Reviewer #1

Reviewer Comment (RC) - This article presents a comprehensive analysis of environmental forcing upon the distribution and abundance of dominant diatoms and coccolithophores in the Great Calcite Belt, a region of high importance for marine biogeochemical cycles. The study has been carefully conducted and the results are presented clearly and concisely. This work will contribute to improve our knowledge of the factors that control the biogeography of phytoplankton in the Southern Ocean. I support publication of this material in BG, provided the authors address some uncertainties in their analyses and conclusions.

Author Response (AR) - We thank the reviewer for their constructive comments and recommendation for publication pending our responses and further development of the manuscript. We address their comments below.

RC1 - Reading the description of BGC at the beginning of the Introduction, one may be tempted to infer that the biogeochemical importance of the GCB (e.g. a region of net CO2 uptake) stems from the fact that it is a region of high PIC. However, its importance is probably more related to its being a region of generally increased plankton abundance and productivity. In fact Fig. 1 suggests that the region could be equally defined in terms of enhanced chla levels.

AR1 - We are in agreement with the reviewer – the Great Calcite Belt is an area of both elevated chlorophyll-*a* and particulate inorganic carbon associated with increased seasonal production. The recent confirmation of the GCB as a significant coccolithophore phenomenon leads to this region being of interest in the context of upper ocean biogeochemistry and changing climate. Acknowledging the reviewers comment we have made the following changes to the introduction to better reflect the generalised increase in plankton abundance and productivity within the GCB, page 2 line 1-2.

"The Great Calcite Belt (GCB), defined as an elevated particulate inorganic carbon (PIC) feature occurring alongside seasonally elevated chlorophyll-a in austral spring and summer in the Southern Ocean (Fig. 1; Balch et al., 2005), plays an important role in climate fluctuations…"

RC2 - On a related note, is the PIC to POC ratio actually higher in this region than it is in tropical and subtropical waters?

AR2 - The GCB spatial extent is set by high satellite-detectable PIC concentrations rather than a change in the PIC to POC ratio – both PIC and POC may increase relative to subtropical waters to get the GCB signal, without necessarily changing the ratio between the two.

RC3 - Some studies have shown that the coccolithophore to diatom biomass ratio actually increases in tropical, unproductive waters (Cermeno et al. PNAS 2008). This study uses

abundance to assess dominance of different phytoplankton species. But, due to interspecific differences in cell size, an assesment based on carbon biomass could have been more reliable, as some of the authors have shown before (Daniels et al. MEPS 2016). For instance, section 3.2 starts by noting that nanoplankton tended to be more abundant than microphytoplankton, but this is always to be expected and cannot be directly translated to ecological dominance patterns. The authors should include a statement, and/or provide some sensitivity tests, on how results could change if dominance were assessed by biomass instead of abundance.

AR3 - Indeed, considering biomass would most likely change the picture and decrease the dominance of coccolithophores (in many cases). 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. In contrast, comparisons across subtropical waters (no diatoms, some coccolithophores) and upwelling zones (many diatoms, few coccolithophores), as in Cermeno et al. (PNAS 2008), is relatively straightforward.

Furthermore, many potentially significant issues over carbon conversions are not straight forward. Although there are now extensive conversion tables for various phytoplankton carbon content, these come with important caveats, as described in detail (e.g.) in Leblanc et al. (2012), which include (but are not limited to) the effect of preservatives on cell size and content (shrinkage), simplistic bio-volume conversions from cell measurements, time of sampling and age of the community or population, and growth conditions (light, nutrients, temperature). The scope of our manuscript was not intended to cover full discussion of these issues.

We have now included at statement to show that we recognise the differences in species dominance if biomass was considered, page 7 line 22-24.

"…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…"

RC4 - page 12 line 20. A reference is needed here to support the value of chla content used for Ehux.

AR4 - We do state the appropriate references (i.e. Haxo, 1985 and Poulton et al., 2013 who applied these estimates previously) used to estimate the E. huxleyi chl-*a* contribution in section 4.2.1 page 12 line 22, and have now restated this in sections 4.2.2 (page 13 line 9) and 4.2.3 (page 13 line 25) to avoid confusion.

RC5 - However, the chla content of algal cells is highly dependent on temperature, light, nutrients, etc. which makes this calculation very uncertain. Carbon biomass is a more reliable metric to estimate relative importance of different species, because the C cellular content is less variable.

AR5 - Cell chl-*a* content is indeed variable with physiological growth conditions. Carbon biomass is possibly a more reliable metric, however this would rely on two requirements: (1) that the entire phytoplankton community (pico-plankton to micro-plankton) be assessed in terms of cell carbon (which few studies undertake), and (2) that there are few errors in estimates of cell carbon from cell size and biovolume (see earlier comment). Literature biovolume to carbon conversions are often generalist across multiple species and many (though not all) are based on culture values under optimum growth conditions rather than realistic in situ conditions (temperature, light, nutrients). Hence, there are also large potentials for cell carbon estimates to be as variable with physiological growth conditions than a rough conversion of cell numbers to chl-*a*. We have now added an appropriate caveat to the text to acknowledge potential issues over variable cell chl-*a* content and the estimates derived from them, page 12 line 26-28.

"It should be noted that the cell Chl a content from Haxo (1985) falls at the lower end of the current range of measurements for E. huxleyi cell Chl a content (e.g., 0.24-0.38 pg Chl a per cell; Daniels et al., 2014) and leads to conservative estimates of Chl a contribution from this species."

RC6 - The conclusion in the Abstract that temperature is the main driver of nanoplankton distribution should be qualified, as it may well be that temperature is co-varying with other factors that are the actual, ultimate drivers.

AR6 - We agree with the reviewer and have now rewritten the final line of the abstract to better reflect the results of the multivariate analysis.

"Multivariate statistics identified a combination of carbonate chemistry and macro-nutrients, co-varying with temperature, as the dominant drivers of biomineralizing nanoplankton in the GCB sector of the Southern Ocean."

RC7 - On p. 10 line 10, what is the basis for statement that nanophytoplankton contribute 40% of total PP? The references provided do not have that kind of evidence (they are reviews on the ecology and biogeochemical role of diatoms). The authors should use instead remote sensing studies (e.g. Uitz et al. 2010 GBC) to support the statement that nanophytoplankton are the largest contributors to global marine PP.

AR7 - We actually refer in the text to the micro-phytoplankton contribution, in order to highlight that the majority of studies in the Southern Ocean have focused on large phytoplankton species (i.e. most often diatoms). We have now inserted the Uitz et al. (2010)

reference in the relevant section to further highlight the contribution of micro-phytoplankton, but also the contribution of nano-phytoplankton, as discussed in the next sentence (starting p.10 line 25).

"Studies of Southern Ocean phytoplankton productivity have generally focused on the micro-phytoplankton (Barber and Hiscock, 2006) as these species contribute around 40% to total oceanic primary production (Sarthou et al., 2005; Uitz et al., 2010). However, nanoplankton and picoplankton are becoming increasingly recognised as important contributors to total phytoplankton biomass, productivity and export in the Southern Ocean (e.g., Boyd, 2002; Uitz et al., 2010; Hinz et al., 2012)…"

RC8 - Minor point 'TOxN' is awkward and seems to suggest organic nitrogen. Better use 'NOx' or just nitrate (indicating in methods that nitrate actually refers to nitrate+nitrite). In any event nitrite concentrations are likely to be negligible, in comparison with nitrate, in these waters.

AR8 - The notation for nitrate+nitrite has now been changed to NOx throughout the manuscript.

AR9 - Additional references used in responses

Cermeño, P., Dutkiewicz, S., Harris, R.P., Follows, M., Schofield, O. and Falkowski, P.G.. The role of nutricline depth in regulating the ocean carbon cycle. P. Natl. Acad. Sci. USA, *105*(51), 20344-20349, 2008.

Daniels, C.J., Sheward, R.M. and Poulton, A.J.. Biogeochemical implications of comparative growth rates of Emiliania huxleyi and Coccolithus species. Biogeosciences, *11*(23), 6915-6925, 2014.

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

Uitz, J., H. Claustre, B. Gentili, and Stramski *D.* Phytoplankton class-specific primary production in the world's oceans: Seasonal and interannual variability from satellite observations, Global Biogeochem. Cy., 24, GB3016, doi:*10.1029/2009GB003680*, 2010.

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 reprensentative of the nanoplankton group (3 diatoms, 1 coccolithophore), that the key drivers of community 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 deducted 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 mispelled 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 µm, 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:

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^{-1}) 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 µm) or its width (2-5 µm). 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 2000 cells mL^{-1}, and present in low numbers (< 5 cells mL^{-1}) 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 (see page 7 line 31).

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

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.

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 pico-plankton, 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 at statement to show that we recognise the differences in species dominance if biomass was considered, page 7 line 22-24.

"…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 from cell size to biomass is not straightforward for diatoms, as highlighted by Leblanc et al. (2012), and to avoid such issues we consider 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. Kopczynska et al., 1986, Cefarelli et al., 2011, Chen et al., 2007, Hinz et al., 2012, Charalampopoulou et al., 2016.

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° and nutrients seem very odd to me. Finally, every bit of conclusion about the different phytoplankton communities and the overarching role of T° 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 Section 3.3 and 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 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.

AC - Comments from sticky notes:
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 <20µm. In your figure 4d, they are about 60 µm if scale bar is correct - or 150 µm if your legend is correct. I would not include them in the nanoplankton group.

AR9 – Now page 2 line 22 - 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. NOx ? or DIN are more standard

AR10 - We have altered the annotation to NOx throughout the manuscript

RC11 - Page 5 Line 15 - I don't understand this sentence. Was a 200 µm mesh placed beneath the 0.8 µm filter on the filtration rig?

AR11 – Now page 5 line 12 - Apologies if this was not clear, the 200 µm mesh was placed beneath the 0.8 µm filter. The sentence has been rewritten as follows.

"Seawater samples were gently filtered through a 25 mm, 0.8 µm Whatman® polycarbonate filter placed over a 200 µm backing mesh 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 µM for nutrients

AR15 - We have amended to µM throughout the paper, tables and figures.

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 NO3 and T° 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 14 *"The SIMPROF routine identified the stations in the GCB that had statistically similar coccolithophore and diatom community composition through a comparison of Bray-Curtis similarities."*

Page 9 Line 23 *"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-1) at one station, whilst they were in limited numbers (<5 cells mL-1) 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 page 7 line 31.

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 – Now page 11 line 11-13 - 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 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 – Now page 13 line 19 - We have now included reference to Leblanc et al. (2009).

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

AR27 – Now page 15 line 20 - 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/or temperature rather than silicic acid dependence."

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

AR28 - Now page 15 line 28 - 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 acid concentrations in the GCB is likely to be *E. huxleyi* 15 specific rather than a coccolithophore-wide phenomena." Why not?

AR29 – Now page 15 line 31 - We have altered the sentence to read and explain better as follows:

"Therefore, low silicic acid in surface waters of the GCB may negatively impact coccolithophore species that have a silicic acid requirement, such as Calcidiscus leptoporus, and favour bloom-forming species that have no silicic acid requirement (e.g., E. huxleyi)."

RC30 - Page 15 Line 25 - Fig. 6

AR30 – Now page 16 line 12 - This has now been amended.

RC31 - Page 16 Line 15 - I would consider pCO2 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 – Now page 17 line 23 - 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 – Now page 17 line 26 As suggested we have changed this to:

"as estimated from cell counts and 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 – Now page 17 line 20 - We have re-written as follows to remove the direct comparison to diatoms.

"This indicates that in the post-spring bloom conditions of the GCB, E. huxleyi is an important contributor to 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 – Now page 17 line 23-25 - We have changed the emphasis of this sentence:

"Out of a wide suite of environmental variables, latitudinal gradients in temperature, macro-nutrients, pCO_2 and $\Omega_{calcite}$ 'best' described statistically the variation of phytoplankton community composition in this study, whereas $\bar{E}_{MLD}$ and pH did not rank as significant factors influencing species 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 Table 2 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 ****

AR37 - Thank you for highlighting this discrepancy, Table 2 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-1) at only one station and in limited abundance (<5 cells mL-1) at only three others, a comment has been inserted at page 7 line 31.

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 the figure caption has now been removed.

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, see page 10 line 1-3.

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

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Hinz, D. J., Poulton, A. J., Nielsdóttir, M. C., Steigenberger, S., Korb, R. E., Achterberg, E. P. and Bibby, T. S.: Comparative seasonal biogeography of mineralising nannoplankton in the Scotia Sea: *Emiliania huxleyi*, Fragilariopsis spp. and Tetraparma pelagica, Deep-Sea Res. Pt II, 59–60, 57–66, doi: 10.1016/j.dsr2.2011.09.002, 2012.

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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.

Reviewer #3

Reviewer Comment (RC) - This work adds incremental knowledge about the environmental forcing of coccolithophores and diatoms distribution in the southern ocean. In the future, the importance of this work may be that it serves as a base line study. The paper is well written and substantial with many references, although I believe it could be shortened by about 25% and still say the same.
Based on the abstract and conclusions there is not much new insight except that the authors are looking at diatoms and coccolithophores at the same time. There is a host of environmental data and they are discussed at length but few significant patterns emerge which is often the case in beyond control "ships of opportunity studies" where the research is constrained by circumstances, timing, and sampling strategy.

Author Response (AR) - We consider that this study presents a comprehensive analysis of environmental forcing upon the distribution and abundance of dominant diatoms and coccolithophores in the Great Calcite Belt, a region of high importance for marine biogeochemical cycles. This work will contribute to improve our knowledge of the factors that control the biogeography of phytoplankton in the Southern Ocean. It may well form a baseline for the standard of analysis required for future studies, in that they will require a comprehensive investigation over a wide suite of environmental data when considering phytoplankton biogeography in the Southern Ocean - there is a need to move beyond single factor analysis.

RC1 - This brings up the next point:
The collections design was perhaps not ideal. In the paper (page 4 line 25) it says that water was collected from the upper 30 m. Apparently, only one liter of water was sampled that integrates the entire 30 m? This is really precious little water unless I am reading this incorrectly in which case it needs to be explained. It is well known that phytoplankton biomass can occur below this level (e.g. Hegseth and Sundfjord, 2008). Why was the collection limited to 30m?

AR1 - The focus of this study was on the upper mixed layer in the Southern Ocean, rather than deeper waters below the productive euphotic zone and noting that few subsurface chlorophyll maxima (SCM) were encountered (limited to sub-tropical waters). Sampling at 30 m is hence suitable for characterising variability in upper ocean phytoplankton communities. Sampling 1 litre of water is standard procedure for SEM identification of phytoplankton on a 25 mm filter area. Higher volumes lead to clogging of the filter and loss of useable filter area for enumeration when cells are covered in additional organic matter and/or other phytoplankton cells.

RC2 - Also the method of identification is not really suited to a detailed morphological analysis of *E.hux* which is important especially in the southern oceans where there exist various morpho/phenotypes of this species.

AR2 - We acknowledge that there are various morphotypes of *E. huxleyi* in the Southern Ocean,

however morphological examination of *E. huxleyi* was not performed as part of this study. Further, differentiating *E. huxleyi* morphotypes for the statistical analysis was not our specific focus, which was on differentiating different coccolithophore and small diatom species.

RC3 - What were the reasons for the magnifications differing at 5kx and 3kx?

AR3 - The difference in magnification for the two transects reflects the overall lower cell densities found in the Indian Ocean versus the Atlantic Ocean and our requirement to enable sufficient filter area for identification and enumeration.

RC4 - What about all the other material on the filter?

AR4 - There were occasionally other material present on the filter, but these were not straightforward to identify and therefore were not quantified. Additional material beyond coccolithophores and diatoms were not the focus of the study and so were not included in the manuscript.

RC - There are many generalities in the paper that could use more explanation. Some of these are defined by G below;
RC5 - G: Page 2 line 5: Takahashi wrote many papers on CO2 sequestration of CO2. How do we know whether the CO2 that is being taken up by areas of the ocean is anthropogenic or natural? Also the North Pacific is also such an area.

AR5 - We have included the North Pacific in this sentence as follows:

"The region between 30-50oS has the highest uptake of anthropogenic carbon dioxide (CO_2) alongside the North Atlantic and North Pacific Oceans (Sabine et al., 2004)."

Also, following back to the original work the anthropogenic uptake was estimated from a carbon tracer technique (Gruber et al, 1996).

Gruber, N., Sarmiento, J. L., & Stocker, T. (1996). An improved method for detecting anthropogenic CO2 in the oceans. *Global Biogeochemical Cycles*, *10*(4), 809–837.

RC6 - G: >Page 2 line 7-9: vague sentences. Poorly constrained, critical? Why

AR6 – Page 2 line 6-11 - We have rephrased this paragraph to read as follows:

"*Our knowledge of the impact of interacting environmental influences on phytoplankton distribution in the Southern Ocean is limited. For example, we do not yet fully understand how*

light and iron availability, or temperature and pH, may interact to control phytoplankton biogeography (Boyd et al., 2010, 2012; Charalampopoulou et al., 2016). Hence, if model parameterizations are to improve (Boyd and Newton, 1999) to provide more accurate predictions of future biogeochemical change, a multivariate understanding of the full suite of environmental drivers is required."

RC7 - G: >Page 2 line 28-30: Why important

AR7 – Page 2 line 30-32 - We have added context at the beginning of the sentence that highlights the importance of studying mineralizing phytoplankton.

"In the context of climate change and future ecosystem function, the distribution of biomineralizing phytoplankton is important to define when considering phytoplankton interactions with carbonate chemistry (e.g., Langer et al., 2006; Tortell et al., 2008) and ocean biogeochemistry (e.g., Baines et al., 2010; Assmy et al., 2013; Poulton et al., 2013)."

RC8 - Page 3 line 1...."south of ~30oS and extends to ~60o" (This has already been stated.

AR8 - This text has now been removed.

RC9 - G: Page 3 line 25 "uncertainties" why?

AR9 – Page 3 line 24 To clarify we have altered the sentence to read as follows:

"... remains a significant issue when considering the impact of future climate change."

RC10 - Page 5 line 11: Why were individual coccoliths not counted? This can also say a lot about the age of the community.

AR10 - Our focus in the present study was on comparative biogeography of coccolithophores and small diatoms rather than coccolithophore growth dynamics. Hence coccolith counts were not included.

RC11 - Page 7 line 5-13. Can all these parameters be displayed graphically?

AR11 - These parameters could be displayed graphically, however, this would look confusing given the north-south and east-west cruise tracks and irregular distances covered between stations. It was decided that retaining the original data in table format also allowed better access to the parameter values.

RC12 - Page 7 line 29. Maybe I missed it but what were ALL of the 28 coccolithophore species?

AR12 - This information will be available as a Pangea dataset, combining the coccolithophore diatom species and their abundances.
https://doi.org/10.1594/PANGAEA.879790

RC13 - Page 11 line 28 "occurrences" instead of "features"

AR13 - Now on Page 12 line 13 This has now been altered.

RC14 - Page 12 line 7. Where is the rest of the Chl coming from? This section (lines 7-9) is not clear

AR14 – Now page 12 line 24 - The remaining fraction of the Chl-a is most likely to represent phytoplankton not enumerated in this study such as small picoplankton, non-mineralising nanoplankton (e.g. naked flagellates), dinoflagellates and other diatoms. We have included a statement to clarify this point.

"This estimate is similar to that estimated in an identical way by Poulton et al. (2013) and highlights the significant contribution of phytoplankton other than coccolithophores (flagellates, diatoms) to phytoplankton biomass and production during coccolithophore blooms."

RC15 - Page 13 line 4 "coccolithophores IN this region"

AR15 – Now page 13 line 22 We have noted this and corrected.

RC16 - Page 14 line 10 What is the "theoretical species abundance"?

AR16 – Now page 14 line 25 We have removed this comment and amended the sentence as follows.

"Nanoplankton are subject to high grazing pressure (Schmoker et al., 2013), with the growth and mortality of a species both directly influencing cell abundances (Poulton et al., 2010), which could result in nanoplankton abundance patchiness additional to the influence of temperature and/or other environmental gradients."

RC17 - Page 15 line 12. "However A FEW non-blooming

AR17 – Now page 15 line 30 We have noted this and corrected it.

RC18 - Page 15 line 13 “conspicuously absent” why is this conspicuous?

AR18 – Now page 15 line 32 - We have removed conspicuously from this sentence.

RC19 - Page 15 line 14-15 “to be a *E. huxleyi* specific rather than a coccolithophore-wide phenomena” not clear what the authors meant to say. I don’t agree.

AR19 – Now page 15 line 30 - We have altered the sentence to read 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 have no silicic acid requirement (e.g E. huxleyi).”

RC20 - Page 16 lines 25-27...There are many studies con and pro for this sentence

AR20 – Now page 17 line 12-14 We are not sure what the reviewer means here. We have removed part of the sentence for clarification.

“In our study, there was no significant correlation between E. huxleyi and Ωcalcite (Pearson’s product moment = 0.093). However, the waters of the GCB remained oversaturated (Ωcalcite> 2) throughout, and furthermore the relationship between coccolithophores, calcification and carbonate chemistry is now recognized as being complex and non-linear…”

The Influence of Environmental Variability on the Biogeography of Coccolithophores and Diatoms in the Great Calcite Belt

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Abstract. The Great Calcite Belt (GCB) of the Southern Ocean is a region of elevated summertime upper ocean calcite concentration derived from coccolithophores, despite the region being known for its diatom predominance. ~~The o~~Overlap of two major phytoplankton groups, coccolithophores and diatoms, in the dynamic frontal systems characteristic of this region, provides an ideal setting to study environmental influences on the distribution of different species within these taxonomic groups. ~~Water s~~Samples for phytoplankton enumeration were collected from the upper mixed layer (~30 m) during two cruises, the first to the South Atlantic sector (Jan-Feb 2011; 60° W-15° E and 36-60° S) and the second in the South Indian sector (Feb-Mar 2012; 40-120° E and 36-60° S). The species composition of coccolithophores and diatoms was examined using scanning electron microscopy at 27 stations across the Sub-Tropical, Polar, and Sub-Antarctic Fronts. The influence of environmental parameters, such as sea-surface temperature (SST), salinity, carbonate chemistry (~~i.e.,~~ pH, partial pressure of CO_2 (pCO_2), alkalinity, dissolved inorganic carbon), macro-nutrients (~~i.e.,~~ nitrate+nitrite, phosphate, silicic acid, ammonia), and mixed layer average irradiance, on species composition across the GCB, was assessed statistically. Nanophytoplankton (cells 2-20 µm) were the numerically abundant size group of biomineralizing phytoplankton across the GCB, with the coccolithophore *Emiliania huxleyi* and ~~the~~ diatoms *Fragilariopsis nana*, *F. pseudonana* and ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. ~~were~~ the most numerically dominant and widely distributed ~~species~~. A combination of SST, macro-nutrient concentrations and pCO_2 were the best statistical descriptors of biogeographic variability of biomineralizing species composition between stations. *Emiliania huxleyi* occurred in ~~the~~ silicic acid-depleted waters between the Sub-Antarctic Front and the Polar Front~~;~~, ~~indicating~~ a favorable environment for this ~~coccolithophore~~ species ~~in the GCB~~ after spring diatom blooms remove silicic acid ~~to limiting levels~~. Multivariate statistics ~~After statistical consideration of the influence of spatial variability in a diverse suite of environmental factors on the distribution of nanoplankton in the GCB, we~~ identified~~y~~ a combination of carbonate chemistry and macro-nutrients, co-varying with temperature, as the dominant drivers of

biomineralizing nanoplankton ~~species biogeography in a large proportion of the modern Southern Ocean~~After in the GCB sector of the Southern Ocean~~. full consideration of variability in carbonate chemistry and temperature on the distribution of nanoplankton in the GCB, we find that temperature remains the dominant driver of biogeography in a large proportion of the modern Southern Ocean~~.

1 Introduction

The Great Calcite Belt (GCB), defined as an elevated particulate inorganic carbon (PIC) feature occurring alongside seasonally elevated chlorophyll *a* in austral spring and summer in the Southern Ocean (Fig. 1; Balch et al., 2005), plays an important role in climate fluctuations (Sarmiento et al. 1998, 2004), accounting for over 60% of the Southern Ocean area (30-60°S; Balch et al., 2011). The region between 30-50°S ~~is recognized as having~~has the highest uptake of anthropogenic carbon dioxide (CO_2) alongside the North Atlantic and North Pacific Oceans (Sabine et al., 2004). Our knowledge of the impact of interacting environmental influences on phytoplankton distribution in the Southern Ocean is limited. For example, we do not yet fully understand ~~, for example~~ how light and iron availability, or temperature and pH, ~~may~~ interact to control phytoplankton biogeography (Boyd et al., 2010, 2012; Charalampopoulou et al., 2016). Hence, if model parameterizations are to improve (Boyd and Newton, 1999) to ~~, and~~ provide ~~more~~ accurate predictions of ~~future~~ biogeochemical change, a multivariate understanding of the full suite of environmental drivers ~~approach~~ is required.~~The impact of future perturbations of ocean chemistry on Southern Ocean phytoplankton biogeography (e.g., Passow and Carlson, 2012) is poorly constrained. Understanding the current environmental influences on phytoplankton biogeography is therefore critical if model parameterizations are to improve (Boyd and Newton, 1999) and provide more accurate predictions of future biogeochemical change.~~

The Southern Ocean has often been considered as a micro-plankton (20-200 µm) dominated system with phytoplankton blooms dominated by large diatoms and *Phaeocystis* sp. (e.g., Bathmann et al., 1997; Poulton et al., 2007; Boyd, 2002). However, since the ~~recent~~ identification of the GCB as a consistent feature (Balch et al., 2005; 2016) and ~~the~~ recognition of ~~the importance of~~ pico- (< 2 µm) and nanoplankton (2-20 µm) importance in High Nutrient Low Chlorophyll (HNLC) waters (Barber and Hiscock, 2006), the dynamics of small (bio-)mineralizing plankton and their ~~subsequent~~ export need to be ~~reconsidered~~acknowledged. The two dominant biomineralizing phytoplankton groups in the GCB are coccolithophores and diatoms. Coccolithophores are generally found north of the PF (e.g., Mohan et al., 2008), though *Emiliania huxleyi* has been observed as far south as 58°S in the Scotia Sea (Holligan et al., 2010), at 61°S across Drake Passage (Charalampopoulou et al., 2016) and 65°S south of Australia (Cubillos et al., 2007).

Diatoms are present throughout the GCB, with the Polar Front marking a strong divide between different size fractions (Froneman et al., 1995). North of the PF, small diatom species ~~(< 20 µm)~~ such as ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. and

Thalassiosira sp. tend to dominate numerically, whereas large diatoms ~~(> 20 µm)~~ with higher silicic acid requirements (e.g. *Fragilariopsis kerguelensis*) are generally more abundant south of the PF (Froneman et al., 1995). High abundances of nanoplankton (coccolithophores, small diatoms, chrysophytes) have also been observed on the Patagonian shelf (Poulton et al., 2013) and in the Scotia Sea (Hinz et al., 2012). Currently, few studies incorporate small biomineralizing phytoplankton to species level (e.g., Froneman et al., 1995; Bathmann et al., 1997; Poulton et al., 2007; Hinz et al., 2012). Rather, the focus has often been on the larger and non-calcifying species ~~of phytoplankton~~ in the Southern Ocean due to sample preservation issues (i.e., acidified Lugol's solution dissolves calcite and light microscopy restricts accurate identification to cells > 10 µm; Hinz et al., 2012). In the context of climate change and future ecosystem function, the distribution of biomineralizing phytoplankton is important to define when considering phytoplankton interactions with carbonate chemistry (e.g., Langer et al., 2006; Tortell et al., 2008) and ocean biogeochemistry (e.g., Baines et al., 2010; Assmy et al., 2013; Poulton et al., 2013). ~~The distribution of mineralizing phytoplankton is important to define when considering phytoplankton interactions with carbonate chemistry (e.g., Langer et al., 2006; Tortell et al., 2008) and ocean biogeochemistry (e.g., Baines et al., 2010; Assmy et al., 2013; Poulton et al., 2013).~~

The GCB ~~begins south of ~30° S and extends to ~60° S covering an area of ~88 × 10^6 km^2 (Balch et al., 2011), spanning~~ spans the major Southern Ocean circumpolar fronts (Fig. 1a): the Sub-Antarctic front (SAF); the Polar Front (PF); the Southern Antarctic Circumpolar Current Front, SACCF); and occasionally, the Southern Boundary of the Antarctic Circumpolar Current (ACC, see Tsuchiya et al., 1994; Orsi et al., 1995; Belkin and Gordon, 1996). The Subtropical Front (STF; at approximately 10° C) acts as the northern boundary of the GCB and is associated with a sharp increase in PIC southwards (Balch et al., 2011). These fronts divide distinct environmental and biogeochemical zones making the GCB an ideal study area to examine ~~the~~ controls on phytoplankton communities in the open ocean (Boyd, 2002; Boyd et al., 2010). High PIC concentration observed in the GCB (1 µmol PIC L^{-1}) compared to the global average (0.2 µmol PIC L^{-1}) and significant quantities of detached *E. huxleyi* coccoliths ~~of the ubiquitous coccolithophore *Emiliania huxleyi*~~ (in concentrations > 20,000 coccoliths mL^{-1}; Balch et al., 2011) both characterize the GCB. The GCB is clearly observed in satellite imagery (e.g., ~~;~~ Balch et al., 2005; Fig. 1b;) spanning from the Patagonian Shelf (Signorini et al., 2006; Painter et al., 2010), across the Atlantic, Indian and Pacific Oceans and completes the Antarctic circumnavigation via the Drake Passage.

~~The waters of the~~ GCB waters ~~have been more specifically~~are characterized as High Nitrate Low Silicate Low Chlorophyll (HNLSiLC; e.g., Dugdale et al., 1995; Leblanc et al., 2005; Moore et al., 2007; Le Moigne et al., 2013), where dissolved iron (dFe) is considered an important control on microplankton (>20 µm) growth (e.g., Martin et al., 1990; Gall et al., 2001; Venables and Moore, 2010). Sea-surface temperature (SST) gradients ~~have long been recognized as~~are a driving factor behind phytoplankton biogeography and community composition (Raven and Geider, 1988; Boyd et al., 2010). The influence of environmental gradients on biomineralizing phytoplankton in the Scotia Sea and Drake Passage has also been assessed (Hinz et al., 2012; Charalampopoulou et al., 2016). However, the controls on the distribution of ~~the~~ biomineralizing

nanoplankton are yet to be established for the wider Southern Ocean and GCB. Previous studies have predominantly focused on a single environmental factor (e.g., Eynaud et al., 1999) or combinations of temperature, light, macronutrients and dFe (e.g., Poulton et al., 2007; Mohan et al., 2008; Balch et al., 2016) to explain phytoplankton distribution. The inclusion of carbonate chemistry as an influence on phytoplankton biogeography is a relatively recent development (e.g., Charalampopoulou et al., 2011, 2016; Hinz et al., 2012; Poulton et al., 2014; Marañón et al., 2016). Furthermore, natural variability in ocean carbonate chemistry and the resulting impacts on in situ phytoplankton populations remains a significant issue when considering the impact of future climate change~~one of the greatest biogeochemical uncertainties~~.

Increasing concentration of dissolved CO_2 in the oceans is resulting in 'ocean acidification' via a decrease in ocean pH (Caldeira and Wickett, 2003). In the high latitudes, where colder waters enhance the solubility of CO_2 and reduce the saturation state of calcite, there may be potential detrimental effects on calcifying phytoplankton (Doney et al., 2009). However, this may be species- (Langer et al., 2006) or even strain-specific (Langer et al., 2011), showing an optimum-response when the opposing influences of pH and bicarbonate are considered in a substrate-inhibitor concept (Bach et al., 2015). The response of non-calcifiers (e.g., diatoms) to ocean acidification is a greater unknown but no less important given their ~40 to 50% contribution to global primary production (e.g., Tréguer et al., 1995; Sarthou et al., 2005). Tortell et al. (2008) observed a switch from small to large diatom species with increasing CO_2, indicating a potential change in future community structure. Large phytoplankton species (>50 µm) may also have ~~the existing~~ physiological traits to withstand changes in ocean chemistry over smaller (<50 µm) celled species (Flynn et al., 2012), as well as potentially being less susceptible to grazing pressure (Assmy et al., 2013). Alternatively, there may be a shift towards small phytoplankton groups due to the expansion of low-nutrient subtropical regions (Bopp et al., 2001; Bopp, 2005). The response of Southern Ocean phytoplankton biogeography to future climate conditions, including ocean acidification, is complex (e.g., Charalampopolou et al., 2016; Petrou et al., 2016; Deppeler and Davidson, 2017) and therefore understanding existing relationships between *in situ* phytoplankton communities and ocean chemistry is an important stepping-stone for predicting future changes.

Here, we assess the distribution of coccolithophore and diatom species in relation to the environmental conditions encountered across the GCB. Diatom and coccolithophore cell abundances were obtained from analysis of scanning electron microscopy (SEM) images, and their distribution statistically assessed in relation to SST, salinity, mixed layer average irradiance, macronutrients and carbonate chemistry. Herein, we examine the spatial differences within the biomineralizing phytoplankton in the GCB, the main environmental drivers behind their biogeographic variability and the potential effects of future carbonate chemistry perturbations.

2 Methods

2.1 Sampling area

Two cruises were undertaken in the GCB during 2011 and 2012 (http://www.bco-dmo.org/project/473206). The Atlantic sector of the Southern Ocean (GCB1) was sampled from 11th January to 16th February 2011 onboard the R/V *Melville*, between Punta Arenas, Chile and Cape Town, South Africa (Balch et al., 2016; Fig. 1). The Indian sector of the Southern Ocean (GCB2) was sampled from 18th February to 20th March 2012 onboard the R/V *Revelle* between Durban, South Africa and Fremantle, Australia (Fig. 1). Water samples were taken at 27 stations across a latitudinal gradient ranging from 38° S to 60° S and a longitudinal gradient ranging from 60° W to 120° E during the GCB cruises, which enabled sampling of the major oceanographic features of this region.

2.1 Physiochemical environmental conditions

Water samples~~, for this study,~~ were collected from the upper 30 m of the water column using a Niskin bottle rosette and CTD profiler for sea surface temperature, salinity, chlorophyll *a* (Chl *a*), nitrate plus nitrite (~~TOxN~~NOx), ammonia (NH_4), phosphate (PO_4), silicic acid ($Si(OH_4)$), and carbonate chemistry. Nutrient analyses of ~~TOxN~~NOx, PO_4, $Si(OH_4)$ and NH_4 were run on a Seal Analytical continuous-flow AutoAnalyzer 3, while salinity was determined using a single Guildline Autosal 8400B stock salinometer (S/N 69-180). Chlorophyll *a* was sampled in triplicate following Joint Global Ocean Flux Studies (JGOFS; Knap. et al, 1996) protocols. Mixed layer depths were calculated from processed CTD data applying a criteria of a 0.02 kg m^{-3} density change from the 5 m ~~depth~~ value (Arrigo et al., 1998). Daily ~~surface~~ Photosynthetically Active Radiation (PAR, ~~) irradiance (~~mol PAR m^{-2} d^{-1}) was estimated from eight-day composite Aqua MODIS data from the closest time and latitude-longitude point (averages were taken where necessary). Mixed layer average irradiance ($\bar{E}_{MLD}$) was calculated from ~~the~~ daily PAR following Poulton et al. (2011).

Water samples were collected for dissolved inorganic carbon (C_T) and Total Alkalinity (A_T) following standardized methods and analyzed using a Versatile Instrument for the Determination of Titration Alkalinity (VINDTA) with precision and accuracy of ±1 µmol kg^{-1} (Bates et al., 1996; Bates et al., 2012). The remaining carbonate chemistry parameters were calculated from the C_T and A_T values using CO2SYS (Lewis and Wallace, 1998) and CO2calc (Robbins et al., 2010), with the carbonic acid dissociation constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987). This includes computation of the saturation state (Ω) for calcite (i.e., $\Omega_{calcite}$).

2.2 Phytoplankton enumeration

Samples for biomineralizing phytoplankton community structure were ~~also~~ taken from the upper 30 m of the water column. ~~At each sampling station~~ One~~1~~ ~~L~~ litre seawater samples were collected and pre-filtered through a 200 µm mesh to remove any large zooplankton. Seawater samples were ~~then~~ gently filtered through a 25 mm, 0.8 µm Whatman® polycarbonate filter

~~with~~ placed over a 200 µm backing mesh ~~as a backing filter~~ to ensure an even distribution of cells across the filter. ~~The~~ ~~f~~Filters were rinsed with ~5 mL potassium tetraborate (0.02 M) buffer solution (pH = 8.5) to prevent salt crystal growth and PIC dissolution, air dried and stored in petri slides in the dark with a desiccant until further analysis.

To identify coccolithophores to the species level, each sample was imaged using the SEM methodology of Charalampopoulou et al. (2011). A central portion of each filter was cut-out, gold-coated and 225 photographs were taken at a magnification of 5000x (equivalent to ~1 mm^2; GCB1) or 3000x (~2.5 mm^2; GCB2) using a Leo 1450VP SEM (Carl Zeiss, Germany). Detached coccoliths and whole coccolithophore cells (coccospheres) were identified following Young et al. (2003). Diatoms and other recognizable protists were identified following Hasle and Syvertsen (1997) and Scott and Marchant (2005). Where a confident species level identification was not possible, cells were assigned to the level of genera (e.g., *Chaetoceros* sp. or *Pappamonas* sp.). Each species identified was enumerated using the freeware ImageJ (v1.44o) for all 225 images or until 300 cells (or coccoliths) were counted. A minimum of 10 random images was picked for enumeration when species were in high abundance (>1000 cells mL^{-1}). The abundance of each species was calculated following Eq. (1):

$$Cells\ mL^{-1} = (C \times F/A)/V \quad (1)$$

where C is the total number of cells (or coccoliths) counted, A is the area investigated (mm^2), F is the total filter area (mm^2) and V is the volume filtered (mL).

2.3 Statistical analysis

Multivariate statistics (PRIMER-E v.6.1.6; Clarke and Gorley, 2006) were used to examine spatial changes in coccolithophore and diatom abundance, species distribution and the influence of environmental variability on biogeography (e.g., Charalampopoulou et al., 2011, 2016). Environmental data was initially assessed for skewness, most likely due to strong chemical gradients across fronts. Heavily left-skewed variables (~~TOxN~~NOx, silicic acid and NH_4) were log(V+0.1) transformed to reduce skewness and stabilize variance. Other environmental data, including SST, salinity, $\bar{E}_{MLD}$, ~~TOxN~~NOx, silicic acid, NH_4, pH, pCO_2 and $\Omega_{calcite}$ was then normalized to a mean of zero and a standard deviation of one, and Euclidean distance was then used to determine spatial changes in these parameters. A principal component analysis (PCA) was used to simplify environmental variability, by combining the more closely correlated variables and the relative influence of the environmental variables within the data (Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley, 2006).

Coccolithophore and diatom species diversity was assessed as the total number of species (S), and Pielou's evenness index (J') which assesses how evenly the count data was distributed between the different species present (before further statistical analysis). Species with cell counts of less than 1 cell mL^{-1}, and/or consistently representing less than 1% of the total cell abundance, were excluded from multivariate statistical analysis to reduce the influence of rare species. Analysis of coccolithophore and diatom community structure was carried out on standardized and square root transformed cell

abundance (to reduce the influence of numerically abundant species) using a Bray-Curtis similarity matrix. Bray-Curtis similarity describes the percentage similarity (or dissimilarity) between different communities according to their relative species composition. To identify which stations had a statistically similar biomineralizing phytoplankton community across the GCB a SIMPROF routine (1000 permutations, 5% significance level) was applied to the Bray-Curtis similarity matrix. SIMPROF identifies, based on pairwise tests of the calculated Bray-Curtis percentage similarity, whether the similarities between samples are smaller and/or larger than those expected by chance, grouping those which are statistically distinct (Clarke et al., 2008). The phytoplankton species driving the differences between the groups were identified through a SIMPER routine and presented using non-metric multidimensional scaling (nMDS; Clarke, 1993; Clarke and Warwick, 2001; Clarke and Gorley, 2006). SIMPER allows statistical identification of which species are primarily responsible for differences between groups of samples and breaks down the Bray-Curtis similarity into individual species contributions.

A BEST routine was applied to environmental and plankton data to determine the combination of environmental variables that 'best' described the variability in coccolithophores and diatoms across the GCB. The BEST routine searches statistically for relationships between the biotic and abiotic patterns and to identify which environmental variable(s) explained most of the variation in species distribution. Spearman's rank correlations were used to further investigate the relationship between key environmental variables identified in the BEST routine and selected coccolithophore and diatom species.

3 Results

3.1 General Oceanography

The GCB cruises crossed various biogeochemical gradients associated with the Antarctic Circumpolar Current (ACC) fronts and currents, with most parameters following a recognizable latitudinal (or zonal) pattern. The position of oceanic fronts referred to in the ~~following~~ text relates to those defined in Fig. 1 (see also Balch et al., 2016). Sea-surface temperature decreased southwards from 21° C north of the STF to 1.1° C close to 60° S (Table 1). Calcite saturation state ($\Omega_{calcite}$) decreased from 5.2 north of the subtropical front to 2.6 close to 60° S (Table 1). Macronutrient concentrations generally increased southwards with a distinct divide across the SAF. ~~TOxN~~NOx ranged from below detection limits (<0.1 ~~µmol L^{-1}~~µM) to as high as 28 ~~µmol L^{-1}~~µM, with higher concentrations generally south of the Sub-Antarctic Front (>12 ~~µmol L^{-1}~~µM), and lower concentrations (<7 ~~µmol L^{-1}~~µM) north of the Sub-Antarctic Front (Table 1). PO_4 followed a very similar pattern with concentrations generally greater than 1 ~~µmol L^{-1}~~µM south of the Sub-Antarctic Front and <0.6 ~~µmol L^{-1}~~µM to the north. Silicic acid concentrations were divided by the PF, being generally less than 2 ~~µmol L^{-1}~~µM to the north and up to 78.5 ~~µmol L^{-1}~~µM to the south (Table 1). $\bar{E}_{MLD}$ was highest on the Patagonian Shelf (~40 mol ~~photons~~ PAR m^{-2} d^{-1}) and generally less than 10 mol ~~photons~~ PAR m^{-2} d^{-1} south of the Sub-Antarctic Front (Table 1). There was no distinct latitudinal trend in pH or pCO_2. Surface water pH was generally greater than 8.06, ranging from 8.03 on the Kerguelen plateau to 8.13 in the Sub-Tropical Front south-west of Australia (Table 1). Surface water pCO_2 ranged from 299 µatm to 444 µatm with

both extremes in the vicinity of the Atlantic STF (Table 1). Chl *a* concentrations were variable across the oceanic gradients, highest on the Patagonian Shelf (2.78 mg m^{-3}) and on average less than 1 mg m^{-3} in the South Atlantic compared with less than 0.5 mg m^{-3} in the South Indian Ocean (Table 1).

3.2 Coccolithophores and diatoms

The most frequently occurring and abundant size group within the coccolithophore~~s~~ and diatom counts were the nanoplankton (cells 2-20 µm). Large diatom species (cells >20 µm) were found in higher numbers (up to 50 cells mL^{-1}) south of the PF ~~but were 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 from cell size to biomass is not straightforward for diatoms, as highlighted ~~in~~by Leblanc et al. (2012), and to avoid ~~these~~such ~~potential caveats~~issues we ~~have~~ consider~~ed~~ species abundance only. Total cell abundances were less than 1000 cells mL^{-1} at most stations (Table 2), which are indicative of late summer, non-bloom conditions. In the South Atlantic, the highest abundance of coccolithophores was on the Patagonian Shelf (station GCB1-16; 1,636 cells mL^{-1}) and the highest abundance of diatoms was east of the South Sandwich Islands (station GCB1-77; 6,~~787~~893 cells mL^{-1}; Table 2). ~~These were also the highest total abundances of coccolithophores and diatoms encountered across the entire GCB.~~ In the South Indian Ocean, coccolithophore abundance was highest near the Crozet Islands (station GCB2-27; 472 cells mL^{-1}) and ~~the~~ diatom abundance was highest at the most southerly station (station GCB2-73; ~~514~~538 cells mL^{-1}; Table 2). There were no stations in the South Indian Ocean ~~with~~ where coccolithophore and diatom abundances were greater than 1,000 cells mL^{-1} (Fig. 2, Table 2). Additionally, the silicifying chrysophyte *Tetraparma sp.* was particularly abundant east of the South Sandwich Islands (~~at~~ station GCB1-77), at a cell density of 2000 cells mL^{-1}, though they were present in low numbers (< 5 cells mL^{-1}) at three more stations in the South Atlantic and absent throughout the rest of the GCB.

Coccolithophores dominated the biomineralizing ~~plankton~~ community at twelve stations in terms of abundance north of the PF (Fig. 2, Table 2). On average coccolithophores contributed approximately 38% to total (coccolithophore and diatom) abundance in the GCB. Coccolithophores were greater than 75% of total abundance at only one station, north of South Georgia (station GCB1-59), and never accounted for 100% of total cell numbers. Twenty-eight species of coccolithophores were identified as intact coccospheres across the GCB. Coccolithophore diversity decreased south towards 60° S, with the highest coccolithophore diversity (19~~3~~ species) found in the vicinity of the STF in the eastern part of the South Indian Ocean (station GCB2-106), while ~~species contributions to total~~ coccolithophore abundance was more evenly distributed between the different species in the lower latitudes (i.e., high J'; Table 2). *Emiliania huxleyi* was the most numerically abundant coccolithophore at all but four stations and ~~was~~ encountered in the mixed layer at all stations except one (station GCB2-73, the most southerly station in the Indian Ocean). Other coccolithophore species (e.g., *Syracosphaera* sp. and *Umbellosphaera* sp.) were present north of the PF throughout the GCB and were most abundant north of the STF. At stations south of the SAF (50° S) only one (*E. huxleyi*) or two species (*E. huxleyi* and *Pappamonas* sp.) were observed as intact coccospheres.

Diatoms dominated 15 stations in terms of biomineralizing plankton ~~numerical~~ abundance across all environments sampled (Fig. 2, Table 2), being ~~and were~~ found in every sample ~~analyzed~~analysed and , contributing 62% (on average) to ~~the~~ total ~~cell~~ (coccolithophores + diatoms) abundance. Diatoms made up 100% of the total cell counts at the most southerly station in the South Indian Ocean (station GCB2-73) and 99.7% east of the South Sandwich Islands (station GCB1-77; Fig. 2). Seventy-six species of diatom were identified as intact cells across the entire GCB. The most frequently occurring species in the GCB were small (< 5 µm in length) *Fragilariopsis* ~~ssp~~spp.. The highest abundance of diatoms in the South Atlantic Ocean (6,~~787~~ 893 cells mL^{-1}) was dominated by *F. nana* east of the South Sandwich Islands (station GCB1-77). The highest diatom abundance in the South Indian Ocean (~~514~~ 538 cells mL^{-1}) was dominated by *F. pseudonana* at the most southerly station (station GCB2-73) sampled. Another frequently dominant diatom was ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp., which ~~that~~ was most abundant north of the PF (Table 2).

Diatom species richness increased south towards 60° S with the contribution of the different diatom species to total biomineralizing plankton abundance fairly even (J' > 0.5, Table 2), except at stations (stations GCB1-70, GCB1-77, GCB2-27 and GCB2-63) where *Fragilariopsis* ~~ssp~~spp. <5 µm were dominant (>70% of the diatom population, J' < 0.5). The highest diatom species richness (~~15~~ 32 species) was found in the GCB south of the SAF (station~~s GCB1-85 and~~ GCB2-36) at a temperature~~s~~ of ~~5°C to~~ 8°C, in HNLSiLC conditions (~~TOxN~~NOx ~~>~~18.9 ~~µmol L~~$^{-1}$µM, silicic acid ~~<2~~1.7 ~~µmol L~~$^{-1}$µM, ~~Chl a~~ 0.21~~-1.11~~ mg Chl *a* m^{-3}).

3.3 Statistical Analysis

Three of the environmental variables were removed from the statistical analysis following a Spearman's rank (r_s) correlation analysis (Table S1). ~~TOxN~~NOx and PO_4 had a strong significant positive correlation (r_s = 0.961, p < 0.0001) and so ~~TOxN~~NOx was deemed representative of the distribution of both nutrients. Sea-surface temperature displayed significant negative correlations with both C_T (r_s = -0.981, p < 0.0001) and A_T (r_s = -0.953, p < 0.0001), and so sea surface temperature was taken as being representative of these two variables of the carbonate chemistry system.

The variation in environmental variables across the GCB was examined using a Principal Component Analysis (PCA)~~-~~, which simplifies environmental variability~~,~~ by combining ~~the more c~~closely correlated variables into principal components~~,~~ in order to account for the greatest variance in the data with the fewest ~~principal~~ components. The first principal component (PC1) accounted for 58% of the variation in environmental variables, with an additional 17% of environmental variation described by PC2 (Table 3). PC1 describes the main latitudinal gradients of environmental changes across the GCB (decreasing SST, increasing macronutrients). PC1 is a predominantly linear combination of SST, salinity, ~~TOxN~~NOx, silicic acid, NH_4, and $\Omega_{calcite}$, where there is a significant positive correlation of PC1 with SST and salinity and a significant negative correlation with all other variables (Table 3). PC2 represented the environmental variation in the GCB occurring

independently of latitude, and was driven predominantly by variation in pCO_2, with weaker influences from $\bar{E}_{MLD}$ and pH (Table 3). PC2 had significant positive correlations with pCO_2 and $\bar{E}_{MLD}$ and a negative correlation with pH.

~~A~~The SIMPROF routine ~~was used to~~ identified~~y~~ the stations in the GCB that had statistically similar coccolithophore and diatom community ~~structures~~composition through a comparison of Bray-Curtis similarities~~, before examining detail of the species within the groups.~~ ~~Variability in coccolithophore and diatom species composition across the GCB was assessed using a SIMPROF routine, comparing the abundance and diversity across all stations, to define groups with statistically similar community composition.~~ Six statistically significant groups ($p < 0.05$) were defined across the GCB (Fig. 3). Three groups of these groupings (A, B, C) were specific to the South Atlantic Ocean (Fig. 3). For example, groups A and B represented individual stations GCB1-46 and GCB1-117 respectively, in the sub-tropical region of the South Atlantic Ocean. The most southerly stations in the South Atlantic Ocean (stations GCB1-70 and GCB1-77) defined group C (Fig. 3). Groups D, E and F included stations across the GCB in both ocean regions. Here, group D was defined by eight stations sampled predominantly north of the SAF, while group F was defined by 11 stations predominantly sampled south of the SAF (Fig. 3). These statistically defined similar community structures indicate that although the GCB covers a wide expanse of ocean, the community structure is consistently latitudinal defined across its longitudinal range.

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).~~The species driving the differences in mineralizing plankton community structure across the GCB were identified through a SIMPER routine (Table 4).~~ The abundance and distribution of four phytoplankton species (*E. huxleyi*, *Psuedo-nitzschia* sp., *F. nana* and *F. pseudonana*; Fig. 4), were identified as having the most significant contribution to differences in community structure across the GCB (Table 4). *Emiliania huxleyi* and *F. pseudonana* were the most numerically dominant coccolithophore and diatom species, respectively, across the GCB (Table 2). *Fragilariopsis pseudonana* was the numerically dominant diatom (> 30%) at seven stations in the South Indian Ocean (Table 2). The diatom with the highest abundance, *F. nana* (6,797 cells mL^{-1}), was almost exclusively found in the South Atlantic Ocean (Table 2) and the more frequently occurring *Pseudo-nitzschia* sp. was present at all but one station.

The non-metric Multi Dimensional Scaling (nMDS) plot of the Bray-Curtis similarities (Fig. 5) shows the station distribution with respect to the SIMPROF defined groups (Fig. 5a), the four main species (Fig. 5b-e) and also holococcolithophores (Fig. 5f). The more closely clustered the stations, the more similar their biomineralizing ~~phytoplankton community~~species composition. Groups A and B were defined by the absence of *E. huxleyi* (Fig. 5b) and the presence of either holococcolithophores (group A; Fig. 5f) or the diatom *Cylindrotheca* sp. (group B). Group C was defined by the ~~presence~~ dominance of *F. nana* (Table 4; Fig. 5d) and low contributions from *E. huxleyi* and ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp.~~, with low diversity overall~~ (~~total of 9 mineralizing species;~~ Table 2; Fig. 5b,e), resulting in a significant difference from the other groups. Group D had high~~er~~ total species diversity overall (19-41 species; ~~i.e., 12-23 species;~~ Table 2) and was defined

by similar relative abundances of *E. huxleyi* and ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp., which were not found elsewhere (Fig. 5b,e) ~~(Table 4)~~. Group E, ~~including~~ composed of stations north of the SAF (Fig. 3, Fig. 5a), included *E. huxleyi*, *U. tenuis* and holococcolithophores (Table 4, Fig. 5b,f). The low abundance and diversity ~~of diatoms~~ (3-125 cells mL^{-1}; ~~Table 2)~~, 7-11 species; Table 2) of diatoms within group E separated it from the other groups ~~(Table 4)~~. The combination of *E. huxleyi*, *F. pseudonana* and ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. that defined group F (Table 4, Fig. 5b,c,e) represented stations on the Patagonian Shelf and south of the SAF (Fig. 3, Fig. 5a). The almost mono-specific *E. huxleyi* coccolithophore community (Table 2) in group F highlights its strong dissimilarity from the other community structure groups identified (Fig. 5) ~~(Table 4)~~.

~~The abundance and distribution of four nanophytoplankton species (*E. huxleyi*, *Psuedonitzschia* sp., *F. nana* and *F. pseudonana*) (Fig. 4), were identified as having the most significant contribution to differences in community structure across the GCB (Table 4, Fig. 5). *Emiliania huxleyi* and *F. pseudonana* were the most dominant coccolithophore and diatom species, respectively, across the GCB (Table 2). *Fragilariopsis pseudonana* was the numerically dominant diatom (> 30%) at seven stations in the South Indian Ocean (Table 2). The diatom with the highest abundance, *F. nana* (6,797 cells mL^{-1}), was almost exclusively found in the South Atlantic Ocean (Table 2; Fig. 5) and the more frequently occurring *Pseudonitzschia* sp. was present at all but two stations (Fig. 5).~~

The influence of environmental variables on the biogeography of coccolithophores and diatoms in the GCB was assessed using the BEST routine. The strongest Spearman's rank correlation ($r_s = 0.55$, $p < 0.001$) between all possible environmental variables and the biogeographical patterns observed came from a combination of five variables, including: (1) SST; (2-4) macronutrients (~~TOxN~~NOx, silicic acid, NH_4); and (5) pCO_2. This was followed by a correlation of $r_s = 0.54$ ($p < 0.001$) that included these parameters as well as $\Omega_{calcite}$. Salinity was included in the third highest correlation, whereas $\bar{E}_{MLD}$ and pH did not rank as significant factors in the BEST analysis.

4 Discussion

4.1 Biogeography of coccolithophores and diatoms in the Great Calcite Belt

Studies of Southern Ocean phytoplankton productivity have generally focused on the micro-phytoplankton (Barber and Hiscock, 2006) as these species contribute around 40% to total oceanic primary production (~~Tréguer et al., 1995;~~ Sarthou et al., 2005; Uitz et al., 2010). However, nanoplankton and picoplankton are becoming increasingly recognised as important contributors to total phytoplankton biomass, productivity and export in the Southern Ocean (e.g., Boyd, 2002; Uitz et al., 2010; Hinz et al., 2012), both as the dominant size group in post-bloom (Le Moigne et al., 2013) and non-bloom conditions (Barber and Hiscock, 2006).

In this study, coccolithophores were generally numerically dominant at stations sampled north of the PF, particularly around the Sub-Antarctic Front, whereas diatoms were ~~observed to be~~ dominant at stations south of the PF (Fig. 2). There was also a significantly different species distribution (*a priori* ANOSIM; R = 0.227, $p < 0.01$) north and south of the Sub-~~-~~Antarctic Front, which has been previously identified as the divider between calcite and opal dominated export in the Southern Ocean (e.g., Honjo et al., 2000; Balch et al., 2016). Diatoms were more abundant (~570 cells mL^{-1}) than coccolithophores (~160 cells mL^{-1}) on average in the entire GCB. This contrasts to ~~a study by~~ Eynaud et al. (1999) in the South Atlantic Ocean at a similar time of year ~~that~~ who reported a peak in coccolithophore cell abundance in the vicinity of the PF (a feature that was not observed in this study). These differences are likely due to ~~be due to the~~ variability of Southern Ocean plankton on short temporal scales (Mohan et al., 2008), including variability in the seasonal progression of the spring bloom (Bathmann et al., 1997).

The coccolithophore *E. huxleyi* and diatoms *F. pseudonana*, *F. nana* and ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. (Fig. 4) were all identified as being central to defining the statistical similarities within, and the differences between, the different biomineralizing phytoplankton groups (Table 4, Fig. 5). 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 *(Pseudo-nitzschia* sp. *is* > 20 µm in length but < 5 µm in width) and~~These four species are all part of the nanoplankton and at the lower end of the size range of the microplankton (*Pseudonitzschia* sp. is ~20 µm in length), which can~~ contributes significantly to biomass in Southern Ocean ~~the~~ HNLC regions ~~of the Southern Ocean~~ (Boyd, 2002). *Emiliania huxleyi* and *Fragilariopsis* sp. ~~less~~ smaller than 10 µm have been identified as two of the most abundant biomineralizing phytoplankton further south in the Scotia Sea (Hinz et al. 2012). ~~The~~ Our results ~~presented here~~ further ~~indicate~~ highlight that nanoplankton ~~do~~ have the potential to contribute a significant proportion to GCB community composition alongside ~~the~~ larger phytoplankton (including large diatoms) typical of ~~ly associated with the~~ HNLC regions.

~~The a~~Abundance of HNLC diatoms such as *F. kerguelensis* (<10 cells mL^{-1}), *T. nitzschioides* (<20 cells mL^{-1}) and large *Chaetoceros* sp. (<10 cells mL^{-1}) were ~~generally~~ lower than those observed in other studies (e.g., Poulton et al., 2007; Armand et al., 2008; Korb et al., 2010, 2012). Furthermore, the ~~virtual~~ absence of the diatom *Eucampia antarctica* (<1 cell mL^{-1}) in this study does not reflect the typical assemblage (sometimes > 600 cells mL^{-1}) found in previous studies (e.g., Kopczyaska et al., 1998; Eynaud et al., 1999; de Baar et al., 2005; Poulton et al., 2007; Salter et al., 2007; Korb et al., 2010). Low abundances of the large-celled diatoms in the silicic acid replete regions may partly relate ~~could be influenced by~~to the small filter area analyzed using SEM; in this study the area imaged equates to a relatively small volume of water (~~i.e.,~~ 2-6 mL depending on magnification) relative to the larger volumes (10-50 mL) often examined for light microscopy in other studies. Large, rare cells may not be enumerated from such small sample volumes, however the numerically abundant nanoplankton groups were well represented in SEM images. Conversely, samples preserved in acidic Lugol's solution for light microscopy analysis are biased towards larger species since small diatoms (<10 µm) are not clearly visible and

coccolithophores are not well preserved (Hinz et al., 2012). ~~Therefore, i~~In future a combination of both imaging techniques is recommended to fully express the ~~should be used when examining the~~ phytoplankton community structure of the ~~wider~~ Southern Ocean.

4.2 *Emiliania huxleyi* in the Great Calcite Belt

The importance of coccolithophores in the GCB was examined via species ~~community~~ composition and abundance of intact cells, focusing on areas identified as having high PIC reflectance from underway sampling and satellite observations (Balch et al., 2014, 2016; Hopkins et al., 2015). Higher species diversity of coccolithophores occurred north of the STF (i.e., ~~46-13~~ 19 species; Table 2). ~~-~~Coccolithophores are diverse in the stratified and low-nutrient waters associated with lower latitudes (Winter et al., 1994; Poulton et al., 2017). Only a few species are found in the colder waters south of the STF (Mohan et al., 2008), the most successful being *E. huxleyi*, which was observed at an abundance of 103 cells mL^{-1} at 1ºC in this study in the South Atlantic (station GCB1-70). The 2°C isotherm has been previously assumed to represent the southern boundary of *E. huxleyi* (e.g., Verbeek, 1989; Mohan et al., 2008) and inter-annual variability could be influenced by movement of the southern front of the Antarctic Circumpolar Current (Holligan et al., 2010). The Southern Ocean *E. huxleyi* morphotype (Cook et al., 2011; Poulton et al., 2011) may therefore have a wider temperature tolerance than its northern hemisphere equivalent (Hinz et al., 2012) and has been observed poleward of 60° S further east in the Southern Ocean (Cubillos et al., 2007) and across Drake Passage (Charalampopoulou et al., 2016). There were three distinct *E. huxleyi* ~~features~~ occurrences (the Patagonian Shelf, north of South Georgia and north of the Crozet Islands) within the GCB where *E. huxleyi* contributed > 50% of the total cell counts of biomineralizing phytoplankton. *Emiliania huxleyi* was most abundant (1,636 cells mL^{-1}) on the Patagonian Shelf and was the most frequently occurring coccolithophore across the entire GCB. The main *E. huxleyi* ~~features~~ occurrences are discussed further below to examine ~~understand~~ why this species is so widely distributed in the GCB.

4.2.1 Patagonian Shelf

The Patagonian Shelf is a well-known region for *E. huxleyi* blooms, as observed in satellite imagery between November and January (~~i.e.,~~ Signorini et al., 2006; Painter et al., 2010; Balch et al., 2011; Garcia et al., 2011; Balch et al., 2014). The *E. huxleyi* cell abundance observed in this study (~1,600 cells mL^{-1}) was similar to that found by Poulton et al. (2013; >1,000 cells mL^{-1}). Using a value of 0.2 pg Chl *a* per cell (Haxo, 1985), following the approach in Poulton et al. (2013), such *E. huxleyi* abundance levels are equivalent to estimated contributions of only ~12% to the total ~~phytoplankton~~ Chl *a* signal (~2.8 mg m^{-3}). This estimate is~~, which is a~~ similar ~~contribution~~ to that estimated in an identical way by Poulton et al. (2013) and highlights the significant contribution of phytoplankton other than coccolithophores (flagellates, diatoms) to phytoplankton biomass and production during coccolithophore blooms. It should be noted that the cell Chl *a* content from Haxo (1985) falls at the lower end of the current range of measurements for *E. huxleyi* cell Chl *a* content (e.g., 0.24-0.38 pg Chl *a* per cell; Daniels et al., 2014) and leads to conservative estimates of Chl *a* contributions from this species. This data, combined with ~~the~~ satellite observations, supports the hypothesis of a repeating~~similar~~ phytoplankton structure ~~repeating~~ on

an inter-annual basis, although the contribution of *E. huxleyi* to ~~net~~ primary production may vary. The optimum range for *E. huxleyi* blooms on the Patagonian Shelf has been identified as between 5-15° C at depleted silicic acid levels relative to nitrate (Balch et al. 2014; 2016). During this study, silicic acid~~).~~ was at almost ~~was drawn down to~~ undetectable levels on the Patagonian Shelf (Table 1), with the source water for this region being Southern Ocean HNLSiLC waters transported northwards via the Falklands current (Painter et al., 2010; Poulton et al., 2013). The persistent low silicic acid availability and residual nitrate (defined as $[NO_3^-]$ - $[Si(OH)_4]$) on the Patagonian Shelf is therefore an ideal environment for *E. huxleyi* to outgrow ~~without the competition of~~ large, fast growing diatoms (Balch et al., 2014).

4.2.2 South Georgia

South Georgia is renowned for intense diatom blooms of over 600 cells mL^{-1} with Chl *a* over 10 mg m^{-3} and integrated primary production up to 2 g C m^{-2} d^{-1} (Korb et al., 2008). However, *E. huxleyi* was the dominant species (>75% of total cell numbers) within the diatom and coccolithophore population at the station north of South Georgia (Table 2, Fig. 2). The associated calcite feature can also be identified from the satellite composite in Fig. 1 (38° E, 51° S). *Emiliania huxleyi* contributed approximately 15%, applying ~~(~~a value of 0.2 pg Chl *a* per cell (Haxo, 1985) following Poulton et al. (2013)~~using 0.2 pg Chl a per cell,)~~ to the total Chl *a* signal (0.71 mg m^{-3}) around South Georgia. The high calcite feature at South Georgia was found at a SST of 5.9°C, which is below the considered 'optimum' growth conditions for *E. huxleyi* previously cultured (Paasche, 2001). This population of *E. huxleyi* was most likely an adapted cold water morphotype (Cook et al., 2011; Poulton et al., 2011; Cook et al., 2013). The dominant diatom species here was *Actinocyclus* sp. and highly silicified *Thalassionema nitzschioides* with silicic acid concentrations likely limiting (1.7 µmol Si L^{-1}; Paasche 1973a & b), whereas ~~TOxN~~NOx concentrations (17.5 µmol N L^{-1}) and PO_4 concentrations (1.22 µmol P L^{-1}) can be considered replete. The low silicate concentrations could explain why *Eucampia antarctica* was not observed in this study, ~~but~~ though it has been observed north of South Georgia ~~previously~~ (Korb et al., 2010, 2012). This indicates that preceding diatom growth ~~event had~~ depleted silicic acid (and other nutrients such as dissolved iron), allowing *E. huxleyi* to become more dominant in the population with a similar residual nitrate environment as found on the Patagonian Shelf (this study, Balch et al., 2014; Balch et al., 2016) and also in the North Atlantic (Leblanc et al., 2009).

4.2.3 Crozet Islands

The *E. huxleyi* feature north of the Crozet Islands with an abundance of 472 cells mL^{-1} (highest in the South Indian Ocean) confirms the presence of coccolithophores in this region. Coccolithophore abundances have not previously been reported in this region, although elevated PIC had been observed and attributed to *E. huxleyi* (Read et al., 2007; Salter et al., 2007). Chl *a* was lowest (0.47 mg m^{-3}) at Crozet out of all three high PIC features, ~~though~~ with *E. huxleyi* contributinged ~20% of this signal, applying a value of 0.2 pg Chl *a* per cell (Haxo, 1985) following Poulton et al. (2013)~~(based on 0.2 pg Chl *a* per cell)~~, proportionally higher than on the Patagonian Shelf and near South Georgia. Previous studies around the Crozet Islands and plateau (2004-2005) have found evidence ~~indications~~ of coccolithophores in sediment trap samples (Salter et al. 2007) and

~~associated~~ large (>30 mmol C m^{-2} d^{-1}) calcite fluxes (Le Moigne et al., 2012), though surface cell counts were unavailable (Read et al., 2007). The satellite-derived calcite signal was observed to increase after the main Chl *a* event in this study (Fig. S1) and in previous years (Salter et al., 2007). An increase in coccolithophore abundance following a diatom bloom is also observed in ~~other~~ similar oceanic regions from satellite-derived products (Hopkins et al., 2015) and is associated with depletion of dissolved iron and/or silicic acid (Holligan et al., 2010) in addition to a stable water column and increased irradiance (Balch et al., 2014).

4.2.4 Summary of biogeochemical characterization of coccolithophore occurrence and abundance

The Southern Ocean ~~was previously~~has been considered to have a biomineralizing phytoplankton community dominated by diatoms. This study highlights that *E. huxleyi* can form distinct features within the GCB and contribute up to 20% towards total Chl *a* in these features compared to an average of less than 5% of Chl *a* across the rest of the GCB. Hence, *Emiliania huxleyi* is likely to have a more important role in biogeochemical processes in the GCB than previously thought. This is particularly important to consider when assessing the impact on calcium carbonate associated export (e.g., Honjo et al., 2000; Balch et al., 2010; Balch et al., 2016) in the Southern Ocean. If *E. huxleyi* is migrating poleward with time (Winter et al., 2013) then the dynamics of the carbon system in the GCB may change, particularly south of the SAF, where silicic acid derived export has historically been dominant (Honjo et al., 2000; Pondaven et al., 2000). Thus, it is essential to gain an understanding of the environmental factors driving the distribution of *E. huxleyi* (Winter et al., 2013, Charalampopoulou et al., 2016) amongst other phytoplankton in the GCB to better understand ~~predict the future~~ biogeochemistry of the Southern Ocean.

4.3 Environmental controls on biogeography

The environmental variables that best describe coccolithophore and diatom species distribution in this study were SST, macronutrients (~~TOxN~~NOx, silicic acid, NH_4) and pCO_2 (Spearman's rank correlation = 0.55, $p < 0.001$), with the second highest correlation (Spearman's rank correlation = 0.54, $p < 0.001$) including calcite saturation state ($\Omega_{calcite}$). The inclusion of pCO_2 and $\Omega_{calcite}$ as important factors indicates a potential influence of carbonate chemistry on coccolithophore and diatom distribution (and *vice versa*) in the GCB. However, $\Omega_{calcite}$ had a very strong positive correlation (r = 0.964, $p < 0.0001$) with SST (Table S1), and therefore separating the influences of the two variables was impossible in this study due to the tight coupling between carbonate chemistry and temperature (as also observed by Charalampopoulou et al., 2016).

4.3.1 Temperature

Temperature is recognized as a strong driving factor behind plankton biogeography and community composition (Raven and Geider, 1988; Boyd et al., 2010). The abundance of two of the dominant species, *E. huxleyi* and *F. pseudonana*, did not significantly correlate (Pearson's product moment correlation = 0.147, $p = 0.493$ and r = -0.247, $p = 0.357$ respectively) with SST, which does not agree with previous work (e.g., Mohan et al., 2008) and implies that *E. huxleyi* distribution is not solely

determined by latitudinal variations in temperature. Nanoplankton are subject to high grazing pressure (Schmoker et al., 2013), with the growth and mortality of a species both directly influencing cell abundances (Poulton et al., 2010), which could result in nanoplankton ~~abundance~~ patchiness additional to the influence of temperature and/or other environmental gradients.~~Nanoplankton are subject to high grazing pressure (Schmoker et al., 2013) and the growth and mortality of a species both directly influence cell abundances (Poulton et al., 2010), which could result in patchiness and deviation away from the theoretical species abundances relative to temperature or other environmental factors.~~ In contrast, the negative correlation of *F. nana* (Pearson's product moment correlation = -0.976, $p < 0.05$, n = 4) versus the positive correlation of ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. (Pearson's product moment correlation = 0.544, $p < 0.05$, n = 19) with SST indicates that these two species have distinctly different physiological tolerances. Southern Ocean diatoms are often ~~mostly~~ observed to have negative relationships with temperature (e.g., Eynaud et al., 1999; Boyd, 2002). ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. was predominantly found in waters north of the PF in this study, as seen by Kopczynska et al. (1986), and is likely to be out competed by other diatom species (e.g., *Chaetoceros* sp. and *Dactyliosolen* sp.) further south due to different nutrient affinities and requirements (Kopczynska et al., 1986), particularly for dissolved iron and silicic acid.

4.3.2 Nutrients

Macronutrient gradients, particularly silicic acid, are considered one of the key driving factors between the differences in community structure in the Southern Ocean (Nelson and Treguer, 1992). ~~TOxN~~NOx (and PO_4 by association) was identified in the BEST test as an important factor in the variability of biomineralizing species ~~phytoplankton~~ distribution, but did not significantly correlate with the four statistically dominant ~~phytoplankton~~ species (Fig. 4) contributing over 50% to changes in species composition in the GCB.

Nitrate drawdown by Southern Ocean diatoms is limited by dissolved iron (dFe) availability south of the STF (Sedwick et al., 2002), which may explain the dominance of the nanoplankton (with lower dFe and macronutrient requirements; Ho et al., 2003) in this study as they are not affected by low dFe concentrations as severely as the microplankton. The low silicic acid concentrations in the region between the SAF and the PF indicate that there was sufficient dFe to allow silicification and diatom growth, but either one or both of the macronutrients were then depleted to limiting concentrations (Assmy et al., 2013). As an essential nutrient for diatoms, silicic acid concentrations less than 2 µM~~mol Si L^{-1}~~ were most common in the GCB, a level which is considered limiting for most diatom species (Paasche, 1973a & b; Egge and Asknes, 1992). However, even at stations with greater than 5 µM ~~mol Si L^{-1}~~ silicic acid, the small diatom species (<10 µm) were still dominant and represented over 40% of the total coccolithophore and diatom assemblage (numerically). ~~There was a~~A significant positive correlation occurred between silicic acid and the small (<5 µm) diatom *F. nana* (Pearson's product moment correlation = 0.986, $p < 0.05$, n = 4)~~., although *Fragilariopsis*~~ *nana* ~~is likely to~~may have a low cellular silicate requirement similar to *F. pseudonana* (Poulton et al., 2013) relative to larger diatom species, so the high abundance of *F. nana* in the high silicic acid waters could be indicative of a seasonal progression driven by light and/or temperature rather than silicic acid dependence.

Fragilariopsis sp. have been observed at high abundances near the Ross Sea ice shelf (Grigorov and Rigual-Hernandez, 2014) and high abundances of large diatoms in ~~the~~ silicic acid- (and dFe-) replete waters may occur~~have been found~~ further south than we sampled~~the sampling strategy of this study allowed~~. In the South Atlantic and the South Pacific Ocean, silicic acid depletion moves southwards as spring to summer progresses, with ~~a~~ maximum diatom biomass observed in late January at 65°S (Sigmon et al., 2002; Le Moigne et al., 2013).

A significant negative correlation between *E. huxleyi* and silicic acid (Pearson's product moment correlation = -0.410, $p < 0.05$, n = 24) ~~was found~~ in this study~~, as~~ has also been identified in the Scotia Sea (Hinz et al., 2012) and Patagonian Shelf (Balch et al., 2014) in the Southern Ocean, as well as in the North Atlantic (Leblanc et al., 2009). Low silicic acid may be considered a positive selection pressure for coccolithophores (Holligan et al. 2010), especially when other macronutrients (and dFe) are replete. However, a few non-blooming coccolithophore species are now recognized as having silicic acid requirements, though this requirement is ~~conspicuously~~ absent ~~from~~ in *E. huxleyi* (Durak et al., 2016). ~~-~~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 have no~~ne~~ silicic acid requirement ~~do not require silicic acid (i.e~~e.g., *E. huxleyi*)~~.Therefore the positive selection pressure at low silicic acid concentrations in the GCB is likely to be *E. huxleyi* specific rather than a coccolithophore wide phenomena.~~ To the south of the PF, silicic acid increased (from < 1 to > 3 µM~~mol Si L^{-1}~~) with five stations between the SAF and PF (and one south of the PF, station GCB1-59), all numerically dominated by *E. huxleyi*, while other stations to the south of the PF were dominated by diatoms (Fig. 2).

These results from the GCB indicate a progression of biomineralizing phytoplankton southwards during spring as irradiance conditions become optimal and macronutrients are depleted. Low silicic acid is often associated with a high residual nitrate concentrations (defined as $[NO_3^-] - [Si(OH)_4]$), as has been observed on the Patagonian Shelf (Balch et al., 2014). The highest coccolithophore abundances in this study (excluding the Patagonian Shelf) were ~~indeed~~ observed in regions with 'residual nitrate' concentrations greater than 10 µ~~mol NO$_3$ L^{-1}~~M (Balch et al., 2016). ~~-~~As silicic acid ~~is~~ becomes depleted in the more northerly surface waters in spring, diatoms progressively become more successful further south as irradiance conditions allow, thereby producing a large HNLSiLC area between the Sub-Antarctic Front and Polar Front; an ideal environment for late summer *E. huxleyi* ~~dominated~~ communities to develop (~~Figure~~ Fig. 6).

Dissolved iron (dFe) ~~is recognized~~acts as a strong control on phytoplankton growth, community composition and species biogeography (e.g., Boyd, 2002~~,~~ Boyd et al., 2015). In this study, dFe measurements were only made at a small number of sampling stations (n = 6; Twining, unpublished data, Balch et al., 2016) limiting their use in the multivariate statistical analysis of community composition. For these stations, dFe showed a statistically significant negative correlation (Pearson's product moment = -0.957, $p < 0.01$) with PC2 from the environmental analysis (Fig. S2). PC2 described the environmental variables least related to latitude (pH, pCO_2 and $\bar{E}_{MLD}$), indicating that dFe was also decoupled from the strong latitudinal

gradient in ~~the~~ environmental parameters (i.e. SST, $\Omega_{calcire}$, macronutrients) ~~in the GCB~~ in ~~the~~ austral spring/summer. Interestingly, dFe concentrations did positively correlate with coccolithophore abundance (Pearson's product moment correlation = 0.858, p <0.05) rather than diatom abundance (p = 0.132, ns) (Fig. S2). Overall, these data support the hypothesis that coccolithophores occupy a niche unoccupied by large diatoms when dFe is replete and silicic acid is depleted (Balch et al., 2014; Hopkins et al., 2015). The numerical dominance of small diatoms less than 20 µm in the GCB during austral spring and summer, alongside the coccolithophore *E. huxleyi*, is thus potentially due to the reduced impact of nutrient limitation (dFe, silicic acid) on small cells with high ratios of surface area to volume (e.g., Hinz et al., 2012; Balch et al., 2014).

4.4 Relating the Great Calcite Belt to carbonate chemistry

Relating carbonate chemistry to phytoplankton distribution, growth and physiology is an important step when considering the potential effects of climate change and ocean acidification on marine biogeochemistry. In this study, no significant correlation (Spearman's r = 0.259, p = 0.164, n = 27) occurred between pH and Chl *a*. The inclusion of pCO_2 and $\Omega_{calcite}$ as influential factors in the statistical results describing ~~the~~ GCB species biogeography highlights the importance of understanding phytoplankton responses to carbonate chemistry as a whole rather than as individual carbonate chemistry parameters (Bach et al., 2015). Of the four major species driving the differences in biomineralizing plankton community composition and biogeography across the GCB, only *F. pseudonana* abundance was positively correlated with pCO_2 (Pearson's product moment coefficient = 0.577, p < 0.05, n = 16).

The response of diatoms to increasing pCO_2 is not straight forward (e.g., Boyd et al., 2015), with some studies implying that large diatoms may be more successful in future climate scenarios (e.g., Tortell et al., 2008; Flynn et al., 2012), although changes in nutrient and light availability (via stronger stratification) may prevent a permanent switch in phytoplankton community structure (Bopp, 2005). The carbonate chemistry system is complex as biological activity also impacts ~~on~~ the concentration of each of the components. Organic matter production reduces dissolved inorganic carbon (C_T) and hence pCO_2 via photosynthesis, as well as increasing alkalinity (A_T) through nutrient uptake, while subsequent respiration and remineralisation of organic matter has the opposite impact. The simultaneous actions of biological and physical processes result in seasonal and localized changes in the carbonate system, which are often difficult to decouple.

In our study, there was no significant correlation between *E. huxleyi* and $\Omega_{calcite}$ (Pearson's product moment = 0.093). However, the waters of the GCB remained oversaturated ($\Omega_{calcite}$ > 2) throughout, and furthermore the relationship between coccolithophores, calcification and carbonate chemistry is now recognized as being complex and non-linear~~In our study, there was no significant correlation between *E. huxleyi* and $\Omega_{calcite}$ (Pearson's product moment = 0.093), which may be viewed as somewhat surprising given the potential detrimental effects on calcifiers at low saturation states (e.g. Riebesell et al., 2000). However, the waters of the GCB remained oversaturated ($\Omega_{calcite}$ > 2) throughout, and furthermore the relationship~~

~~between coccolithophores, calcification and carbonate chemistry is now recognized as being complex and non-linear~~ (e.g., Beaufort et al., 2011; Smith et al., 2012; Poulton et al., 2014; Rivero-Calle et al., 2015; Bach et al., 2015; Charalampopoulou et al., 2016; Marañón et al., 2016). Hence, significant gaps remain in our understanding of the *in situ* coccolithophore response to increasing pCO_2, reduced pH or decreasing $\Omega_{calcite}$. Notably, a significant positive correlation between ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. and $\Omega_{calcite}$ also existed (Pearson's product moment correlation = 0.5924, $p < 0.01$, n = 19) across the GCB despite there being presently no known detrimental effect on diatoms of low saturation states. However, due to the tight coupling of temperature and $\Omega_{calcite}$ (and *Pseudo-nitzshia* sp. and temperature)~~,~~ the correlation is more likely to be temperature driven.

5 Summary

This study of the GCB further highlights the importance of understanding the environmental controls on the distribution of biomineralizing nanoplankton in the Southern Ocean. The results of this study suggest that ~~four~~ three ~~nanoplankton~~ nano- (<20 µm) and one micro- (>20 µm) phytoplankton species (three diatoms and one coccolithophore; *F. pseudonana*, *F. nana,* ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp.*,* and *Emiliania huxleyi*) numerically dominated the compositional variation in biomineralizing phytoplankton biogeography across the GCB. The contribution of *E. huxleyi* to phytoplankton biomass (as ~~measured~~ estimated from cell counts and ~~by~~ Chl~~orophyll~~ *a*) was generally less than ~~-~~5%, although it increased up to 20% in association with high reflectance PIC features found on the Patagonian Shelf, north of South Georgia in the South Atlantic Ocean, and north of the Crozet Islands in the South Indian Ocean. This indicates that in the post-spring~~non-~~ bloom conditions of the GCB, *E. huxleyi* is an ~~could be as~~ important contributor to ~~as diatoms for~~ phytoplankton biomass and primary production at localized spatial scales.

Out of a wide suite of environmental variables, latitudinal gradients in temperature, macro-nutrients, pCO_2 and $\Omega_{calcite}$ 'best' described statistically the variation of phytoplankton community composition in this study, whereas $\bar{E}E_{MLD}$ and pH did not rank as significant factors influencing ~~phytoplankton community~~species composition.~~Latitudinal gradients in temperature, macronutrients and carbonate chemistry 'best' describe the variation of phytoplankton community composition in this study.~~ However, not all species were directly sensitive to the same environmental gradients as determined to be influencing the overall biogeography. The negative correlation between *E. huxleyi* and silicic acid highlights the potential for a seasonal southward movement of *E. huxleyi* once diatom blooms have depleted silicic acid.

These results highlight that the Southern Ocean is a highly dynamic system and further studies examining environmental controls on community distribution earlier in the productive season would greatly enhance overall understanding of the progression of phytoplankton community biogeography. The phytoplankton dynamics of the GCB are also more complex than first considered, with ~~the~~ nanophytoplankton (e.g., *F. pseudonana*) numerically dominant in non-bloom conditions (as

opposed to microphytoplankton), which has further implications for modelling carbon export and projecting phytoplankton changes in future oceanic scenarios.

Competing interests

The authors declare that they have no conflict of interest.

Data availability

The coccolithophore and diatom abundance data can be accessed via the PANGAEA database: https://doi.pangaea.de/10.1594/PANGAEA.879790

Acknowledgements

We would like to thank all the scientists, officers and crew on board the R/V Melville and R/V Revelle, and Matt Durham (Scripps Institution of Oceanography) in particular. Nutrient and salinity data are presented courtesy of the Oceanographic Data Facility, Scripps Institute of Oceanography (ODF/SIO), with thanks to shipboard techs Melissa Miller and John Calderwood. The GCB cruises were supported by the National Science Foundation (OCE-0961660 to WMB and BST, OCE-0728582 to WMB, OCE-0961414 to NRB) and National Aeronautical and Space Administration (NNX11AO72G, NNX11AL93G, NNX14AQ41G, NNX14AQ43A, NNX14AL92G, and NNX14AM77G to WMB). HEKS and AJP were supported by the Natural Environmental Research Council (NERC), including a NERC Fellowship to AJP (NE/F015054/1) and the UK Ocean Acidification Research Program (NE/H017097/1) with a tied studentship to HEKS and an added value award to AJP.

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Tables

Table 1: Details of Great Calcite Belt sampling stations including station and cruise identifier; date of sample collection (DD.MM.YYYY); station position decimal latitude (Lat) and ~~Longitude~~ longitude (Long); sea surface temperature (SST); surface salinity (Sal); mixed layer average irradiance ($\bar{E}_{MLD}$); surface macronutrient concentrations (nitrate and nitrite, ~~TOxN~~NOx; phosphate, PO_4; silicate, $Si(OH)_4$; ammonia, NH_4) surface carbonate chemistry parameters (normalized total alkalinity, A_T; dissolved inorganic carbon, C_T; pH; partial pressure of carbon dioxide (pCO_2); calcite saturation state ($\Omega_{calcite}$); and surface chlorophyll *a* (Chl *a*, mg m^{-3}). Bold type indicates those used in the statistical analyses.

Table 2: Whole cell abundances of coccolithophores and diatoms in surface samples of the Great Calcite Belt, number of species in each group (S), Pielou's evenness (J', **** denotes that J' was not calculated because only one species was present), the dominant species and its percentage contribution to the total numerical abundance of coccolithophores (%Co) or diatoms (%D). $^+$ denotes where one species had almost total numerical dominance (> 99.8%), with only one or two cells of a separate species enumerated, and was therefore rounded up to 100%. Holococcolithophores are abbreviated as Holococco*. Position denotes the location relative to the Southern Ocean fronts and zones (Z; north of the defined front) as defined by Orsi et al. (1995), letters after the front abbreviation denote specific locations and proximity to landmasses: Patagonian Shelf (PS); north of South Georgia (n SG); South Sandwich Islands (SS); Crozet Island (Cr), Kerguelen Island (K); Heard Island (H).

Table 3: Principal component (PC) scores, percentage variation described (%V) and the Pearson's product moment correlation associated with each variable and its significance level: **p <0.0001*****, **p<0.001****, **p<0.005***, **p< 0.01**, p<0.05.

Table 4: Phytoplankton assemblage groups identified, using the SIMPROF routine at $p < 0.05$, in the GCB (see also ~~Figure~~ Fig. 3), from the South Atlantic (GCB1) and the South Indian (GCB2) Oceans. Location is indicated as in ~~Figure~~ Fig. 2. Group Average Similarity (Group Av.Sim%) defines the percentage similarity of the community structure in all the stations within each group. The defining species contributing >50% to the species similarity for each group as identified through the SIMPER routine are presented alongside the average similarity for each species in each group (Average Similarity), where higher Similarity SD indicates more consistent contribution to similarity within the group. The percentage contribution per species to the group similarity (Contribution %) was also calculated.

Table 1

Station	Date	Lat	Long	SST	Sal	$\bar{E}_{MLD}$	NOx	PO_4	$Si(OH_4)$	NH_4	A_T	C_T	pH	pCO_2	Ω_{calc}
		°S	°E	°C		mol PAR m^{-2} d^{-1}		~~µmol L^{-1}~~ µM						µatm	
GCB1-6	14.01.2011	51.79	-56.11	8.6	34.0	17.8	14.2	1.05	1.7	0.64	2336	2138	8.09	367	3.3
GCB1-16	17.01.2011	46.26	-59.83	11.8	33.8	39.8	6.5	0.54	0.0	0.15	2333	2100	8.12	407	3.8
GCB1-25	20.01.2011	45.67	-48.95	16.1	35.1	25.5	0.0	0.23	0.2	0.16	2320	2047	8.12	390	4.6
GCB1-32	22.01.2011	40.95	-45.83	20.0	35.6	36.7	0.1	0.11	1.1	0.05	2307	2029	8.07	444	4.8
GCB1-46	26.01.2011	42.21	-41.21	18.3	34.9	16.0	0.2	0.19	0.3	0.00	2328	2050	8.09	356	4.7
GCB1-59	29.01.2011	51.36	-37.84	5.9	33.8	7.9	17.5	1.22	1.7	0.67	2368	2184	8.10	325	3.1
GCB1-70	01.02.2011	59.25	-33.15	1.1	34.0	9.7	22.3	1.74	78.5	1.54	2388	2235	8.10	407	2.6
GCB1-77	03.02.2011	57.28	-25.98	1.4	33.9	11.9	20.7	1.55	68.8	1.00	2386	2225	8.12	405	2.7
GCB1-85	05.02.2011	53.65	-17.75	4.1	33.9	8.9	19.1	1.33	0.7	0.30	2369	2191	8.12	363	3.0
GCB1-92	07.02.2011	50.40	-10.80	5.9	33.8	9.5	17.5	1.27	1.4	0.37	2362	2182	8.10	351	3.0
GCB1-101	09.02.2011	46.31	-3.21	11.0	34.0	17.1	12.5	0.95	0.6	0.16	2345	2134	8.08	400	3.5
GCB1-109	11.02.2011	42.63	3.34	15.1	34.4	20.0	5.3	0.56	0.8	0.00	2332	2098	8.07	359	4.0
GCB1-117	12.02.2011	39.00	9.49	18.8	35.0	19.4	0.0	0.20	0.7	0.06	2321	2047	8.08	299	4.7
GCB2-5	21.02.2012	37.09	39.48	21.0	35.5	11.2	0.0	0.05	1.1	0.07	2310	2005	8.10	340	5.2
GCB2-13	23.02.2012	40.36	43.50	18.4	35.3	13.7	0.1	0.17	0.2	0.02	2307	2032	8.09	351	4.7
GCB2-27	26.02.2012	45.82	51.05	7.7	33.7	5.8	20.1	1.35	2.9	0.14	2344	2194	8.00	425	2.6
GCB2-~~35~~36	28.02.2012	46.74	57.48	8.1	33.7	8.7	18.9	1.40	1.7	0.49	2363	2175	8.08	355	3.1
GCB2-43	01.03.2012	47.52	64.04	6.5	33.7	5.9	21.7	1.53	0.5	0.38	2358	2197	8.04	387	2.8
GCB2-53	02.03.2012	49.30	71.32	5.1	33.7	8.5	23.8	1.66	7.1	0.17	2359	2210	8.03	396	2.6
GCB2-63	04.03.2012	54.40	74.56	3.5	33.8	3.0	25.3	1.70	10.5	0.21	2363	2210	8.07	360	2.6
GCB2-73	06.03.2012	59.71	77.75	1.1	33.9	4.3	28.0	1.91	40.4	0.34	2372	2233	8.07	360	2.4
GCB2-87	10.03.2012	54.25	88.14	3.4	33.9	4.3	24.2	1.69	9.0	0.45	2367	2216	8.06	367	2.6
GCB2-93	12.03.2012	49.81	94.13	7.8	34.0	5.9	17.5	1.27	1.5	0.26	2345	2149	8.10	333	3.3
GCB2-100	13.03.2012	44.62	100.50	13.0	34.8	4.7	6.4	0.55	0.2	0.15	2328	2083	8.11	326	4.1
GCB2-106	15.03.2012	40.13	105.38	17.0	35.4	12.8	0.1	0.14	0.3	0.03	2318	2029	8.13	313	4.9
GCB2-112	17.03.2012	40.26	109.60	15.8	34.9	11.1	3.6	0.43	0.2	0.00	2323	2060	8.11	332	4.4
GCB2-119	20.03.2012	42.08	113.40	13.8	34.8	11.2	5.3	0.55	0.2	0.01	2320	2080	8.10	342	4.1

Table 2

		Coccolithophores (Co)					Diatoms (D)				
Station	Position	Cell mL^{-1}	S	J'	Dominant species	% of Co	Cell mL^{-1}	S	J'	Dominant species	% of D
GCB1-6	SAF, PS	243	2	0.02	*E. huxleyi*	100^{+}	127	15	0.79	*C. deblis*	26
GCB1-16	SAF, PS	1636	2	0.00	*E. huxleyi*	100^{+}	4610	5	0.11	*F. pseudonana*	96
GCB1-25	SAFZ	55	9	0.67	*S. mollischi*	38	28	10	0.84	*Pseudo-nitzschia* sp.	37
GCB1-32	STF	23	8	0.83	*U. tenuis*	31	19	8	0.70	*Nitzschia* sp.	55
GCB1-46	STF	3	1	****	Holococco*	100	4	3	0.91	*Chaetoceros* sp.	56
GCB1-59	sPF, n SG	565	1	****	*E. huxleyi*	100	183	30	0.72	*T. nitzschioides*	29
GCB1-70	sPF	103	1	****	*E. huxleyi*	100	720	24	0.29	*F. nana*	81
GCB1-77	sPF, SS	2	1	****	*E. huxleyi*	100	6893	18	0.04	*F. nana*	98
GCB1-85	sPF	28	1	****	*E. huxleyi*	100	151	30	0.77	*C. aequatorialis* sp.	22
GCB1-92	PFZ	77	2	0.13	*E. huxleyi*	98	111	28	0.73	*Pseudo-nitzschia* sp.	32
GCB1-101	SAFZ	92	7	0.57	*E. huxleyi*	68	52	11	0.57	*F. pseudonana*	59
GCB1-109	SAFZ	39	9	0.90	*E. huxleyi*	25	129	17	0.55	*Pseudo-nitzschia* sp.	61
GCB1-117	STF	15	6	0.88	*U. tenuis*	35	209	9	0.13	*C. closterium*	95
GCB2-5	STFZ	37	15	0.69	*E. huxleyi*	46	6	8	0.76	*Nanoneis hasleae*	47
GCB2-13	STFZ	51	17	0.61	*E. huxleyi*	57	28	7	0.57	*Nitzschia* sp.<*20µm*	67
GCB2-27	SAF, Cr	478	6	0.04	*E. huxleyi*	99	375	24	0.28	*F. pseudonana*	83
GCB2-36	SAF	166	8	0.32	*E. huxleyi*	83	155	32	0.69	*F. pseudonana*	33
GCB2-43	PFZ	12	4	0.18	*E. huxleyi*	95	90	25	0.57	*F. pseudonana*	54
GCB2-53	sPF, K	51	3	0.90	*E. huxleyi*	56	512	28	0.39	*F. pseudonana*	47
GCB2-63	sPF, H	132	1	****	*E. huxleyi*	100	254	24	0.38	*F. pseudonana*	71
GCB2-73	sPF	0	0	****	n/a	n/a	538	24	0.55	*F. pseudonana*	56
GCB2-87	sPF	106	1	****	*E. huxleyi*	100	184	29	0.55	*F. pseudonana*	42
GCB2-93	PFZ	100	11	0.33	*E. huxleyi*	80	75	29	0.67	*Pseudo-nitzschia* sp.	37
GCB2-100	SAFZ	123	13	0.26	*E. huxleyi*	86	164	26	0.44	*Pseudo-nitzschia* sp.	67
GCB2-106	STF	90	19	0.77	*E. huxleyi*	29	80	22	0.58	*Pseudo-nitzschia* sp.	54
GCB2-112	STF	123	12	0.35	*E. huxleyi*	80	257	27	0.38	*Pseudo-nitzschia* sp.	74
GCB2-119	SAFZ	121	17	0.32	*E. huxleyi*	82	68	21	0.55	*Pseudo-nitzschia* sp.	47

Table 3

Variable	PC1 - EV 5 (58%)		PC2 - EV 1.5 (17%)	
Temp	0.42	**(0.97***)**	0.08	(-0.10)
Salinity	0.36	**(0.90***)**	0	-
$\bar{E}_{MLD}$~~EML~~	0.24	**(-0.55*)**	0.5	**(0.62**)**
~~TOXN~~NOx	-0.4	**(-0.91***)**	-0.05	(-0.06)
~~SIL~~$Si(OH)_4$	-0.35	**(-0.77***)**	0.02	(-0.03)
NH_4	-0.35	**(-0.81***)**	-0.07	(-0.09)
pH	0.18	**(-0.39)**	-0.42	**(-0.50*)**
pCO_2	-0.15	(-0.33)	0.75	**(0.89***)**
$\Omega_{calcite}$	0.43	**(-0.99***)**	-0.02	(-0.02)

Table 4

Group	Station	Location	Group Av.Sim%	Defining Species	Average Similarity	Similarity SD	Contribution %
A	GCB1-46	STF	n/a	Holococco*	n/a	n/a	n/a
B	GCB1-117			*Cylindrotheca* sp.			
C	GCB1-70	SBDY	54.5	*F. nana*	53.3	n/a	97.8
	GCB1-77						
D	GCB1-25	N of PF	47.6	*E. huxleyi*	13.9	2.68	29.3
	GCB1-109			*Pseudo-nitzschia* sp.	12.7	3.6	26.7
	GCB2-36						
	GCB2-93						
	GCB2-100						
	GCB2-106						
	GCB2-112						
	GCB2-119						
E	GCB1-32	N of SAF	42.3	*E. huxleyi*	18.9	3.8	44.8
	GCB1-101			Holococco*	8.45	4.01	20
	GCB2-5						
	GCB2-13						
F	GCB1-6	PS	40.6	*E. huxleyi*	15.1	1.51	37.3
	GCB1-16			*F. pseudonana*	14.2	1.25	35
	GCB1-59	S of SAF					
	GCB1-85						
	GCB1-92						
	GCB2-27						
	GCB2-43						
	GCB2-53						
	GCB2-63						
	GCB2-73						
	GCB2-87						

Figures

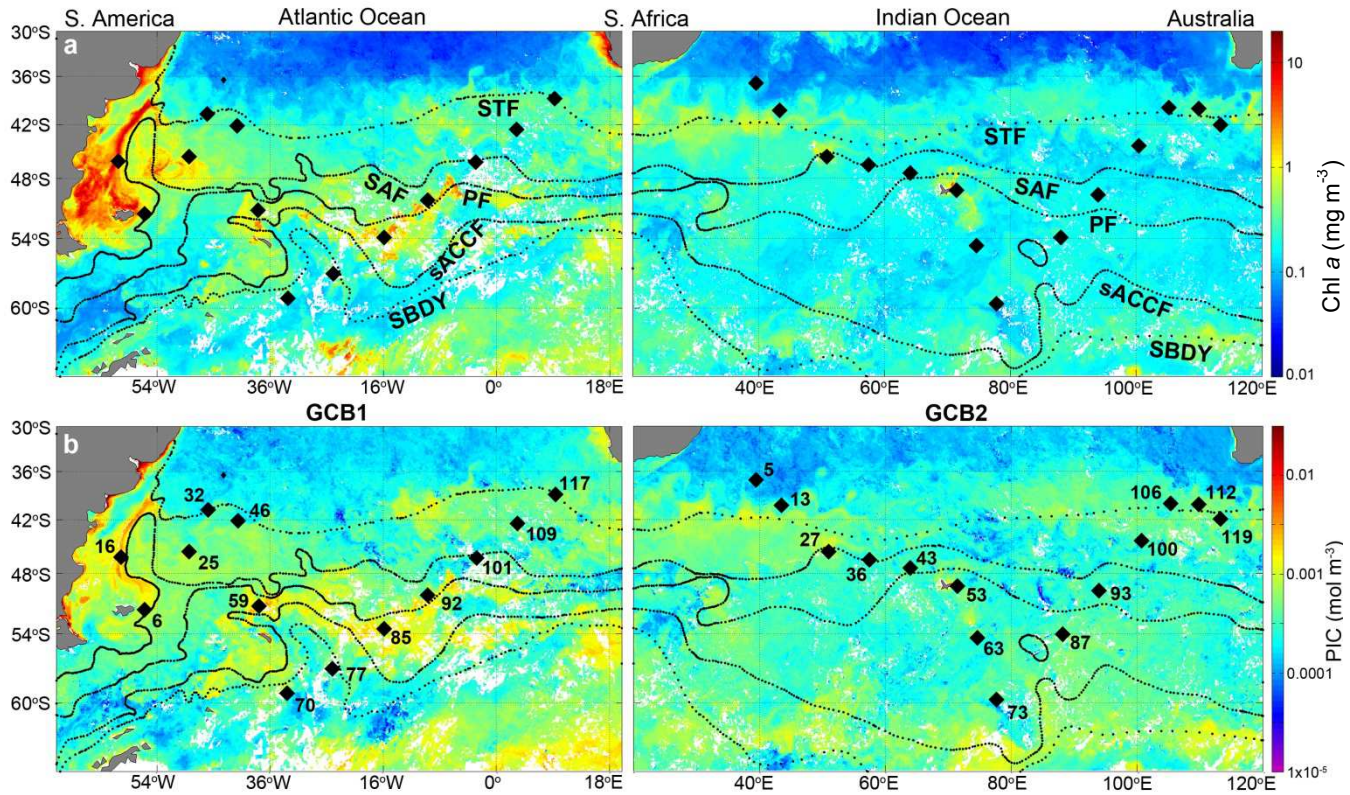

Figure 1 Rolling 32 day composite from MODIS-Aqua for both (a) Chlorophyll a (mg m-3) and (b) PIC (~~µmol L-1~~µM) for the South Atlantic sector (17th January to 17th February 2011) and the South Indian sector (18th February to 20th March 2012). Station number identifiers and averaged positions of fronts as defined by Orsi et al. (1995) are superimposed: Sub-~~t~~Tropical front (STF), Sub Antarctic front (SAF), Polar Front (PF), Southern Antarctic Circumpolar Current Front (SACCF) and Southern Boundary (SBDY).

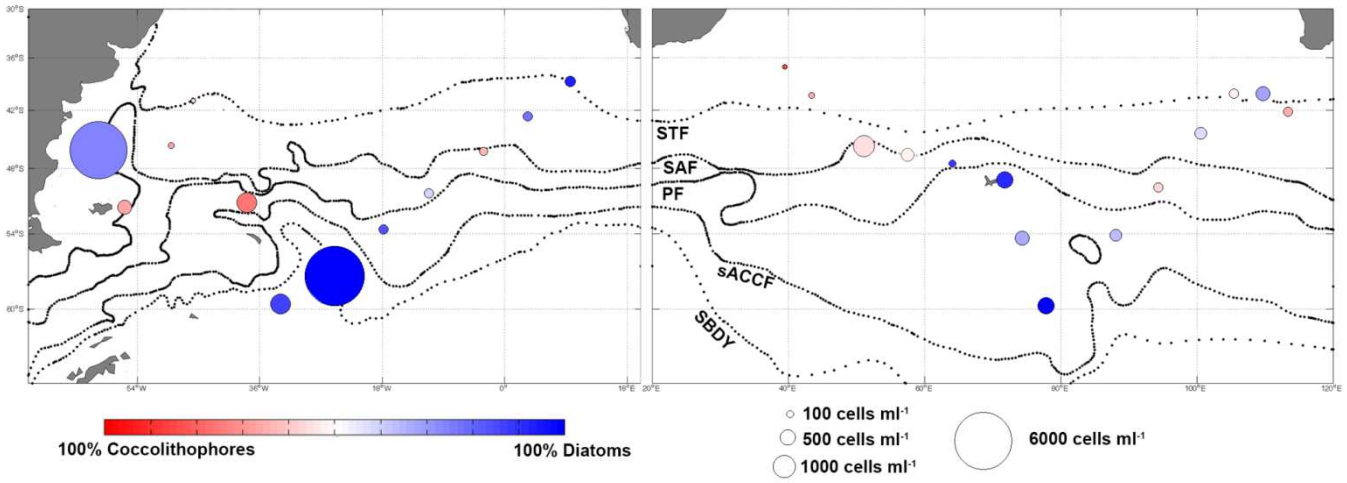

Figure 2 Coccolithophore and diatom abundance and dominance information. The area of the circles denotes abundance while shading denotes percentage contribution of each phytoplankton group, where red denotes coccolithophore dominance and blue denotes diatom dominance. Fronts are defined as in ~~Figure~~ Fig. 1

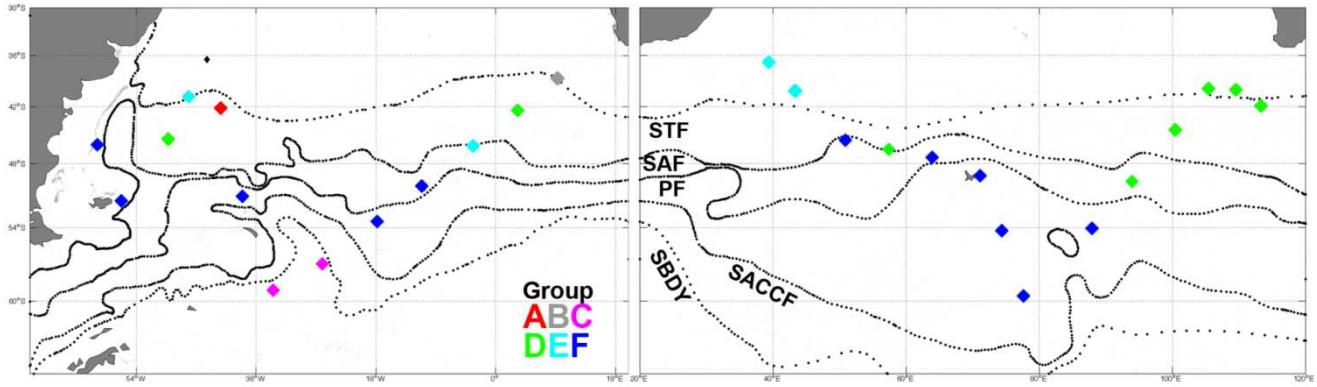

Figure 3 Statistically significant groups of coccolithophore and diatom communities in the Great Calcite Belt as identified by the SIMPROF routine. The colors designate which statistical group defines the coccolithophore and diatom assemblage at each station as shown in the group key. Fronts are defined as in ~~Figure~~ Fig. 1. See Table 4 for full group species descriptions.

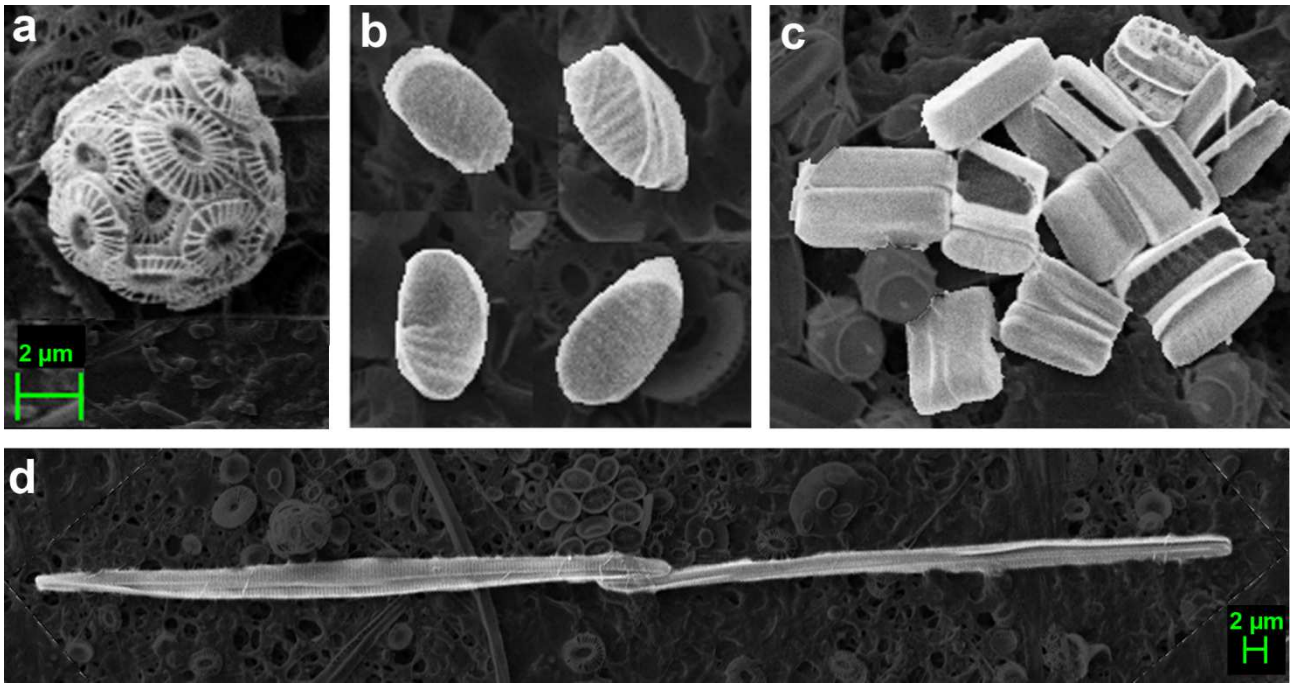

Figure 4 SEM images of the four phytoplankton species identified by the SIMPER analysis as characterizing the significantly different community structures. (a) *E. huxleyi*; (b) *F. pseudonana*; (c) *F. nana*; and (d): ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp.. ~~Scale bar 2 um for a-c and 5 um for d.~~

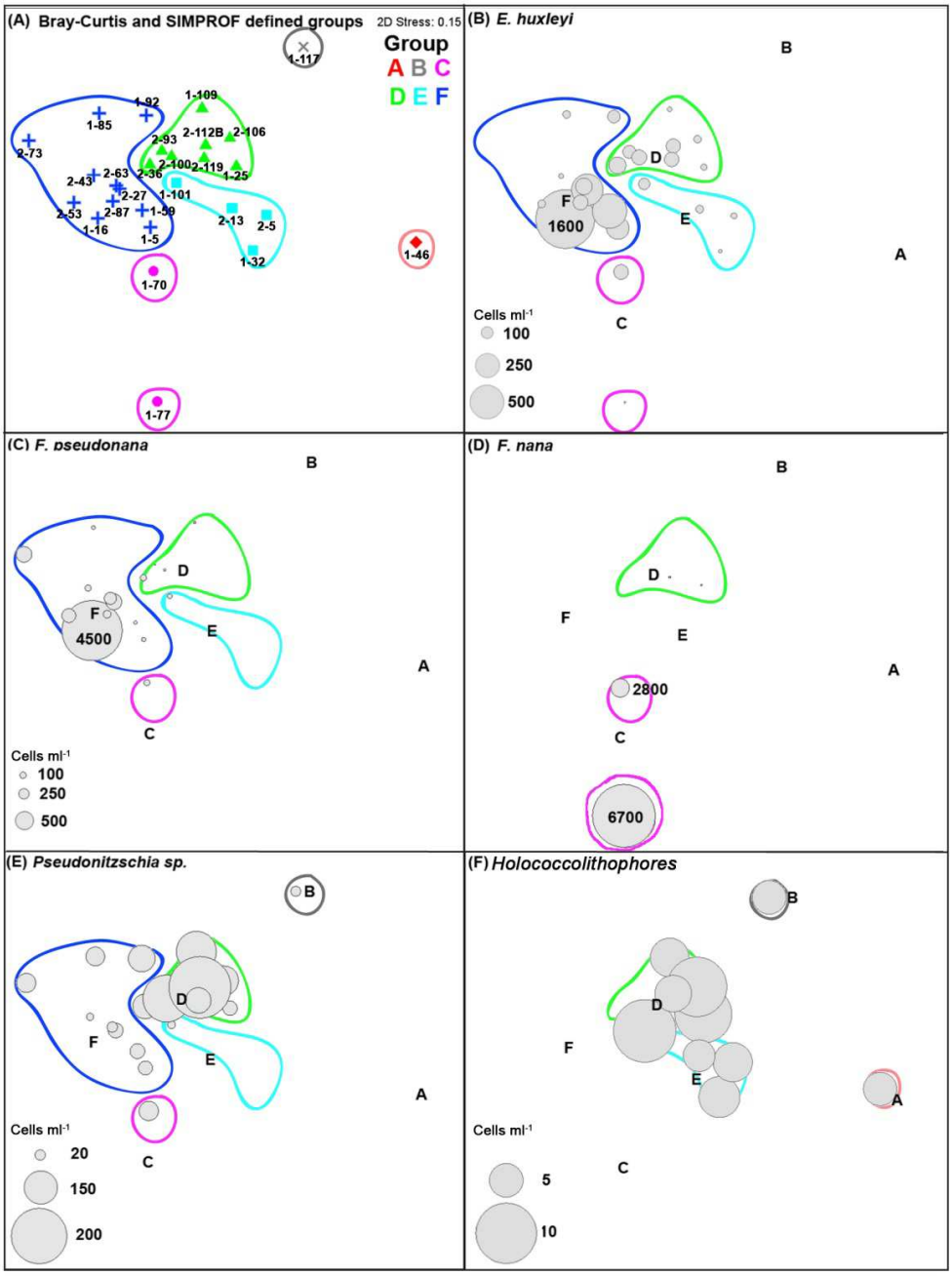

Figure 5 Two dimensional non-metric multidimensional scaling (nMDS) ordination of station groupings A) as defined by the SIMPROF routine, with group color identifiers as in ~~Figure~~ Fig. 3, where relative distances between samples represent the similarity of species composition between phytoplankton communities. Stations with statistically similar species composition are clustered together, whereas stations with low statistical similarity in terms of species composition are more widely spaced. Overlay of bubble plots of the defining species abundance (cells mL^{-1}) characterizing the statistically significant groups in the GCB (see also Table 4; (B) *E. huxleyi* abundance; (C) *F. pseudonana* abundance; (D) *F. nana* abundance; (E) ~~*Pseudonitzschia*~~*Pseudo-nitzschia* sp. abundance; and (E) Holococcolithophore abundance. The two-dimensional stress of 0.15 gives a 'reasonable' representation of the data in a 2-D space (Clarke and Warwick, 2001).

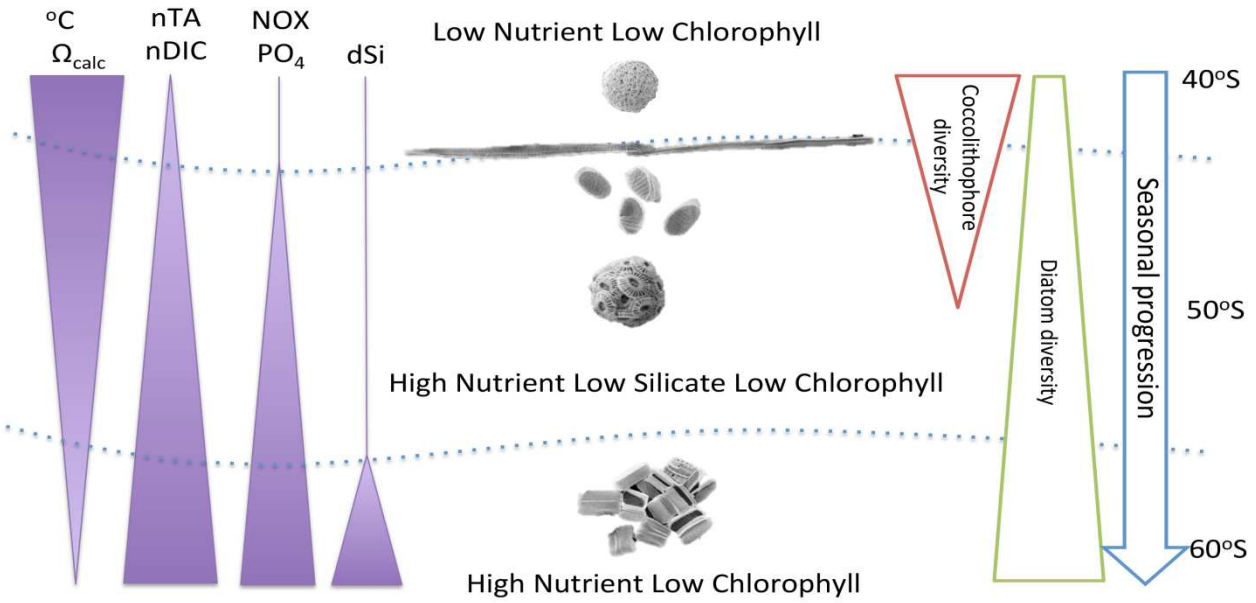

Figure 6 Schematic of the potential seasonal progression occurring in the Great Calcite Belt, allowing coccolithophores to develop after the main diatom bloom. Note phytoplankton example images are not to scale.