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Interactive comment on "Effects of elevated CO_2 and temperature on phytoplankton community biomass, species composition and photosynthesis during an autumn bloom in the Western English Channel" *by* Matthew Keys et al.

Matthew Keys et al.

mke@pml.ac.uk

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Dear reviewer and editors, The authors would like to thank the reviewer for the helpful comments provided in order to improve our manuscript. The manuscript has been revised based on the reviewers feedback. Our responses to the detailed comments are listed below.

The manuscript 'Effects of elevated CO2 and temperature on phytoplankton community biomass, species composition and photosynthesis during an autumn bloom in the

Western English Channel' by Keys et al. presents much needed data on the combined effects of two stressors of ocean change on the base of the marine food-web. Furthermore, a twenty year long monitoring record of phytoplankton community composition at the experimental site is shown. However, in its present form, I do not agree with the way the experimental data was analyzed and hence with certain conclusions. Major comments and suggestions: 1) The time-series data on the natural variability of phytoplankton community composition and biomass does not complement the experimental data set and is actually disconnected. It is not clear how both data sets would complement each other which is also reflected in the fact that the time-series data is not mentioned in the abstract. It hence appears to be an unnecessary add-on.

Response - The time-series in the results/discussion section has now been removed from the manuscript. We have restructured the manuscript by shifting portions of this section describing the natural variability at the L4 study site, ahead of the microcosm experiment in the introduction.

2) Most critically, however, I consider the choice of the authors to restrict their analysis of the experiments to the last data point at the end of incubations on day 36 as being problematic. This appears to be arbitrary as many of the main conclusions would be different for the sampling days before, and most likely for the ones to come, if the experiments would have been run for a longer period of time. A different approach is needed.

Response - We presented analysis in the form of generalised least squares model results on all of the data (i.e. at all time points) for chlorophyll a concentrations (L 284), estimated total biomass (L 296 – L298), POC, PON and POC:PON (L307 – L317). The results of these analyses were also presented in Table 1 (page 40). However, we wanted to illustrate the abrupt regime shifts in community composition between T24 and T36, when the control community switched to diatoms, and the combination treatment (elevated pCO2/elevated temperature) switched to the most diverse experimental community with significant contributions from dinoflagellates and Synechococcus.

For this reason, we have focussed the community structure analysis on differences in composition between experimental treatments at T36. Critically, this highlights the importance of the experimental incubation period (i.e. extending to 36 days following significant shifts in taxonomic composition and biomass beyond 24 days in this study and in a previous pilot study (data not published). We have updated the manuscript to provide additional analysis on all time points to evaluate the relative shifts of the community through time across the different treatments.

3) I found it interesting that phytoplankton biomass in the combined high CO2 and temperature treatment, and the control did decrease from day 20 and 25 onwards (Fig. 3B). Why is this not reflected in Chl a development and what is the cause? In the semicontinuous culturing set-up of the experiments with a daily dilution of about 10% of the incubation volume with fresh media containing 8 and 0.5 LôĂĂĂ1 nitrate and phosphate, respectively, a decline in phytoplankton biomass suggests that net growth has slowed down and is lower than the dilution rate. The PON data, however, suggests that there should be ample amounts of dissolved inorganic nutrients left for phytoplankton growth, thus it appears that there are indirect effects at work which should be discussed.

Response – Biomass in the control peaked at T25 followed by decline to T36. In line with this biomass trend, Chl a also peaked at T25 in the control (3.9 mg m-3) and declined to 3.3 mg m-3 by T27, remaining close to this value until T36. Biomass in the combination treatment (high CO2 and high temperature) peaked at T20 followed by decline to T36 whereas Chl a in this treatment declined from T20 (3.8 mg m-3) to T25 (3.1 mg m-3) followed by an increase at T27 (to 5.4 mg m-3) before further decline in line with biomass. Chl a peaked in this treatment again at T36 (6.8 mg m-3). We attribute the increase in Chl a between T25 – T27 (coincident with an overall biomass decrease) to lower species specific carbon:chlorophyll ratios (C:Chl a) since dinoflagellates, Synechococcus and picophytoplankton biomass increased in this treatment from T25. Sampling throughout the experiment was conducted by peristaltic pumps linked

to sampling tubes positioned in the centre of each incubation bottle. At T36 however, incubation bottles were removed from the incubator and samples were poured after gentle mixing directly from the incubation bottles. We cannot exclude the possibility of non-phytoplankton particles (e.g. build up on incubation bottle walls) influencing the final high Chl a value at T36 in this treatment. The manuscript has been amended to show C:Chl a data and this (including published literature on C:Chl a values) together with potential bottle effects have been added to the discussion. We further attribute declining biomass under nutrient replete conditions in the combination treatment to changes in community structure in the context of differential species-specific growth rates in respect of increasing dinoflagellate biomass.

4) The photosynthesis versus irradiation curves for the four treatments are based on the assumption that the electron requirement for carbon uptake is independent of seawater CO2 concentration, temperature and species composition. Since this is most likely not the case, any conclusions drawn (if any) would need to be discussed with much more caution.

Response - We applied the same electron requirement parameter for carbon uptake across all treatments and we acknowledge that in nature and between species, there exists significant differences in this parameter (e.g. variation of 1.15 to 54.2 mol e- (mol C)-1, Lawrenz et al, 2013; Hancke et al, 2015) which co-vary with temperature, nutrients, Chl a, irradiance and community structure. We have amended our manuscript to consider this variability relative to our method and results within the discussion.

Additional comments and suggestions: 1) P2, L36: I assume you mean 'concentrations of CO2', not 'uptake of atmospheric CO2'.

Response – We have amended this sentence.

2) P2 L63-66: The grammar in the last sentence of this page seems wrong.

Response – We have amended this sentence.

3) P3, L70: Citing only Engel et al. (2008) and Moutaka-Gouni et al. (2016) here is very selective. Response – We have amended the manuscript to discuss further trends in the response of nano- and picophytoplankton with appropriate references to expand this paragraph.

4) P4, L127: It should read 'was' not 'were'.

Response – We have amended this sentence.

5) P6, L170: Strictly speaking, the calculation of carbonate system parameters from DIC and TA require to account for the contribution of silicate and phosphate to the latter. Have dissolved inorganic nutrients been measured in the incubations?

Response - This research was from a PhD thesis. I did not have the resources or training to measure nutrients for each treatment, replicate and time point over the course of the experiment, hence no data of nutrient concentrations are shown in the manuscript beyond T0. Since phosphate was amended and held constant at 0.5 μM L-1, this value was used in the calculation of carbonate system parameters.

6) P6, L187: How was size determined for the particles measured by flow cytometry, e.g. forward scatter calibrated with fluorescent beads of known size?

Response - Phytoplankton data acquisition was triggered on both chlorophyll fluorescence and forward light scatter (FSC) using prior knowledge of the position of Synechococcus sp. to set the lower limit of analysis. Density plots of FSC vs. CHL fluorescence, phycoerythrin fluorescence vs. CHL fluorescence and side scatter (SSC) vs. CHL fluorescence were used to discriminate Synechococcus sp., picoeukaryote phytoplankton (approx. 0.5–3 μm), coccolithophores, cryptophytes, Phaeocystis sp. single cells and nanophytoplankton (eukaryotes >3 μm, excluding the coccolithophores, cryptophytes and Phaeocystis sp. single cells).

7) P14, L446: Most phytoplankton also uses CO2 as an inorganic carbon source, not only HCOôĂĂĂ3 . Furthermore, 'more efficient' use of CO2 in comparison to what?

Finally, I did not understand the rational behind the notion that Phaeocystis would have an advantage at higher CO2 levels.

Response – We have amended this paragraph to better reflect our experimental findings that under elevated CO2, Phaeocystis spp. dominated the community and this is likely due to variability in the carbon acquisition strategy between the species present, in favour of Phaeocystis spp.

8) P14, L458: I agree, but what could be an explanation for this finding (see also comment above)?

Response – Please refer to our response to your major comment No. 3) above.

9) P14, L467: The four references on CO2 effects on phytoplankton community biomass appear to be a very selective choice. Furthermore, this paragraph lacks any conclusions.

Response – We have amended the manuscript to discuss further cited studies on CO2 effects and conclude upon the trends.

10) P16, L501: The temperature for maximum photosynthetic rates should be species specific.

Response – We have amended the manuscript to acknowledge species-specific maximum photosynthetic rates.

11) P16, L517: I would assume that almost any autotrophic organisms growing at pH levels above 9 would slow down in growth because of inorganic carbon limitation, not only dinoflagellates. Response – We have amended the manuscript to reflect effects of high pH on the growth of all phytoplankton species, not only dinoflagellates which were used in this particular section of the discussion. 12) P18, L578-609: This discussion on Prorocentrum is unconnected to the experimental data and I do not see any added benefit or conclusions to be drawn. Response – We have removed the discussion on Prorocentrum cordatum. 13) P19, L618: It is not clear to me what the authors

mean with the 'present upper limit of the pCO2 threshold increase'. Response – Since we have removed the time-series analysis from the manuscript, the section containing this line (p19, L611-645) has been removed. 14) P20, L648: Why are there potential positive feedbacks? Response – The increased photosynthetic rates observed in our individual treatments of elevated CO2 and elevated temperature suggest these single factors may lead to removal of more CO2 from the surface ocean than under current ambient conditions. This may therefore lead to a more rapid exchange of CO2 between the surface ocean-atmosphere boundary layer, leading to positive feedbacks on atmospheric CO2. We have updated the manuscript to make this statement clearer. 15) P20, L651: Why is there a potential for negative impacts on ecosystem functioning? Response - Dense blooms of Phaeocystis spp. in some ecosystems can be responsible for fish and shell-fish mortality (Levasseur et al, 1994; Peperzak & Poelman, 2008). Phaeocystis spp. colony mucous matrix can inhibit copepod grazing, and therefore affect food web structure through predator-prey size mis-match. Additionally, carbohydrates excreted by Phaeocystis spp. that coagulate to form transparent exopolymer particles (TEP) have strong inhibitory feeding effects on both nauplii and adult copepods (Dutz et al., 2005). Phaeocystis spp. can also be inadequate as a food source for some copepods (e.g. Calanus helgolandicus, Temora stylifera and Acartia tonsa), which can lead to negative effects on fecundity and egg production (Tang et al., 2001; Turner et al., 2002). Exotoxins produced by Phaeocystis spp. during the spring bloom in the northern Norwegian coast can also induce stress in cod larvae (Gadus morhua) (Eilertsen and Raa, 1995). Mass fish mortalities have been linked to Phaeocystis spp. blooms in the Irish Sea (Rogers and Lockwood, 2009) and south-eastern Vietnamese coastal waters (Tang et al., 2004). In addition, the odorous foam produced by Phaeocystis spp. blooms can wash up on beaches and create anoxic conditions in the surface sediment which can lead to mortality of the intertidal benthic community (Desroy and Denis, 2004; Spilmont et al., 2009). Our microcosm experiment suggests that future high CO2 scenarios could increase Phaeocystis spp. blooms at station L4 in the WEC which could adversely affect ecosystem functioning, food web structure and fisheries.

16) P20, L556: Why do 'little response and no effects' suggest 'negative feedbacks'? Response – We assume you refer to L656 in relation to our statement on negative feedbacks. No significant increase in community biomass or photosynthetic rates were observed in our combination treatment of elevated CO2 and temperature. This suggests no change in the removal of CO2 via photosynthesis from the surface ocean relative to current ambient conditions. Under conditions of future increased atmospheric CO2, no change in the surface ocean uptake of CO2 would therefore lead to a negative feedback on atmospheric CO2 concentrations.

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