Dear Editor,

We are grateful for both your comments and the constructive suggestions on our manuscript from the two reviewers. Please find below our point by point replies to the comments and suggestions as well as the revised version of our manuscript 'Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community'.

As suggested, the manuscript, in particular the discussion, has been condensed and the number of figures and tables reduced to improve readability. Changes made in the text are indicated in blue.

We look forward to hearing a response on the manuscript soon.

Yours Sincerely,

Allanah Paul
*Corresponding author, on behalf of all co-authors*

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**Response to review by L. Yebra:**

We thank Lidia Yebra for her review and constructive comments on our manuscript. We considered all comments and suggestions when revising the manuscript and have responded below with our comments and description of changes made to the manuscript.

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Specific comments:

**Comment 1 by L. Yebra:** *The MS states that no nutrients were added during the experiments. However in Fig. 4 several 15N2 additions are shown. These additions are not mentioned elsewhere in the MS. This is a very important aspect that needs clarification.*

**Author response:** We thank the reviewer for highlighting this point. As correctly identified by the reviewer, two $^{15}N$-$N_2$ additions were made, as is indicated in Fig. 3. However $^{15}N$-$N_2$ (gas) is an isotope tracer specific for $N_2$-fixing organisms and is not a nutrient which is accessible for the wider plankton community. Hence this was not described in the Methods section as a nutrient addition. The timing, and not the nature of the tracer addition, was relevant for the data and sampling schedule presented in this manuscript (see also response to comment 3 from L. Yebra below). We have shifted a statement that no nutrients were added in this mesocosm study to the methods section (p.5, line 5) from the results section. In addition, we have clarified this in the text to read 'no dissolved inorganic or organic nutrients ...'. A brief comment to describe the addition has been added to explain this clearly in the manuscript (p. 5, lines 2 - 5). Further details on the $^{15}N$-$N_2$ isotope tracer addition will be provided in an accompanying manuscript by Paul et al. in this Special Issue which focuses on diazotroph activity and abundance in this mesocosm study. A citation to this manuscript (Paul et al., in prep.) has been added (Methods, section 2.1, p. 5, line 4).

**Comment 2 by L. Yebra:** *In section 2.5.3. Methodology for POM sampling does not include pre-screening of water to remove zooplankton. How was this dealt with?*

**Author response:** No pre-screening to remove zooplankton was conducted for the total particulate matter sampling. We sampled for particulate matter <55 µm to remove large zooplankton and particles, as described in '2.5.3 Particulate material (C, N, P, Si)' (p. 9). The total particulate carbon (TPC) concentrations in the total and <55 µm size fractions was almost identical with an average difference between size fraction of 0.4 µmol $L^{-1}$ (~ 2% of total TPC pool) across all mesocosms and all sampling days.

**Comment 3 by L. Yebra:** *In general variables sampled and period of sampling is not clear. For example, PON<10 data are shown only from day 20 onwards but not in all MC, but nothing is mentioned in Methods. Also, zooplankton community was sampled and is not mentioned until half way into the Discussion. A clearer explanation of what was collected/analysed and when is needed, a summary table would be useful.*

**Author response:** Thank you for pointing out this ambiguity in the methods description. All data present for particulate organic matter (POM) <10 µm was shown in Fig. 15 (submitted 'Biogeosciences Discussion' manuscript, now Fig. 12). Sampling for this size fraction, however, only occurred for particulate nitrogen (and carbon) from *t23* onwards and only for four mesocosms (M5, M3, M6, M8). This is related to the timing of the $^{15}N$-$N_2$ tracer addition on *t22* to only these four mesocosms. We wanted to exclude filamentous cyanobacteria from the particulate matter pool to observe tracer uptake or transfer from larger diazotrophs into smaller organisms in PON pool < 10 µm. A more detailed description of this sampling regime has been added to the Methods section in the revised version of the manuscript (p. 9 , lines 16 – 18) and a summary table of all sampled variables and respective sampling frequencies and methods are also included (Table 3). Further details about the response of the zooplankton community and relevant methods used in this study will be presented in Almén et al., Lischka et al., and Vehmaa et al., also under preparation for submission to this same Special Issue in Biogeosciences.

**Comment 4 by L. Yebra:** *In section 3 and Figs. 6-7, M8 is selected as representative for all MC. Why? Please provide statistical data to support your choice.*

**Author response:** We thank the reviewer for pointing out that this was not described sufficiently previously in the manuscript. One mesocosm was chosen arbitrarily (here M8) to show vertical profiles of temperature, salinity and density because all mesocosms reacted similarly based on integrated water column temperature and salinity. As can be seen in Fig. 4, the average water column temperature in each mesocosm was practically identical. We calculated a standard deviation on a daily basis for the average temperature for all mesocosms (excluding the Baltic) which ranged between 0.01 and 0.33°C (*t39*, absolute difference between highest and lowest temperature was 0.9°C and is visible in Fig. 5). The same calculation for average water column salinity gave a range of daily standard deviation of 0.00 to 0.02 with a maximum range in salinity between mesocosms of 0.05. The typical daily

difference between mesocosms for temperature and salinity was 0.04°C and 0.01, respectively. This information has been included in the Methods section in the revised version of the manuscript (p. 13, lines 1 - 4).

**Comment 5 by L. Yebra:** *Also in Results, there are several statements about similarities, increases and decreases but no statistical data are provided. Please specify if they are statistically significant or not. E.g. P6878, L22, P6881, L11, P6882, L18.*

**Author response:** As $fCO_2$ was the key independent variable in this study, detected $CO_2$-related differences between mesocosms in each phase were considered to be represented in the linear regression analyses completed (see Tables 4-6). Additional background statements made about increases or decreases in a particular variable (eg. P6881, L11, dissolved silicate concentrations, see also Fig. 10 in revised manuscript) were considered to be clearly distinguishable by looking at the figures. Hence no statistical tests were carried out to determine the specific effect of time as we do not consider this to be critical to the interpretation of the data set and the response to the manipulated variable of interest, $fCO_2$.

**Comment 6 by L. Yebra:** *P6881, L5-10: Given that a profound increase in zooplankton abundance occurred in Phase II (P6888) how do you explain the decrease/stable values in ammonium?*

**Author response:** If we assume bottom-up control of phytoplankton growth by inorganic macronutrient concentrations, nitrogen (N) was in high demand as this plankton community had low fixed N concentrations present and there was no substantial bloom in $N_2$-fixing filamentous cyanobacteria. Hence we would generally assume that ammonium released through organic matter respiration by zooplankton would have been almost immediately assimilated by the phytoplankton community as it is highly bioavailable.

**Comment 7 by L. Yebra:** *Section 3.6. L28, 'in all MC up to 90% of POM was attributed to TPC<10 (data not shown)', looking at Fig. 15 it seems that POM<10 was analysed not in all MC, and data of C:N in POM are shown only from day 20 onwards. Please clarify.*

**Author response:** We have added more precise details about the sampling time period and mesocosms that were sampled for POM < 10 µm in the methods section (p. 9, lines 16 - 18; see also response to reviewer comment 3 above) and also refer to the sampling summary table (Table 3).

**Comment 8 by L. Yebra:** *How do you explain that TPC total correlates with CO2 but not TPC < 55um or TPC < 10 in Phase III? Also given its importance, why (are) TPC <10 data not shown?*

**Author response:** We have modified Fig. 12 in the revised version of the manuscript to include data for size fractions of TPC (total, <10 µm). We are confident in this correlation between $CO_2$ and $TPC_{tot}$ as this positive effect was also detected in Chl *a* and TPP concentrations, two independent analyses. We show the <10 µm data to highlight the importance of this small size fraction, containing picoplankton, to the total TPC pool (see p. 16, lines 5 – 7). There is no clear (biological) reason that we could identify from the data to explain why $CO_2$ correlates with the total fraction but not with the smaller size fractions in Phase III. It is also important to recognise the small size of the $CO_2$ effects detected in this study in terms of absolute concentrations. Hence, if there was an additional source of noise in the analyses, e.g. during the pre-filtration step, this may blur or mask any effect that was truly present in the sample.

**Comment 9 by L. Yebra:** *Section 3.7. According to Fig. 16, cyanobacteria abundance was highest during both Phases II and III. Please rephrase.*

**Author response:** This has been modified in the revised version of the manuscript.

**Comment 10 by L. Yebra:** *Fig. 17. Given that your mass balance calculations give % of pigments > 100% and > 0 % in some cases, how reliable are these calculations and their results.*

**Author response:** As correctly pointed out, % of pigments in different size classes were over 100% and less than 0% based on our calculations. This is described in the caption of Fig. 15 (revised manuscript). We have now added a comment (p. 17, lines 10 - 16) to acknowledge this in the text and have identified factors (nature of size fractionation filtration, problems with low concentrations, particularly in the size fraction > 20 µm) which may explain this discrepancy in the mass balance. However, we put emphasis on the increase in the proportion of Chl a <2 µm rather than the absolute concentrations. Both the increase in % Chl a <2 µm and decline in Chl a 2 - 20 µm are supported by flow cytometry data with an increase in picoeukaryotes abundance, as mentioned in the manuscript (p. 17, lines 19 – 20), and a decline in nanoeukaryote abundances during Phase II (*t17 – t30*), which will be presented in

Crawfurd et al. (in preparation) in this Special Issue. Hence we believe our use and interpretation of the results of this calculation are robust despite short-comings in the mass balance calculation.

**Comment 11 by L. Yebra:** *Discussion, it is very difficult to review this section. Not one but 7 papers in prep. are cited, that contain additional variables/information that has not been mentioned before in the text. For example in P6884 zooplankton is suggested to be partly responsible for an increase in POM during Phase I, however no sampling or assessment of zooplankton variables is mentioned in the text until P.6888 (4 pages later), when the authors cite a work in prep to state that zooplankton abundance increased in Phase II. The same occurs or the abundance of picoeukaryotes (P6883), bacterial activity (P6889), carbon fixation or respiration (P6890), etc. A full list of variables sampled during the experiment is needed in Methods, even if they are not presented in this MS, in particular those that are used to support the Discussion.*

**Author response:** A table summarising all variables in this study and the respective manuscripts is now included in the revised version of this manuscript (Table 3).

**Comment 12 by L. Yebra:** *P6886, L3-5, 'the correlation between temperature and organic matter pools will be discussed', however no statistical data are presented relating temperature with the mentioned variables in the following sections. Please add this information, eg. P6888, L27.*

**Author response:** During restructuring of section 4.3 in the Discussion, this particular reference to temperature (P6888, L27, submitted BGD manuscript) was removed. However we are grateful for the reviewer comments on the use of the term 'correlation' without the supporting statistics. These statements were made in reference to the obvious temperature decreases and increases (see Fig. 4) which occurred around the same time as a decrease in Chl *a*. However we did not complete any statistical tests to confirm a correlation with temperature as this was not the independent variable of interest in this study. We have now checked for references to temperature (e.g. Section 4.1) and have clarified these in the revised version of the manuscript to remove any ambiguity of statistical correlation.

**Comment 13 by L. Yebra:** *P6888, L13, by 'non-chl containing organisms' do you mean non-autotrophic microplankton? Please specify.*

**Author response:** We used this term to incorporate all organisms that do not contain chlorophyll *a* (Chl *a*) but contain carbon without specifying a size range as this ratio was calculated from TPC and Chl *a* concentrations in the total particulate matter fraction. We have changed this to read 'from autotrophic to heterotrophic organisms' to better describe the plankton community referred to here.

**Comment 14 by L. Yebra:** *Section 4.4., Zooplankton is suggested as grazer controlling the phytoplankton pool (P.6890, L16) and picoplankton 'must aggregate and be eaten by zooplankton in order to sink' (P6891, L8-11), hence in a future scenario the authors hypothesize that organic matter is retained in the upper column and not exported downwards. My question is: how does microplankton grazers fit in your hypothesis? Have they been considered in the experiment or in Lischka et al. In prep.?*

**Author response:** We thank the reviewer for bringing this up as microplankton grazers were not considered explicitly in this particular manuscript. Microzooplankton abundances (ciliates) will be presented in Lischka et al. in this Special Issue. Rates of microplankton grazing on phytoplankton will be presented and discussed in Crawfurd et al. along with lysis rates, specifically of picoeukaryotes (see also Table 3 in revised manuscript). While microplankton grazing presents an interesting point to ponder with respect to sinking flux, in complex plankton assemblages, such as that in the mesocosm study presented here, there are many possible explanations for retention of organic material in the upper water column. One such example is due to changes in DOC bioavailability (as suggested by Referee #2). However we do not have any concrete mechanistic evidence for a particular hypothesis at this stage.

**Comment 15 by L. Yebra:** *And diel migrant zooplankton?*

**Author response:** This is indeed also an interesting point to reflect on, but we do not have any detailed or conclusive information about these patterns. It is also not possible to resolve how diel vertical migration may have affected sinking material flux due to the vertical and sampling resolution in this study. We can only comment on the temporal variations in zooplankton abundances in relation to phytoplankton abundance and collection of sinking material with a temporal resolution of two days and mesozooplankton sampling weekly. Hence, as any conclusions would be highly speculative based on the available data, we did not incorporate this discussion point in the manuscript.

**Comment 16 by L. Yebra:** *Conclusions, first sentence states that 'fluctuations in temperature correlated well to Chl...' but no statistical data related to temperature are provided in Results or Discussion.*

**Author response:** Please see response to reviewer comment 12 above.

**Comment 17 by L. Yebra:** *POM repackaging by zooplankton mediated sinking flux', see comment 14.*

**Author response:** Please see response to reviewer comment 14 above.

Technical corrections:

**L. Yebra:** *P6868, L26: According to Fig. 4 t5 should be t-5.*

**Author response:** This has been corrected accordingly.

**L. Yebra:** *P6881, L24: It is true that both POM and Chl were higher in Phase I than in II-III, but POM did not 'mirrored Chl' in Phase I. Please rephrase.*

**Author response:** This sentence was rephrased and now reads 'Particulate C, N and P concentrations were higher in Phase I than in Phase II and III, (Fig. 12), as also observed for Chl *a*.'

**L. Yebra:** *P6884, L25, to my knowledge, there is no need to state the year of a personal communication.*

**Author response:** This citation has now been updated as this data set is included in another manuscript which is under preparation for this Special Issue.

**L. Yebra:** *Fig. 9, M2 was discarded, remove its pH panel.*

**Author response:** The pH panel for M2 has been discarded from Fig. 9 (now Fig. 8) in the revised version of the manuscript.

**L. Yebra:** *Fig. 10, panel a and b are equal. Move Baltic data to right Y axis in Fig. 10a and delete panel b.*

**Author response:** Figure 10 has been modified in the revised version of the manuscript (see Fig. 9).

**L. Yebra:** *Fig. 14a/15a, Fig. 14a and 15a are equal. I suggest removing panel 14a as in the text these data are more related to the other panel in Fig. 15 than to Fig. 14.*

**Author response:** Through restructuring of the discussion, Figure 15 is no longer referred to in the Discussion section and so has been removed.

**L. Yebra:** *Fig. 16, 'Baltic pigment concentrations are not shown because of different scale required'. Please use right Y axis to add those data.*

**Author response:** Through restructuring of the discussion, references to the Baltic phytoplankton pigment concentrations have been removed and so no longer need to be added using a right Y axis.

**L. Yebra:** *Some MS in prep. are cited as (in prep) and other as (2015), please amend.*

**Author response:** All cited articles in preparation, apart from Schulz et al., are for submission for this Special Issue and were originally cited as in prep. in the submitted manuscript. In the editing process, this was changed to 2015. We have changed these back in the text as well as in the reference list to 'in prep.' or 'in preparation' respectively. When these manuscripts are submitted and accepted for review before the final submission of this manuscript, this will be adjusted accordingly.

**Response to review by Anonymous Referee #2:**

We thank the reviewer for their useful and constructive comments on this manuscript which helped in particular to focus and refine the discussion. Our responses to reviewer comments, including modifications to the manuscript, are detailed in the following:

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**Comment 1 by Reviewer #2:** *Paul et al. present an enormous amount of data from the KOSMOS mesocosm experiment. In fact they probably present too much data in that this manuscript reads as if it was pulled directly from a PhD dissertation with little distillation. Indeed a paper with 5 weighty tables and 18 figures is too much. Part of the reason for delay in getting this review turned around is directly related to trying to understand what the story was with the data. Specifically many of the Discussion sections read like rewrites of the results and thus are way too long for what is said. For example, Section 4.1 remove the 'environmental' statements as this is really about closed mesocosms, and the link to the environment isn't that strong and just proves a distraction.*

**Author response:** As the reviewer highlights, this manuscript contains a lot of data and figures. We believe that these provide important biogeochemical, chemical and physical information which together build a solid picture of the study. The influence of $CO_2$ on particulate and dissolved matter pools and fluxes, analysed in details in this manuscript, sets the scene for more specialised manuscripts which are currently under preparation (see Table 3). Nonetheless we agree with the reviewer that some parts of the Discussion would better fit in the Results section. For example, as suggested by the referee, we have shifted the 'environmental' statements regarding the initial conditions in the Tvärminne Storfjärden from Discussion section 4.1 to the Results section 3.1. We have also condensed the discussion by approximately three pages to focus more on the mesocosms and removed redundant environmental statements as well as removed two figures.

**Comment 2 by Reviewer #2:** *Phase 1 (section 4.2), there are no differences in contrast to expectations, don't need 1+ pages to say that.*

**Author response:** We have now restructured and condensed this section to focus the discussion on the flux of carbon into the DOC pool and into sinking particle flux in Phase I.

**Comment 3 by Reviewer #2:** *Section 4.3 ends with picoplankton were always affected by CO2 but were not abundant enough early on to impact the total. However, C:N ratio in the total was much greater than C:N in the <10 fraction so how is it possible that their increase relative importance, with a lower C:N, accounted for even the higher C:N in the total?*

**Author response:** As the reviewer correctly states, picoplankton appeared to be affected (positively) by $CO_2$ from early on in the experiment but were not abundant enough to influence particulate or dissolved matter pools. We are not sure if we correctly understood the reviewer's comments here, but as this is unclear we decided to remove this statement from the discussion (P6889, L28/29; P6890, L1) to avoid confusion and instead show TPC concentrations for the total and < 10 µm size fractions (Fig. 12).

**Comment 4 by Reviewer #2:** *Section 4.4 the discussion of flow into the DOC pool is weakened without rates of DOC production or consumption, which seem like they are presented in a companion paper in this issue?*

**Author response:** Bacterial production rates and respiration rates will be presented and discussed in accompanying papers (Hornick et al., in prep; Nausch et al., in prep; Spilling et al., in prep., Table 3 in the revised manuscript).

**Comment 5 by Reviewer #2:** *While DOC concentrations are higher in the CO2 treatments how do we know it isn't due to a reduction in its bioavailability, or is this assessment related to the hypothetical reduction in respiration?*

**Author response:** This is a valuable point that the reviewer raises about DOC bioavailability that was not explicitly considered in the manuscript. Higher DOC concentrations may have been, at least in part, due to a reduction in bioavailability. Unfortunately we have no information about DOC lability and so we cannot confirm this with the available data from this study. However, lower respiration and bacterial remineralisation rates (observed during the experiment, data presented in Spilling et al. in prep. and Hornick et al. in prep.) under elevated $CO_2$ could also explain the measured higher DOC concentrations in the higher $CO_2$ treatments. We have included this point in the discussion (p. 21, lines 15 – 17).

**Comment 6 by Reviewer #2:** *The discussion seems to focus on the channelling of carbon from POM to DOM cycling but isn't really clearly presented.*

**Author response:** The discussion was focussed on $CO_2$-related differences in particulate and dissolved matter pools and fluxes with reference to the size structure of the plankton community. We have reworked and restructured the discussion and hope that this message is now more clearly presented in the revised version of the manuscript.

**Comment 7 by Reviewer #2:** *Section 4.5, seems like it should be in the conclusions more than its own stand alone section as it is all just about the hypothesis that high natural variability has selected for a community that doesn't respond in a dramatic way to CO2 enrichment – no data related to this topic is actually presented.*

**Author response:** We thank the reviewer for highlighting this. We tried to integrate this information into the conclusions, as suggested, however felt that the discussion about this point was too detailed to be included solely in the conclusions and thus justify inclusion as a separate section in the discussion.

**Comment 8 by Reviewer #2:** *I would strongly recommend the authors refocus the discussion and clearly state the story they are making. I think that the idea of a muted response to OA when nutrients are low is really important and so the basis of their study is really exciting and provides a great 'end-member' to the continuum of OA responses.*

**Author response:** We thank the reviewer for their encouraging thoughts on this study and its potential contribution to the literature on the responses to ocean acidification. Please also see response to reviewer #2 comments 1, 2, and 6.

**Comment 9 by Reviewer #2:** *I do have a question about the removal of outliers, specifically that it seems there is a high amount of outlier exclusion. I'm not a statistician but is it acceptable to remove so many data points? Is there a belief that this was a sampling issue? Should we be concerned about the broader dataset or is this telling us something?*

**Author response:** In many variables no outliers were removed, for example Chl *a*, pH, DIC, dissolved silicate, sinking particle flux. The decision to remove a small number of outliers in some data sets was made carefully and based on a statistical test for outliers (Grubb's test). While in a few data sets numerous outliers were removed (e.g. dissolved $NO_3^-$ + $NO_2^-$ , see Fig. 10A), these variables are often prone to errors in measurements because concentrations are low (nanomolar range) and are challenging to measure (e.g. dissolved inorganic nutrients). Other data sets are the result of a mass balance or calculation (e.g. dissolved organic nutrients,

particulate matter stoichiometry) which combines the errors of two measurements. However we do not believe that these select examples bring the whole data set into question nor compromises conclusions presented in the manuscript.

Limited specific comments:

**Anonymous Reviewer #2:** *Table 1: Lomas et al. reference is North Atlantic, not Pacific.*

**Author response:** This has been corrected accordingly.

**Anonymous Reviewer #2:** *Is Figure 1 really necessary – information in there seems tangential at best to the story.*

**Author response:** Figure 1 was included as this gives a clear depiction of natural variability in pH in the Baltic Sea compared to other oceanographic regions. However, in light of the reviewer's criticism concerning the large number of figures and tables, we have removed this from the revised version of the manuscript.

**Anonymous Reviewer #2:** *Figure 4, useful but not really necessary.*

**Author response:** Here, we disagree with the reviewer that Fig. 4 (experiment timeline, now Fig. 3) is not really necessary as this provides an important and coherent overview of various manipulations and see this as a useful element in the manuscript. In particular, this manuscript is considered an 'overview paper' guiding the other publications in this special issue with background experimental design, sampling regime and biogeochemical setting.

**Anonymous Reviewer #2:** *Figure 6, symbols horizontally – issue in upload or trying to show something?*

**Author response:** These horizontal symbols are the values of average water column salinity, temperature and density from the CTD profiles. These have now been removed from Fig. 5 (was Fig. 6) and Fig. 8.

# Effect of elevated $CO_2$ on organic matter pools and fluxes in a summer Baltic Sea plankton community

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## Abstract

Ocean acidification is expected to influence plankton community structure and biogeochemical element cycles. To date, the response of plankton communities to elevated $CO_2$ was studied primarily during nutrient-stimulated blooms. In this $CO_2$ manipulation study, we used large-volume (~55 $m^3$) pelagic in situ mesocosms to enclose a natural summer, post spring-bloom plankton assemblage in the Baltic Sea to investigate the response of organic matter pools to ocean acidification. The carbonate system in the six mesocosms was manipulated to yield average $fCO_2$ ranging between 365 and ~1230 µatm with no adjustment of naturally available nutrient concentrations. Plankton community development and key biogeochemical element pools were subsequently followed in this nitrogen-limited ecosystem over a period of seven weeks. We observed higher sustained chlorophyll *a* and particulate

matter concentrations (~25 % higher) and lower inorganic phosphate concentrations in the water column in the highest $fCO_2$ treatment (1231 µatm) during the final two weeks of the study period (Phase III), when there was low net change in particulate and dissolved matter pools. Size-fractionated phytoplankton pigment analyses indicated that these differences were driven by picophytoplankton (<2 µm) and were already established early in the experiment during an initial warm and more productive period with overall elevated chlorophyll *a* and particulate matter concentrations. However the influence of picophytoplankton on bulk organic matter pools was masked by high biomass of larger plankton until Phase III when the contribution of the small size fraction (<2 µm) increased to up to 90 % of chlorophyll *a*. In this phase, $CO_2$-driven increase in water column particulate carbon did not lead to enhanced sinking material flux but was instead reflected in increased dissolved organic carbon concentrations. Hence ocean acidification may induce changes in organic matter partitioning in the upper water column during the low nitrogen summer period in the Baltic Sea.

## 1 Introduction

The Baltic Sea is a semi-enclosed, brackish epicontinental sea with a substantial freshwater catchment area which is approximately four times larger than the water body itself. In addition, the Baltic Sea has limited and infrequent saline deep water inputs from the North Sea through the Danish Straits which form an important oxygen supply for the Baltic Sea bottom waters. Weak circulation, vertical mixing and water mass exchange in the Baltic Sea leads to strong horizontal and vertical salinity gradients (surface waters from north (< 5) to south (~20) Baltic, and surface (~7) to deep (~12) at station BY15 at Gotland Deep (The International Council for the Exploration of the Sea, 2014)). Consequently, the enclosed nature of the water body and minimal water exchange mean that terrestrial and anthropogenic activities have a considerable influence on water quality, biogeochemistry and ecosystems in the Baltic Sea.

Global change is expected to have pronounced effects on the physical and chemical conditions in the Baltic Sea. Warming, decreasing pH, and increasing freshwater inputs are expected to affect primary productivity and decrease oxygen concentrations in the deeper basins (HELCOM, 2013). In combination with higher nutrient loads from changes in agricultural activity, this may lead to increased hypoxia or even anoxia in sub-surface waters (Meier et al., 2011) with feedbacks on biogeochemical element cycles (Sutton et al., 2011),

and ecosystem structure and functioning particularly at higher trophic levels (Ekau et al., 2010; Turner, 2001; Wu, 2002). Changes in the Baltic Sea environment have already been detected. Regular monitoring of the Baltic Sea over the past 100 years has indicated higher rates of temperature increase (0.08 to 0.11°C per decade) than the global average, along with a 20 % decrease in annual maximum ice extent (HELCOM, 2013). Observed shifts in the spring and summer phytoplankton community dynamics have been primarily associated with warming in northern Baltic Sea regions over the past three decades (Suikkanen et al., 2013).

Ocean acidification is another anthropogenic process of potential relevance for Baltic plankton communities. As $CO_2$ dissolves in seawater, the carbonate system shifts with an associated decrease in pH. Ocean acidification therefore adds to the decrease in seawater pH as a result of nitrogen and sulphate deposition in the form of acid rain (Doney et al., 2007). Between 1993 and 2012, pH in the Baltic Proper decreased on the order of 0.1 pH units (The International Council for the Exploration of the Sea, 2014) which is more than two times faster than observed in the Pacific Ocean (~0.04 pH decrease between 1992 and 2012 in surface 30 m, Station ALOHA, Hawaii Ocean Time-Series (Dore et al., 2009)). Changes in $fCO_2$ and pH influence phytoplankton physiology, growth rates, and carbon fixation with some phytoplankton functional groups, such as calcifying organisms, more sensitive than others such as diatoms (Riebesell and Tortell, 2011; Rost et al., 2008). Thus the relative fitness of each functional group determines the response of the plankton community as a whole. Changes in physiological processes in phytoplankton on a cellular level can cascade through trophic levels and induce shifts in the structure of the planktonic food web.

To date, the majority of ocean acidification experiments have utilised nutrient replete starting conditions or added nutrients to investigate effects of high $CO_2$ on plankton communities and biogeochemical cycles (nutrient replete/addition e.g. Biswas et al., 2012; Engel et al., 2005, 2008, 2014; Feng et al., 2010; Hama et al., 2012; Hare et al., 2007; Hopkins et al., 2010; Hopkinson et al., 2010; Hoppe et al., 2013; Kim et al., 2006; Nielsen et al., 2010, 2011; Richier et al., 2014; Rossoll et al., 2013; Schulz et al., 2008, 2013; Tatters et al., 2013a, 2013b; Yoshimura et al., 2013, 2014) vs. (nutrient deplete e.g. Law et al., 2012; Lomas et al., 2012; Losh et al., 2012; Yoshimura et al., 2010). These studies mimic the productive spring bloom where nutrient concentrations are relatively high and relatively low light levels initially limit phytoplankton growth. However for considerable parts of the year, the opposite is the case. Growth is not limited by light but by nutrient concentrations and biomass tends to be

low. This is also the case during summer in the Baltic Sea. Here, a diatom-dominated spring bloom in April/May usually draws down dissolved inorganic nutrients so that concentrations remain low from early summer. Diazotrophic filamentous cyanobacteria then commonly bloom in July and August when surface water temperatures peak, calm weather conditions induce water column stratification and low nitrogen in a bioavailable form limits growth in the non-diazotrophic phytoplankton (Gasiūnaitė et al., 2005; Kanoshina et al., 2003; Stal et al., 1999).

We undertook a pelagic in situ mesocosm study on a summer Baltic Sea plankton community to investigate the response of this low nutrient ecosystem to projected changes in $fCO_2$. Using this approach, many different trophic levels from bacteria and viruses through to zooplankton can be investigated over extended periods of time. Using the KOSMOS mesocosm system (Kiel Off-Shore Mesocosms for future Ocean Simulations, Riebesell et al. (2013)), we were able to enclose large volumes containing whole plankton communities with a low level of disturbance and thereby utilising natural variability in light and temperature.

## 2 Methods

### 2.1 Study area, deployment site, and mesocosm set-up

On 12 June 2012 (day -10 = *t-10*, 10 days before $CO_2$ manipulation), nine floating, pelagic mesocosms (Fig. 1, KOSMOS, volume ~ 55 $m^3$) were deployed and moored at 59° 51.5' N, 23° 15.5' E in the Tvärminne Storfjärden, an open archipelago area on the eastern side of the Hanko peninsula on the south-west coast of Finland (Fig. 2). The water depth at the mooring site was approximately 30 m. The bottom ends of the mesocosm bags were lowered to a depth of 17 m below the surface to enclose the plankton community with minimal disturbance to the water column. A mesh of 3 mm was attached to the top, which was submerged ~0.5 m below the surface, and bottom of the bag, at 17 m deep, to exclude any large organisms or particles with patchy distribution in the water column. Initially the mesocosm bags were kept open and covered with only the 3 mm nets at the top and bottom openings for five days to allow for rinsing of the mesocosm bags water and free exchange of plankton (< 3 mm). On *t-7*, the nets were removed, sediment traps (2 m long, Fig. 1) were then attached to close the bottom of the mesocosms and the top ends of the bags were pulled up to 1.5 m above the water surface thereby isolating the water in the mesocosms from the surrounding Baltic Sea.

To ensure a homogeneous water column in each mesocosm at the start of the experiment, the halocline present was destroyed by bubbling each mesocosm with compressed air for three and a half minutes on *t-5*. A video profile taken in one of the mesocosms on *t-4* shows the plankton community present at the beginning of the study period (Boxhammer et al., 2015). Figure 3 indicates the experiment timeline including important manipulations. Mesocosm bags were cleaned occasionally inside and outside throughout the experiment to minimise wall growth and keep the biofilm biomass at a minimum (see Fig. 3 and Riebesell et al. (2013) for further details). An isotope tracer ($^{15}N$-$N_2$ gas) specific to the nitrogen fixing organisms present was injected in two additions (*t22* and *t26*) to four mesocosm bags (M3, M5, M6, M8). Further details about the addition are described in Paul et al. (in preparation). No dissolved inorganic or organic nutrients were added to the mesocosms in this study. At the end of the experiment, the volume of each mesocosm (0 – 19 m) was determined through addition of a calibrated salt solution as described by Czerny et al. (2013). Final mesocosm volumes ranged between 53.1 and 55.1 $m^3$ with an estimated uncertainty of 2 %. Unfortunately three mesocosms (M2, M4 and M9) were lost because of extensive and unquantifiable water exchange with the surrounding seawater due to a welding error on the mesocosm bags, and were thus excluded from sampling and analyses.

## 2.2 $CO_2$ manipulations

$CO_2$ treatments were achieved by equally distributing filtered (50 µm), $CO_2$-saturated seawater into the mesocosm as described by Riebesell et al. (2013) in four separate additions (see Table 1 for details). The first addition of $CO_2$-enriched seawater defined the beginning of the experiment and took place on *t0* following sampling activities. Seawater for the additions was collected from 10 m depth by a pipe connected to the laboratory in the research station. Different amounts of $CO_2$-saturated seawater were added to four mesocosms to set-up an initial gradient in $fCO_2$ treatments from ambient (~240 µatm) up to ~1650 µatm. On *t15*, $CO_2$ was manipulated in the upper 7 m to counteract pronounced outgassing in the mesocosm. Two mesocosms were selected as controls with no addition of $CO_2$-enriched seawater. Instead unenriched filtered seawater (50 µm) was added for the initial manipulations. For the later smaller addition, the water distributor (‘spider’, Riebesell et al. (2013)) was pulled up and down in each mesocosm to simulate water column mixing and manipulation side effects caused by the device on *t15*.

## 2.3 CTD and light measurements

CTD casts in each mesocosm and in the surrounding water were made with a hand-held self-logging CTD probe (CTD60M, Sea and Sun Technology) from 0.3 m down to ~18 m (mesocosms) and to ~30 m (surrounding water in Archipelago = Baltic) between 13:30 and 14:30 local time (LT) daily until *t31*, and then every second day until *t46*. Temperature, pH, dissolved oxygen and PAR (photosynthetic active radiation) sensors were deployed on the CTD as well as a conductivity cell. Details on the sensors, their accuracy and precision and corrections applied are described in Schulz and Riebesell (2013). The potentiometric CTD pH was corrected to spectrophotometric measurements (see Section 2.5.1). The depth of average water column light intensity in metres was calculated by averaging all water column PAR data and relating this to the depth where this intensity of PAR occurred.

A PAR sensor (LICOR LI192) was placed unobstructed at the end of a 2 m pole on the roof of Tvärminne Zoological Station (~1 km from mesocosm mooring site) to record incoming PAR for the mesocosms. Incoming PAR was recorded from 14:43 LT, on 14 June 2012 continuously as the mean of integrated 60 second intervals until the end of the experiment at 11:23 LT on 7 August 2012.

## 2.4 Sampling procedures

Water samples were collected regularly from each mesocosm and the surrounding water using depth-integrated water samplers (IWS, HYDRO-BIOS Kiel). Unless otherwise reported, all samples are from the entire water column (0 to 17 m). For example, inorganic dissolved nutrient and fluorometric Chl *a* samples were also taken regularly for the upper water column (0 to 10 m). Full details of mesocosm sampling procedures and equipment are described in Riebesell et al. (2013) and Schulz et al. (2013). There were two intensive sampling periods where sampling took place every day (*t-3* to *t5*, *t29* to *t31*), otherwise most variables were sampled every second day. Table 2 presents sampled variables including sampling frequency and respective manuscripts which report each data set. Samples for carbonate chemistry variables and trace gas analyses were the first to be sampled and were taken from the IWS directly on board the sampling boat. Other samples (e.g. particulate matter, Chl *a*, phytoplankton pigments) were collected into 10 L carboys and stored in the dark. Carboys were stored at in situ temperature on-shore and sub-sampling from these carboys was usually within one hour and up to a maximum of five hours after sampling. Care was taken to mix the

water samples in the carboys well before taking subsamples to ensure homogeneous sampling for all parameters.

The sediment trap was emptied every second day using a manual vacuum pump system to acquire the settled material via a silicon tube reaching down to the collection cylinder of the sediment trap (Boxhammer et al., in prep., Riebesell et al., 2013). This material was used to quantify and characterise particle sinking flux. Subsamples of the particle suspension (<6 % in total) were taken before the material was concentrated. Particles and aggregates were allowed to settle down within two hours at in situ temperature before separation of the supernatant. Collected particulate material was then centrifuged, while subsamples of the supernatant were filtered and analysed analogous to water column samples for particulate matter. Centrifuged material was subsequently frozen, lyophilised and ground to a fine powder of homogeneous composition. From this powder small subsamples of between 0.7 and 1.5 mg were weighed and analysed for carbon, nitrogen, phosphate and biogenic silica content as described in this manuscript for water column samples (see section 2.5.3). Concentrations of particulate material were calculated based on total mesocosm volume (in L). Mesocosm volume determined on *t45* by salt addition in kg (Section 2.2) was converted using mean mesocosm temperature and salinity over 0 – 17 m between *t-3* and *t43* (mean temperature = 11.42 °C, mean salinity = 5.70) and the algorithms described by Fofonoff and Millard Jr. (1983). A more in-depth description of sampling and processing of particles collected in the sediment traps of the KOSMOS setup will be presented in Boxhammer et al. (in prep.).

## 2.5 Sample analyses

### 2.5.1 Carbonate system parameters (DIC, TA, $pH_T$)

Samples for total alkalinity (TA), dissolved inorganic carbon concentrations (DIC) and total pH (on the total pH scale: $pH_T$) were gently pressure-filtered (Sarstedt Filtropur PES, 0.2 µm pore size) using a membrane pump (Stepdos) to exclude calcareous particles and particulate organic material before analysis. Presence of particulate matter can influence precision of carbonate chemistry measurements. In addition, the sterile filtration eliminates the influence of biological processes on pH and DIC during sample storage by phytoplankton or bacteria.

Total pH was determined by spectrophotometry as described in Dickson et al. (2007). Samples were analysed on a Cary 100 (Varian) spectrophotometer in a temperature controlled

10 cm cuvette using a low ionic strength m-cresol indicator dye matching the salinity of the sample water and an appropriate low salinity pK (Mosley et al., 2004). CTD pH measurements were corrected to $pH_T$ by daily linear correlations of mean water column potentiometric pH measurements to spectrophotometric $pH_T$ measurements.

DIC concentrations were determined by infrared absorption using a LICOR LI-7000 on an AIRICA system (MARIANDA, Kiel). Measurements were made on four replicates of 2 mL sample volume and DIC was calculated as the mean of the best three out of four measurements. The precision was typically better than 1.5 µmol $kg^{-1}$. Dissolved calcium concentrations in seawater were determined by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a VARIAN 720-ES and quality controlled with IAPSO reference material.

TA was analysed by potentiometric titration using a Metrohm 869 Sample Changer and a 907 Titrando Dosing unit according to the open cell method described in Dickson et al. (2007). Due to unaccounted contributions to TA in the range of 20 and 25 µmol $kg^{-1}$ by components such as organic acids and bases, spectrophotometric $pH_T$ and DIC were used to calculate carbonate chemistry speciation using the stoichiometric equilibrium constants for carbonic acid of Mehrbach et al. (1973) as refitted by Lueker et al. (2000). Buffering by organic compounds is not accounted for in the traditional TA definition (Dickson, 1981) and depends on unknown concentrations and acid/base equilibria of certain DOM components. Thus, using TA for carbonate chemistry speciation calculations would have resulted in errors (Koeve and Oschlies, 2012). Both TA and DIC measurements were calibrated using measurements of the certified reference material batch, CRM 115 (Dickson, 2010).

### 2.5.2 Dissolved inorganic nutrients

Samples for nutrients were collected in acid-cleaned (1 mol $L^{-1}$ HCl) 60 mL low density polyethylene bottles (Nalgene), stored at 4°C in the dark following sampling and analysed within 12 hours of collection. Dissolved silicate (DSi) concentrations were determined using standard colorimetric techniques (Grasshoff et al., 1983) at the micromolar level using a nutrient autoanalyser (Seal Analytical, Quattro). Nanomolar levels of dissolved nitrate + nitrite (hereafter nitrate) and dissolved inorganic phosphate (DIP) were determined with a colorimetric method using a 2 m liquid waveguide capillary cell (LWCCs) (Patey et al., 2008; Zhang and Chi, 2002) with a miniaturised detector (Ocean Optics Ltd). Detection limits were 2 nmol $L^{-1}$ for nitrate and 1 nmol $L^{-1}$ for DIP, with a linear range up to

300 nmol $L^{-1}$. All samples for inorganic nutrient measurements were filtered using glass fibre filters (GF/F, nominal pore size of 0.7 µm, Fisher Scientific) prior to analysis. This was done to reduce the dissolution of nutrients from particulates during analysis, and also to avoid particles blocking the LWCCs and interfering with the spectrophotometric measurements. Ammonium ($NH_4^+$) measurements were undertaken following the method by Kérouel and Aminot (1997) with fluorimetric detection (Trilogy, Turner), and featuring a detection limit of 5 nmol $L^{-1}$.

### 2.5.3 Particulate material (C, N, P, Si)

Total particulate carbon, particulate organic nitrogen and total particulate phosphorus (TPC, PON, TPP) samples were collected onto combusted GF/F filters (Whatman, nominal pore size of 0.7 µm) using gentle vacuum filtration (<200 mbar) and stored in glass petri dishes at -20°C directly after filtration until analysis. Filters and glass petri dishes were combusted at 450°C for 6 hours before use. Filters were not acidified to distinguish between inorganic and organic particulate carbon before analyses hence we measured TPC. However, microscopy counts and total alkalinity drawdown indicated pelagic calcifying organisms were not abundant and there was no significant calcification, thus it was probably mostly particulate organic carbon. In addition to the total particulate matter fraction, gauze pre-filters were used to separate size-fractionated samples for C and N analyses (0.7 to 10 µm = $TPC/PON_{<10}$, 0.7 to 55 µm = $TPC/PON_{<55}$). Filtration volumes ranged from 500 mL for the total fraction ($POM_{tot}$) to up to 1500 mL for <55 µm size fraction to ensure sufficient biomass on the filter for analyses. Sampling for $TPC_{<10}$ and $PON_{<10}$ only occurred after isotope tracer addition on *t23* in the four mesocosms where tracer was added (M3, M5, M6, M8). This size fraction was sampled to exclude large filamentous diazotrophic cyanobacteria.

Filters for TPC/PON were dried at 60°C, packed into tin capsules and stored in a dessicator until analysis. TPC and PON measurements were made on an elemental analyser (EuroEA) according to Sharp (1974), coupled by either a Conflo II to a Finnigan Delta$^{Plus}$ isotope ratio mass spectrometer or a Conflo III to a Thermo Finnigan Delta$^{Plus}$ XP isotope ratio mass spectrometer. Sub-samples of sediment material powder (1 – 2 mg) were weighed directly into tin capsules using an electronic microbalance (Sartorius M2P) with an accuracy of 0.001 mg. In addition to the standard calibration at the beginning of each run, standard materials (caffeine, peptone, acetanilide, nicotinamide, glutamic acid) were also included within runs to identify any drift and ensure accuracy and full combustion of the samples during analysis.

Selected samples for sediment material TPC and PON were reanalysed on an elemental analyser (EuroEA) not coupled to a mass spectrometer using the same method and standard materials. Total sinking particle flux is the sum of both the particulate matter concentrations determined in sediment powder and supernatant.

Filters for total particulate phosphorus (TPP) were placed in 40 mL of deionised water (MilliQ, Millipore) with oxidising decomposition reagent (MERCK, Catalogue no. 112936) and autoclaved for 30 minutes in a pressure cooker to oxidise the organic phosphorus to orthophosphate. Samples were allowed to cool before concentrations were determined by spectrophotometric analysis as for dissolved inorganic phosphate concentrations according to Hansen and Koroleff (1999).

For biogenic silica (BSi), samples were collected on cellulose acetate filters (0.65 µm Whatman) as described above for TPC, PON and TPP. Particulate silicate was leached from filtered material using 0.1 mol $L^{-1}$ NaOH at 85°C for 2 hours and 15 minutes, neutralised with $H_2SO_4$ (0.05 mol $L^{-1}$, Titrisol) and analysed as dissolved silicate by spectrophotometry according to Hansen and Koroleff (1999).

Content of TPP and BSi in finely ground sediment trap samples was determined from subsamples and analysed according to methods described for water column samples.

### 2.5.4 Dissolved organic matter (C, N, P)

For dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) analyses, 35 mL of sample was filtered through pre-combusted GF/F filters (450°C, 6 h) and collected in acid cleaned and combusted glass vials (450°C, 6 h), acidified with HCl to pH 1.9 and then flame sealed, and dark-stored in a fridge (4°C) for subsequent analysis. DOC and TDN concentrations were determined using a high-temperature catalytic combustion technique with a Shimadzu TOC-TN V analyser following Badr et al. (2003). Acidified deep Sargasso Sea water, preserved in glass ampoules and provided by D. Hansell (University of Miami), served as a certified reference material. Our analytical precision, based on the coefficient of variation (standard deviation/mean) of consecutive measurements of a single sample (generally between 3 and 5 injections), was typically <1 %. Dissolved organic nitrogen (DON) concentrations were calculated from TDN by the subtraction of the inorganic nitrogen concentrations.

Dissolved organic phosphorus (DOP) samples were collected as for DOC and TDN but stored at -20°C in acid-rinsed, high density polyethylene (HDPE) bottles. Total dissolved phosphate was decomposed to inorganic phosphate using an oxidising solution and microwave radiation (MARS 5X microwave, CEM) before analysis according to Hansen and Koroleff (1983). DOP concentrations were calculated from total dissolved phosphate by subtracting dissolved inorganic phosphate concentrations. Samples for DOP were only taken until *t30*. For further details, please refer to Nausch et al. (in prep.).

### 2.5.5 Phytoplankton pigments

Samples for fluorometric chlorophyll *a* determination (Chl *a*) and for phytoplankton pigment analyses by reverse phase high performance liquid chromatography (HPLC) were collected as described for POM with care taken to minimise exposure to light. Size fractionation for HPLC samples was achieved by pre-filtration using a 20 µm mesh and 2 µm membrane filters (Nuclepore) and was sampling was undertaken every 4$^{th}$ day, except for between *t31* and *t39* where sampling occurred only on *t31*, *t33* and *t39* (Table 2). Filtration volume for the total and <2 µm fraction as well as for Chl *a* was 500 mL whereas for the large fraction (>20 µm) volume ranged between 3000 and 5000 mL. All HPLC samples were stored at -80°C for under 6 months and Chl *a* samples at -20°C overnight until analysis.

Pigments from both fluorometric and HPLC analyses were extracted in acetone (90 %) in plastic vials by homogenisation of the filters using glass beads in a cell mill. After centrifugation (10 min., 800 x g, 4°C) the supernatant was analysed on a fluorometer (TURNER 10-AU) to determine Chl *a* concentrations (Welschmeyer, 1994). Samples for phytoplankton pigment analyses were also centrifuged (10 min., 5200 rpm, 4°C) and the supernatant was filtered through 0.2 µm PTFE filters (VWR International). Phytoplankton pigment concentrations were determined in the supernatant by reverse phase high performance liquid chromatography (HPLC; WATERS HPLC with a Varian Microsorb-MV 100-3 C8 column (Barlow et al., 1997; Derenbach, 1969)) and peaks were calibrated with the help of a library of pre-measured commercial standards. Relative contributions of phytoplankton groups to total Chl *a* were calculated using the CHEMTAX matrix factorisation program (Mackey et al., 1996). Pigment ratios were adapted accordingly to those reported for Baltic Sea phytoplankton (Eker-Develi et al., 2008; Schluter et al., 2000; Zapata et al., 2000). The size fraction 2 – 20 µm was calculated as <2 µm and >20 µm subtracted from the total size fraction.

### 2.6 Statistical data treatment

As in previous mesocosm experiments, an $fCO_2$ gradient was chosen for reasons as outlined in Schulz et al. (2013). Linear regression analyses were used to determine the relationship between average $fCO_2$ and average response of the variables during each experimental phase. Outliers were detected based on Grubb's test ($p < 0.05$). This test was applied to all treatments by experiment phase to account for temporal development of each variable. Detected outliers were not included in the calculation of experiment phase average. Exceptions to outlier exclusion include a) biogenic silicate concentrations in M8 on *t23* because all data was higher on this particular sampling day, and b) C:N in total POM on *t19* in M8 because the C:N in this treatment was also markedly higher than other treatments on the following sampling day (*t21*) and c) the contribution of cryptophytes to total Chl *a* M8 on *t17* and d) all five outliers in contribution of euglenophytes to total Chl *a* detected in Phase III for the same line of reasoning as b). All data points are included in the figures with excluded outliers clearly marked. Linear regression analyses and outlier detection and exclusion were undertaken using R Project for Statistical Computing (http://www.r-project.org/).

## 3 Results

### 3.1 Variations in temperature, salinity and oceanographic conditions

Conditions in the Tvärminne Storfjärden at the beginning of the experiment and during mesocosm closure were typical for the early summer season. Daily solar irradiance was at the annual peak (summer solstice) and surface water temperatures were ~10°C. Daily average water column temperature was highly variable over the experiment ranging from 8.0 – 8.5°C at the beginning of the experiment to 16°C on *t16* (Fig. 4). Temperature variations as well as the first $CO_2$ manipulation on *t0* were used to define different experimental phases, (Phase 0 = *t-5* to *t0*, Phase I = *t1* to *t16*, Phase II = *t17* to *t30*, Phase III = *t31* to *t43*). Warming occurred over the first 15 days and average water column temperatures peaked at 16°C (Phase I). A cooling phase (Phase II) occurred until *t31* (~ 8°C), followed by a second warming period (Phase III) which continued until the end of the experiment reaching around 12°C on average in the water column (Fig. 4 and 5C). The cooling in Phase II occurred around the same time as a period of lower incoming PAR between *t15* and *t25* (land based PAR measurements, Fig. 6A). Surface water temperatures reached a maximum of 18°C with a surface-to-depth

gradient of 6°C. The water column in the mesocosms remained thermally stratified throughout the study according to daily CTD profiles. Stratification strength, defined here as the potential density anomaly ($\sigma_T$) difference between the surface 10 m and bottom 7 m above the sediment trap in each mesocosm, was variable but lower in Phase I than in II and III. Detected changes in density over time were largely driven by changes in temperature within the mesocosms as there was only a minimal increase in salinity during the experiment probably due to evaporation (Fig. 5). Here, M8 was arbitrarily selected as representative for all mesocosms in Figs. 5 and 6. A typical daily difference in measured average water column temperature and salinity between mesocosms was 0.04°C and 0.01, respectively. The increase in salinity on *t45* is from addition of a calibrated salt solution for mesocosm volume determination. A notable decrease in temperature and increase in salinity in the archipelago between *t15* and *t31* coincided with a period of stormy weather and a change in wind direction from north-easterly to a more westerly direction, indicating a period of upwelling. During this period, there was slightly lower incoming PAR indicating higher cloud cover (Fig. 6). The depth of average light intensity was relatively stable between 3.7 and 4.7 m inside the mesocosms and very similar between treatments over time (Fig. 6).

### 3.2 Temporal variations in carbonate system

All mesocosms had a similar $pH_T$ of around 8.0 prior to $CO_2$ perturbations. Initial $CO_2$ enrichment reached target values on *t4* ranging from ~240 µatm in the two ambient control mesocosms up to ~1650 µatm in the highest treatment, corresponding to a $pH_T$ range of ~7.45 to 8.2 (Fig. 7). Aside from the $CO_2$ addition on *t15*, $fCO_2$ was allowed to vary naturally and treatments remained well separated over the entire experiment. The decrease in $fCO_2$ over time in the high $CO_2$ treatment mesocosms was mostly driven by outgassing rather than biological uptake as productive biomass remained relatively low in this experiment (see section 3.3). The effect of outgassing is evident in the rapid increase in surface $pH_T$ in all treatment mesocosms (Fig. 8). Surrounding water $pH_T$ (0 – 17 m) ranged from 8.30 initially to 7.75 during the experiment. The profound $pH_T$ variability outside the mesocosms was due to upwelling of deeper, $CO_2$-rich seawater. Within each mesocosm, $CO_2$ manipulations over the entire depth were relatively homogeneous initially. However a decrease in pH in the ambient control mesocosms below 5 m depth was detected from around *t15* onwards, suggesting heterotrophic activity at depth involving respiration of organic matter to $CO_2$ (Fig. 8). DIC increased in the control mesocosms due to gas exchange, which counteracted losses through

uptake by the plankton community which left the water column undersaturated in $CO_2$ compared to the overlying atmosphere (~230 µatm in control mesocosms vs. ~400 µatm in atmosphere (Schernewski, 2011)). Undersaturation of $CO_2$ is typical for post-spring bloom conditions such as those in the Tvärminne Storfjärden before the first $CO_2$ enrichment in this study on *t0*.

Calcium concentration was 2.17 mmol $kg^{-1}$ which was higher than calculated from a typical mean ocean salinity relationship of 1.67 mmol $kg^{-1}$ (Dickson et al., 2007), because of high riverine calcium carbonate inputs in the Baltic Sea (Feistel et al., 2010). We accounted for this in the calculation of the calcium carbonate saturation state in the water (Fig. 7D). All mesocosms apart from the two ambient controls during Phase 0 and I were undersaturated with respect to aragonite (Fig. 7D) and the highest three $fCO_2$ treatments were also undersaturated with respect to calcite (data not shown) during the entire experiment.

### 3.3 Effects of elevated $CO_2$

Out of 105 linear regressions applied to particulate and dissolved material from the water column and the accumulated sediment trap material to analyse the effect of $CO_2$, we detected a significant correlation in 18. These are summarised in Table 3 and highlighted in the following sections. The majority of detected responses (14) indicated a positive effect of $CO_2$ whereas only four indicated a negative effect of $CO_2$.

In this study, the low number of $fCO_2$ treatments (six) due to the exclusion of three mesocosms limited the statistical power of our conclusions. However the effect of $CO_2$ was consistent across biogeochemical element pools with higher sustained particulate matter concentrations and lower dissolved phosphate under high $CO_2$. This gives us confidence that the results of our study are indicative of the response of this particular plankton community in the Baltic Sea to ocean acidification.

### 3.4 Chlorophyll *a* dynamics

Chl *a* concentrations were low but typical of a post-spring bloom period. An increase in Chl *a* began after *t1* and signified a phase characterised by higher Chl *a* concentrations (~2 µg $L^{-1}$) until *t16* (Fig. 9, Phase I: *t1* to *t16*). Chl *a* concentrations decreased by ~0.8 µg $L^{-1}$ in the mesocosms during Phase II and remained low and relatively stable in Phase III (~0.9 to 1.2 µg $L^{-1}$). Between 50 % and 80 % of Chl *a* was in the upper water column (IWS samples 0 – 10

m, Fig. 9C). Chl *a* concentrations were in general lower (0.9 to 2.5 µg $L^{-1}$) in the mesocosms than in the surrounding water (1.2 to 5.5 µg $L^{-1}$, Fig. 9). $CO_2$ related differences first developed during Phase II and remained stable during Phase III with 24 % higher Chl *a* in the highest $fCO_2$ treatment in Phase III (Table 3).

### 3.5 Dissolved inorganic and organic matter dynamics

No dissolved inorganic or organic nutrients were added to the mesocosms in this study and nutrient concentrations remained relatively stable with low inorganic nitrogen concentrations throughout the entire experiment. There was low inorganic nitrogen (~50 nmol $L^{-1}$ nitrate and ~200 nmol $L^{-1}$ ammonium) relative to phosphate (~150 nmol $L^{-1}$) in all mesocosms at the start of the study period compared to the canonical Redfield nutrient stoichiometry (Fig. 10, C:N:P = 106:16:1, Redfield (1958). These concentrations are within the natural range for this region in a post-spring/early summer bloom phase (Fig. 10). Fixed nitrogen availability primarily limited the development of phytoplankton biomass in this system. This is common in the Baltic Sea following the spring bloom (Matthäus et al., 1999). Temporal dynamics between phosphate and nitrate showed decoupling. Nitrate concentrations increased from ~20 nmol $L^{-1}$ up to ~80 nmol $L^{-1}$ from *t1* until the end of the experiment (*t43*), whereas phosphate concentrations were slightly more dynamic, decreasing in Phase I and increasing in Phases II and III (Fig. 11). Around *t30*, differences in phosphate concentrations between $fCO_2$ treatments became visible with a significant negative relationship between $fCO_2$ and phosphate concentration in Phase III (Table 3). For further details and discussion on phosphorus pool sizes, uptake rates and cycling, see Nausch et al. (in prep.).

Ammonium concentrations decreased from between ~170 and ~280 nmol $L^{-1}$ on *t-3* to between 40 and 150 nmol $L^{-1}$ on *t39* with a small increase until *t43* in all mesocosms (Fig 10C). Samples for $NH_4^+$ concentration were lost on *t27* and *t29* for all mesocosms. The strongest decrease occurred during Phase I and concentrations remained relatively stable in Phase II and III. No significant $fCO_2$ effect was detected during any experimental phase above the variability in the data. Inside the mesocosms, dissolved silicate concentrations decreased minimally from around 6.2 µmol $L^{-1}$ on *t-1* to between 5.5 and 5.8 µmol $L^{-1}$ at the end of the initial productive Phase I on *t16* (Fig. 10D). Thereafter, dissolved silicate remained relatively constant until the end of the experiment. No significant effect of $fCO_2$ on dissolved silicate concentrations was detected in any phase.

DOC concentrations ranged between 410 and 420 µmol $L^{-1}$ on *t2* and increased by ~30 µmol $L^{-1}$ up to between 440 and 450 µmol $L^{-1}$ on *t43* (Fig. 11A). In Phase III, DOC positively correlated with $fCO_2$ (Table 3). There was no statistically significant correlation of $fCO_2$ with DON or DOP concentrations in any experimental phase. No clear temporal trends were distinguished in DOP concentrations although DON decreased during Phase I (Fig. 11). Where data points are missing, DON could not be corrected for $NH_4^+$ concentrations hence are excluded from the data set.

### 3.6 Particulate matter dynamics

Particulate C, N and P concentrations were higher in Phase I than in Phase II and III, (Fig. 12), as also observed for Chl *a* (Fig. 9A). The importance of small particles was even more pronounced in Phase III, where up to ~90 % of total particulate organic matter was attributed to the fraction $TPC_{<10}$ in the four mesocosms sampled for this size fraction (M3, M5, M6, M8, Fig. 12). In Phase III, there was a significant positive correlation between $fCO_2$ and average total TPC, PON and TPP (Table 3).

C:N and C:P ratios in $POM_{tot}$ (Fig. 13) were above the Redfield ratio ($C:N:P_{tot}$ = 106:16:1) during the productive phase, peaked at the beginning of Phase I ($C:N_{tot}$ = 7 – 8.5, $C:P_{tot}$ = 110 - 160) then decreased and became stable during Phase II ($C:N_{tot}$ = 5.8 – 7.0, $C:P_{tot}$ = 80 - 140). Differences between $fCO_2$ treatments were first observed in Phase III with higher $C:N_{tot}$ in the highest $fCO_2$ treatment (Table 3). No significant effect of $fCO_2$ on N:P or C:P was detected in any experiment phase or in any size fraction.

BSi decreased from around 1.0 µmol $L^{-1}$ at the beginning to ~0.3 µmol $L^{-1}$ at the end of the experiment (Fig. 12). During Phase II, there was a statistically significant correlation of BSi with $fCO_2$, however this was absent in Phases I and III (Table 3).

### 3.7 Phytoplankton succession

The contribution to Chl *a* by different phytoplankton groups varied over time although the temporal trends in all mesocosms appeared remarkably similar (Fig. 14). Results from CHEMTAX analyses of the phytoplankton community present indicate that cryptophytes and chlorophytes had the highest contribution to total Chl *a* during Phase I and Phase II/III, respectively. The total abundances of cryptophytes decreased from *t-3* to *t17* in all mesocosms, succeeded by a brief euglenophyte peak around *t15*, with chlorophytes being the

dominant contributor to Chl *a* from *t17* on (Fig. 14). Total abundances of cyanobacteria, probably non-diazotrophic *Synechococcus*, were highest during both Phase II and III. Diatoms made up a relatively small proportion of the plankton assemblage and contributed to less than 10 % of Chl *a* in Phases I and II and between 10 – 25 % in Phase III. Other key groups detected included dinoflagellates and prasinophytes, however, they made up minor proportions (below 15% of total Chl *a*) of the plankton community throughout the entire experiment (dinoflagellate data not shown).

We analysed the relationship between $fCO_2$ and the contribution of phytoplankton groups to Chl *a* by linear regression for each experimental phase (Table 4). These analyses indicated small differences in plankton community composition between $CO_2$ treatments. There was a significant negative correlation between $CO_2$ and total diatom contribution to Chl *a* in Phase III. In Phase III, $fCO_2$ was also negatively correlated to the contribution of cryptophytes to Chl *a* and a significant positive effect on the contribution of prasinophytes to Chl *a*.

Linear regression of the absolute concentrations of a number of phytoplankton pigments in the size fraction <2 µm indicated primarily a positive correlation to $fCO_2$ during Phase I (i.e. Chl *a*, Violaxanthin, Neoxanthin) although a statistically significant effect was not detected in all pigments (Table 5). In Phase III, where the highest Chl *a* concentrations were in the size fraction <2 µm, mass balance calculations indicated more than 100% of total Chl *a* in this size range which is not physically possible. These unbalanced Chl *a* measurements are the result of measurement uncertainties at such low absolute concentrations, particularly in the >20 µm size fraction and of mass balance calculations between three independent filtrations. As the increase and decline in Chl *a* <2 µm and 2 – 20 µm fractions respectively are supported by flow cytometry data for picoeukaryote and nanoeukaryote abundances, we still consider the observed temporal variations to be robust. A positive correlation between picoeukaryote abundance and $CO_2$ treatment was also already detected in Phase I (Crawfurd et. al, in prep.). Absolute concentrations of Chl *a*, Chl *b*, Prasinoxanthin, Violaxanthin and Neoxanthin in the total fraction had a statistically significant positive correlation with $fCO_2$ during Phase III (see Table 5). Fucoxanthin concentrations (key pigment in diatoms but also present in dinoflagellates) and $fCO_2$ were also positively correlated in the fraction >20 µm during Phase III. Size fractionation of HPLC pigment analyses indicated a higher proportion of Chl *a* in all treatments in biomass <2 µm during Phases II and III (Fig. 15).

### 3.8 Sinking material flux

The amount of material collected in the sediment traps in each phase reflected biomass (here POM and Chl *a*) build-up from the water column. We calculated that >84 % of total carbon sinking into the sediment trap was collected during Phases I and II and less than 16 % during Phase III (Fig. 16). This corresponds to average accumulation rates (± standard deviation) of 0.303 ± 0.011, 0.203 ± 0.033 and 0.094 ± 0.029 µmol C $L^{-1}$ $day^{-1}$ across all mesocosms in Phases I, II and III respectively. No significant $CO_2$ trends were detected during any phase with regards to the total amount of C, N, P and BSi in the sediment trap material.

# 4 Discussion

## 4.1 Phase I: productive phase with high organic matter turnover

Phase I (*t1* to *t16*) was characterised by the highest sustained Chl *a* and particulate matter concentrations in the water column. Relatively high light availability, particularly between *t6* and *t15* (Fig. 6A), accompanied by increasing water column temperatures likely supported autotrophic growth. However, no increase in particulate matter pool size was observed in any treatment during this productive phase. Instead carbon was diverted into the sinking particle flux and DOC pool (Fig. 11) with a net daily accumulation of DOC of between 10 to 15 % of the total TPC pool between *t3* and *t13*. As inorganic nitrogen availability was very low, we assume this is due to carbon overconsumption (Toggweiler, 1993). Thus, organic matter turnover in the system appeared to be high during this period, although overall phytoplankton biomass production was limited by low inorganic nitrogen availability.

Although phytoplankton carbon fixation is expected to be stimulated by increased $CO_2$ availability (Hein and Sand-Jensen, 1997; Losh et al., 2012; Riebesell et al., 2007), previous $CO_2$ enrichment experiments using natural plankton assemblages under various conditions of nutrient repletion in different regions have shown no consistent response of primary production to elevated $CO_2$ (Engel et al., 2005; Hopkins et al., 2010; Hopkinson et al., 2010; Nielsen et al., 2011; Riebesell et al., 2007; Schulz et al., in prep.; Yoshimura et al., 2013). During high organic matter turnover in Phase I, we detected no statistically significant differences in bulk organic matter concentrations or elemental stoichiometry between $CO_2$ treatments. No effect $CO_2$ treatment could be detected in the most abundant, and presumably most productive, phytoplankton size class (2 – 20 µm, Fig. 15). Instead, detected differences between $fCO_2$ treatments in particulate matter in Phase I were mostly confined to pigment

concentrations in the smallest size fraction (<2 µm). Here, pigment concentrations were generally higher in the highest $CO_2$ treatment (Table 5). This is in line with flow cytometry counts which revealed a positive effect of $CO_2$ on the abundance of picoeukaryotes (Crawfurd et. al, in prep.) and is in agreement with studies in the Arctic (Brussaard et al., 2013), the sub-arctic North Pacific (Endo et al., 2013), and North Atlantic Ocean (Newbold et al., 2012) but contrasts the results from Richier et al. (2014) from shelf seas in the northeast Atlantic Ocean. The positive influence of $CO_2$ on phytoplankton pigment concentrations was also detected in the largest size fraction (>20 µm) in Phase I, however this size class made up only a small portion of total Chl *a* (<10 % Fig. 15, size fractionated pigment analyses). Thus, small $CO_2$-driven differences in plankton community structure in the smallest and largest phytoplankton were not relevant for biogeochemical element cycling in this plankton assemblage during this productive phase.

### 4.2 Phase II: decline in autotrophic biomass and organic matter turnover

The distinct changes in the phytoplankton communities in the mesocosms coincided with the decrease in temperature during the upwelling even in the Archipelago in Phase II (*t17* to *t30*). Temperature decreases of greater than 10°C in surface water, as observed in this study, have been reported for upwelling events during periods of thermal stratification (Lehmann and Myrberg, 2008) with considerable influence on the ecosystem productivity (Nômmann et al., 1991). Here we assume that the combination of higher grazing pressure, lower PAR and cooler temperatures likely slowed down phytoplankton productivity and contributed to decreased phytoplankton biomass, observed here as a decrease in Chl *a*, during this period (Fig. 9).

An increase in $TPC_{tot}$:Chl *a* from ~10 µmol $µg^{-1}$ on *t17* to over 15 µmol $µg^{-1}$ on *t29* indicates that carbon was being shifted from autotrophic to heterotrophic organisms, assuming that the Chl *a* content of the autotrophs remained constant. CTD profiles showed a decrease in $pH_T$ below 10 m in both control mesocosms (Fig. 8) at the same time as surface Chl *a* (0 to 10 m) decreased between *t18* and *t30*. This pH decrease (i.e. $CO_2$ increase) could indicate a possible change in the equilibrium between dominance of autotrophic ($CO_2$ uptake) to heterotrophic ($CO_2$ release) processes during a phase of strong cooling in the lower water column. Higher organic material availability seemed to stimulate bacterial activity up until *t23* (Hornick et al., in prep.). Furthermore, higher zooplankton abundances after *t17* (Lischka et al., in prep.), as well as a peak in abundance of a potential mixotroph around *t17* (Euglenophycaea) also likely

contributed to higher organic matter remineralisation and $CO_2$ release. Hence Phase II is defined by increased heterotrophy and organic matter remineralisation. Carbon was primarily channelled into sinking material flux and higher trophic levels rather than accumulating in the DOC pool, mediated by increased zooplankton grazing pressure on primary producers.

Differences between $CO_2$ treatments in the dissolved and particulate matter pools developed during the Chl *a* decrease and apparent increase in net heterotrophy in Phase II. In addition, size-fractionated pigment analyses indicated a shift in phytoplankton community size to smaller organisms with up to ~90 % of Chl *a* in phytoplankton <2 µm at the end of Phase II. This was not caused by a remarkable gain in Chl *a* in the smaller size class but instead due to Chl *a* loss in the larger size class, which we think was driven by high grazing pressure from abundant zooplankton at this time (Lischka et al., in prep.). This removal of larger phytoplankton unmasked the underlying positive $CO_2$ response of picoplankton that was already present since Phase I but now became clearly visible. In other words, a positive $CO_2$ effect on picophytoplankton seemed to be present throughout the entire experiment. However, their ecological and biogeochemical relevance within the plankton community was too small initially, so that the $CO_2$ effect was not detectable in the other bulk biogeochemical element pools.

Interestingly, measured carbon fixation rates did not show any fertilising effect of $CO_2$ (Spilling et al., in prep.), whereas both respiration (Spilling et al., in prep.) and bacterial production rates between *t14* and *t23* (Hornick et al., in prep., Nausch et al., in prep.) were lower at higher $CO_2$. This suggests slower net particulate matter loss rather than increased production under ocean acidification (see Hornick et al. in prep. and Spilling et al. in prep. in this issue for more on this topic).

### 4.3 Phase III: inactive plankton community

While temperature increased again during Phase III, there did not seem to be any recovery of phytoplankton biomass to the same level as in Phase I. In Phase II autotrophic growth was apparently dampened so severely that it could not recover within the duration of this study and was likely strongly controlled by high zooplankton grazing pressure. There was very little change in the amount or stoichiometry of the particulate or dissolved matter pools suggesting that production and loss of particulate matter in the water column were either very low or relatively well balanced in Phase III. Only a small amount of TPC (~1 µmol $L^{-1}$, ~16% of

total suspended TPC) was collected in the sediment traps implying low particulate matter sinking flux strength in this phase. The positive (picoplankton-mediated) effect of $CO_2$ on particulate and dissolved pools unmasked in Phase II was sustained throughout Phase III in Chl *a*, TPC, PON, TPP and DIP. Thus in this study, higher autotrophic biomass was sustained under elevated $CO_2$ in this plankton community during the post-bloom phase and had a significant influence on biogeochemical pool sizes.

Variations in water column particulate matter concentrations did not translate into statistically significant differences in the amount of accumulated sediment trap material between $CO_2$ treatments. This may be because the response of $CO_2$ was the strongest in phytoplankton <2 µm, which taxonomically were likely to be chlorophytes and prasinophytes (Fig. 14B and 14F, Table 4). The unicellular organisms are, however, too small to sink as individual cells. Instead picoplankton contribute indirectly to carbon export through secondary processing of sinking picoplankton material (Richardson and Jackson, 2007). The positive effect of $CO_2$ on particulate matter pools was reflected positively in the DOC pool suggesting that a higher proportion of freshly produced organic matter was directed into the microbial food web, rather than being exported during the period of low organic matter turnover in Phase III. A similar channelling of carbon and the positive $CO_2$ response in the DOC pool was observed during nutrient-deplete conditions in an Arctic $CO_2$-enrichment mesocosm study (Engel et al., 2013). Here, this could be a consequence of continued reduced organic matter remineralisation at elevated $CO_2$ (Spilling et al., in prep.), as hypothesised for Phase II (see also section 4.2), although unfortunately no respiration data for Phase III is available.

Based on our results, we hypothesise that under future ocean acidification the Baltic Sea in low nitrogen, summer periods may shift towards a system where more organic matter is retained for longer time-periods in the upper water column but may not result in increased particulate matter sinking flux.

### 4.4 Potential ecosystem resilience under elevated $CO_2$

Although a significant, but small, response to $CO_2$ was detected in a number of particulate and dissolved matter pools, in numerous others no significant effect of $CO_2$ was detected in any phase (e.g. DON and DOP concentration, N:P and C:P in POM). The muted response of the plankton community and biogeochemistry to elevated $CO_2$ observed in this experiment might be linked to higher tolerance or resilience of the plankton community. The Baltic Sea is a

highly dynamic system with much larger annual temperature, light period, inorganic nutrient, pH, and salinity fluctuations than in many other major water bodies and the open ocean. Thus the community present in this study may have considerable physiological plasticity through exposure to large natural diurnal and annual fluctuations in carbonate chemistry speciation and pH (see also Joint et al. (2011) and Nielsen et al. (2011)). Low nitrogen availability in this study may have dampened underlying trends particularly in larger phytoplankton size classes. In past $CO_2$ enrichment experiments, nutrient addition amplified the existing effect of $CO_2$ between treatments, for example Schulz et al. (2013). This is one of few plankton community experiments, where nutrient concentrations were very low initially and concentrations and nutrient ratios were not manipulated. Such conditions are representative of a steady-state stratified water column present in many ecosystems for most of the year.

## 5 Conclusions

We observed higher post-bloom Chl *a*, particulate organic matter and DOC concentrations under elevated $fCO_2$ in this low nitrogen plankton community. No effect of $CO_2$ was identified in larger organisms (2 to 20 µm) which were dominant in the phytoplankton community during the period of higher productivity in Phase I. Hence their dominance masked the $CO_2$ signal from picophytoplankton in bulk particulate and dissolved pools. As a result of the shift in phytoplankton community size structure towards dominance of smaller phytoplankton size classes around three weeks after initial $CO_2$ enrichment, the underlying positive effect of $CO_2$ present on picophytoplankton (<2 µm) biomass since Phase I was revealed in particulate and dissolved matter pools. This signal could not be explained by a detectable increase in carbon fixation in this study (Spilling et al., in prep.).

Differences in water column biomass did not directly translate into increased particle sinking flux at higher $fCO_2$. Instead higher organic matter concentrations are more likely due to decreased net respiration at higher $fCO_2$ with the positive $CO_2$ effect on biomass channelled into the DOC pool. Alternatively secondary processing of sinking material may have removed the $CO_2$ signal present in the water column particulate matter, driven by picophytoplankton so that it was not reflected in the collected sinking material during the study period. Hence we suggest $CO_2$-induced changes in productivity in the upper water column may be decoupled from particle sinking flux.

In this study, it took almost four weeks until we first observed $CO_2$-related differences in the size and stoichiometry of some bulk biogeochemical pools. In many other variables, simulated ocean acidification did not have any significant effect at all. This slow response or lack of detected effect to ocean acidification may have been modulated by overall low inorganic nitrogen availability and high natural pH variability in the ecosystem. Therefore we recommend future experiments run for as long as practically feasible, focus on the vast oligotrophic regions and avoid nutrient additions. Changes in the abundance of key phytoplankton groups in steady-state systems due to higher $CO_2$ may underpin sustained fundamental changes in biogeochemical cycling in these regions.

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## Tables and figures

Table 1. Volumes of $CO_2$-enriched seawater added for the $CO_2$ manipulation indicating day of addition and total manipulation volumes. Symbols and colours indicated here indicated here are used in all following figures.

| Mesocosm | | **M1** | **M5** | **M7** | **M6** | **M3** | **M8** | **Baltic** |
|---|---|---|---|---|---|---|---|---|
| Target $fCO_2$ (µatm) | | ambient/ control | ambient/ control | 600 | 950 | 1300 | 1650 | ambient |
| Average $fCO_2$ (µatm) *t1 – t43* | | 365 | 368 | 497 | 821 | 1007 | 1231 | 417 |
| Average $fCO_2$ (µatm) *t1 – t30* | | 346 | 348 | 494 | 868 | 1075 | 1333 | 343 |
| Symbol | |  |  |  |  |  |  |  |
| Day | *t0* | - | - | 20 L | 50 L | 65 L | 75 L | - |
| | *t1* | - | - | 10 L | 40 L | 50 L | 65 L | - |
| | *t2* | - | - | 10 L | 30 L | 45 L | 50 L | - |
| | *t3* | - | - | 5 L | 8 L | 9 L | 10 L | - |
| | *t15* | - | - | - | 9 L | 12 L | 18 L | - |
| | **Total** | - | - | 45 L | 137 L | 181 L | 218 L | - |

Table 2. Summary of sampled variables for this study, including a brief description of method used, sampling frequency and corresponding manuscript in this Special Issue where data set and further details of methods used can be found.

| Variable | Method/Instrument | Sampling frequency | Corresponding manuscript |
|---|---|---|---|
| ATP and phosphate uptake rates | $^{33}$P incorporation | Every 2nd day until *t29* | Nausch et al. in prep. |
| Bacteria and virus abundances | Flow cytometry | Daily until *t31*, then every 2nd day until *t43* | Crawfurd et al. in prep. |
| Bacterial production | $^{14}$C-Leucine incorporation | *t-3, t0*, from *t2* every 3rd day until *t26*, from *t29* every 2nd day until *t43* | Hornick et al., Nausch et al. in prep |
| Biogenic silica | Spectrophotometry | Every 2nd day until *t43* | This manuscript |
| Chlorophyll *a* | Fluorometry | Daily until *t30*, every 2nd day until *t39* | This manuscript |
| Community respiration | $O_2$ consumption | Daily until *t33*, excluding *t2*, *t14*, *t32* | Spilling et al. in prep. |
| Copepod (*Acartia bifilosa*, *Eurytemora affinis*) reproduction | Incubations, microscopy counts | Weekly *(t3, t10, t17, t24 + t45 for A. bifilosa)* | Almén et al. in prep, Vehmaa et al. in prep. |
| Copepod adult female size (*A. bifilosa*) | Microscopy measurements | Weekly (*t3, t10, t17, t24, t45*) | Vehmaa et al. in prep. |
| Copepod antioxidant capacity | ORAC | Weekly (*t3, t10, t17,t31*) | Almén et al. in prep, Vehmaa et al. in prep. |
| Dissolved inorganic carbon (DIC) | IR absorption | Daily until *t30*, every 2nd day until *t43* | This manuscript |
| Dissolved organic carbon and nitrogen | Shidmadzu TOC/TDN analyser | Every 2nd day until *t43* | This manuscript |
| Dissolve organic phosphorus | Microwave digestion, spectrophotometry | Every 2nd day until *t29* | This manuscript, Nausch et al. in prep. |
| Fatty acid concentrations (phytoplankton, copepods: *A.bifilosa*, *E.affinis*) | GC-MS | Phyto.: every 4th day until *t29*, Copepods: weekly (*t3, t10, t17, t24*, t31, *t38*) | Almén et al. in prep, Bermudez et al. in prep. |
| Fatty acid concentrations (*E.affinis* adults and eggs from reproduction incubations) | GC-MS | Weekly (*t7, t14, t21, t28*) | Almén et al. in prep |
| Inorganic nutrient concentrations | Colorimetry (LWCC) | Every 2nd day until *t43* | This manuscript |
| Light intensity (PAR) | LICOR sensor | Daily between *t-5* and *t45* | This manuscript |
| Mesozooplankton abundances | Stereomicroscopy counts | *t-3, t-2, t-1, t0, t3, t10, t17, t24, t31, t38, t43* | Lischka et al. in prep. |
| Microzooplankton abundances | Microscopy counts | *t-3, t0,t2, t4, t7,t9, t11, t13, t15,t17, t21, t23, t25, t27, t29, t31, t33, t35, t37, t39, t41, t43* | Lischka et al. in prep. |
| $N_2$-fixation rates | $^{15}$N incorporation, EA-IRMS | Every 2nd day until *t43* | Paul et al. in prep. |
| pH | Spectrophotometry and CTD sensor for mesocosm profiles | Daily until *t30*, every 2nd day until *t43* | This manuscript |
| Phytoplankton abundances | Microscopy counts | Every 2nd day until *t43* | Bermudez et al. in prep, Paul et al. in prep. |
| Phytoplankton abundances | Flow cytometry | Daily until *t31*, then every 2nd day until *t39* | Crawfurd et al. in prep |
| Phytoplankton pigments | HPLC | Every 2nd day until *t43*, size fractions every 2nd sampling day excluding *t37* and *t39* | This manuscript |
| Primary production | $^{14}$C incorporation | Every 2nd day until *t30*, excluding *t1, t2, t3, t6, t7, t8* | Spilling et al. in prep. |
| Salinity, Temperature | CTD sensor | Daily until *t30*, every 2nd day until *t43* | This manuscript |
| Sediment trap material – amount and elemental characterization (C,N, P, BSi, pigment concentration) | EA-IRMS, HPLC, spectrophotometry | Every 2nd day until *t43* | This manuscript, Paul et al. in prep. |
| Total alkalinity | Potentiometric titration | Daily until *t30*, every 2nd day until *t43* | This manuscript |
| Total particulate carbon (including $\delta^{13}$C), particulate organic nitrogen (including $\delta^{15}$N), size fractions (total, <55 µm, <10 µm) | EA-IRMS | Every 2nd day until *t43*,except for <10 µm fraction every 2nd day from *t23* until *t43* | This manuscript, Paul et al. in prep. ($\delta^{13}$C unpublished) |
| Total particulate phosphorus | Spectrophotometry | Every 2nd day until *t43* | This manuscript |
| Trace gas concentration | GC-MS | Every 2nd day until *t17* then daily until *t30* | Webb et al. in prep. |
| Viral lysis and grazing of bacteria | Incubations, Flow cytometry | *t-3, t0, t4, t7, t11, t14, t18, t21* | Crawfurd et al. in prep |
| Viral lysis and grazing of phytoplankton | Incubations, Flow cytometry | *t1, t3, t6, t10, t13, t17, t20, t24, t31* | Crawfurd et al. in prep |

Table 3. Summary of linear regression analyses of $CO_2$ effects on particulate and dissolved matter and sediment trap material including elemental stoichiometry in different size fractions for each experimental phase. $fCO_2$ and the parameter were averaged for each phase and using a linear model, a regression analysis was done to test for statistical significance of a potential $CO_2$ effect. Significant positive effects detected are in bold, significant negative effects of $CO_2$ are in italics. Degrees of freedom = 4, apart from particulate matter size fraction <10 µm where n = 2.

| | **Particulate matter** | | | | **Dissolved matter and Chl *a*** | | | | **Sediment material** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Parameter | *p* | Multiple $R^2$ | F-statistic | Parameter | *p* | Multiple $R^2$ | F-statistic | Parameter | *p* | Multiple $R^2$ | F-statistic |
| Phase I | TPC total | 0.152 | 0.438 | 3.113 | Nitrate (0 – 17 m) | 0.547 | 0.098 | 0.433 | Total accumulated material | 0.265 | 0.296 | 1.680 |
| Phase II | | 0.902 | 0.761 | 12.760 | | 0.602 | 0.074 | 0.320 | | 0.593 | 0.078 | 0.336 |
| Phase III | | **0.011** | **0.834** | **20.070** | | 0.768 | 0.034 | 0.105 | | 0.945 | 0.001 | 0.005 |
| Phase I | TPC < 55 µm | 0.580 | 0.083 | 0.363 | Nitrate (0 – 10 m) | 0.709 | 0.085 | 0.185 | Total accumulated material in phase | 0.265 | 0.296 | 1.680 |
| Phase II | | 0.536 | 0.103 | 0.458 | | *0.033* | *0.718* | *10.170* | | 0.799 | 0.018 | 0.074 |
| Phase III | | 0.759 | 0.026 | 0.108 | | 0.540 | 0.101 | 0.448 | | 0.372 | 0.202 | 1.010 |
| Phase I | TPC < 10 µm | -- | -- | -- | DIP (0 – 17 m) | 0.486 | 0.128 | 0.589 | Cumulative TPC in phase | 0.752 | 0.028 | 0.115 |
| Phase II | | **0.036** | **0.929** | **26.120** | | 0.076 | 0.587 | 5.679 | | 0.902 | 0.004 | 0.017 |
| Phase III | | 0.187 | 0.661 | 3.899 | | *0.003* | *0.910* | *40.170* | | 0.386 | 0.191 | 0.947 |
| Phase I | PON total | 0.668 | 0.051 | 0.214 | DIP (0 – 10 m) | 0.651 | 0.056 | 0.239 | Cumulative PON in phase | 0.848 | 0.010 | 0.042 |
| Phase II | | 0.490 | 0.126 | 0.576 | | 0.075 | 0.589 | 5.737 | | 0.662 | 0.052 | 0.222 |
| Phase III | | **0.001** | **0.940** | **62.890** | | *0.030* | *0.732* | *10.950* | | 0.309 | 0.253 | 1.357 |
| Phase I | PON < 55 µm | 0.640 | 0.060 | 0.255 | $NH_4^+$ (0 – 17 m) | 0.225 | 0.340 | 2.058 | Cumulative TPP in phase | 0.621 | 0.067 | 0.286 |
| Phase II | | 0.516 | 0.113 | 0.508 | | 0.297 | 0.265 | 1.439 | | 0.749 | 0.028 | 0.117 |
| Phase III | | 0.381 | 0.195 | 0.968 | | 0.217 | 0.349 | 2.147 | | 0.358 | 0.212 | 1.079 |
| Phase I | PON < 10 µm | -- | -- | -- | Dissolved silicate | 0.389 | 0.189 | 0.930 | Cumulative BSi in phase | 0.950 | 0.001 | 0.005 |
| Phase II | | 0.207 | 0.630 | 3.401 | | 0.272 | 0.288 | 1.617 | | 0.850 | 0.010 | 0.041 |
| Phase III | | 0.098 | 0.813 | 8.703 | | 0.642 | 0.059 | 0.252 | | 0.108 | 0.515 | 4.255 |
| Phase I | TPP | 0.084 | 0.567 | 5.240 | P* | 0.554 | 0.094 | 0.416 | | | | |
| Phase II | | 0.363 | 0.208 | 1.050 | | 0.549 | 0.096 | 0.427 | | | | |
| Phase III | | **0.004** | **0.897** | **34.690** | | *0.003* | *0.918* | *44.470* | | | | |
| Phase I | Biogenic silica (BSi) | 0.070 | 0.601 | 6.032 | DOC | 0.324 | 0.240 | 1.262 | | | | |
| Phase II | | **0.034** | **0.717** | **10.120** | | 0.230 | 0.334 | 2.006 | | | | |
| Phase III | | 0.553 | 0.095 | 0.419 | | **0.005** | **0.882** | **29.920** | | | | |
| Phase I | C:N in total POM | 0.653 | 0.056 | 0.236 | DON | 0.652 | 0.056 | 0.236 | | | | |
| Phase II | | **0.020** | **0.779** | **14.080** | | 0.358 | 0.212 | 1.079 | | | | |
| Phase III | | **0.050** | **0.659** | **7.716** | | 0.926 | 0.002 | 0.010 | | | | |
| Phase I | C:N in POM < 55 µm | 0.487 | 0.128 | 0.587 | DOP | 0.914 | 0.003 | 0.013 | | | | |
| Phase II | | 0.208 | 0.360 | 2.249 | | 0.391 | 0.188 | 0.924 | | | | |
| Phase III | | **0.037** | **0.704** | **9.516** | | 0.812 | 0.016 | 0.065 | | | | |
| Phase I | C:N in POM < 10 µm | -- | -- | -- | Chl *a* (0 – 17 m) | 0.796 | 0.019 | 0.076 | | | | |
| Phase II | | **0.009** | **0.982** | **105.800** | | **0.020** | **0.780** | **14.180** | | | | |
| Phase III | | 0.164 | 0.699 | 4.643 | | **0.022** | **0.766** | **13.070** | | | | |
| Phase I | N:P in total POM | 0.707 | 0.039 | 0.163 | Chl *a* (0 – 10 m) | 0.227 | 0.337 | 2.037 | | | | |
| Phase II | | 0.848 | 0.010 | 0.042 | | **0.034** | **0.714** | **9.995** | | | | |
| Phase III | | 0.397 | 0.184 | 0.900 | | **0.008** | **0.859** | **24.320** | | | | |
| Phase I | C:P in total POM | 0.507 | 0.117 | 0.529 | | | | | | | | |
| Phase II | | 0.582 | 0.082 | 0.358 | | | | | | | | |
| Phase III | | 0.056 | 0.641 | 7.133 | | | | | | | | |
| Phase I | C:BSi in total POM | 0.989 | 0.000 | 0.000 | | | | | | | | |
| Phase II | | 0.127 | 0.480 | 3.695 | | | | | | | | |
| Phase III | | 0.307 | 0.255 | 1.370 | | | | | | | | |

Table 4. Results of linear regression analyses of $CO_2$ and percentage contribution of phytoplankton groups to chlorophyll *a*.

| Phytoplankton group | **Phase I** | | | **Phase II** | | | **Phase III** | | |
|---|---|---|---|---|---|---|---|---|---|
| | *p* | Multiple $R^2$ | F-statistic | *p* | Multiple $R^2$ | F-statistic | *p* | Multiple $R^2$ | F-statistic |
| Prasinophytes | 0.645 | 0.058 | 0.248 | 0.095 | 0.543 | 4.751 | **0.025** | **0.754** | **12.270** |
| Cryptophytes | 0.995 | 0.001 | 0.004 | 0.463 | 0.141 | 0.657 | *0.041* | *0.687* | *8.789* |
| Chlorophytes | 0.631 | 0.063 | 0.269 | 0.244 | 0.317 | 1.860 | **0.008** | **0.857** | **24.020** |
| Cyanobacteria | 0.224 | 0.341 | 2.067 | 0.421 | 0.167 | 0.803 | 0.153 | 0.437 | 3.110 |
| Diatoms | 0.866 | 0.008 | 0.324 | 0.515 | 0.113 | 0.508 | *0.009* | *0.849* | *22.560* |
| Euglenophytes | 0.962 | 0.001 | 0.003 | 0.438 | 0.156 | 0.741 | 0.976 | 0.000 | 0.001 |

Table 5. Summary of linear regression analyses done on absolute concentrations of phytoplankton pigments for the three experiment phases in different size fractions. Bold indicated significant positive effect and italics indicates significant negative effect of $CO_2$ concentration. ND indicates pigment was not detected. Where no pigment was detected in any phase in any size fraction, results were not included in this table.

| Pigment | Size fraction | Phase I | | | Phase II | | | Phase III | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *p* | Multiple $R^2$ | F-statistic | *p* | Multiple $R^2$ | F-statistic | *p* | Multiple $R^2$ | F-statistic |
| Chlorophyll *a* | total | 0.470 | 0.137 | 0.636 | **0.008** | **0.854** | **23.440** | 0.081 | 0.573 | 5.377 |
| | < 2 µm | **0.014** | **0.815** | **17.650** | 0.658 | 0.053 | 0.228 | 0.659 | 0.057 | 0.227 |
| | > 20 µm | **0.009** | **0.850** | **22.720** | **0.011** | **0.836** | **20.440** | 0.273 | 0.288 | 1.616 |
| Chlorophyll *b* | total | 0.143 | 0.454 | 3.321 | **0.034** | **0.713** | **9.920** | 0.885 | 0.006 | 0.024 |
| | < 2 µm | 0.815 | 0.015 | 0.063 | 0.726 | 0.034 | 0.141 | 0.369 | 0.204 | 1.025 |
| | > 20 µm | **0.001** | **0.944** | **66.940** | **0.004** | **0.896** | **34.320** | ND | ND | ND |
| Chlorophyll C2 | total | 0.283 | 0.278 | 1.538 | *0.026* | *0.750* | *12.010* | 0.371 | 0.202 | 1.015 |
| | < 2 µm | 0.877 | 0.007 | 0.027 | 0.437 | 0.157 | 0.745 | 0.876 | 0.007 | 0.028 |
| | > 20 µm | ND | ND | ND | 0.094 | 0.544 | 4.765 | ND | ND | ND |
| Canthaxanthin | total | **0.031** | **0.726** | **10.590** | ND | ND | ND | ND | ND | ND |
| | < 2 µm | 0.078 | 0.582 | 5.576 | ND | ND | ND | 0.973 | ND | 0.001 |
| | > 20 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Fucoxanthin | total | 0.876 | 0.007 | 0.028 | 0.420 | 0.168 | 0.807 | 0.371 | 0.202 | 1.012 |
| | < 2 µm | 0.131 | 0.472 | 3.581 | 0.374 | 0.200 | 1.000 | 0.257 | 0.304 | 1.743 |
| | > 20 µm | 0.649 | 0.057 | 0.242 | 0.370 | 0.201 | 1.020 | **0.037** | **0.705** | **9.560** |
| Myoxoxanthophyll | total | 0.056 | 0.642 | 7.157 | 0.755 | 0.027 | 0.112 | ND | ND | ND |
| | < 2 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| | > 20 µm | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| Neoxanthin | total | 0.940 | 0.002 | 0.007 | **0.006** | **0.880** | **29.310** | 0.089 | 0.555 | 4.986 |
| | < 2 µm | **0.030** | **0.730** | **10.820** | 0.660 | 0.053 | 0.225 | 0.820 | 0.015 | 0.059 |
| | > 20 µm | **0.005** | **0.890** | **32.470** | **0.003** | **0.907** | **39.090** | ND | ND | ND |
| Prasinoxanthin | total | **0.040** | **0.691** | **8.947** | **0.001** | **0.945** | **68.540** | ND | ND | ND |
| | < 2 µm | 0.517 | 0.112 | 0.504 | 0.072 | 0.595 | 5.883 | 0.503 | 0.119 | 0.539 |
| | > 20 µm | **0.001** | **0.951** | **77.440** | **0.003** | **0.917** | **44.360** | ND | ND | ND |
| Violaxanthin | total | **0.030** | **0.731** | **10.840** | **0.002** | **0.929** | **52.580** | **0.035** | **0.711** | **9.839** |
| | < 2 µm | **0.017** | **0.797** | **15.710** | 0.854 | 0.01ß | 0.038 | 0.882 | 0.006 | 0.025 |
| | > 20 µm | **0.002** | **0.926** | **49.770** | **0.002** | **0.925** | **49.480** | 0.982 | ND | 0.001 |

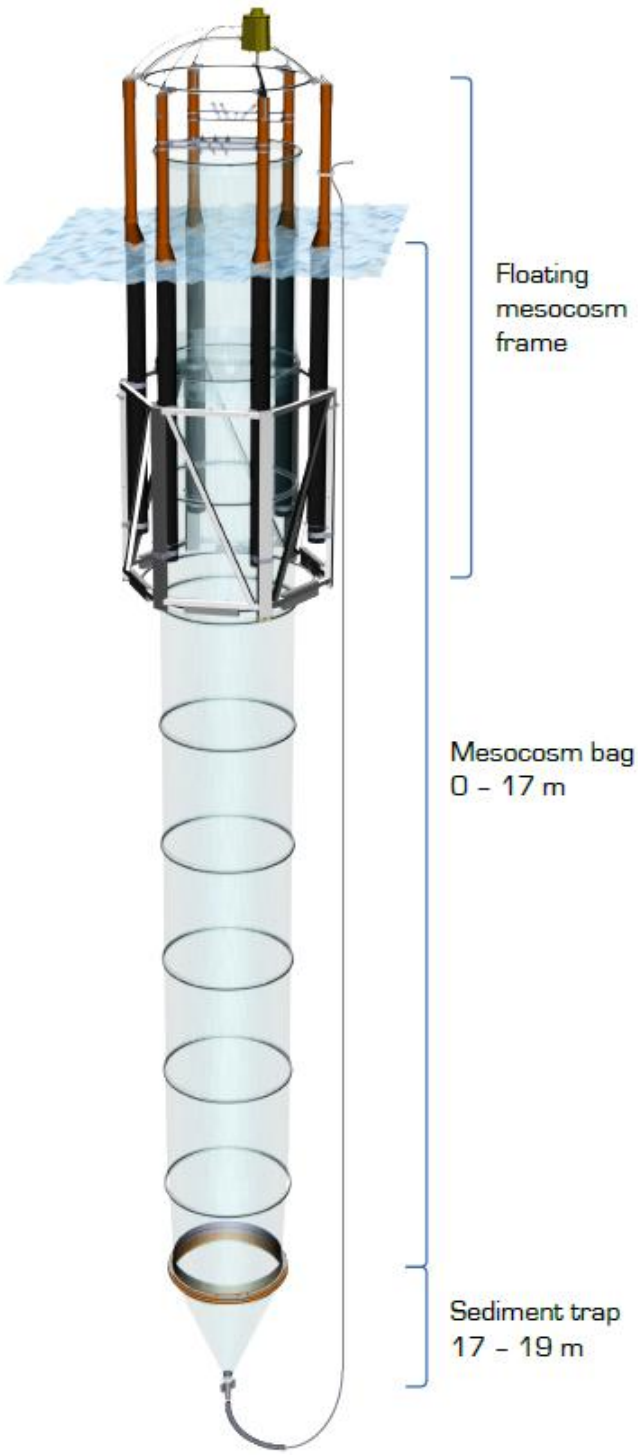
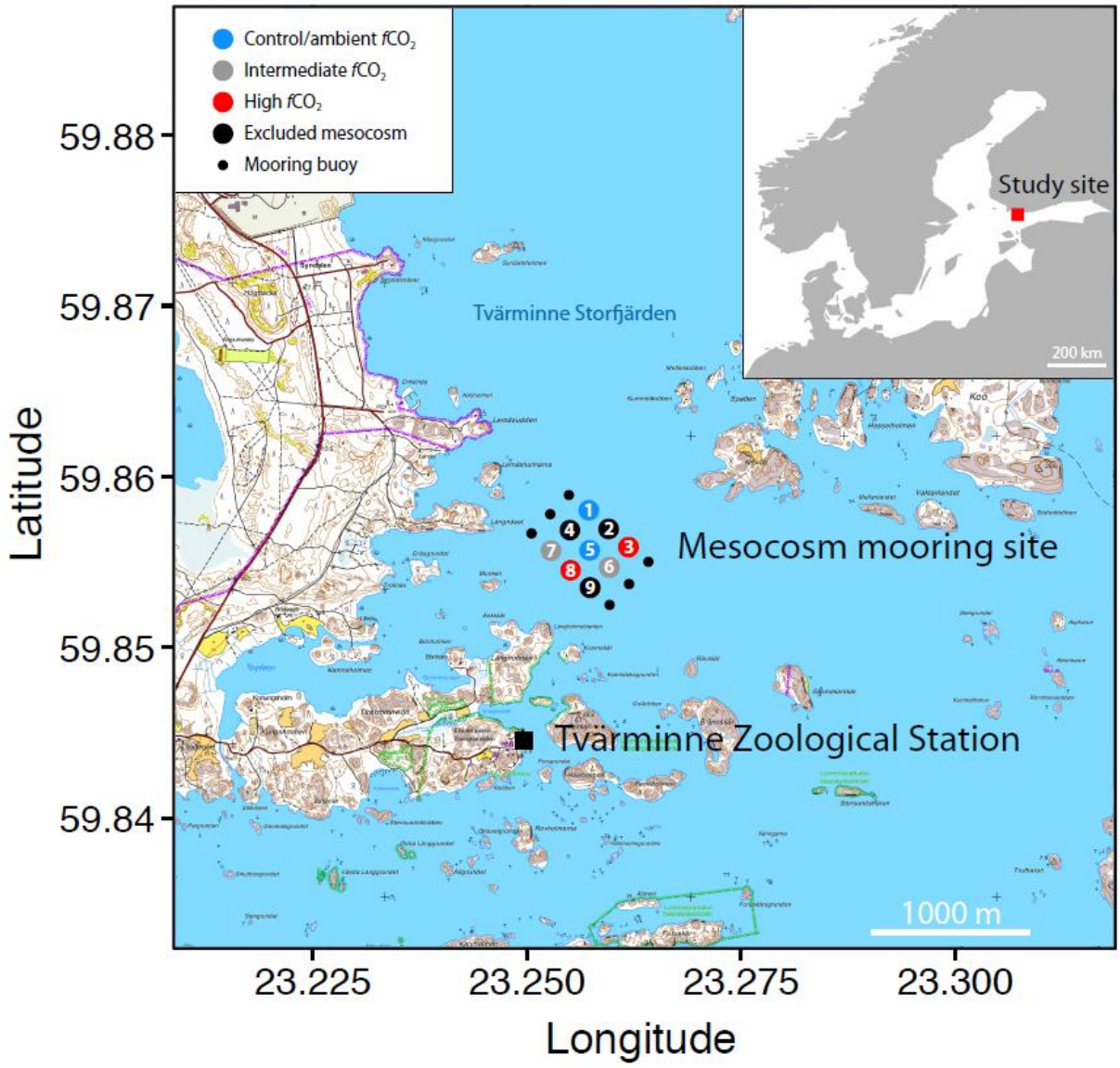



Figure 1. Diagram of Kiel Off-Shore Mesocosm for future Ocean Simulations showing floating frame, mesocosm bag and attached sediment trap. Source: GEOMAR




1

2 Figure 2. Map of study area (inset) and mesocosm mooring site in the Tvärminne Storfjärden,
3 off the Hanko Peninsula close to the entrance to the Gulf of Finland in the Baltic Sea.
4 Mesocosm representation is not to scale. Map contains data from the National Land Survey of
5 Finland Topographic Database, accessed March 2015.

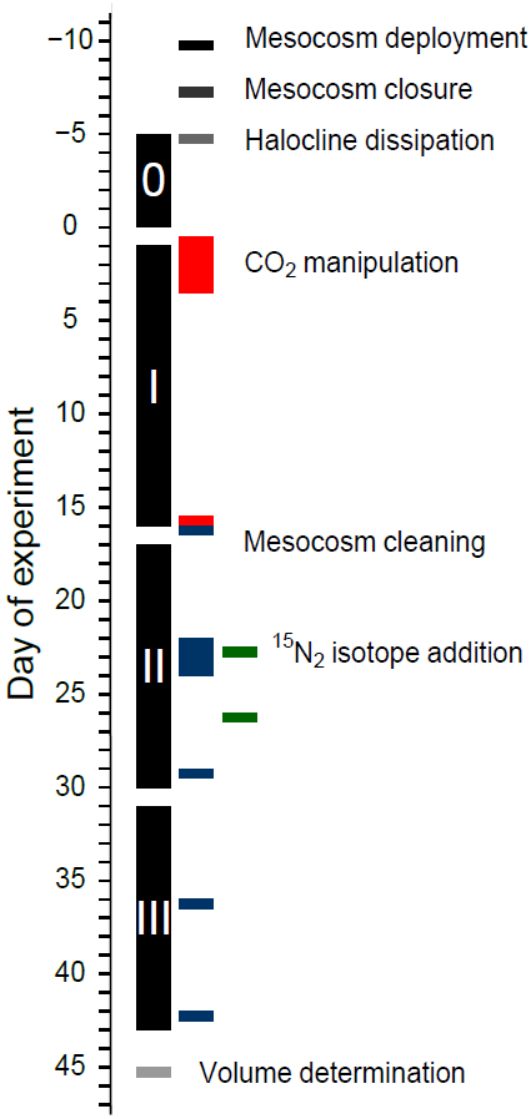



Figure 3. Experiment timeline indicating important activities such as $CO_2$ manipulations (red), cleaning (dark blue), phases (black labelled with 0, I, II and III for Phases 0, I, II and III respectively), volume determination (light grey) and isotope addition (dark green). Distinction of experimental phases is described in section 3.1.

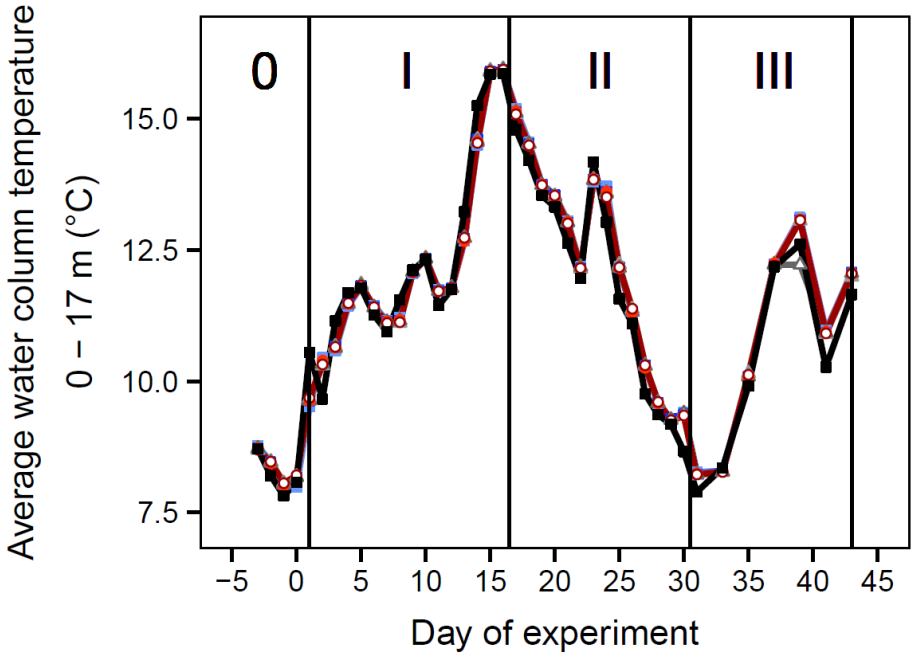



Figure 4. Variation in average water column temperature for all mesocosms and surrounding water during the study period. $CO_2$ enrichment (after *t0*) and temperature variations defined experimental phases. Phase 0 = no $CO_2$ treatments, Phase I = warming, Phase II = cooling, Phase III = 2nd warming phase until end of the experiment at *t43*. Colours and symbols are described in Table 1.

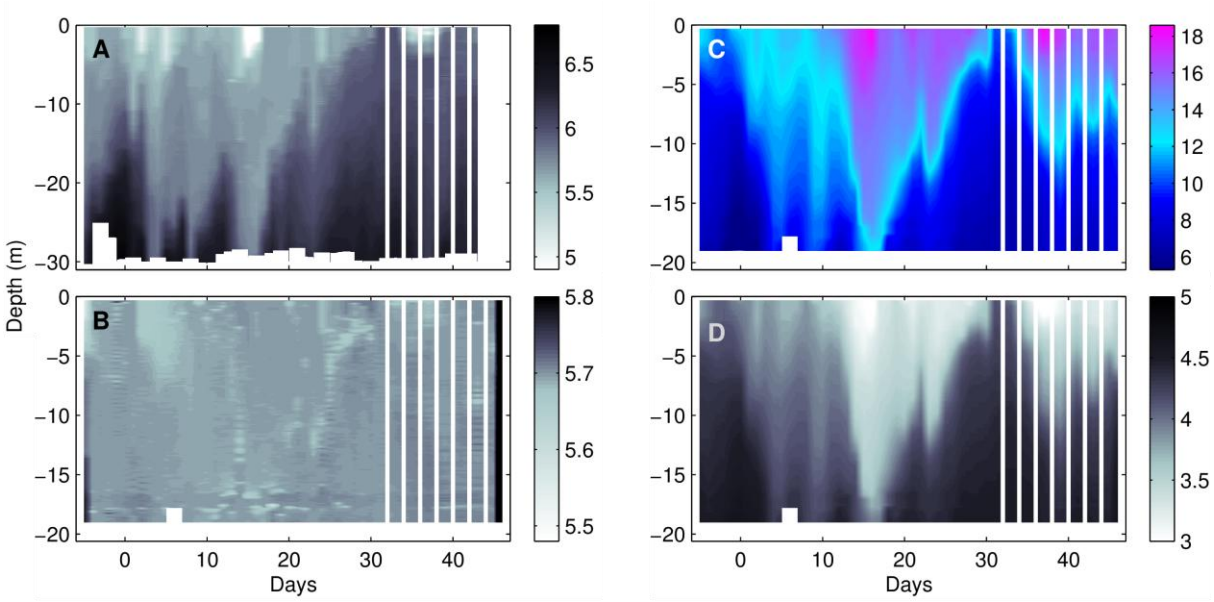



Figure 5. CTD profiles taken between *t-5* and *t46* for A) salinity of surrounding water (Baltic), and B) salinity, C) temperature (°C), and D) density anomaly of M8 ($\sigma_T$ in kg $m^{-3}$). M8 profiles are representative for all mesocosms. White vertical lines indicate CTD profiles were taken every second day after *t31*.

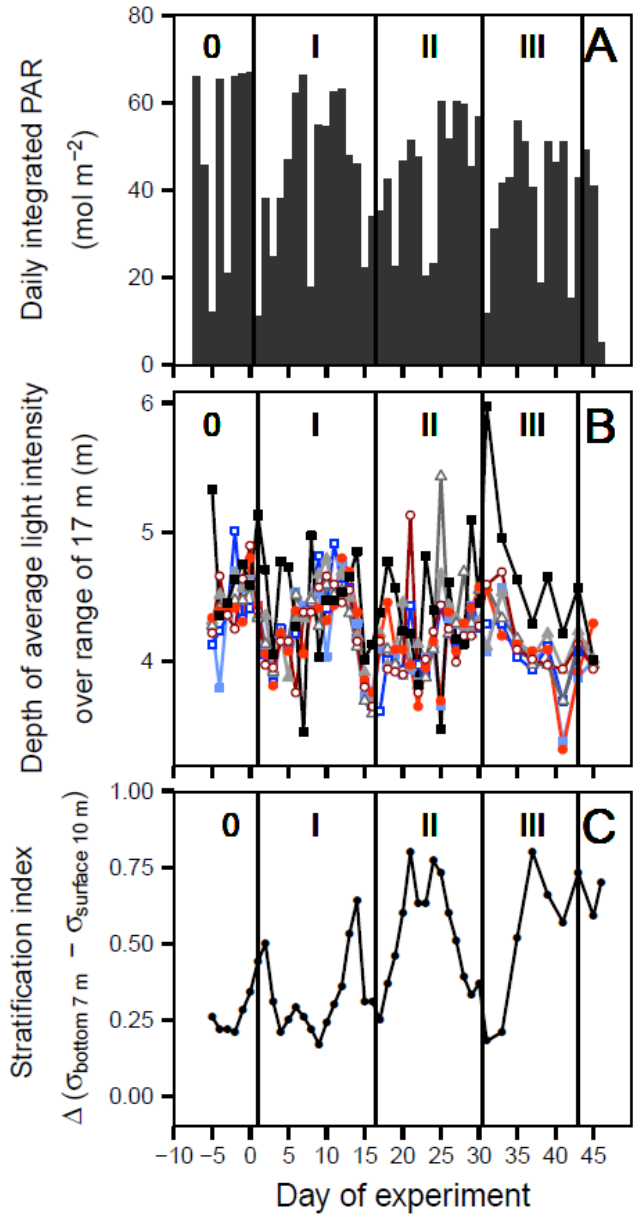



Figure 6. A) Daily integrated incoming photosynthetically active radiation (PAR) measured by a unobstructed sensor on land during the study period, B) depth of average water column light intensity calculated from CTD PAR sensor profiles between 0 and 17 m deep, and C) stratification index calculated from $\sigma_T$ difference between the top 10 m and bottom 7 m in M8 as representative for all mesocosms. Symbols and colours are described in Table 1.

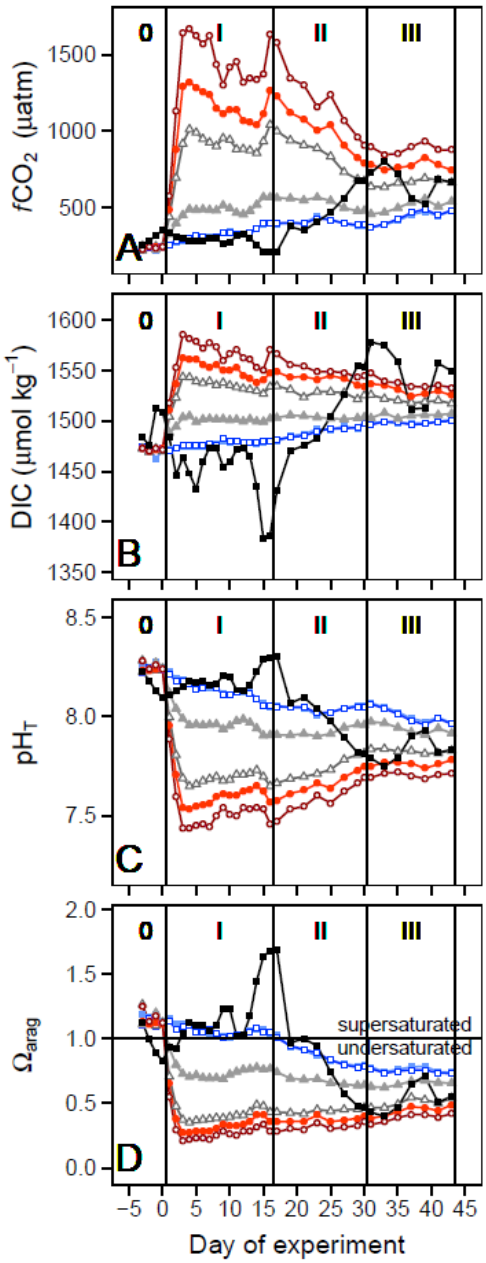



Figure 7. Dynamics in carbonate chemistry speciation with A) calculated fugacity of $CO_2$, B) measured dissolved inorganic carbon concentrations, C) measured pH on total scale and calculated for in-situ temperatures, and D) calculated saturation state (Ω) of calcium carbonate (aragonite). $\Omega_{arag}$ and $fCO_2$ were calculated from DIC and TA using the stoichiometric equilibrium constants for carbonic acid of Mehrbach et al. (1973) as refitted by Lueker et al. (2000). Colours and symbols are described in Table 1.

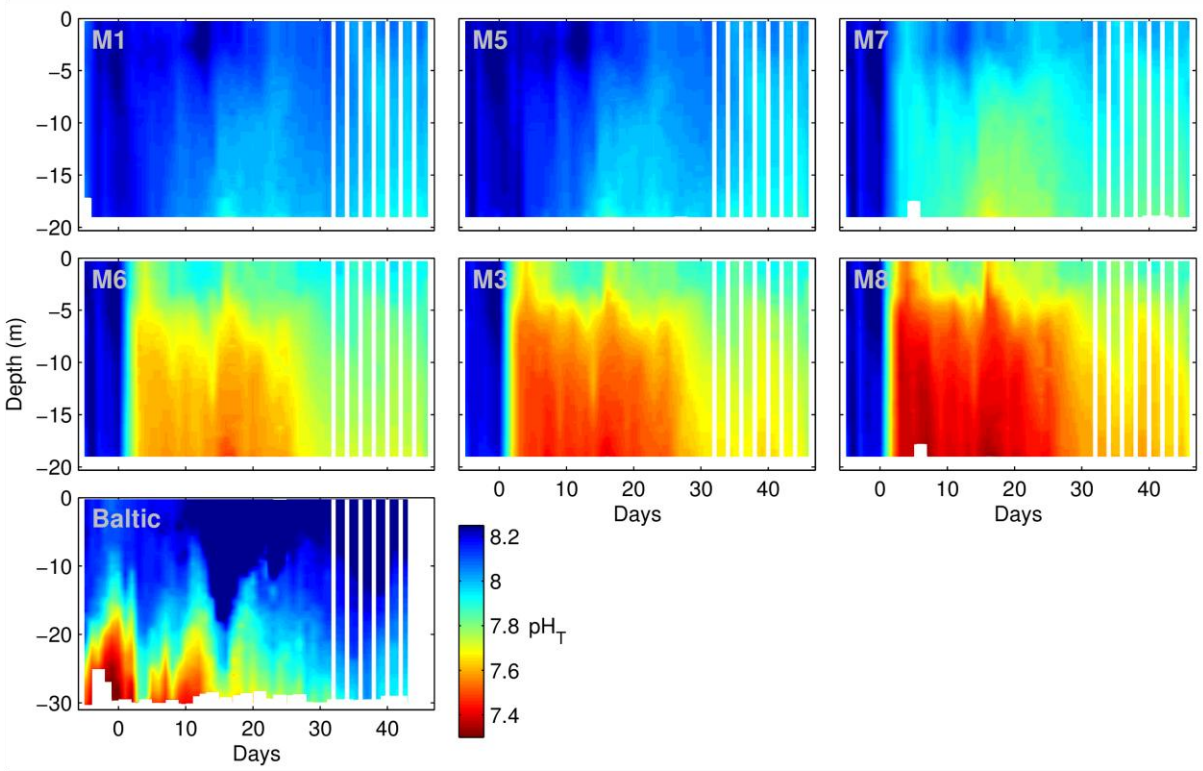



Figure 8.Vertical $pH_T$ profiles taken using a pH sensor on a hand-operated CTD during the experiment in the mesocosms and in the surrounding water, here named 'Baltic'. For details of CTD operations and $pH_T$ calculations, see section 2.5.1. White vertical lines indicate CTD profiles were taken every second day after *t31*.

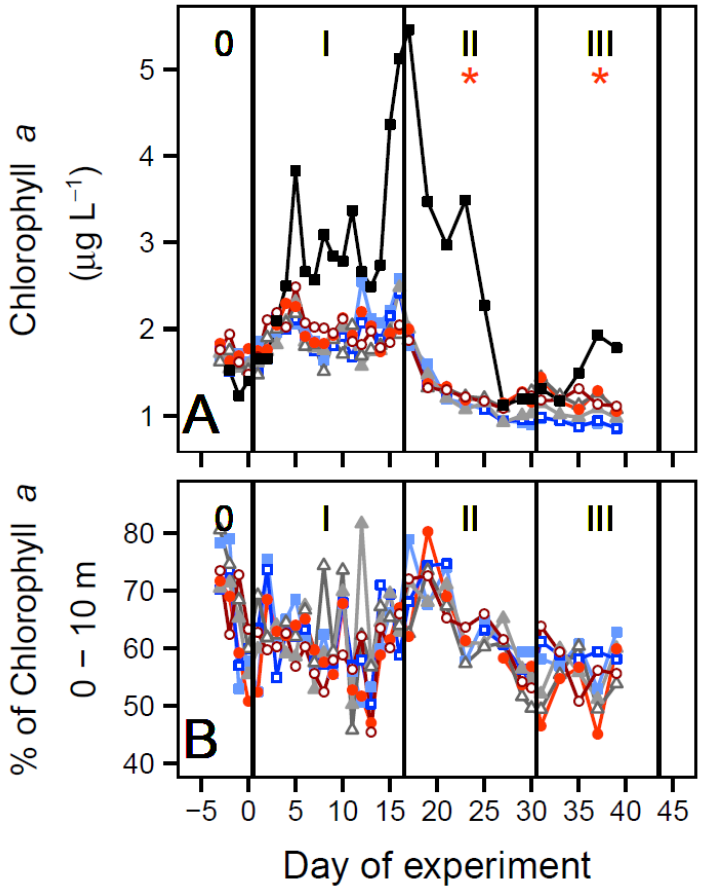



Figure 9. Temporal dynamics in A) chlorophyll *a* (0 – 17 m) including surrounding water and B) percent of total chlorophyll *a* in the upper 10 m. Colours and symbols are described in Table 1. Red asterisk denotes significant positive effect of $CO_2$ (* = $p < 0.05$).

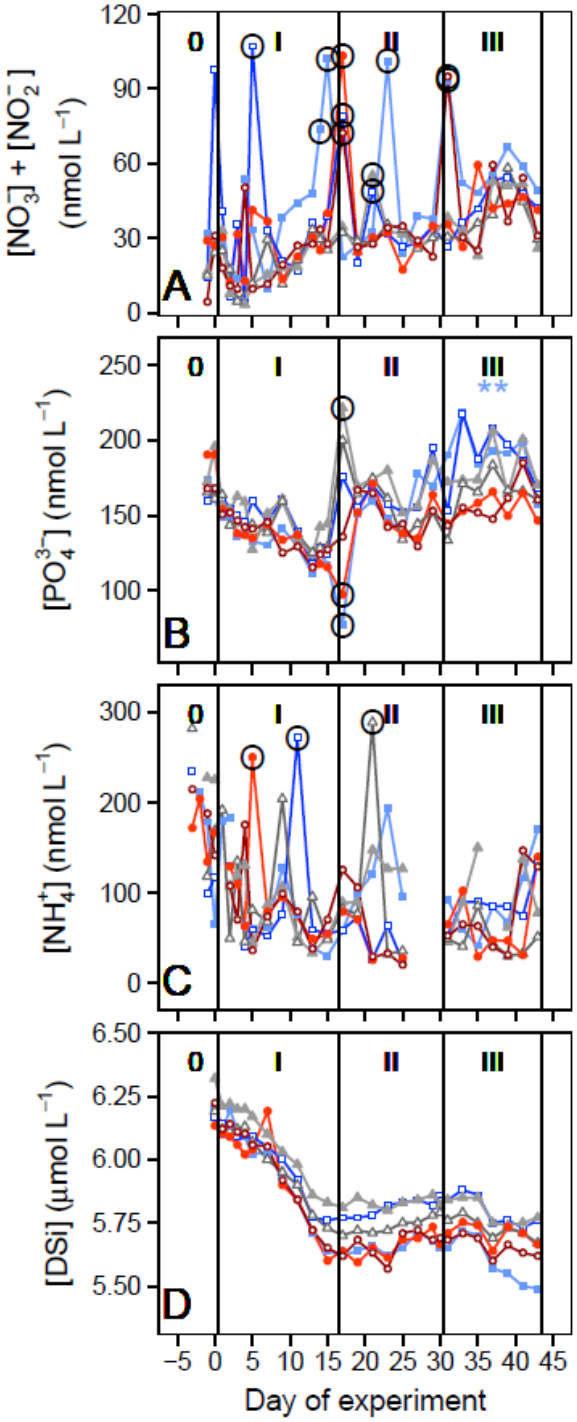



Figure 10. Temporal variation in concentrations of A) dissolved nitrate + nitrite, B) dissolved inorganic phosphate, C) ammonium, and D) dissolved silicate. Colours and symbols are described in Table 1. Blue asterisk denotes a statistically significant negative effect of $CO_2$ (** = $p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

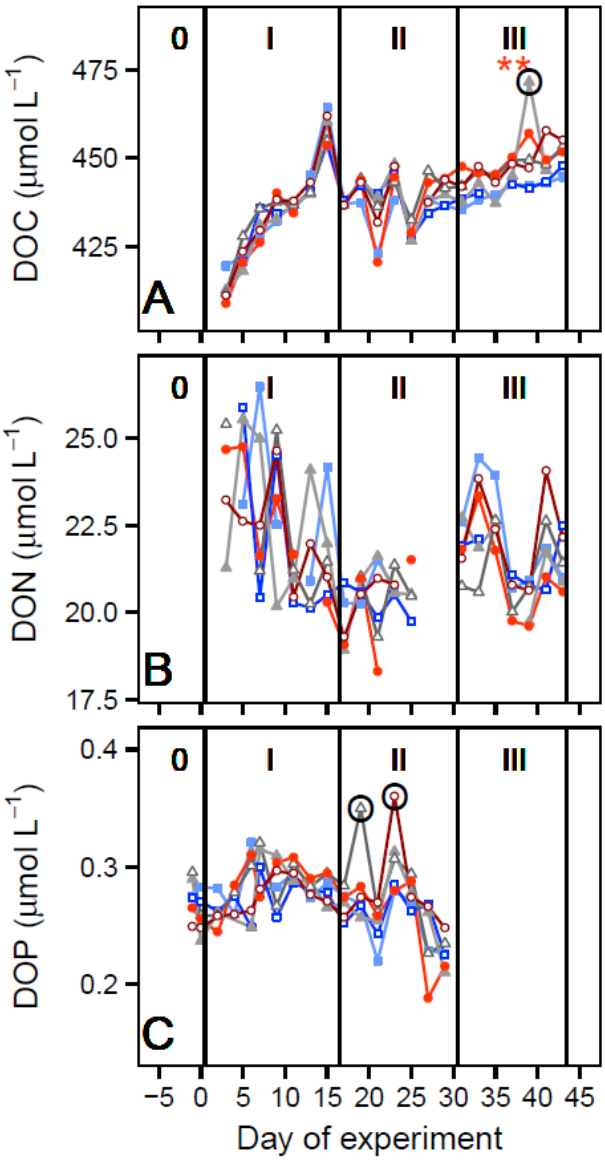



Figure 11. Temporal variation in concentrations of A) dissolved organic carbon, B) dissolved organic nitrogen, and C) dissolved organic phosphorus. $CO_2$ treatments are indicated by colours and symbols described in Table 1. Red asterisks denotes a statistically significant positive effect of $CO_2$ (** = $p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

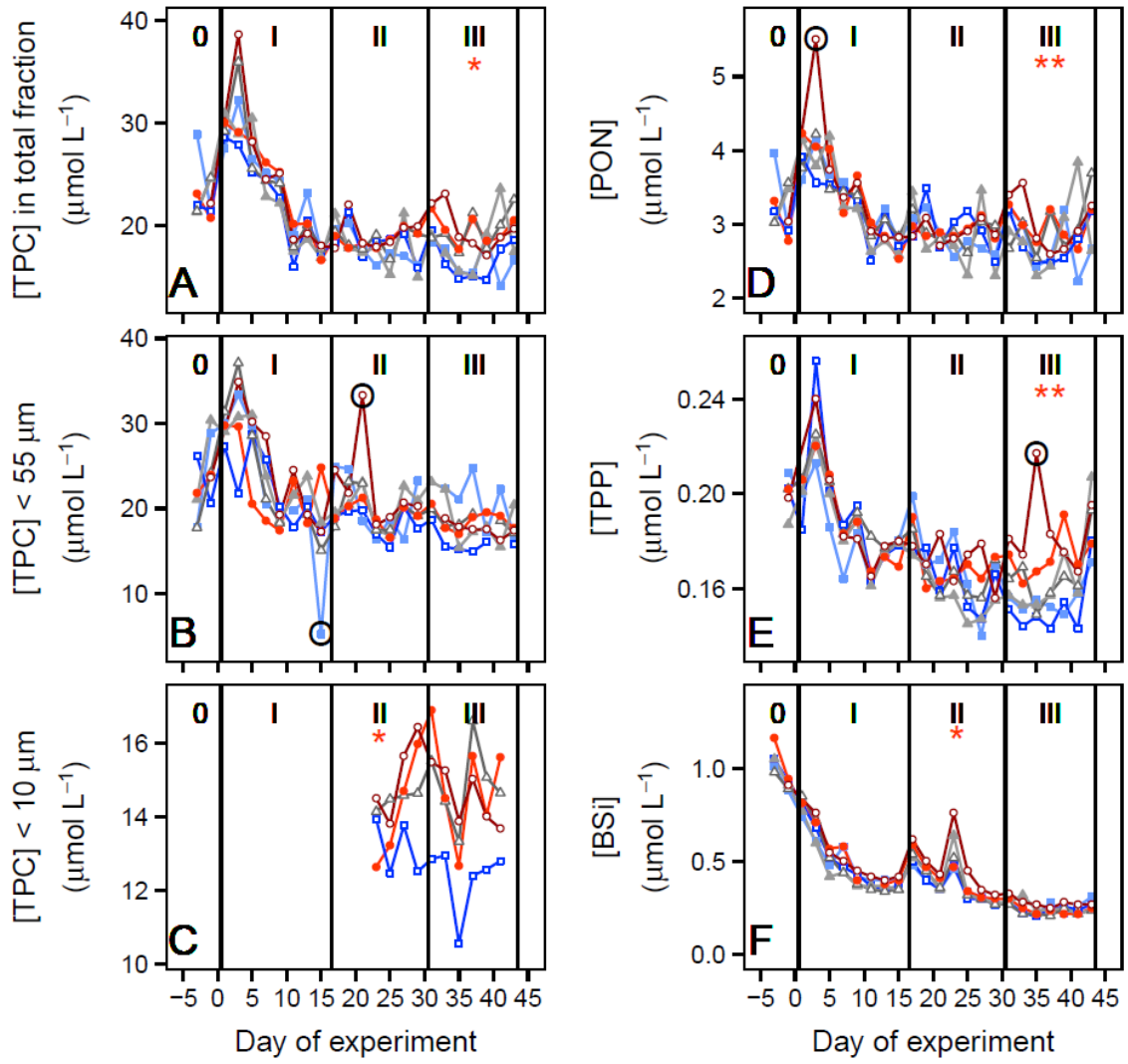



Figure 12. Temporal dynamics in concentrations of A) total particulate carbon, B) particulate carbon < 55 µm, C) particulate carbon < 10 µm, D) particulate organic nitrogen, E) total particulate phosphorus, and F) particulate biogenic silica. Colours and symbols are described in Table 1. Red asterisk denotes significant positive effect of $CO_2$ (* = $p < 0.05$, ** = $p < 0.01$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

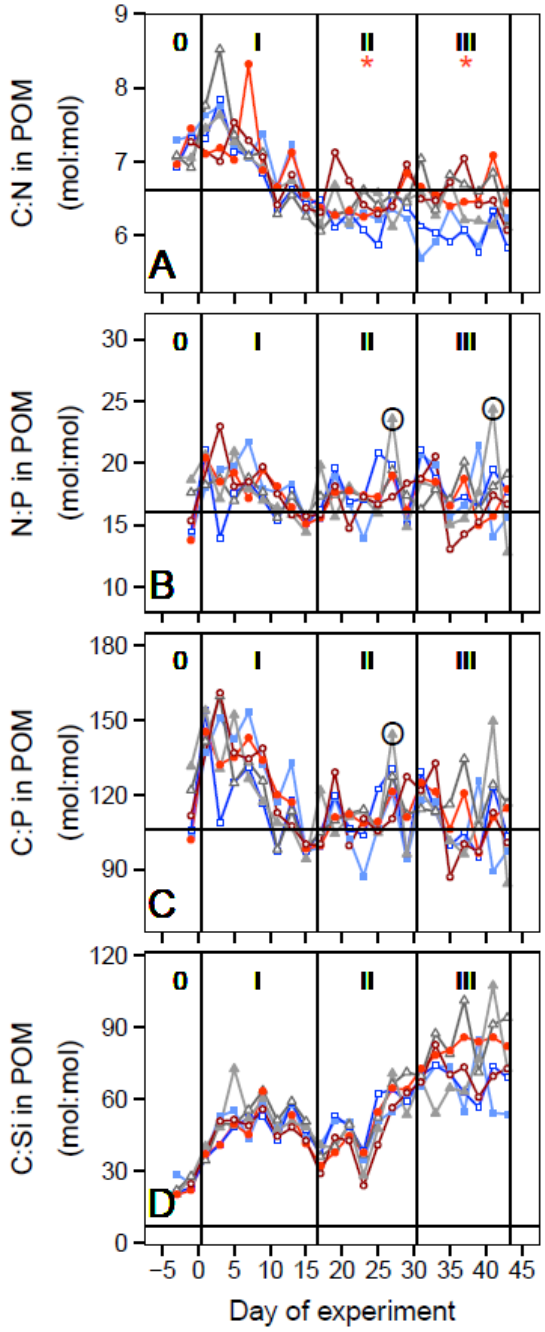



Figure 13. Temporal dynamics of elemental stoichiometry in particulate organic matter: A) carbon to nitrogen, B) nitrogen to phosphorus, C) carbon to phosphorus, D) carbon to biogenic silica. Horizontal lines indicate Redfield stoichiometry (C:N:P:Si = 106:16:1:15, Redfield (1958)). Colours and symbols for different treatments are described in Table 1. Red asterisk denotes significant positive effect of $CO_2$ (* = $p < 0.05$). Outliers (Grubb's test; see methods) are indicated by black circles and were excluded from linear regression analyses.

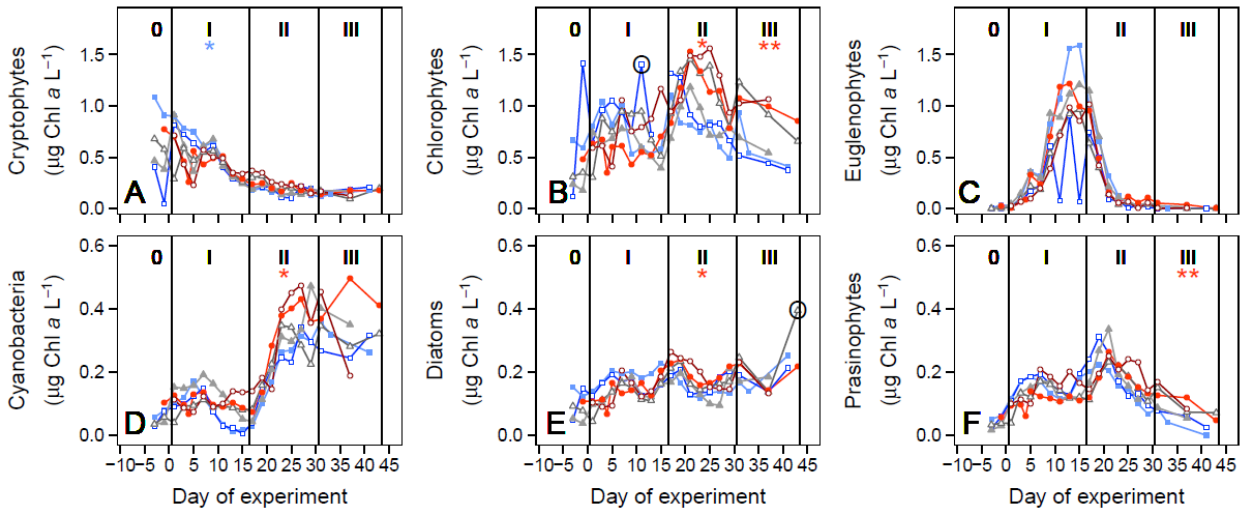



Figure 14. Contribution to total chlorophyll *a* by different phytoplankton groups as calculated by CHEMTAX from HPLC pigment analyses: A) cryptophytes, B) chlorophytes, C) euglenophytes, D) cyanobacteria, E) diatoms, and F) prasinophytes. Colours and symbols for each $CO_2$ treatment are described in Table 1. Red asterisk denotes significant positive effect and blue asterisk a significant negative effect of $CO_2$ (* = $p < 0.05$, ** = $p < 0.01$). Outliers are indicated by black circles and were excluded from linear regression analyses.

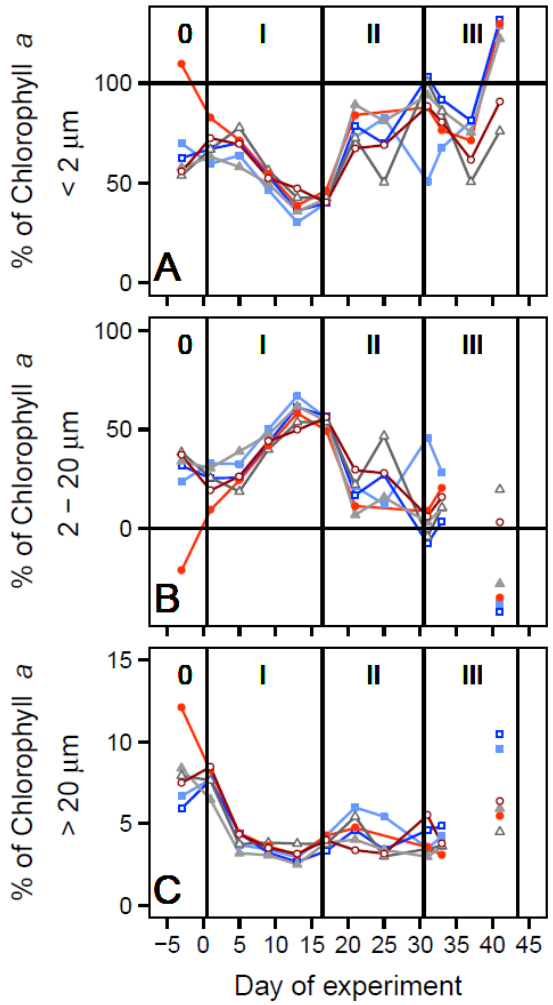



Figure 15. Relative contribution of different size fractions to total chlorophyll *a*. Size fraction 2 – 20 µm was calculated as a mass balance from total fraction and the two size fractions <2 µm and > 20 µm. Colours and symbols for different treatments are described in Table 1. Values larger than 100% or smaller than 0% are due to errors in mass balance calculation.

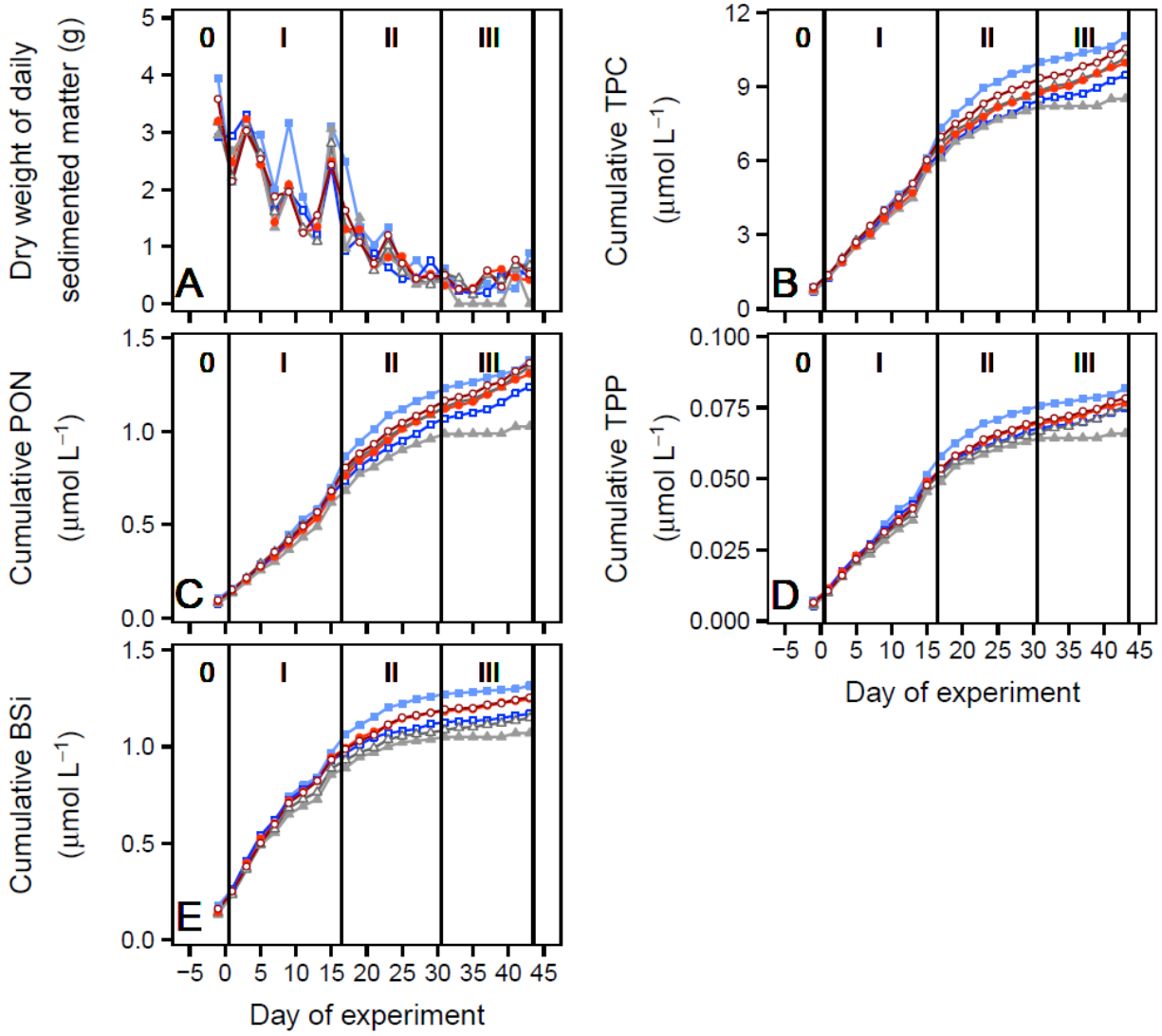



Figure 16. Temporal dynamics in A) collected sediment trap material mass and cumulative B) total particulate carbon, C) particulate organic nitrogen, D) total particulate phosphorus, and E) particulate biogenic silica. Concentrations in B-E were calculated based on individual mesocosm volumes determined at the end of the study. Colours and symbols for different treatments are described in Table 1.