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.

Anonymous Referee #1

Received and published: 20 December 2017

The manuscript from Keys and collaborators deals with the impacts of ocean acidification and warming on the composition and biomass of the phytoplankton community in Autumn in the Western English Channel. The authors conducted a 36-day experiment in microcosms filled with seawater sampled in the declining phase of the autumn phytoplankton bloom at station L4 on October 7th 2015, where a long-term dataset of nutrient, chlorophyll a and community composition (among many other parameters) is available. This sampled seawater (sieved onto 200 microm) was used to fill 16 borosilicate bottles (2.5 L) corresponding to 4 replicates of 4 treatments. Treatments were 1) control (no modification of pH, T of 14.5 _C), 2) high CO2 (800 microatm, T of 14.5_C), 3) high T (ambient pCO2, T of 18.6 _C), and 4) high CO2 and T. The high T treatment appeared to be applied at once (seawater placed in a temperature regulated outdoor incubation system, while pCO2 was increased gradually to 800 microatm over 8 days. Each bottle was linked to reservoirs filled with filtered seawater in which nutrient concentrations were modified (NOx from 0.24 microM in situ to 8 microM, PO4 from 0.09 microM in situ to 0.5 microM, silicate maintained at in situ concentrations). pCO2 were also controlled in the reservoir for high pCO2 bottles in order to maintain constant pCO2 in the bottles. After 2 days conducted in batch mode, 10-13% of each experimental bottle were replaced with the medium contained in the reservoirs. Various parameters were sampled during the experiment at different frequencies. While chlorophyll a and carbonate parameters were sampled every 2-3 days, phytoplankton community biomass (biomass calculated based on flow cytometry data) was estimated on 5 occasions (T0, T10, T17, T24 and T36), and POC/PON were measured on 3 occasions (T0, T15, T36). Finally, the photosynthetic efficiency was investigated based on samples taken only at the end of the experiment (T36). Based on this experiment, the authors conclude that 1) in all treatments the phytoplankton community shifted from dinoflagellates to nanophytoplankton, 2) large nano-flagellates dominated in the control treatment, while smaller species dominated in the high CO2 and high T treatments, 3) combining these 2 “stressors”, led to a different community with a higher proportion of dinoflagellates and especially of the HAB species Prorocentrum cordatum and 4) finally the authors conclude that “future increases in temperature and pCO2 do not appear to influence coastal phytoplankton productivity during autumn in the WEC which would have a negative feedback on atmospheric CO2”.

Although this manuscript deals with the very important question being “how coastal phytoplankton will respond to global anthropogenic stressors and will coastal plankton community exert a feedback on atmospheric CO2 increase and global warming”, I definitely cannot recommend this manuscript for Biogeosciences in its present form.

My main concerns are 1) related to the experimental protocol considered and how it could be used as a tool for projecting future evolutions of coastal plankton community in this area, 2) the way data have been used and conclusions that have been drawn based on those data, and 3) the way the manuscript is organized mixing results from an experiment and long-term in situ data, with both parts being in my opinion poorly related.

1) Experimental set-up

The authors mention that the effects of pCO2 and temperature on phytoplankton succession in autumn is presently unknown, which is the reason for their experimental

investigation. However, I am really concerned about the experimental choices that have been made and I would like the authors to explain much better the rationale behind these choices. First of all, seawater was sampled at the end (let's say the declining phase) of the autumn bloom leading to low nitrate concentrations of 0.2 microM. My question is, is it realistic to force this system again to high levels of nitrate (8 microM, while the long-term average of NOx at station L4 is of 4.1 microM in October/ November)? Don't you think your results are biased because of this, and prevent you from extrapolating your experimental results to the "real world"? The second experimental choice that is of concern to me is that, during the whole experiment, while chlorophyll a concentrations vary from 0.1 to 3-7 microg/L, the conditions of the carbonate chemistry have been maintained constant. Again, I do not understand the reason behind this choice. In situ, surface pCO2 is extremely dependent on biological activity (by far the main reason for the ocean being a sink of atmospheric CO2). So I have the same question that really needs to be fairly discussed in the paper: Don't you think your results are biased because of this, and prevent you to extrapolate your experimental results to the "real world"? Related to this, it seems to me that nutrient concentrations have been maintained constant during the experiment, although absolutely no data of nutrient concentrations are shown in the manuscript, which is not acceptable to me. Certainly much more than C availability, N and P (and Si) availability structure the composition of phytoplankton communities and control their productivity. What is the reason for maintaining these parameters constant? Another experimental choice is to sieve the sampled seawater onto 200 microm, removing mesozooplankton grazers. Grazing is certainly a very important process shaping phytoplankton communities, what are the consequences of this choice, does it hamper your conclusions? Another missing (in my opinion) very important compartment is the heterotrophic prokaryotic community for which no data are shown in the manuscript (bacterial abundance at least could help). Finally, concerning these experimental choices, why did you conduct an experiment over 36 days? I will come back on that later in the second part, but this choice needs to be explained. This is a very important point since you base a lot of your conclusions on the interpretation of results obtained at T36 only.

Response - It is realistic to force this system from low levels (0.2 microM) to high levels (8 microM) of nitrate, which can frequently occur after heavy rainfall during autumn (see Barnes et al. 2015a; Fig 6) from August to December. In addition, during a pilot study experiment in which we kept nitrate low in all treatments, the phytoplankton populations in all treatments crashed within 7 days. Of course, it depends on what the research question is and for these experiments we are asking: what the long-term trend is to elevated CO_2 and temperature. We are not asking the question about adaptation to nutrients, hence why it has been kept replete in all treatments. This has now been highlighted in the methods section. This research was from a PhD thesis. Whilst I had the expertise and resources to measure the carbonate chemistry, biological and photo-physiological parameters during the experiments 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 why no data of nutrient concentrations are shown in the manuscript (following T0). This has now been highlighted in the methods section.

The seasonality in pH and TA are fairly stable at L4 with high pH and low DIC during summer, and low pH, high DIC during winter (Kitidis et al. 2012 CSR), whereas Chl *a* is much more variable with high values in spring and summer and low values in both summer and winter (see Smyth et al. 2010; Widdicombe et al. 2010; Barnes et al. 2015a). By maintaining the carbonate chemistry, we are mimicking natural events at L4. This has now been highlighted in the discussion section.

We agree with the reviewer that grazing could certainly be important in shaping the phytoplankton community, however our question was, what were the combined effects of CO_2 and temperature on phytoplankton biomass and photosynthesis, not what were the combined effects of CO_2 and temperature on zooplankton grazing. This has now been highlighted in the methods section.

We did not sample for bacterial abundance.

To provide sufficient time for changes in the phytoplankton community to occur and to achieve an ecologically relevant data set, a primary experimental goal was to extend the incubation period beyond the short-term acclimation phase. Exactly how long the acclimation phase is with natural populations may be the subject of some debate. However, previous pilot experiments using the same experimental protocols (unpublished data) have highlighted that after 24 days of incubation, significant changes in community structure and increases in biomass were observed. These pilot study results were used to inform a more relevant incubation period (i.e. 36 days).

2) Data analysis
The authors considered an experimental set-up in which they sampled their experimental bottles on a regular (yet variable depending on the parameter) basis. Nonetheless, many of their conclusions are based only on the analysis of data obtained at T36. For instance, they discuss on L456 to 462, that "chlorophyll a was significantly higher in the combination treatment at T36, : : :, but Chl a was significantly lower in the high pCO2 treatment: : :". I apologize but this interpretation does not make sense at all. Actually, T36 seems to be the only sampling point for which these conclusions are valid. At the penultimate sampling point, there is as much Chl a in the combination treatment than in the high CO2 while high T and control lead to lower concentrations. If you choose another time point, you reach another conclusion: : : and so on: : : The entire dataset MUST be used instead of single points. This is actually even more problematic for parameters that have been sampled with a lower frequency (especially POC and PON), again conclusions have been drawn based on the last sampling point. At T10, the biomass is the highest in the high T, followed by high T/high COP2, then high CO2 and control with the lowest biomass. At T17, you can draw another conclusion etc: : : This is also true for community composition, for which you insist a lot on the abrupt increase in dinoflagellate abundance in the high T/high CO2 treatment at T36, while their abundance is much lower at T24: : :. Again, what is the rationale in using data obtained after 36 days of incubation in a small volume with all the artefacts associated with this incubation technique: : :?

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 pCO_2/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) Structure of the manuscript
I am not convinced about the way this manuscript that combines analyses of in situ long-term data and experimental data, is structured. In my opinion, the analysis of in situ data should be put upfront in the manuscript, before describing and analyzing the experimental results. Furthermore, I am not convinced about the relevance of these observational data in this manuscript. Analyzing the distribution of the abundance of phytoplankton species just based on temperature and pCO2 is a huge simplification.

Response - We agree that analysis of the time-series natural variability based on temperature and pCO_2 alone is a simplification of a complex natural system. 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. Any further analysis and reference to the time-series in the results/discussion section has now been removed from the manuscript.

Minor comments.
Introduction. L36 to 58. Citing 10 years old (at least) papers in this section is not acceptable and suggests (wrongly I suppose) that the authors are not aware of recent literature. L43: please add "surface" pH of 0.3 units: : : L81: what is the reason of this warming? L83: this sentence makes no sense. If no significant trend, there is no increase: : :.
Mat and Met L131: what were the PAR levels during the experiment?
Results L254: please add: "with a mean concentration over this period, of : : : or equivalent
Please provide data of nutrients and data of TA and DIC. Regarding TA and DIC, I am extremely surprised by Figure 2. How can you have similar pCO2/pH and CO32-/HCO32- between control T and high T? With a difference of 4 _C, this appears unreal: : :. If you keep pCO2 constant as you mention, you should have several hundreds of microM difference between HCO3- at 14_C compared to 18_C: : :. or am I wrong?
On several occasions, please add "atmospheric CO2 increase" when you refer to the potential negative or positive feedbacks on atmospheric CO2.

Response - We have incorporated all of your minor comments into the manuscript. You have rightly pointed out that we presented the wrong CO32-/HCO32- data in error (Fig. 2). We thank you for drawing our attention to this error and have updated this figure in the manuscript. We have also included an additional table of all carbonate system parameters in the supplementary material. We are however unable to provide further nutrient data as discussed above.

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.

Anonymous Referee #2

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The manuscript '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 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 pCO_2/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 CO_2 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 L□1 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 CO_2 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. 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. The manuscript has been revised accordingly.

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 CO_2 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 CO_2', not 'uptake of atmospheric CO_2'.

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 CO_2 as an inorganic carbon source, not only HCO_3^-. Furthermore, 'more efficient' use of CO_2 in comparison to what? Finally, I did not understand the rational behind the notion that Phaeocystis would have an advantage at higher CO_2 levels.

Response – We have amended this paragraph to better reflect our experimental findings that under elevated CO_2, *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 CO_2 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 CO_2 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 CO_2 and elevated temperature suggest these single factors may lead to removal of more CO_2 from the surface ocean than under current ambient conditions. This may therefore lead to a more rapid exchange of CO_2 between the surface ocean-atmosphere boundary layer, leading to positive feedbacks on atmospheric CO_2. 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 CO_2 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 CO_2 and temperature. This suggests no change in the removal of CO_2 via photosynthesis from the surface ocean relative to current ambient conditions. Under conditions of future increased atmospheric CO_2, no change in the surface ocean uptake of CO_2 would therefore lead to a negative feedback on atmospheric CO_2 concentrations.