

We greatly appreciate the valuable comments made by J. Henderiks and a second reviewer to improve the scientific quality of the manuscript. Please find below our answers (red font) to the reviewers comments (black font) and the changes made in the manuscript (red italics).

General re-evaluation of data:

Apart from the changes made to address the reviewers' comments, we also slightly improved data analysis by re-evaluating the Coulter Multisizer measurements and analysing the data assuming a normal distribution. This resulted in slightly lower average coccolith volumes. The results for coccosphere and cell diameter were not influenced by the re-evaluation. This analysis (assuming normal distribution) has the advantage that signals of coccoliths which are attached/sticked to each other (therefore measured as one particle with a double volume) have a minor influence on the average coccolith volume. This is stated in the Method section of the manuscript.

Reviewer 2

GENERAL COMMENTS

The sensitivity of calcite producing plankton to pCO₂ is an important topic within the current debate over the fate of oceanic carbonate production in a high CO₂ and warming ocean. Müller et al. present results from a series of culture experiments of a strain of *Emiliana huxleyi* under different pCO₂ levels and conditions of nitrogen availability (N⁻, N⁺). The focus of this manuscript is on changes in the size of the cell, coccosphere and coccolith under these conditions. It is worth highlighting that such (culture) studies are not only the remit of palaeo-oceanographic research, but of significant importance and relevance to the interpretation of trends in present-day coccolithophore populations. Missing from the article (e.g. abstract) is a significant conclusion that can be drawn from Figures 2 and 3 (scatter plots of pCO₂ and coccosphere/cell/coccolith diameter): the sensitivity of cell/coccosphere/coccolith to nutrient (nitrogen in this case) availability is much greater than the sensitivity to pCO₂. For example, coccolith volume (um³) changes from 0.76-0.89 under nutrient deplete conditions to 2.09-3.43 under nutrient replete conditions (Fig. 3, Table 4). This observation should be included in the abstract and conclusions.

This is a very important conclusion pointed out by the reviewer and we emphasised this observation in the abstract and conclusion of the manuscript.

Abstract:

"The conducted experiments revealed that the coccolith volume of *E. huxleyi* is variable with aquatic CO₂ concentration but its sensitivity is rather small in comparison with its sensitivity to nitrogen limitation. Comparing coccolith morphological..."

Conclusion:

"It is demonstrated that the coccolith volume of *Emiliana huxleyi* varies with changes in the seawater carbonate chemistry but the effect is minor compared to a moderate nitrogen limitation."

As ocean acidification research continues it is worth noting the relative sensitivity of different processes to multiple stressors, especially when we are dealing with a multivariate ocean environment. The 3-4 fold differences in coccolith volume (and estimated mass) and calcification rates between nutrient treatments (2-2.4 vs. 5.7-9.3 pg CaCO₃ coccolith-1 and 2-4 vs. 12-21 pg C cell-1 d-1, respectively) is another very interesting observation. Such modifications of cellular levels of calcite and coccolith production rates have implications for how we interpret the mass of individual

coccoliths (either in the modern ocean or fossil record). At this time there are relatively few observations of such a trend (i.e., changes in coccolith mass under different environmental conditions which are independent of changes in species/morphotype). Following on from this, the authors should include a few SEM images from the different nitrogen treatments to support this finding (i.e. showing large (>4.5 μm) coccoliths in the nutrient replete cultures) and also indicating the level of malformation in the culture experiments.

As mentioned earlier, we experienced unusual preservation problems but we added now an additional SEM image from the chemostat experiment (Fig. 5D). The picture indicates some coccoliths which are partly or nearly completely disintegrated (please see text for details).

SPECIFIC COMMENTS

1. Role of irradiance. In terms of determining growth rates of oceanic populations, light availability is as important as nutrient supply/availability and the introduction should better reflect this.

We included the importance of light availability for phytoplankton growth in the upper ocean:

*“Studies on *E. huxleyi* under nutrient limited conditions and elevated $p\text{CO}_2$ are rare (Sciandra et al., 2003; Leonardos and Geider, 2005; Borchard et al., 2011) whereas light, macro- and micronutrient supply in the upper ocean are the main factors limiting phytoplankton growth (Davey et al., 2008; Moore et al., 2008; Marinov et al., 2010).”*

2. Cell quota. Although it may be obvious, there is no mention in the methods or results of how the cell quota (of PIC, POC, POP, TPN etc) was calculated. This is a central part of the calculation of various parameters (including calcification rates) and it would be good if the authors commented on how it was performed and the possible errors associated with it.

We included the calculation of particulate matter cell quota in the Method section and estimated the error which emerges from Coulter (cell number) and gas chromatograph measurements.

3. SEM images. The SEM images are a key part of this manuscript, and although no quantitative analysis is possible (due to preservation problems?), a few representative images would support various conclusions. This is especially important in the large scale differences in coccolith volume (driven by differences in coccolith size, as concluded by the authors) between nutrient conditions/ $p\text{CO}_2$ - images showing >4.5 μm coccoliths would support their observations. The images (Fig. 5) are difficult to interpret from only the $p\text{CO}_2$ treatments. Images from the nutrient replete and deplete treatments are needed.

Unfortunately, we lack SEM images for analyses. However, we compared now the coccolith volume measurements with recent field and laboratory data from the literature by transforming the coccolith volume to coccolith distal shield length (DSL).

*“This study presents direct coccolith volume measurements from culture experiments and data for comparison are rare. However, coccolith distal shield length (DSL) has been described to correlate with the coccosphere diameter in field and fossil samples (Henderiks et al., 2012; Henderiks, 2008). Converting the measured coccolith volume (V) to distal shield length (DSL) by applying equation (2) with the species specific constant $k_s = 0.02$ (as given for normal calcified coccoliths of *E. huxleyi**

morphotype A, Young and Ziveri (2000)) results in an average coccolith DSL ranging from 2.9 to 3.2 μm and 4.1 to 5.5 μm for the chemostat and batch experiments, respectively.

Equation (3) – please refer to the manuscript.

*Corresponding to the estimates for coccolith mass the calculated DSLs from the batch experiments (nutrient replete) are higher than average DSL of $\approx 3.5 \mu\text{m}$ derived from field samples of *E. huxleyi* morphotype A (Henderiks et al., 2012; Poulton et al., 2011; Triantaphyllou et al., 2010; Young and Ziveri, 2000). Visual inspection of coccoliths from the batch culture experiments via scanning electron microscopy confirmed the presence of coccoliths with $\text{DSL} > 4.1 \mu\text{m}$ (Fig. 5A-C) while coccoliths from the nitrogen limitation experiments (C1-C3) were found to be partly or completely disintegrated due to preservation problem (Fig. 5D).*

*Previous observations of *E. huxleyi* morphotype A with $\text{DSL} > 4.1 \mu\text{m}$ (Cubillos et al., 2007) and the present SEM pictures let us assume that the calculated DSL (and consequently the coccolith volume) from the batch and chemostat experiments is valid and comparable to previous applied methods measuring the DSL of coccoliths.*

*A comparison of field and laboratory data on the relationship between coccosphere diameter and coccolith DSL of *E. huxleyi* (Fig. 6) reveals that results from laboratory experiments (Bach et al. (2012) and this study) have a distinct pattern from field data (Henderiks et al., 2012; Triantaphyllou et al., 2010). The difference between laboratory and field data is not surprising. Laboratory studies are commonly conducted with one single strain of *E. huxleyi* and environmental parameters are kept constant and optimised, except for one variable parameter (e.g. carbonate system or nutrient concentration). Field studies, on the other hand, are investigating whole *E. huxleyi* populations (assemblages of multiple strains) and several environmental parameters can change with time and space, amplifying or balancing their effect on physiology and coccolith formation. Additionally, environmental parameters can either influence directly physiology and coccolith formation or alter the strain distribution in one population towards a strain with different coccolith geometry/morphology.”*

4. Cell shrinkage. The authors used an acid treatment to dissolve off the coccoliths from the cells and then measured the cell size. How did they know that (a) all the coccoliths dissolved, and (b) there was no associated shrinkage of the inner organic cell with acidification?

(a) Visual inspection using a cross-polarized light microscope did not indicate any coccoliths left after treatment with acid. Second, Fig. 1A displays that the vast amount of coccoliths were dissolved after acid addition (disappearance of the first peak, black line). Even if parts of the coccoliths were not completely dissolved it should not significantly interfere with the measurement of more than 10.000 coccoliths per sample.

(b) In true, we cannot exclude cell shrinkage. However, excess addition of acid was avoided by calculating the amount needed to lower the carbonate saturation state below 1 (using CO₂ sys). Observation under the microscope while adding acid did not reveal any unusual cell shrinkage. We tested the acid addition on a non-calcifying strain of *E. huxleyi* (at the Institute for Marine and Antarctic Studies, IMAS) and could not detect significant cell shrinkage by comparing Coulter measurements before and after acid addition. However, a time series-measurement of the cell diameter indicated cell shrinkage when incubating the acidified samples for longer than 1 hour. The samples of the present study were acidified and directly measured (within minutes). Additionally, the

difference of approx. 0.5 um between cell and coccosphere diameter is comparable to common observations of the coccolith layer surrounding the cell.

We are confident that no significant cell shrinkage occurred by treating the samples with acid as long as the samples were processed quickly.

5. Treatment of POC filters. Although minor, it would be good for future reference to know how the GFF filters for POC analysis were "treated with HCl" - rinsing? a few drops? overnight fuming? What concentration and what was the duration. There are several different methods of removing the PIC from filters for POC analysis.

We added the treatment of the POC filters in the Method section:

"The filter for POC analysis was treated with fuming HCl ($\approx$ 10 hours) to remove all inorganic carbon."

6. Changes in cell diameter and coccosphere diameter. On pg 4990, lns 3-5: These values are averages and although the standard errors are reported in the Table (Table 4) it would be useful if they were reported in the text as well. This makes it clearer to the reader that these are significant differences.

We added the standard deviation in the text section to make the difference clearer.

7. Morphotype E Following Young et al. (2003), what is morphotype E? In Beaufort et al. (2011) this is referred to as morphotype R. Changing terminology is confusing.

We apologise for the typo and the confusion caused. We changed the specification to morphotype R.

TECHNICAL CORRECTIONS

Generally the article needs proof reading for grammar.

We improved overall wording and structure of the manuscript.

Abstract – best studied or most studied?

Corrected.

pg 4981, ln 4 – missing "as" between "referred to" and "ocean carbonation/ acidification".

Corrected.

Table 4 – use correct terminology for final column, "volume of coccoliths".

Corrected.

Correct spelling of Young in Poulton et al. (2011) reference.

Corrected.

