

## ***Interactive comment on “Effect of elevated CO<sub>2</sub> on organic matter pools and fluxes in a summer, post spring-bloom Baltic Sea plankton community” by A. J. Paul et al.***

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We thank Lidia Yebra for her review and taking the time to give 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.

### Specific comments:

REVIEWER 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

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needs clarification.

Author response: We thank the reviewer for highlighting this point. As correctly identified by the reviewer, two 15N-N2 additions were made, as is indicated in Fig. 3. However 15N-N2 (gas) is an isotope tracer specific for N2-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 15N-N2 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).

REVIEWER 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  $\mu\text{m}$  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  $\mu\text{m}$  size fractions was almost identical with an average difference between size fraction of 0.4  $\mu\text{mol L}^{-1}$  ( $\sim 2\%$  of total TPC pool) across all mesocosms and all sampling days.

REVIEWER 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

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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)  $<10 \mu\text{m}$  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  $^{15}\text{N}$ -N<sub>2</sub> 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  $< 10 \mu\text{m}$ . 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.

REVIEWER 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

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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).

REVIEWER 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<sub>2</sub> was the key independent variable in this study, detected CO<sub>2</sub>-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<sub>2</sub>.

REVIEWER 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<sub>2</sub>-fixing filamentous cyanobacteria. Hence we would generally assume that am-

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monium released through organic matter respiration by zooplankton would have been almost immediately assimilated by the phytoplankton community as it is highly bioavailable.

REVIEWER 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  $\mu\text{m}$  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).

REVIEWER COMMENT 8 by L. Yebra: How do you explain that TPC total correlates with CO<sub>2</sub> 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  $\mu\text{m}$ ). We are confident in this correlation between CO<sub>2</sub> and TPC<sub>tot</sub> as this positive effect was also detected in Chl a and TPP concentrations, two independent analyses. We show the <10  $\mu\text{m}$  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<sub>2</sub> 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<sub>2</sub> 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.

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

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

REVIEWER 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  $\mu\text{m}$ ) which may explain this discrepancy in the mass balance. However, we put emphasis on the increase in the proportion of Chl a <2  $\mu\text{m}$  rather than the absolute concentrations. Both the increase in % Chl a <2  $\mu\text{m}$  and decline in Chl a 2 - 20  $\mu\text{m}$  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.

REVIEWER 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 P6888 (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

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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).

REVIEWER 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.

REVIEWER 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.

REVIEWER COMMENT 14 by L. Yebra: Section 4.4., Zooplankton is suggested as grazer controlling the phytoplankton pool (P6890, L16) and picoplankton 'must aggre-

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gate 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.

REVIEWER 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.

REVIEWER COMMENT 16 by L. Yebra: Conclusions, first sentence states that 'fluctuations in temperature correlated well to Chl...' but no statistical data related to temper-

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ature are provided in Results or Discussion.

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

REVIEWER 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

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(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.

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Interactive comment on Biogeosciences Discuss., 12, 6863, 2015.

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