Dear Prof. Young,

Thank you very much for your detailed review of our manuscript, which has been very helpful. In the following, we address all comments step by step.

With best regards,

Prof. Christina Scheu

-On behalf of all authors-

Reviewer 1 – Prof. J. Young:

General comments

This is the first application of focussed ion beam sectioning of coccospheres and is a remarkable demonstration of the potential of the technique which will likely stimulate further studies. As well as providing a demonstration of the method it contains useful methodological data.

One particular application I would like to see is comparative study of type A and type B E. huxleyi strains as this method should be ideal for demonstrating the morphological differences between them - and indeed for retrieving the data needed to make 3D models of coccoliths. The extended analysis of coccosphere size and its effect on cell density is valuable both for calcification studies of PIC/POC ratios and for ecological study of coccolithophores. In this respect I would urge the authors to take the final step and calculate sinking velocities as well as densities.

I have made some specific comments below and the text is also in need of a detailed revision by a native english speaker. Given this, however, I think this is an excellent contribution which merits publication in Biogeosciences. Finally I would note that the movie which is currently in an appendix is well worth presenting much more prominently and if possible as part of the main publication.

Our response:

We thank the Reviewer for his positive reception of our work and the helpful suggestions which are addressed below. As recommended we have calculated the sinking velocities and provide now this information in the revised manuscript (new figure 7 below).

We agree that the comparison of type A and type B is very interesting and we hope to perform these experiments in the future.

Concerning the English, a native speaker has now corrected it.

Reviewer 1

Notes on some specific points

p12776 line17 "Clonal cultures of E. huxleyi (strain RCC1238) were grown " This is an E. huxleyi type A strain (as is clear from the SEMs), it is worth stating this because this technique

would actually be a perfect way to demonstrate the difference between type A and type B in terms of coccolith shape in profile. You can probably quote a previous paper of Langer as a source for the identification.

Our response:

In the revised version we state that RCC1238 is a type A. We use the following reference: Langer, G., Probert, I., Nehrke, G. and Ziveri, P. (2011) The morphological response of *Emiliania huxleyi* to seawater carbonate chemistry changes: an inter-strain comparison, Journal of Nannoplankton Research 32 (1), pp. 29-34

Reviewer 1

p12780 line7 "in image 1.6 the organic residues are visible in the upper part of the coccosphere." As well as the organic residues an intracellular coccolith can be seen inside the cell. This is fairly clear in figs 1.4 and 1.5 and is confirmed by the video. It is worth commenting on, also since one possible application of the technique would be study of intracellular coccoliths.

Our response:

Indeed, we often observe remainings of the organic material. However, for a detailed study of intracellular coccoliths, it would be best if beam damage of the organic material would be reduced by using e.g. a He source instead of a Ga source in the FIB. But at this point we have no access to such a machine but hopefully we will have in future.

Reviewer 1

p12781 line4 "sectioning was performed at 27 different" should be sectioning was performed on

Our response:

We have corrected this in the revised manuscript.

Reviewer 1

p12781 lines 9-12 "The closed circles denote the outer diameter and the open circles the inner sphere diameter" Should be "the filled circles indicate the inner diameter and the unfilled circles the outer diameter."

Our response:

Thank you! We have made the changes in the revised manuscript.

Reviewer 1

p12782 lines 13-15 "The contrast differences of the coccoliths, which can be seen in image 5.2 (brighter area at the lower right side of the coccosphere) can be caused by orientation

differences of the calcite platelets. do you mean differences in crystallographic orientation or in the angle of the exposed face to the beam? Also it would be useful if you indicated with an arrow the area referred to.

Our response:

It could be caused by both effects: differences in the crystallographic orientation or in the angle of the exposed face relative to the beam. We have added this in the revised version and also added an arrow in the figure.

Reviewer 1

p12784 lines 19-20 "It has been put forth that coccoliths act as ballast stones in the cell's buoyancy control (Winter and Siesser, 1994)" better "It has been suggested that coccoliths may have a ballasting function by increasing the cell's density." Also Winter & Siesser is an edited volume and the reference should be to a specific paper, or papers, within it.

Our response:

We changed the sentence as suggested and added the reference: Young, J. Functions of coccoliths, pp 63-82, in Coccolithophores (1994), Winter and Siesser, Eds.

Reviewer 1

p12785 "It is simply not possible to obtain the required information on architecture in the context of a standard culture experiment, because the number of analyses required is at least an order of magnitude bigger than the one performed in the present study. This is far too time-consuming to fit the scope of a standard culture experiment, which usually focuses on other parameters such as organic carbon production" This is not necessarily true since with light microscopy it is easy to measure both cell diameter and coccosphere diameter - see Gibbs et al. (2013 - in your bibliography) for an example of this.

Our response:

We agree and changed the text. It now reads:

"In the context of a standard culture experiment, the number of analyses required is at least an order of magnitude bigger than the one performed in the present study. This is far too time-consuming to fit the scope of a standard culture experiment, which usually focuses on other parameters such as organic carbon production. However, density and sinking rate estimates might alternatively be based on light microscopy data (Gibbs et al. 2013), which are easier to obtain than FIB-SEM data. It would be worthwhile to perform a comparative study to figure out whether densities based on light microscopy agree with those based on FIB-SEM data."

Reviewer 1

p12785 "Taken together with individual cell PIC/POC ratios, this sheds new light on the old question of the relationship between coccolithophore nutrient limitation and sinking rates." Why don't you calculate the sinking rates? You can do this using Stokes Law (discussed in

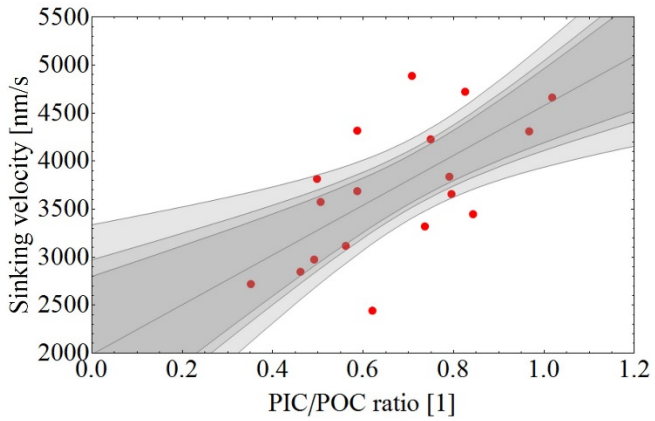
Young 1994 - in Winter & Siesser 1994 - where I also made predictions of density based on much less precise data).

Our response:

We agree and calculated sinking rates using Stokes Law. The Discussion was changed accordingly. It now reads:

"The fossil material used by Gibbs et al. (2013) would in fact be ideal, because it features, quite unusually, many complete coccospheres. Thus this material would additionally render it possible to apply the FIB-SEM method to fossil material. PIC and POC quotas as well as overall cell density and sinking rate are a very interesting amendment to the data presented by Gibbs et al. (2013), because the authors showed that *Coccolithus* displays peak-PETM-specific cell geometry, namely higher coccolith quota and bigger coccospheres. PIC/POC ratios would allow for assessing this important calcifier's feedback on carbon cycling over the PETM; the closest geological approximation to current climate change. Sinking rates would give insights into the nutrient limitation-sinking rate debate (Baumann et al. 1978), because nutrient availability during the PETM was presumably considerably altered (Gibbs et al. 2013)."

……."At any rate, overall cell density is not sufficient to make statements about sinking rate. This is illustrated by the much better correlation between PIC/POC ratios and sinking rates (as opposed to the correlation between PIC/POC ratios and density, Fig. 7). The reason for this is that Stokes Law, which was used to calculate sinking rate, features not only particle density, but also particle diameter. Hence only the combination of the latter two parameters allows statements about sinking rate. So it seems as if the PIC/POC ratio is a bad indicator for density, but possibly a useful one for sinking rate. That would vindicate the conclusion, if not the reasoning, of Benner (2008). …….. Next: Page 12785, line 12: Is the method of estimating density…..."



Reviewer 1

Fig 3 The caption appears to be mislabelled - the filled blue dots are presumably the inner diameter and the open red dots the outer diameter. I do not understand what the text at top left of the diagram refers to The caption for fig 3b is confusing and needs to be rewritten.

Our response:

We have changed this in the revised manuscript.

Reviewer 1

Fig. 4 Caption of y axis should be "coccosphere thickness"

Our response:

Thank you! We have made the changes to the figure in the revised manuscript.