Interactive comment on **"Carbon cycling and phytoplankton responses within highly-replicated shipboard carbonate chemistry manipulation experiments conducted around Northwest European Shelf Seas"** ***by*** **S. Richier et al.**

We are grateful for the comments of the two anonymous reviewers. 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.

Generally, in relation to many of the points raised by both reviewers, we maintain that progress in investigating and understanding the sensitivities of organismal physiology to variability in carbonate chemistry over a wide range of timescales is of fundamental scientific value; ultimately it may also prove to be a productive route by which to address the wider questions related to changes (both natural and anthropogenic) which occur within the natural system. It is in this context that the study was conceived and presented. We further argue that no individual study, including ours, should be considered to represent a robust demonstration of the changes in marine microbial communities, which will occur as a consequence of anthropogenic ocean acidification (AOA).

Anonymous Referee #1

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The manuscript describes the general set-up, experimental design and phytoplankton community's dynamic. The manuscript is well written however some parts of the results section are confusing. The general discussion and conclusion are globally coherent, well justified and brought to the reader even if some considerations are (I think) missing.

We acknowledge this and will revise the manuscript in order to clarify the results section, mainly based on the comments below.

General comments and suggestions:

1. You really managed to emphasize the fact that the major interest was on replicate number and not time and the discussion point about this is convincing. The preferred short time experiment versus wide geographic area has been a nice risk and it was challenging to choose this approach. It is unfortunate that at one location you could not (for technical reason obviously) extend the experiment period of few days. This might be a point on the discussion, of what could have been done to add justification on the short-term effect. It remain exceptional to have so good replicates on the biology, which is a major results and strength of your study.

We thank the reviewer for appreciating the level of replication contained within the presented work. We understand the reviewer's point concerning the interest in running a longer-term experiment. As stated in the original manuscript, we acknowledge that short-term incubations cannot address the effect of slow changes in carbonate chemistry on the adaptation/evolution of the natural phytoplanktonic populations and communities. We reiterate that our approach and results should be

considered complementary to, rather than a replacement for, the large body of work which has been performed with less replication (in terms of number of experiments), but over longer time periods. Within the manuscript we remain extremely cautious about extrapolating from experimental work performed over any time period, out to the longer scales and slower alterations which will be associated with ongoing AOA (see general statement above and further details below).

2. Your justification of small cell size being more sensitive to H+ is based only on one publication (Flynn et al 2012). As doing experiments it would be interesting to compare with previous experiments performed in situ in similar conditions as yours. For example, you do not cite Yoshimura et al. 2013, in which the different response to pCO2 of two locations (Fe limited) was attributed to different community composition. For them large cells dominated the community that respond to pCO2 while the location with small cell size did not show pCO2 effect. The experimental conditions and method to increase CT wasn't the same as your but this could have been a point of the discussion to compare with contradictory results obtained from in situ experiments.

We will expand our discussion of previously observed cell size related effects to manipulation of carbonate chemistry, adding the suggested recent paper by Yoshimura et al. 2013, alongside others including Eggers et al. 2014 and Wu et al., 2014.

Our mechanistic explanation, for the significant physiological responses evidenced in the study, was indeed largely based on the theoretical study by Flynn et al. 2012. We maintain that this represents the most likely explanation for our observations. Moreover, we argue that the perspective we develop may also be reconcilable with previous work based on longer timescale experiments where, nonetheless, the carbonate chemistry system is still significantly perturbed over a reasonably short timescale compared to, for example, AOA. For example, if our arguments hold, we might expect a component of the small cell sized phytoplankton community to be negatively impacted in the early stages of any such experiment and subsequently outcompeted and potentially replaced by larger sized groups. Indeed we potentially observe such a succession in at least one of our experiments and will discuss this further in a revised version of the manuscript (see E4, Fig. 8).

Looking in the literature and the increasing number of publications the last years at community level, it seems that biological answer will depend on the region. You had a large geographical distribution showing some differences. The nutrient status and generalities about in situ conditions could have been interesting to be discussed also to place in the local environmental context before generalise to all oceanic provinces.

We agree with the reviewer that the biological response of the microbial community likely depends on both environmental setting and community structure. We originally acknowledged this in the results section (paragraph 3.1) and reiterated in the conclusion paragraph (page 3511 lines 17-21), but will now expand further in the discussion. We further note that we would strongly caution about generalization of our study, or others, to areas outside those sampled until better mechanistic understanding of the underlying ecophysiological drivers is established.

Specifics comments:

1. The title suggest for that we'll have information on the whole carbon cycle (including DOC production, grazing, particles sinking, ...) which isn't the target of the manuscript. I would rather suggest to use "carbon net production and phytoplankton responses . . ." or something restricting the "carbon cycle" term.

We never meant to imply that we investigated all observable components of the carbon cycle. However, we feel that changing the title to "carbon net production and phytoplankton response" as suggested by the reviewer would not indicate the true scope, which includes, for example, details of changes in the carbonate chemistry system. By reference to the accompanying paper by MacGilchrist et al., we further note that, as stated in the original manuscript, no significant changes in bulk DOC production were observed as a function of treatment. Similarly, in response to reviewer 2 (below), our title states that the study considers 'Carbon cycling', it does not refer to 'Complete carbon budgeting'. However, we acknowledge both reviewers concerns and as such we propose to amend the title to:

'Phytoplankton responses and associated carbon cycling within highly-replicated shipboard carbonate chemistry manipulation experiments conducted around Northwest European Shelf Seas'

2. *For the introduction there might have too many publication cited for one concept, it might be interesting to reconsider some cited papers.*

We acknowledge this and will address in the revision.

3. P.3495, L20: the total depth of the water column is not shown; it is disturbing especially for the E3 location. I made the assumption to read the manuscript assuming that the depth was much important at that site than the others, as stratification is deeper.

We will add the total depth of the water column in the Table 2.

4. For the light, it might be interesting (in the idea to repeat and compare experiments), to know which percentage of the surface irradiance the 100 µmol photons m-2 s-1 represents for each locations (in Table 2?).

We will now quote the integrated daily light dose within the experiments alongside the integrated mixed layer irradiance as presented by Poulton et al. (same issue), the latter being most relevant for comparison with *in situ* conditions (paragraph 2.1.3). We note that surface irradiance conditions would be 3-4 fold higher than this and would thus significantly overestimate the irradiance experienced by a natural phytoplankton population/community being advected vertically within a mixed layer. Overall, daily light dose within the experiments was chosen to be as representative as possible of that likely to be experienced by the phytoplankton community under in situ conditions.

P 3501, L10: For coherence with the first part of the sentence, I would prefer to read, $\hat{An}'$ with E2 being the exception (20 % < 10 μm Chl a).

We agree with the suggestion and will change accordingly in the text.

5. For results section, paragraphs 3.2 and 3.3 nothing is mentioned about the additional locations. Do we have to assume they behave the same as the main locations in term of carbonate chemistry and reproducibility?

We can confirm that the carbonate chemistry in the incubation bottles offer the same reproducibility and evolve similarly to the main experiments (results not shown). Information of the evolution of basic parameters such as pH_T, pCO_2 and Ωc, through the 48 h incubation, is given in Poulton et al. (this issue) and we will cross reference in the revised manuscript.

6. P 3502 paragraph 3.4 I would suggest to reorganise this part, as there is some repetition. L16 should be later in the paragraph.

We acknowledge this and will change in revision.

7. Table 2: the depth column is the sampling depth? Why some of them have < 10 or < 20?

The depth column in Table 2 corresponds to the sampling depth and we will amend the table to include the total depth of the water column for each of the bioassay location as recommended by the reviewer. Specific depths for each of the additional bioassay experiments will also be included as suggested.

8. Figure 4 b); do you take into account the E1 location for this? In the text it is written "highly reproducible" (P3502 L7) but on the figure the area in the middle of the graph is spreading. Is that E1 effect?

We did take E1 data into account in the analysis, which explain the spreading of the data away from the 1:1 line. We will clarify the text in revision.

9. Figure 6: I suggest decreasing the symbols' size to make them clearer and coherent with other figures (such as Fig 4 or 3).

Agreed, we will amend the suggested figure as suggested.

10. Figures 7: for the significant difference indicated by "", does it mean "at least one treatment was statistically different", as it is for figure 8? You have decided to not have any paragraph about statistics in the manuscripts, so it should be very precisely specified in the legend what "*" mean. If the other manuscripts of the special issue use the same statistical tests it might be interesting to mentioned in which the statistics are explained.*

In response to both this comment and that of the other reviewer we will add a paragraph on statistical analysis. To be explicit here, yes '*' means at least one treatment was statistically different.

11. References: change “Klause” to “Krause”

Noted and will be changed in revision.

Added