



Biogeosciences
RESPONSE LETTER

Manuscript **Biogeosciences Discuss.**, 11, 12773, 2014.

Dear Editor,

Thank you very much for passing on the recommendations of the reviewers, which were very helpful. In the following, we list and comment on the reviewers suggestions step by step. The changes that have been made in the text are indicated by the use of a yellow background color. We hope that we have adequately considered all the issues raised by the reviewers.

With best regards,

Prof. Christina Scheu
-On behalf of all authors-

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MAX-PLANCK-INSTITUT FÜR EISENFORSCHUNG

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:



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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 *Emiliana 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



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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).

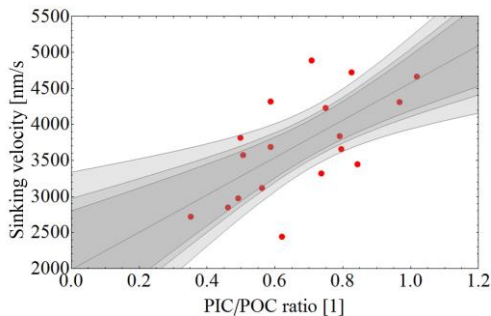
Our response:

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

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“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.



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Dear Reviewer,

Thank you very much for the careful reading of our manuscript. In the following, we address all comments step by step.

With best regards,

Prof. Christina Scheu
-On behalf of all authors-

Reviewer 2:

General Comments:

This manuscript represents a very interesting application of FIB SEM to the study of coccospheres. As pointed out by the authors, the ability to more accurately calculate the PIC/POC has implications for CO₂ sequestration, as well as trying to understand why the algae make platelets in the first place. The images and the movie are quite amazing, and I will definitely like to use the movie in lectures on the application of FIB SEM to natural materials.

Our response:

We thank the reviewer for the positive evaluation of our work.

Reviewer 2:

However, there are two points that detract from this work. The first is the language. The manuscript uses lots of “empty” words, and strange phrasing, which detracts from the story. Please remove all uses of the word “latter”. The way the figures are used is also not helpful- they are presented sort of like a report: Figure 1 shows this. Figure 2 shows that. I think the story would be better served if the figures were more organically woven into the text.

Our response:

Thank you for the advice. We have removed such empty words and have rewritten the sentences in particular where the figures are mentioned.

Reviewer 2:

The second point is that there is a little disjoint between the material presented in the introduction and the overall discussion. The introduction discusses climate change and whether the coccoliths would be a sink or source of CO₂, and if we could use the marine archives to better understand climate in the past. I don't feel the results fully come back to these points. I would like a paragraph or two explaining the implications of the calculated PIC/POC values, and if the authors think this method could be used to section whole coccospheres found in chalks.

Our response:



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See reply to Reviewer 1, p12785 above

Reviewer 2:

However, I definitely agree that this manuscript warrants publication in Biogeosciences. Specific points: P12774 L16- I don't think you mean to imply that the ecosystem itself knows about global climate change. I would change the first sentence to read " In the context of the current climate change debate, understanding ecosystem response to environmental disturbances has become... "

Our response:

Thank you, we made this change.

Reviewer 2:

P12774 L17- remove "In order to be able". You use a lot of empty words like this, which detracts from your message.

Our response:

As mentioned above we have removed such words.

Reviewer 2:

P12775: L8: I am not sure what is meant by "morphological abberations possible". P12775 L9-11: replace " Some features complete coccospheres" with " Up until recently, it was only possible to image coccoliths using conventional SEM, using conventional sample preparation methods- either smearing coccoliths onto sample holders, or using the microtome to create single cross sections through the cells. However, advances in technology now allow us to both serially image and cross section through the coccospheres, opening up a whole new way of observing coccosphere architecture."

Our response:

Thank you, we followed your suggestion in the revised manuscript.

Reviewer 2:

P12276 L1: You cannot get crystallographic information from BSE, unless you are using a back-scattered diffraction detector. Back-scattered electrons will absolutely display atomic number contrasts, if the energy of the incoming electrons is high enough to generate an x-ray from the material in question.

Our response:

Clearly, BSE imaging does not provide any information on the crystallographic phase as for instance backscatter electron diffraction can do. However, BSE channeling contrast



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mechanisms which depend on the crystallographic orientation of the investigated volume allow for discrimination of differently aligned grains/crystals with the same mass-contrast.

Reviewer 2:

P12777 L 15: You give us your polishing current, but do you mean to say you did all the FIB work just using 240 pA? If so, technically it is not a polishing current. Polishing is the last step, usually, when making a TEM sample. If you only used 240 pA, then remove the word “polishing”. If you used some other settings in the FIB, please let us know what they are.

Our response:

The reviewer is correct. We have therefore removed the “polishing” in the revised manuscript.

Reviewer 2:

P!2778 L14-15: The fact the n per cell and the inner coccosphere diameter can be determined from FIB sectioning is really part of the new science in this paper. Using the FIB to get these values has not been attempted before. Unfortunately these points are buried in the methods section! I would suggest stating this very clearly in the introduction. Something along the lines “PIC and POC values are often used to evaluate a coccospheres response to climate change. However, it can be difficult to accurately calculate the number of coccoliths per cell, and the inner coccosphere diameter. These are two parameters that are needed to calculate the PIC and POC values. The FIB SEM presents an elegant way to obtain these values ...”

Our response:

We agree with the reviewer and have added the following to the Introduction. Inserted after page 12775, line 11: “The coccolith quota is needed to calculate particulate inorganic carbon (PIC) quota from SEM images. The inner coccosphere diameter, which equals the cell diameter, can be used to calculate particulate organic carbon (POC) quota. Both coccolith quota and inner coccosphere diameter cannot be determined using conventional SEM, but can be obtained accurately by means of FIB-SEM. The PIC/POC ratio determines if coccolithophores act as a source or a sink of CO₂ relative to the atmosphere (Balch et al., 1991; Holligan et al., 1993; Buitenhuis et al., 1996) and therefore is an important variable for modeling carbon-cycling in the oceans (Ridgwell et al., 2009). Moreover, coccolithophore’s response to climate change is often expressed in terms of PIC and POC quotas.”

Page 12776, lines 7-12, were deleted. Page 12775, lines 12-17 were changed to: “FIB-SEM (Inkson et al., 2001; Williams et al., 2005; Uchic et al., 2006; Holzapfel et al., 2007; Kato et al., 2007; Mc Grozther and Munroe, 2007) enables bulk samples to be locally sectioned.....”

Page 12775, line 11: was changed to Langer et al. 2006

Page 12785, line 2: was changed to Benner 2008



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Reviewer 2:

P12779: I think the results can be presented much better. For example: “FIB SEM images taken at different stages of milling (for the whole processes, readers are encouraged to see the video in the supplementary information), illustrate the beautiful and complex structure of E. Hux (Figure 1). When moving from a single complete coccosphere (Fig. 1.1), into the milling (Fig 1.2) and then into the interior of the cell, one begins to see how the individual coccolith platelets are layered (Fig 1.3 and 1.4). In some cases the layers of coccoliths is uneven (Fig 1.5 and 1.6), however this is not often revealed until the middle of the coccosphere is milled. These results imply that whole coccospheres need to be milled, and it is not sufficient just to mill part of the organism. Additionally, it is not sufficient to mill only one sample. The coccospheres in this study are quite heterogenous (Figure 2). Some have four layers of coccoliths (Fig 2.1), whilst some have only three (Fig 2.2). Some have unequal layers (Fig 2.3 and 2.4), which may correspond to the growth direction. Some of the cross sections also show that the shell thickness varies with the number of observable coccoliths (Fig 2.5 and 2.6)”.

Our response:

We thank the reviewer and have rearranged our sentences.

Reviewer 2:

P12781 L21: Replace with “It is difficult to calculate the number of coccoliths layers, since in this study we calculated that only 1 in 3 coccospheres have coccoliths that are evenly distributed. In a case where the layers are not even all around the sphere, the value corresponding to the maximum number of layers was used.”

Our response:

Thank you, we made this change.

Reviewer 2:

P 12782 L13: I do not see the contrast differences in Figure 5.2. What I do see is a lot of charging. I disagree that the bright colors are from orientation of the calcite crystals, as this is not something a back-scattered detector would show. In BSE images, materials with different atomic number would appear brighter, but calcite is calcite. It should be the same greyscale.

Our response:

We do not agree with the reviewer. As mentioned above, BSE channeling contrast mechanisms which depend on the crystallographic orientation of the investigated volume allow for discrimination of differently aligned grains/crystals with the same mass-contrast and composition. We therefore correlate the different colors to channeling and not to charging.

Reviewer 2:

Overall, I think the figure captions are a bit long, and information gets lost.



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Figure 5: Replace caption with “SEM images of the same coccosphere cross-section taken using secondary electrons (5.1) and back-scattered electrons (5.2). Secondary electrons are generated closer to the surface, so 5.1 shows a lot more of the finer surface features. Back-scattered electrons are sensitive to chemical composition, and are generated deeper in the sample. Brighter areas here are charging.

Our response:

Thank you for the advice. We have made the changes except for the last sentence (see discussion above about the channeling).

Reviewer 2:

Technical corrections: Overall, I think the text needs a bit of revision. But here are some definite mistakes.

P12775 L4: replace “were used to gain” with “provide”. P12775 L24: remove the “y” from difficult. P12777 L3: Replace “spatle” with “spatula”. P12781 L4: replace “at” with “on

Our response:

We have made the corrections.