~ of key importance during the experiment. Already at the start of the experiment ~~less~~ more than 95% of the phytoplankton community was ~~larger~~ smaller than 20 µm cell diameter (~~Paul et al. 2016~~), and by day 5, 70% was smaller than 2 µm (Paul et al., 2015). The -picoeukaryotic photoautotrophs Pico I and II showed a very strong fertilization effect with enhanced $f\text{CO}_2$, 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 $f\text{CO}_2$. These shifts in the size structure of the community ~~structure~~ could be explained by examining the gross growth rates in combination with the losses of the individual groups.

Overall, microbial temporal dynamics in the ~~microbial communities 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 abundances ~~in the surrounding water~~ whilst all the picoplankton abundances were lower in the surrounding waters. Higher abundances of nano-sized phytoplankton in the surrounding water ~~was~~ were likely due to upwelling of cold, CO_2 -rich deep water to the surface, bringing in inorganic nutrients, silicate particularly silicate (Paul et al., ~~2016~~ 2015). Average temperatures in all the mesocosms and surrounding waters were similar, with the upwelling ~~event~~ also being responsible for reducing the temperature ~~decrease~~ from around 15 to 8°C during phase II. Variation in temperature Along with reduced PAR (Paul et al., 2015) may have affected this generally reduced gross growth of the different phytoplankton groups ~~but our results do not show clear patterns, however no synergistic effects with $f\text{CO}_2$ could be ascertained. nor do the fluctuations relate to the differences in phytoplankton group responses to enrichment with CO_2~~ . The microbial population dynamics in the surrounding water more closely resembled those in the ambient $f\text{CO}_2$ mesocosms, and more importantly the differences were in contrast to the shifts in phytoplankton

group dynamics in response to CO₂ enrichment. This implies that enhanced fCO₂ was indeed responsible for the changes seen.

4.1 Phase 0 (days -5 to 0), before CO₂ 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 CO₂ manipulation. The flow cytometric phytoplankton community was dominated by cyanobacteria *Synechococcus* spp. (SYN) and the smallest picoeukaryotes (Pico I; both around 1 µm). (Brussaard et al., 2013), in which smaller sized algae, which are better competitors for nutrients tend to dominate (Raven, 1998; Veldhuis et al., 2005). From the start of the experiment the flow cytometric phytoplankton community (<20 µm cell diameter) was dominated by *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 $0.18\text{--}0.53 \text{ d}^{-1}$, Kuosa, 1991). ~~The net growth rates of the total prokaryotic community~~ ~~The net growth rates of the prokaryotes (0.19 d^{-1})~~ also increased, at rates of 0.22 d^{-1} and 0.14 d^{-1} for the high and low DNA (HDNA prokaryote and LDNA prokaryote), respectively ~~were also comparable, similar~~ to rates reported for this region (Kuosu, 1991). ~~Because the losses (strongly dominated by grazing) Grazing rates were between around $0.3\text{--}0.5 \text{ d}^{-1}$, the with viral lysis rates of $<2\% \text{ d}^{-1}$, indicating that bacterial their gross growth production~~ rates must have been around $0.5\text{--}0.6\text{--}0.7 \text{ d}^{-1}$.

4.2 Phase I (days 1-16)

According to Paul and coauthors (2015) ~~this phase~~ 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 ~~during phase I~~, responsible for the referred high turnover of organic matter. ~~Certainly~~ ~~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 $f\text{CO}_2$ as demonstrated by their significantly ($p < 0.05$) higher gross growth rates. Net growth rates of ~~P~~ 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 $f\text{CO}_2$ 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 $<5 \text{ }\mu\text{m}$ 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 $f\text{CO}_2$ 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 $f\text{CO}_2$ (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 have reported enhanced abundances of the picoeukaryotic ~~photoautotroph~~ ~~prasinophyte~~ *Micromonas pusilla* at higher $f\text{CO}_2$ (Engel et al., 2007; Meakin and Wyman, 2011; ~~Maat et al., 2014~~). 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 $f\text{CO}_2$ (Brussaard et al., 2013). Furthermore, Schaum et al. (2012) found that 16 ecotypes of *Ostreococcus tauri* ~~(also another prasinophyte~~ similar in size to Pico I) increased in growth rate by 1.4-1.7 fold at 1,000 ~~$\mu\text{atm pCO}_2$ compared to 400 $\mu\text{atm pCO}_2$~~ . All ecotypes increased their photosynthetic rates and those with most plasticity, most able to vary their photosynthetic rate in response to changes in $f\text{CO}_2$, were most likely to increase in frequency in the community. It is likely that the picoeukaryotes in our study, which show stimulation by $f\text{CO}_2$ 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. In this experiment nanoeukaryotes may have been outcompeted during phase I.~~

~~A net loss of 60% of the mean standing stock of Pico I at low, and 42% at high $f\text{CO}_2$ after day 5 was likely due to grazing, on average 0.26 d^{-1} , and lysis 0.18 d^{-1} (lysis in M3 only). 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 abundances of most of the phytoplankton groups increased, corresponding with a~~

decline in abundance of this ciliate (Lischka et al. 2015). Occasionally grazing rates between the high $f\text{CO}_2$ (M3) and present-day, low $f\text{CO}_2$ (M1) mesocosms differed significantly although no general trend could be observed.

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 $f\text{CO}_2$, 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 $f\text{CO}_2$ 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 $f\text{CO}_2$ (M3) and present-day low $f\text{CO}_2$ (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 $f\text{CO}_2$. Nonetheless, this did not seem to hold for the high abundance groups SYN and Pico I, nor for Pico II with comparable~~

1 abundances to Pico III. Alternatively the significantly reduced gross growth rates at high $f\text{CO}_2$ are the
2 more likely cause for the clear differences in population dynamics between high and low $f\text{CO}_2$
3 treatments.

4 In contrast, higher gross growth rates alongside a predominance of viral lysis at high $f\text{CO}_2$ was seen in
5 both Pico II and Nano I during phase I. Metabolically active cells ~~have been~~ were reported to be
6 infected at higher rates and phytoplankton growing at higher growth rates produced more viral
7 progeny, which could explain this observation (Bratbak et al., 1998; Weinbauer, 2004; Maat et al.,
8 2014). Direct effects of higher $f\text{CO}_2$ on viruses themselves are not expected as marine virus isolates
9 ~~have been~~ were found to be quite stable (both particle and infectivity) over the range of pH obtained
10 in the present study (Danovaro et al., 2011; Mojica and Brussaard, 2014). Besides lytic infection,
11 there is the potential for a lysogenic viral life cycle, during which viral DNA is integrated in the host as
12 a prophage (Weinbauer, 2004). We found, however, no evidence that the share of lysogeny
13 compared to the lytic cycle was affected. In fact, the percentage lysogeny was found insignificant
14 during the entire campaign. Mean viral abundances were higher under CO_2 enrichment towards the
15 end of phase I, which is expected to be in response to increased phytoplankton and prokaryote
16 biomass.

17 -

18 During Phase I the high turnover of phytoplankton biomass led to increasing growth of heterotrophic
19 prokaryotes (Hornick et al., 2016). The enhanced net abundances (this study) were -heavily grazed
20 and additionally viral lysis became increasingly important (to 60% of total losses at the end of phase
21 I). Bermúdez et al. (2016) reported highest biomass of protozoans around t15. This was
22 predominantly ~~the~~ heterotrophic choanoflagellate *Calliakantha natans* (Hornick et al.,
23 2016). *Calliakantha natans*- feeds selectively only on particles $<1\ \mu\text{m}$ in diameter (Marchant and
24 Scott, 1993) and so could graze on heterotrophic bacteria. During the second half of phase I
25 significantly more- prokaryotes were recorded in the high $f\text{CO}_2$ mesocosms, which was likely due to

increased availability of dissolved organic carbon at high $f\text{CO}_2$ 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 $237 \text{ fg C } \mu\text{m}^{-3}$ (Worden et al., 2004) and $196.5 \text{ fg C } \mu\text{m}^{-3}$ (Garrison et al., 2000) for pico- and nano-sized plankton, respectively, we calculated that viral lysis of phytoplankton resulted between days 9 and 13 resulted in the release of 1.1 and $12.4 \text{ ng C ml}^{-1}$ 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.

~~The high turnover of phytoplankton biomass led to increasing Phase I total prokaryote abundances. These were also heavily grazed and additionally viral lysis became increasingly important (to 60% of total losses at the end of phase I). The significantly more abundant prokaryotes were significantly more abundant at in the high $f\text{CO}_2$ mesocosms during the second half of phase I was likely due. This may be due to increased availability of dissolved organic carbon at high $f\text{CO}_2$ due to by higher rates of viral lysis of phytoplankton. We measured higher lysis rates in the high CO_2 mesocosm (M3) than in the control (M1) Pico II and Nano I initially (day 6) but consecutively also Pico I and Nano II (day 10) on day 6 for Pico II and Nano I and on day 10 for Pico I and II, Nano I and II at higher $f\text{CO}_2\text{CO}_2$. The increased prokaryote standing stock did not sustain and was mainly grazed down to abundances comparable to the present-day $f\text{CO}_2$ mesocosms at day 16. Stimulation of prokaryote abundances was seen in a previous mesocosm campaign apparently due to higher availability of TEP and aminopeptidase activity (Endres et al., 2014). Increased TEP production is often associated with low nutrient, high $f\text{CO}_2$ conditions (Weinbauer et al., 2011).~~

Mean virus abundances increased ~~were higher under CO₂ enrichment at higher fCO₂ levels~~
~~towards the end of during phase I, which is expected to be in likely a response to increased~~
~~phytoplankton and prokaryote biomass. During previous mesocosm studies no direct or indirect~~
~~effects of CO₂ addition on the abundance of viruses/bacteriophages were seen (Paulino et al., 2007;~~
~~Larsen et al., 2008; Brussaard et al. 2013). Direct effects of higher fCO₂ on viruses themselves are not~~
~~expected as marine virus isolates have been found to be quite stable (both particle and infectivity)~~
~~over the range of pH obtained in the present study (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. examined whether increased fCO₂ concentrations affect~~
~~the type of viral life cycle but found no evidence lysogeny was affected.~~

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 ~~net~~ 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. ~~Furthermore, higher abundances of the~~
~~smallest size class of the ciliate *M. rubra* were seen in the higher fCO₂ mesocosms (days 19-31,~~
~~Lischka et al., 2015) which may explain the lower abundances of Pico III due to its ability to “rob”~~
~~chloroplasts from cryptophytes (Lischka et al. 2015). *M. rubra* abundance decreased from day 3 to~~
~~day 17, which may be due to the decreased Pico III cryptophyte prey abundances.~~

Synechococcus bloomed during phase II, however with significantly ~~reduced-lower~~ abundances at higher $f\text{CO}_2$. So although ~~the~~ 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 pPicoeukaryotes, needing extremely efficient CCMs due to the inefficiency of its Rubisco. Able to, which can concentrate CO_2 to up to 1000-fold higher than the external medium (Badger and Andrews, 1982), they may attain maximal growth rates. Thus at the present-day current CO_2 concentrations they may attain maximal growth rates (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 $f\text{CO}_2$ in Phase I, we found during phase II during Phase II (from days 16-25) decreased abundances of HDNA-prokaryotes at high $f\text{CO}_2$, which 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 $f\text{CO}_2$. 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 $f\text{CO}_2$ 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 ~~still~~ clearly reflected in the Chlorophyll a concentration, particulate organic carbon and phosphorus, ~~but and~~ also in the dissolved organic carbon (DOC) pools in Phase III (Paul et al., 2015). This increase in DOC at high $f\text{CO}_2$ (Paul et al., 2015), may originate from viral lysis of prokaryotes and phytoplankton (Suttle 2005, Lønborg ~~Lønborg et al.~~, 2013). We measured ~~indeed~~ higher viral lysis rates for SYN, Pico II and Nano I, and similar lysis rates but higher standing stock of Pico I at high $f\text{CO}_2$ ~~on day 31~~. ~~Additionally after day 22 total viral abundances increased steadily until the end of the experiment.~~ Alternatively, increased $f\text{CO}_2$ coupled with low nutrient ~~availabilities may also have~~ can stimulated photosynthetic release of DOC and ~~subsequently~~ 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, may and is thought to~~ promote aggregation and sinking of particulate organic matter (Brussaard et al., 2008; Lønborg et al., 2013), ~~and~~ 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~~ $f\text{CO}_2$ treatments for the current study ~~indicating that the change in phytoplankton community composition did not result in altered transport of POC (live or dead) (Paul et al., 2015).~~ Still, 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., 20165). 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 ($>20\text{ }\mu\text{m}$) (Paul et al., 20165). 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 and in combination with the relative share of grazing and viral lysis (Brussaard et al., 2008).

Additionally a significant negative correlation was reported between diatom abundance and fCO_2 during phase III (Paul et al., 2015), which is similar to a previous ocean acidification mesocosm experiment (Brussaard et al., 2013). Brussaard et al. (2013) suggested that diatom growth was reduced due to increased uptake of the growth limiting nutrients by the picoeukaryotes at high fCO_2 and may result in reduced sedimentation.

5 ~~Future perspectives~~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 $<20\text{ }\mu\text{m}$ size fraction (Paul et al., 20165). 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 ($0.2\text{--}3\text{ }\mu\text{m}$) and nano-sized ($3\text{--}20\text{ }\mu\text{m}$) photoautotrophs were seen during the experiment which were also reflected in size-fractionated Chl *a* concentrations (Paul et al., 20165). 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 CO₂ enrichment favours the net growth of the very small-sized (1 µm) picoeukaryotic phytoplankton. This positive response with fCO₂ is very specific, as neither *Synechococcus* spp., Pico III, nor ~~nor~~ of the nanoeukaryotic phytoplankton groups displayed enhanced growth. Increasing atmospheric fCO₂ leads to a number of further global changes, e.g. increasing sea surface temperatures (SST) which in turn strengthens vertical increase stratification and, ~~shallowing the mixed layer reducing nutrient supply to in surface waters depth~~ (Sarmiento et al., 1998; Toggweiler and Russell, 2008). Such changes in physicochemical conditions have been reported to favour small cells, largely because of reduced nutrient supply to the surface waters (Cermeno et al., 2008; Riebesell et al., 2009; Li et al., 2009; Craig et al., 2013; ~~Li et al., 2009~~; Mojica et al., ~~2015~~2016 a, b; Riebesell et al., 2009). 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 additional increase in abundance of specifically the small picoeukaryotes in direct response to increased levels of CO₂ has been reported a few times earlier () and as such seems a general feature.~~

Also the overall activity of prokaryotes is expected to be affected not only by viral lysis of phytoplankton and prokaryotes themselves, ~~but~~ but also by higher SST. This ~~due to results in~~ increased enzyme activities, bacterial production ~~but 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; Piontek et al., 2009; Wohlers et al., 2009; 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; ~~Riebesell and Tortell, 2011; Riebesell et al., 2009~~). At the same

time, through viral lysis and subsequent microbial respiration ~~are the main natural sources of~~
atmospheric CO₂ ~~that negatively affect~~ the biological pump is negatively affected by the production
of atmospheric CO₂ (del Giorgio and Duarte, 2002). ~~Overall, the evidence presented in the current~~
~~study indicates~~ suggests that ~~besides~~ CO₂ enrichment favours small-sized picoeukaryotic
phytoplankton, which is further strengthened by multiple other factors increased SST and
strengthened enhanced vertical stratification. By and large these findings changes will tend to reduce
carbon sequestration ~~promote a more regenerative system with potential consequences for~~
~~ecosystem production and functioning~~.

Author Contribution

Design and overall coordination of research by CB. Organization and performance of analyses in the
field by KC. Data analysis by KC and CB. Design and cCoordination of the overall KOSMOS mesocosms
project by UR. All authors contributed to the writing of the paper.

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Table 1. $f\text{CO}_2$ concentrations (μatm) as an average for the duration of the experiment following CO_2 addition and specification of this CO_2 level as low, medium or high. *denotes mesocosms sampled for grazing and viral lysis assays







<i>Mesocosm</i>	<i>M1*</i>	<i>M5</i>	<i>M7</i>	<i>M6</i>	<i>M3*</i>	<i>M8</i>
<i>CO₂ Level</i>	<i>LOW</i>	<i>LOW</i>	<i>INTERMEDIATE</i>	<i>INTERMEDIATE</i>	<i>HIGH</i>	<i>HIGH</i>
<i>Mean $f\text{CO}_2$ (μatm) days 1-43</i>	365	368	497	821	1007	1231
<i>Symbol</i>						

Figure captions

Fig. 1. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) total phytoplankton and **b)** total eukaryotic phytoplankton, ie. all except the prokaryotic photoautotroph *Synechococcus* spp.. Lines indicate the start and end of phase II. The colours and symbols used in the legend are consistent throughout subsequent figures and, in parenthesis, is shown the mean $f\text{CO}_2$ across the duration of the experiment ie. days 1-43 .

Fig. 2. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) total prokaryotic phytoplankton, *Synechococcus* spp., whereby the lines indicate the different phases (I-III). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (~~control~~ low $f\text{CO}_2$, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rates derived from exponential regression of the net SYN abundances, versus average $f\text{CO}_2$ for days 4-7.

Fig. 3. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) picophytoplankton I (Pico I). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (~~control~~ low $f\text{CO}_2$, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rates derived from exponential regression of the net Pico I abundances, versus average $f\text{CO}_2$ for days 1-5; **e)** days 5-9; **f)** days 18–t21, a negative growth rate indicates cell loss. **g)** Phytoplankton cell abundance versus actual $f\text{CO}_2$ for Pico I on days 5; **h)** 13 **i)** 21.

Fig. 4. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) picoeukaryotic phytoplankton II (Pico II). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. A rate of zero is displayed as a 0 in the colour of the mesocosm it relates to. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (control, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rate determined from the net Pico II abundances, versus average $f\text{CO}_2$ for days 12-17. **e)** Phytoplankton cell abundance versus actual $f\text{CO}_2$ for Pico I on day 17.

Fig. 5. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) picoeukaryotic phytoplankton III (Pico III). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. No data indicates a failed experiment and a rate of zero as a 0 in the colour of the mesocosm it relates to. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (~~control~~ low $f\text{CO}_2$, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rate determined from the net Pico III abundances, versus average $f\text{CO}_2$ for days 1-2. **e)** Phytoplankton cell abundance versus actual $f\text{CO}_2$ for Pico I on day 24.

Fig. 6. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) nanoeukaryotic phytoplankton I (Nano I). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. No data indicates

a failed experiment and a rate of zero as a 0 in the colour of the mesocosm it relates to. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (~~control~~ low $f\text{CO}_2$, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rate determined from the net Nano I abundances, versus average $f\text{CO}_2$ for days 10-12, a negative growth rate indicates cell loss **e)** Phytoplankton cell abundance versus actual $f\text{CO}_2$ for Nano I on day 17.

Fig.7. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) nanoeukaryotic phytoplankton II (Nano II). **b)** Gross growth rates and total loss rates in mesocosms M1 and M3. Gross growth displayed as bars above the X- axis and total losses as bars below the X-axis. No data indicates a failed experiment and a rate of zero as a 0 in the colour of the mesocosm it relates to. Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1$. **c)** Abundances for mesocosm M1 (~~control~~ low $f\text{CO}_2$, blue line) and mesocosm M3 (high CO_2 , red line). **d)** Specific growth rate determined from the net Nano II abundances, versus average $f\text{CO}_2$ for days 6-17 (M1, days 6-16) **e)** Phytoplankton cell abundance versus actual $f\text{CO}_2$ for Nano II on day 17 (M1, day 16).

Fig.8. POC calculated from mean cell abundances applying conversion factors of $237 \text{ fg C } \mu\text{m}^{-3}$ (Worden et al.2004) and $196.5 \text{ fg C } \mu\text{m}^{-3}$ (Garrison et al. 2000) for pico- and nano-sized plankton, respectively according to Mojica et al. (2015), cellular carbon was calculated based on the average cell diameters. **a)** Temporal dynamics of Pico I and II **b)** Temporal dynamics of POC for all other eukaryotic groups ie. Pico III, Nano I and II.

Fig.9. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) total heterotrophic prokaryotes (HP) **b)** High DNA fluorescence heterotrophic prokaryotes (HDNA-HP) **c)** Low DNA fluorescence heterotrophic prokaryotes (LDNA-HP) .

Fig.10. a) M1 (low $f\text{CO}_2$) and M3 (high CO_2) temporal dynamics of total heterotrophic prokaryotes (HP) abundances **b)** grazing rates (d^{-1}) (bars below the X-axis). Significant differences between mesocosms are marked: $p \leq 0.001^{***}$, $p \leq 0.01^{**}$, $p \leq 0.05^*$, $p \leq 0.1^{\cdot}$. **c)** Viral lysis as percentage of HP standing stock in mesocosm M1 (low $f\text{CO}_2$, blue) and M3 (high $f\text{CO}_2$, red) **d)** Total HP cell abundance versus actual $f\text{CO}_2$ on day 13. **e)** Mean prokaryote abundances in high (3,6,8) and low CO_2 mesocosms (1,5,7) vs total particulate organic carbon (POC) calculated from total cell abundances, ie. all groups measured by flow cytometry, for both series $R^2=0.7$.

Fig.11. a) Temporal dynamics of depth-integrated upper layer (0.3–10 m) total virus abundances, **b)** Virus group V3, discriminated by its higher green nucleic acid-specific fluorescence.

