

## ***Interactive comment on “The Influence of Environmental Variability on the Biogeography of Coccolithophores and Diatoms in the Great Calcite Belt” by Helen E. K. Smith et al.***

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Received and published: 7 July 2017

Reviewer #2 Reviewer Comment (RC) - This manuscript presents phytoplankton cell counts results from the Southern Ocean from two cruises conducted in the GCB (Great Calcite Belt) together with a number of environmental physico-chemical data that are merged in a statistical analyses to provide causalistic hypotheses to plankton community structure. The main results of this manuscript are that: coccolithophores and diatoms co-occur in the studied area and that coccolithophores in particular extend very far South, that community structure is mainly driven by four representative of the nanoplankton group (3 diatoms, 1 coccolithophore), that the key drivers of community

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structure are both T° and Si depletion which create different ecological niches.

Author Response (AR) - We thank the reviewer for their thorough review of the manuscript. We address their comments below

RC - Overall, I find the methods, results and main conclusions presented here are quite weak, with two main criticism:

RC 1 - My first and main concern regards the phytoplankton cell counts. I find that the method used for cellular abundance determination is not a very robust nor trustable method. Counting very small area of filtered samples in SEM is not usual for nano- or microphytoplankton determination. From what the authors indicate in their method section, I deduced that sample cell counts were determined on only 2 ml sample, which is insufficient in most cases to provide statistically robust results. If I agree with the authors general recommendation to use both SEM and light microscopy in parallel, it should be to count cell numbers in light microscopy on a sufficient volume (50-100 ml usually) and use SEM to improve species determination, and not the other way around. I don't understand why lugol/formol fixed samples were not collected or analyzed here. My second concern is on the large bias towards small species that this method implies, as correctly identified by the authors themselves. The main statement here about nanophytoplankton dominating the mineralizing algae is not trustworthy when large cells can not correctly be assessed by this method. The authors misspelled on several occasions diatom names, and include Pseudo-nitzschia sp. within the nanoplankton size-class which is quite surprising, as this species is most typically much larger than 20  $\mu\text{m}$ , as can be seen very easily in figure 4. Also Figure 4 reveals very interestingly that a number of Parmales were present, they are part of the pico-nano- size fraction of siliceous plankton, so I find very surprising that no mention was made of that in the manuscript.

AR1 - Following these comments, we have identified and respond to the following points:

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1. SEM counting of nano- and micro-plankton versus Light Microscopy: there have been several studies using SEM techniques to count coccolithophores and other nano-plankton, for example Mohan et al. (2008), Cubillos et al. (2007), Leblanc et al. (2009), Hinz et al. (2012) and Charalampopoulou et al. (2011). Though we do acknowledge that using SEM for enumeration is not typical for studying micro-phytoplankton communities (exclusively), our focus is the small diatoms not typically identified by light microscopy. Furthermore, we also aim to put the mineralising nanoplankton in the wider context of the phytoplankton community. To better reflect this we have now amended the manuscript to make this clearer throughout.

2. Limited volume (2 mL) examined: We fully understand the reviewers concerns in terms of the statistically robustness of the count results (though our methods match those listed above). In our study, our pre-treatments of the data before multivariate analysis specifically aim to avoid any potential issues that may arise from low sampling resolution of the species composition of the community. Specifically, we have removed species with low cell densities (in our study  $< 1$  cell mL<sup>-1</sup>) to remove their potentially random influence on the multivariate statistics. We have also standardised our count data (converted to percentage relative abundances) and performed a square-root transformation of the relative abundances to reduce the influence of potential count bias (at both ends of the abundance spectra) on the end results.

3. The cell size of the diatom *Pseudo-nitzschia*: The initial definitions of size-fractions of phytoplankton were based on mesh sizes of plankton nets. In the case of the *Pseudo-nitzschia* in our study, its size affiliation depends on whether one considers its length (30-50  $\mu$ m) or its width (2-5  $\mu$ m). In recognition of the point of the reviewer we have now altered the revised manuscript to make it clear that in this case we have considered *Pseudo-nitzschia* to be at the small end of the micro-phytoplankton group.

4. No mention of the Parmales: The focus in the original manuscript was not on the rarer nanoplankton and hence we chose not to mention them. *Tetraparma* sp. were particularly abundant at only one station, where they were present at a cell density of

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2000 cells mL<sup>-1</sup>, and present in low numbers ( $< 5$  cells mL<sup>-1</sup>) at three more stations in the South Atlantic, whilst they were absent throughout the rest of our sampling of the GCB. We have now added this information to the revised manuscript.

RC2 - SEM observations should also have allowed species determination for the dominant *Pseudo-nitzschia* species, which is not indicated. This suggests an overall lack of expertise for diatoms, and that calcifying algae were initially the focus of the study and that diatoms were only added lately to the analysis.

AR2 - The SEM images could have allowed for species-specific determination of the *Pseudo-nitzschia*, however the resolution and collapsed nature of the cells after filtration (i.e. they were weakly silicified species) was not adequate for high-resolution taxonomic identification. Reliable species-level taxonomic identification on all cells (or a representative majority) in all samples was also not feasible, and so we chose to retain identification at the genus level.

RC3 - I have a hard time believing the low species numbers (1-3) indicated for diatoms at certain stations.

AR3 - We apologise for the slight mistake or mis-understanding in Table 2. In the original version of the manuscript Table 2 presented the post-transformed species data (i.e. the counts minus the rare species prior to multivariate statistical analysis). We have now altered Table 2 (see supplement) to reflect the number of species identified prior to transformation of the data (i.e. removal of the rare species).

RC4 - Another point is the presentation of cellular abundance only. This is absolutely not the best metric to compare with physico-chemical parameters, and C biomass conversions are absolutely needed in this kind of data analysis. This would have allowed a relative estimation of the contribution of mineralizing algae to total POC (or Chla stretching it with POC:Chla ratios) and more robust conclusions regarding the real importance of both coccolithophores and nano-sized diatoms in total phytoplankton summer blooms.

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AR4 - Indeed, a comparison of cell biomass from all species and phytoplankton groups would be the most comprehensive comparison. This would need to include all picoplankton, nano-plankton and micro-plankton, which are not often all enumerated or reliably measured in terms of biomass. There are also issues (for each group), as described in detail (e.g.) in Leblanc et al. (2012), in terms of carbon conversions (from bio-volume or cell sizes), including preservation effects on cell size, variable cell sizes with growth conditions and nutritional strategies (autotrophic or mixotrophic).

Whilst deriving coccolithophore biomass is relatively straightforward (as they are mostly spherical in shape, with no vacuoles or complex cell structures that may include biomass), diatoms are far more morphometrically complex (not spherical, often with setae which may or may not contain cell plasma, and many cells have large internal vacuoles), making direct comparison between the two potentially problematic (especially when the two may be equally abundant) – i.e. small errors in diatom estimates can cause species dominance to radically change.

We have now included a statement to show that we recognise the differences in species dominance if biomass was considered, page 7 line 17.

“...not numerically dominant compared to the nanoplankton species at these locations. Consideration of community biomass would potentially reduce the dominance of the nanoplankton relative to microplankton in the GCB. However, converting cell size to biomass is not straightforward for diatoms, as highlighted in Leblanc et al. (2012), and to avoid these potential caveats we have considered species abundance only. Total cell abundances...”

The suggestion to use comparison to POC, which includes a variable proportion of detrital material, bacteria and zooplankton, would seem to only compound issues over representativeness of the comparisons. Lastly, other previous studies have done the same type of comparisons as presented here; e.g. Kopczyńska et al., 1986, Cefarelli et al., 2011, Chen et al., 2007, Hinz et al., 2012, Charalampopoulou et al., 2016.

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RC5 - My second main concern is about the statistical analyses. Although I will frankly admit that I am not qualified to expertise the tests presented here further than simple correlation matrixes, I really miss the added value of such extensive statistical tests. Quantifying so many environmental variables (such as carbonate chemistry which is very tricky) to collapse them in the end with  $T^{\circ}$  and nutrients seem very odd to me. Finally, every bit of conclusion about the different phytoplankton communities and the overarching role of  $T^{\circ}$  and silicic acid could have been stated by directly looking at the data and the statistics provided here are not at all convincing.

AR5 - The added value of such an extensive statistical test is that the ocean is not univariate, environmental factors vary at the same time, occasionally in the same direction or in a linear fashion (but not always) and a simple correlation matrix completely ignores the importance of a multivariate perspective on phytoplankton ecology. Our analysis also has no a priori assumptions in terms of driving factors and allows the data to identify the key correlating parameters. This is why the environmental variables collapse down to a limited number of factors. Making the conclusion reached in this study by solely looking at the data, with no attempt to statistically examine or balance the significance of the relationships found, goes against our approach to this type of research. In light of the reviewers comments we have now added text directing the reader as to why each statistical test is included (see specific response to Page 9 Line 14) to ensure that the importance of such extensive statistical techniques is made much clearer.

RC6 - The discussion section leaves much to be desired and is a succession of short paragraphs that are very counter-intuitively organized and that should be entirely rewritten. A number of other papers regarding the succession patterns of coccolithophores and diatoms elsewhere are ignored.

AR6 - We are not sure exactly what the reviewer means here by 'counter-intuitively organised'. We have ordered the discussion to reflect the order of the results and tailored the discussion from general trends towards more specific areas of interest that were highlighted by the statistical results. We are also not sure which papers the

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reviewer is referring to, but do recognise that our focus tends to be on Southern Ocean publications rather than ones from the northern hemisphere.

RC - I have several other comments/corrections/questions that are added as sticky notes in the manuscript pdf attached.

RC7 - Page 1 Line 26 – Spelling Pseudonitzschia to Pseudo-nitzschia (and thereafter within document)

AR7 - Thank you for highlighting this error in spelling of the diatom genus Pseudo-nitzschia – this has been amended throughout.

RC8 - Page 2 Line 15 - What about non mineralizing nanoplankton? Are they important?

AR8 - Non-mineralizing phytoplankton are important within the context of the overall function of the oceanic ecosystem and carbon export. However, the focus of this paper was to assess the distribution of the coccolithophores and diatoms in the Great Calcite Belt. As biomineral providers, the biogeographical distribution of mineralising phytoplankton species are of great interest when it comes to the resulting carbon export and surface ocean biogeochemistry.

RC9 - Page 2 Line 20 - Pseudo-nitzschia are very seldom  $<20\mu\text{m}$ . In your figure 4d, they are about  $60\mu\text{m}$  if scale bar is correct - or  $150\mu\text{m}$  if your legend is correct. I would not include them in the nanoplankton group.

AR9 - We have removed the size classification from the sentence to avoid confusion about size classes of diatom species.

“North of the PF, small diatom species (e.g. Pseudonitzschia sp. and Thalassiosira sp.) tend to dominate numerically, whereas large diatoms with higher silicic acid requirements (e.g. Fragilariopsis kerguelensis). . .”

RC10 - Page 4 Line 26 - bizarre annotation. NO<sub>x</sub> ? or DIN are more standard

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AR10 - We have altered the annotation to NO<sub>x</sub> throughout the manuscript

RC11 - Page 5 Line 15 - I don't understand this sentence. Was a  $200\mu\text{m}$  mesh placed beneath the  $0.8\mu\text{m}$  filter on the filtration rig?

AR11 - Apologies if this was not clear, the  $200\mu\text{m}$  mesh was placed beneath the  $0.8\mu\text{m}$  filter. The sentence has been rewritten as follows.

“Seawater samples were then gently filtered through a 25 mm,  $0.8\mu\text{m}$  Whatman<sup>®</sup> polycarbonate filter placed over a  $200\mu\text{m}$  mesh backing to ensure an even distribution of cells across the filter.”

RC12 - Page 5 Line 20 - this is only 1/500 of the surface of a 25 mm filter, this seems to be very little (equivalent to 2 ml of sample counted).

AR12 - Yes, this is a small surface area and equivalent volume, and does have its limitations (as with every sampling or analytical method). We have followed a standard method for enumerating phytoplankton from SEM images and statistically analysing species distributional patterns as applied in (e.g.) Charalampopoulou et al. (2011).

RC13 - Page 6 Line 1 - I am not qualified to review the robustness of the statistical analyses used in this paper

AR13 - We appreciate that unfamiliarity with multivariate statistics has not made this possible for the reviewer. We have endeavoured to make the statistics section as reader-friendly as possible to aid those unfamiliar with this type of statistical approach.

RC14 - Page 6 Line 30 - Date of sampling could have been included in this table (Table 1)

AR14 - We agree with the reviewer and have inserted the date of sampling into Table 1

RC15 - Page 7 Line 1 - use  $\mu\text{M}$  for nutrients

AR15 - We have amended to  $\mu\text{M}$  throughout the paper, tables and figures.

C8

RC16 - Page 7 Line 16 - again I think this is potentially very biased if only fractions of SEM filters were analyzed and if no larger water volumes were counted. Also, this kind of assertion needs to be substantiated by biomass estimates. Picoplankton abundance is most frequently always > nanoplankton > microplankton, but cell abundance conversion to C biomass often reverses these orders. Links with nutrient and light availability should preferably be considered with biomass rather than abundance.

AR16 - Please see response to main comments.

RC17 - Page 8 Line 11 - correct spp. (and occurrences thereafter)

AR17 - Thank you for bringing this to our attention, this was amended where necessary.

RC18 - Page 8 Line 25 - I understand the general assumption here, but it seems very strange to go through all this trouble measuring all parameters and C chemistry, which is tedious, just to collapse everything with NO<sub>3</sub> and T<sup>o</sup> as explanatory variables in the end.

AR18 - Please see earlier comment regarding statistical analysis. The highly dynamic nature of the Southern Ocean requires a more robust approach to analysis. We felt it was best to start with the greatest range of parameters that may influence phytoplankton biogeography, and then let the statistical analysis determine significant patterns and correlations.

RC19 - Page 9 Line 14 - How is that different from the SIMPROF routine and Fig 3?

AR19 - Apologies if this is not clear in the text, we have altered the text to make this clearer. In short, the SIMPROF test statistically identifies groups of samples with more similar community structure, whilst the SIMPER test statistically identifies the specific-species that define these groups.

Page 9 Line 3 "A SIMPROF routine was used to identify the stations in the GCB that had statistically similar coccolithophore and diatom community structures, without examining detail of the species within the groups."

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Page 9 Line 14 "A SIMPER routine statistically identified the species that define the difference between (and similarity within) the statistically different community structures defined by the SIMPROF routine (Table 4)."

RC20 - Page 10 Line 13 - Agreed. This is why I find regrettable a better job was not done on accurate quantification of all size-classes, together with C conversions. Also, from Fig 4, the siliceous armored Parmales, which are spanning over the pico-nano size fractions are present, too bad they were not quantified. That would have strengthened this argument, and brought some new insights to SO communities.

AR20 - The *Tetraparma* sp. were only particularly abundant (2000 cells mL<sup>-1</sup>) at one station, whilst they were in limited numbers (<5 cells mL<sup>-1</sup>) at three more stations in the South Atlantic and absent across the rest of the GCB. Hence we do not think that addition of these counts would add to the statistical analysis. We have now added this information to the results section.

RC21 - Page 10 Line 27 - I really disagree about *Pseudo-nitzschia* being part of the nanoplankton. They are very rarely <20 μm, and definitely much larger than that in your figure 4, no matter which scale is used (the figure's or the legend's which differ).

AR21 - Apologies if this sentence is unclear, we have rephrased it to make clear that we do not include *Pseudo-nitzschia* in the nanoplankton class.

"Three of these species (*E. huxleyi*, *F. nana* and *F. pseudonana*) are part of the nanoplankton, whilst *Pseudo-nitzschia* sp. is at the lower end of the size range of the microplankton (*Pseudonitzschia* sp. is >20 μm in length but <5 μm in width)..."

RC22 - Page 11 Line 2 - Again this argument falls short, when 1 of the 4 species is not attributed to its correct size class, and when accurate cell abundance determinations of the microplankton size class were not made. I have a hard time believing the very low species numbers given for diatoms in Table 2 (between 1 and 3) at several sites.

AR22 - We have now amended Table 2 to reflect the number of species identified in

C10

the sample pre-statistical analysis.

RC23 - Page 11 Line 3 - correct nitzschioides (and occurrences thereafter)

AR23 - Thank you for highlighting this spelling mistake, the spelling has been altered

RC24 - Page 11 Line 9 - most certainly

RC25 - Page 11 Line 15 - If I totally agree with this recommendation, I feel that it should be reversed. Cell counts need to be made in fixed water samples, while correct species determination can be made using SEM, but not the other way around.

AR25 - This would provide a thorough analysis of the micro-plankton, however nanoplankton are rarely observed in light microscopy or accurately enumerated.

RC26 - Page 12 Line 30 - similar studies conducted in the North Atlantic could be cited here.

AR26 - We have now included reference to Leblanc et al. (2009).

RC 27 - Page 15 Line 3 - this argument is unclear to me

AR27 - We have altered the sentence for further clarification as follows.

“...so the high abundance of *F. nana* in the high silicic acid waters could be indicative of a seasonal progression driven by light and temperature rather than silicic acid dependence.”

RC 28 - Page 15 Line 10 - this was also described in Leblanc et al. 2009

AR28 - This reference has now been incorporated into the sentence.

“... has also been identified in the Scotia Sea (Hinz et al., 2012) and the Patagonian Shelf (Balch et al., 2014) in the Southern Ocean, as well as in the North Atlantic (Leblanc et al., 2009).

RC 29 - Page 15 Line 15 – “Therefore the positive selection pressure at low silicic

C11

acid concentrations in the GCB is likely to be *E. huxleyi* 15 specific rather than a coccolithophore-wide phenomena.” Why not?

AR29 – We have altered the sentence to read and explain better as follows:

“Therefore, a low silicic acid concentration in the surface waters of the GCB may negatively impact coccolithophore species that do have a silicic acid requirement, such as *Calcidiscus leptoporus*, and favour bloom-forming species that do not require silicic acid i.e. *E. huxleyi*.”

RC30 - Page 15 Line 25 - Fig. 6

AR30 - This has now been amended.

RC31 - Page 16 Line 15 - I would consider pCO<sub>2</sub> being the result of phytoplankton bloom development, rather than its driver.

AR31 - In general we agree with the reviewer in the context of temporal changes, however our study has little temporal context (and was carried out in summer).

RC32 - Page 17 Line 6 – “...suggest that four nanoplankton...” Three

AR32 - As suggested we have changed this to:

“...suggest that three nano- (<20 μm) and one micro- (>20 μm) phytoplankton species...”

RC33 - Page 17 Line 9 - estimated by cell/chla ratio conversions rather?

AR33 - As suggested we have changed this to:

“as estimated by Chl a”

RC34 - Page 17 Line 13 - I don't find that this is properly demonstrated through similar estimations of cocco and diatom biomass or Chla contributions

AR34 - We have re-written as follows to remove the direct comparison to diatoms.

C12

“This indicates that in the non-bloom conditions of the GCB, *E. huxleyi* could be important for phytoplankton biomass and primary production at localized spatial scales.”

While there are no other estimations of coccolithophore and diatom biomass in this study, for reasons described in earlier comments, a conservative estimate indicating that *E. huxleyi* contributes up to 20% of the measured chl a, does imply that this species is important within the overall phytoplankton community, even if at very local spatial scales and short time scales.

RC35 - Page 17 Line 14 - All right, this could have been hypothesized even before sample collection.

AR35 - We have changed the emphasis of this sentence:

“Out of a wide suite of environmental variables, latitudinal gradients in temperature, macro-nutrients, pCO<sub>2</sub> and Ω<sub>calcite</sub> 'best' described the variation of phytoplankton community composition in this study, whereas EMLD and pH did not rank as significant factors influencing phytoplankton community composition.”

RC36 - Page 27 Line 11 - \*\*\*\* only one species present: this is highly unusual for diatoms, even though close to monospecific abundance can be noted. Probably an artefact linked to the small area of filter analyzed again.

AR36 - We have altered the table to include the number of all species identified. Given that only a few cells of some species were identified in the area imaged, please note that we excluded these species from the statistical analysis given the uncertainty involved estimating abundance from a single cell.

RC37 - Page 27 Line 13 - so why is the dominant species at 100% when the \*\*\*\* code is given for coccos but not for diatoms ? this does not make sense with legend for instance at GCB1-46, S for diatoms =1, there is a \*\*\*\*, but then it is indicated that *Chaetoceros* represents 56% of diatoms ? this occurs again on the other two lines with \*\*\*\*

C13

AR37 - Thank you for highlighting this discrepancy, the table has been altered to include all species observed in the sample. Please see comment above.

RC38 - Page 33 Fig 4 c - I see quite a few Parmales in grey in this picture. They are beginning to be considered as abundant in the SO, did you not count them? They are part of the biomineralizing algae...

AR38 - See previous response to comment regarding Page 10, line 13. In short, yes they were counted and were abundant (2000 cells mL<sup>-1</sup>) at only one station and in limited abundance (<5 cells mL<sup>-1</sup>) at only three others.

RC39 - Page 33 Fig 4 d - the scale bar says 2 μm, your legend says 5 μm, please correct. Also correct *Pseudo-nitzschia*

AR39 - Thank you for identifying this error. The scale bar is correct, the text in figure caption has now been amended to 2 μm.

RC40 - Page 34 Fig 5 - I have a very hard time understanding the utility (and meaning) of this figure

AR40 - Figure 5 is included to visually represent how the specific phytoplankton species (and genus) play a role in defining the statistically different phytoplankton communities. We have now made this clearer in the main text and figure legend.

RC50 - Based on these comments, I suggest either rejection or major revisions including entirely reworking both the dataset and its subsequent analysis.

AR51 - References referred to in the responses

Cefarelli, A.O., Vernet, M. and Ferrario, M.E.. Phytoplankton composition and abundance in relation to free-floating Antarctic icebergs. *Deep-Sea Res. Pt II*, 58(11), 1436-1450, 2011.

Charalampopoulou, A., Poulton, A. J., Tyrrell, T. and Lucas, M. I.: Irradiance and pH affect coccolithophore community composition on a transect between the North Sea

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and the Arctic Ocean, *Mar. Ecol-Prog. Ser.*, 431, 25–43, doi:10.3354/meps09140, 2011.

Charalampopoulou, A., Poulton, A. J., Bakker, D. C. E., Stinchcombe, M., Lucas, M. I. and Tyrrell, T.: Environmental drivers of coccolithophore abundance and cellular calcification across Drake Passage (Southern Ocean), *Biogeosciences* 13, 5917-5935, doi:10.5194/bg-13-5917-5935, 2016.

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Leblanc, K., Arístegui, J., Kopczynska, E., Marshall, H., Peloquin, J., Piontkovski, S., Poulton, A.J., Quéguiner, B., Schiebel, R., Shipe, R. and Stefels, J., 2012. A global diatom database—abundance, biovolume and biomass in the world ocean. *Earth Syst. Sci. Data*, 4, 149-165, 2012

Mohan, R., Mergulhao, L. P., Guptha, M. V. S., Rajakumar, A., Thamban, M., AnilKumar, N., Sudhakar, M. and Ravindra, R.: Ecology of coccolithophores in the Indian sector of the Southern Ocean, *Mar. Micropaleontol.*, 67(1–2), 30–45, doi:10.1016/j.marmicro.2007.08.005, 2008.

Please also note the supplement to this comment:

<https://www.biogeosciences-discuss.net/bg-2017-110/bg-2017-110-AC2-supplement.pdf>

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Interactive comment on *Biogeosciences Discuss.*, <https://doi.org/10.5194/bg-2017-110>, 2017.