Effect of enhanced pCO_2 levels on the production of DOC and TEP in short-term bioassay experiments: response to anonymous reviews.

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Introductory comments

We are grateful for the comments of the three anonymous reviewers. The reviews were very helpful, constructive and generally positive, and in the manuscript we have implemented changes based on the comments. In the following, we address each of these comments in turn, and outline the specific changes and clarifications that we have implemented. Reviewer's comments are in italics, while our responses are in normal typeface.

Anonymous reviewer #1

AR1.1 While I agree that environmental parameters may be the cause of the observed results (this assumption is discussed convincingly), in my opinion, it omits one scenario that could explain not only the observed highly variable effect of pCO_2 on the production of TEP, but also the variability between the studies conducted so far. It has been showed that TEP have a density much lower than that of seawater (Azetsu-Scott and Passow 2004), and that this low density tended to bring them at the surface and to fuel the surface microlayer (Azetsu-Scott and Niven 2005, Wurl and Holmes 2008, Wurl et al. 2009). If such an ascending flux of TEP occurred in the incubation bottles during the present experiments (actually, there is no reason why TEP should not also ascend during these bioassay experiments), one could expect a vertical heterogeneous distribution of TEP in the bottles and an accumulation at the surface. This heterogeneity of distribution may cause a high level of heterogeneity in the measured TEP concentration depending on the sampling procedure and replicability. It is to be noted that DOC concentration should not be influenced by the same density-driven mechanism. Unfortunately, the Material & Methods section (presented in Richier et al. same issue) does not give any details on the sampling procedure, and thus it is not possible to evaluate this potential source of variability in TEP concentration. Therefore, I suggest providing details about the sampling procedure inside the bottles (e.g. sampling depth, sampling equipment, volume sampled) and incubations method (agitation or not), and discuss the possibility of an ascending flux of TEP, likely to introduce a certain level of heterogeneity in the vertical repartition of TEP and subsequently some uncertainty in the measurement of TEP concentration. Until this ascending mechanism is well taken into consideration, any attempt to decipher the effect of OA on TEP production and vertical flux may continue to generate contradictory and puzzling results.

We agree with the reviewer that a heterogeneous vertical distribution of TEP would have influenced our results, and are grateful for the several useful references provided on this. Having realised this in advance of the experiments, bottles were gently agitated throughout the incubation, and particularly immediately before sampling. Thus, the TEP was well mixed in the bottles before sampling, which should have negated the possible aliasing due to an ascending flux of TEP. The volume sampled was 200 ml and it was sampled from the bottom of the incubation bottle. An explanation of this has been included in the Methods section of the revised manuscript (Section 2.1, lines 127 – 128 and Section 2.3, line 174), and that of Richier et al. (same issue).

AR1.2 If the TEP concentration measured in the bottles is converted in terms of TEP-C concentration using the conversion factor (i.e. TEP-C = 0.75 TEPcolor; µg C L-1) provided by Engel and Passow (2001), for the measured range of TEP concentration (Fig. 3b), the TEP-C concentration should range between about 5 to 12 µM. This range for TEP-C concentration is about the same as the measured total POC, i.e. from 8 to 20 µM (Richier et al. same issue). While the TEP-C concentration is only an indirect estimation, I think TEP-C and POC should be compared in the light of the respective sampling procedure for TEP and POC, as it may help understand the fate of TEP in the bottles, and probable different repartition patterns of POC and TEP in the bottles.

As mentioned in our response above, the incubation bottles were well mixed before sampling. Thus, a comparison between TEP-C and POC is not deemed necessary to elucidate the fate of TEP in the bottles.

Following the reviewer's suggestion, however, we did consider the relationship between TEP-C (converted from TEP concentration following Engel and Passow, 2001) and POC. While interesting, we found that it did not greatly add to our understanding of the organic carbon dynamics in the bioassays. In E4, it reinforced the suggestion that TEP was closely correlated to phytoplankton growth, and in E2, it supported the hypothesis that TEP was produced in the aftermath of a bloom, while other organic matter was being degraded. We have not included details of this in the revised manuscript.

AR1.3 Fig. 2c, experiment E2: The results of the chlorophyll a concentrations in the small size fraction are different from those presented for the same experiment in Richier et al. (same issue).

We are grateful to the reviewer for highlighting this discrepancy, which was, in fact, due to an error in the values of total chlorophyll *a* in this manuscript (the small size fraction of chlorophyll *a* was evaluated as the residual of the total and the large size fraction). This has now been amended and the figures redrawn.

AR1.4 Fig. 2 and 3: insert time scale on the x-axis.

This has been done.

AR1.5 I suggest adding Mari et al. (2001) in the carbon overconsumption citation lists (line 469) as they first described the role of TEP production in the carbon overconsumption process.

This has been done.

Anonymous reviewer #2

AR2.1 Data presented in this manuscript could be better compared to observations available in the accompanying manuscripts of the special issue. This information is not readily accessible, because in some of the other manuscripts the stations are named differently; or not the same stations were sampled in the same regions? Information needed to be considered for this manuscript are the initial pCO_2, the pCO_2 evolution during the incubations, phytoplankton species composition, primary production, and if available bacteria abundance. I suggest adding this information to the manuscript.

We have included the time evolution of pCO_2 (which of course also highlights the initial values, Figure 2), referring to it in Section 2.1, lines 140 – 142. Initial pCO_2 values and phytoplankton community composition are also presented in Figure 3 and Table 4 of Richier et al. (same issue), respectively, and discussed more extensively in that manuscript. Figures showing primary production and bacterial abundance will be included as a supplementary figure. Furthermore, we reiterate that all of the data are available from www.bodc.ac.uk.

AR2.2 Page 3706: Were the bottles filled without headspace? Were they aerated during the experiment? How stable was the pCO_2 over the incubation time? Please give actual values.

The bottles were filled without headspace and were not aerated, since they were sacrificed at each sampling point (Section 2.1, line 129, more details in Richier et al., same issue). The pCO_2 evolution can now be observed in Figure 2. The initial achieved pCO_2 were generally close to the target values, though less so at higher pCO_2, presumably due to the lower buffer capacity of the carbonate system at high pCO_2. Levels of pCO_2 varied more significantly in the higher pCO_2 treatments, changing by up to 200 µatm during the course of the incubation in the 750 and 1000 µatm treatments but never more than 50 µatm in the ambient treatments.

AR2.3 What means 'ambient' in this respect? How variable was the initial pCO_2 between stations? Please give actual values.

This information can now be determined from Figure 2. Initial pCO_2 varied by approximately 50 µatm between stations (significantly less than the variation induced by carbonate

chemistry manipulations), and was highest (400 µatm) in E4. In the revised manuscript (Section 2.1, lines 130 – 133), we have clarified that by 'Ambient', we mean that the carbonate chemistry was unperturbed (left at the background level) and the pCO_2 was left to evolve naturally.

AR2.4 TEP method: Alcian Blue adsorbs to surface polysaccharides of coccolithophores that may have been present in the areas studied. Were the filters examined microscopically to ensure that stained material was 'free' TEP and not stained surface polysaccharides? How high was the coccolithophore abundance at each station? Maybe phytoplankton composition could explain the relationship between TEP and Chl-a observed in E3-5? Could it even be that coccolithophores grew better at lower pCO_2? Please add more information on species composition.

We are grateful to the reviewer for raising this interesting point that we had not previously considered, and that required some thinking. The TEP filters were not examined microscopically, and thus it is possible that some staining of coccolithophores occurred and aliased our results. Based on the following calculations, however, we find that this effect was negligible, and did not affect our results or conclusions.

Engel et al. (2004) noted the staining of E. huxleyi cells with Alcian blue, and calculated that this accounted for 2.59±0.4 pg Xanthan equiv. $cell^{-1}$. Previous studies have applied this factor to correct TEP concentrations for the presence of coccolithophores in their samples (Borchard and Engel, 2012; Engel et al., 2009). The number of coccolithophores in our bioassay experiments ranged from ~0 to 250,000 cells l^{-1}. Poulton et al. (same issue) note that around this region of the Northwest European shelf seas, typically >60% of coccolithophores present are E. huxleyi (Table 3). Thus, assuming that all of the coccolithophores in our bioassay experiments were E. huxleyi, the error induced by staining of these cells was between 0 and 0.75 µg Xanthan equiv. The minimum TEP measured in our experiments was 80 µg Xanthan equiv., making this potential error negligible.

We have included a brief explanation of this in the Methods section of the revised manuscript (Section 2.3, lines 188 – 197).

AR2.5 Figures 4 and 5; there are no error bars indicated in the figures. Was the propagation of the analytical error taken into account?

Error bars have now been included in these figures. The errors were calculated as the root sum of squares of the standard deviations of the two measurements used in each calculation.

In Figure 4 (now Figure 5), only error bars for the change in DOC and TEP were included as these are the important variables. Error in initial pCO_2 can be inferred from Figure 2 if

necessary. In Figure 5 (now Figure 6), error bars are provided for both the change in TEP and the change in Total Chl-a.

AR2.6 Fig. 3; please add x-axis legend

This has been done.

AR2.7 Fig. 5; please use same units for TEP and Chl-a (µg L-1)

Units for chlorophyll *a* have been changed to µg L^{-1} throughout the manuscript, to maintain consistency with the other measured variables.

AR2.8 How meaningful is the regression analysis in Fig. 5, given that production and degradation processes likely show different relationships between TEP and Chl-a? Moreover, the data used in Fig. 5 are not independent as the response 0-96 is always the average of the values 0-48 and 48-96. Thus, the number of observations is increased artificially. Regression lines should, if at all, be calculated without 0-96 values. Also consider possibility of direct staining of cells with Alcian Blue at E3-5 (nanoplankton!) mentioned above.

We believe that this regression is meaningful and useful in supporting our hypothesis that, in experiments E3, E4 and E5, TEP variability is primarily controlled by growth. As the reviewer comments, we would perhaps expect a different relationship between TEP and Chl-a during production and degradation processes. An interesting result of these regression analyses (particularly that for E4) is that we do not see a different relationship. This supports the suggestion that TEP is closely coupled to Chl-a (and, by assumption, phytoplankton growth and degradation) in these experiments. We have provided some further detail on this in the revised manuscript, stating "The relationship was maintained even when Chl-a concentrations decreased, suggesting a tight coupling between these two variables during both production and degradation processes" (Section 4.3, lines 403 – 405).

The reviewer is correct that the data in this figure are not all independent, and we are grateful to them for highlighting this oversight. It is in fact the 48 to 96h values that are derived from the other two. As a result, we have removed these data from the figure and from the regression analyses.

As mentioned in our response above, the potential error introduced by the staining of coccolithophore cells was neglible.

AR2.9 Page 3715: The study by Engel (2002) also considered pCO_2 values below present day. The increase in TEP concentration with increasing pCO_2 was clearly observed when going from past to present day pCO_2, whereas there was almost no TEP increase towards future pCO_2. This is a clear difference to the design and outcome of this study.

We are grateful to the reviewer for highlighting this difference between this study and that of Engel (2002). We maintain that, of all the studies considering the effect of ocean acidification on TEP production, this is the most comparable, being short-term experiments on natural phytoplankton assemblages and not amended by nutrients. We have adapted this paragraph to highlight the differences between the studies. It now reads:

"In terms of methodology, our study is most similar to that of Engel (2002) in which carbonate chemistry was manipulated (to both past and future levels of pCO_2) in a natural phytoplankton assemblage with no nutrient addition. The study was carried out using seawater from two locations in the Baltic Sea and found that after 24 h, more TEP was produced at higher pCO_2 up to present day levels, but no effect was observed when pCO_2 was increased further. We did not determine the effect of reducing pCO_2 below Ambient levels. However, the heterogeneity of the responses we observed at increased pCO_2 clearly indicates that the relationship between pCO_2 and TEP production is more variable than observed by Engel (2002)."

AR2.10 The authors should stress that the observed declining situation encountered in E1, E2 and partly E3 is different from earlier studies cited here that mainly considered biomass build-up phases; e.g. blooms, or culture experiments.

We do not believe that the declining Chl-*a* of these experiments makes them unique. Several studies that have initiated phytoplankton blooms consider the organic carbon dynamics both during and after the build up of biomass (Riebesell et al., 2007; Kim et al, 2010; Engel et al., 2014). Indeed this is when many of the most interesting results were observed. We observed similar dynamics in E2, which appeared to capture the declining phase of a bloom, and suggest that this may be the required condition under which an effect of pCO_2 is observed (Section 4.4). What does make these experiments unique (though not entirely, see Engel et al. 2002, Yoshimura et al. 2010, 2013) is that phytoplankton growth was not initiated by nutrient addition, meaning that growth dynamics are as natural as possible in a manipulation experiment, a point that is stressed throughout the manuscript.

Anonymous reviewer #3

AR3.1 The introduction section are well structured and provides the relevant literature on the subject while the results section are very hard to read and some paragraphs need to be improved. The set experiments using triplicate provides the possibility of good replication that lacks sometimes in the other experiments. References are well cited.

We have attempted to make the results section as readable as possible, which is often difficult when describing multiple variables in multiple experiments. We note the positive comments by the other two reviewers about the clarity of the manuscript. Without further information on

which paragraphs require improvement and in what way, we unfortunately cannot take this comment further.

AR3.2 The main issues are that variable effect of pCO_2 on the production of TEP is rather indirect and due to a combination of several environmental factors varying differently according to studied locations (nutrient availability, phytoplankton community structure and growth conditions) is missing here a discussion on how different hydrological context (well-mixed /stratified water) may affect these relationships.

It is difficult to determine how a binomial variable such as the stratification of the water column affected the complex relationship between TEP and pCO_2 that we observed. Furthermore, the hydrological context at the sample station will influence only the initial conditions of the experiments, manifested by differences in e.g. community structure, nutrient availability and growth conditions. Thus, it seems more relevant to focus on how these factors relate to the TEP to pCO_2 relationship, since we can observe how they concurrently evolved in the experiments.

AR3.3 In some experiments the accumulation or degradation of TEP is mainly impacted by the effect of pCO_2 on phytoplankton growth and was dependent on species composition. While some results are given on small-celled phytoplankton, I suggest, if available to include data and on large- phytoplankton species that contributed to net growth in the higher pCO_2 treatments.

The concentration of large celled (>10 μm) phytoplankton in the experiments is presented in Figure 1d of the manuscript and discussed in Section 3.1. The only experiment in which the dynamics of large-celled phytoplankton appears to have responded to pCO_2 treatment is E4. In this experiment, large-celled phytoplankton thrived at higher pCO_2 during the latter half of the incubation. However, as mentioned in Section 3.1, this appears to have been due to nutrient control in these treatments, following the initial preferential growth of small-celled phytoplankton at lower pCO_2, rather than a direct physiological effect of pCO_2 on large-celled phytoplankton. Furthermore, the change in community structure in the latter half of this experiment did not affect the TEP to Chl-a relationship (Figure 5). Thus there is no evidence, in our experiments, for a secondary effect of pCO_2 on TEP production due to its effect on community size structure. We have included further details on this at the end of Section 4.3 (lines 445 – 450) in the revised manuscript.

AR3.4 In experiment E2 it was suggested that TEP production is a function of the initial availability of nutrients and could be treatment dependent. The environmental condition of this location corresponds to the post- blooms and the occurrence of carbon overconsumption (already described as a mechanism increasing TEP pool in oceans). So the hypothesis of a

treatment-dependent increase in TEP needs to be accompanied by the given calculations specifying in what treatment (1000µatm) significant result was observed.

We presume that the reviewer is referring to the calculation of carbon overconsumption, which we describe as "the difference between the concurrent decreases in DIC and nitrate (applying a C:N ratio of 117:16)". We have included the explicit calculation in the revised manuscript (Section 4.4).

As mentioned in the manuscript (Section 4.4, lines 508 – 510), we observed that carbon overconsumption occurred at both time points in E2, but there was no consistent treatment dependence. We argue that the occurrence of this process, while far from conclusive, lends support to the hypothesis that it contributed to the treatment dependent increase in TEP.

AR3.5 Page 3716 Line 15 -30 (and Figure 5) The few presented data do not allow for a conclusion on a future scenario of TEP production through grow as they do show how TEP production would change at different growth rates, growth could no be calculated here. Authors should compare TEP data with primary production available for the same experiment

We unfortunately do not understand the reviewer's comment here. We do not intend, from this figure, to suggest that future increases or decreases in TEP could be calculated from changes in chlorophyll or primary production. The figure is meant to portray the close relationship observed between these two variables during some of the experiments. The suggestion is that, during these experiments, variability in TEP was due to variability in growth. We use chlorophyll a, rather than primary production, because this variable also reflects degradation processes, which we observe to affect TEP in a consistent manner.

AR3.6 Fig. 5 shows that relationship between TEP production and chlorophyll are not treatment dependent and not always positive, it is already suggested by many authors (included in the MS). In the ocean, cell growth rate is highly variable due to nutrient, light, temperature changes.... Moreover I'm not sure about the significant correlation between TEP and Chl-a in experiment E1 and E2. In the methodological settings, "measurements are taken at the start and after 48 and 96 h. You have only 2 time steps; the time 48-96h is a subtraction of the amount of TEP obtained from 0-96 to 0-48h. In this case it cannot be considered in the regression. This figure could be removed as not related to the main question.

The reviewer is correct that there was not a significant correlation between TEP and Chl-a in E1 and E2. The regression lines and statistics were included in the figure to highlight this fact, but have now been removed to avoid this understandable confusion. The points for the time step 48 to 96h, which, as the reviewer rightly points out, are not independent, have also been removed from the figure and the regression calculation. We are grateful to the reviewer for

highlighting this oversight.

We do not agree that this figure could be removed, as it is shows the close relationship between TEP and chlorophyll *a* in E3, E4 and E5. This point is central to our suggestion that treatment dependent TEP changes are due to the effect of pCO_2 on growth, rather than a direct effect on TEP itself.

AR3.7 Initial and final pCO_2 of experiments are not given. This information is crucial to see the range of pCO_2 experienced in different locations.

This information has now been included in Figure 2.

AR3.8 Page 3713 line 10 is better to use "the rate of TEP increase/decrease per day

This grammatical change has been implemented.

AR3.9 The term lower pCO_2 for the 550 µatm could bring to confusion is better is use 550 µatm.

We unfortunately do not know which part of the manuscript the reviewer is referring to here. We have endeavored to use the terms 'lower' or 'higher' only when there is a consistent trend, and adopt the exact treatment value (e.g. 550 µatm) when the response is unique to a specific treatment.

AR3.10 Instead of "TEP destruction" is better to say " TEP losses or TEP decrease (bacterial degradation can be an important pathway of TEP loss, Passow 2002).

This has been done.