Point-by-point response to the Reviews and List of Changes on "Distribution of coccoliths in surface sediments across the Drake Passage and calcification of *Emiliania huxleyi* morphotypes" by Nele Manon Vollmar et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-105-RC1, 2021

RC#1: comments from anonymous Reviewer #1 RC#2: comments from anonymous Reviewer #2

Author's comment (AC): Original answer to the Reviewers comment

Author's revision (AR): Indication of the lines of the corresponding changes in the revised Manuscript and further information and edits incorporated in the revised Manuscript

RC#1: Lines 9-10 Results of the relationship between coccolithophore species and environment are barely discussed. Why not salinity, phosphate or other relevant environmental parameters are mentioned in the discussion? I believe Figure 10 is not even mentioned in the discussion.

AC: With the brief presentation in the abstract, we just want to shortly explain what we have actually done. The findings in Figure 10 are of course taken into account in the discussion, although we have actually forgotten to reference Figure 10 in the text so far (missing in line 467). That has been changed. We thank reviewer #1 for pointing this out.

We did not mention salinity, phosphate, or other environmental parameters because the main outcome of the two independent statistical analysis (Hierarchical Cluster Analysis and Redundancy Analysis) made in this study is that, among the included environmental variables, sample depth is the main driver of differences in the assemblage distribution (see lines 452). The outcome of the RDA is how much variation in the coccolith assemblages can be explained by the environmental variables and if this variation is significant. In this case, the environmental variables together explain 56% of the variation (i.e. by the constraints). The first axis is the only significant explaining 38 % variation and being strongly (negatively) correlated to sample depth, while the second axis explains only 9% of the variation. Further interpretation of less significant parameters as suggested by reviewer #1 (PAR, salinity and phosphate, all at 10m water depth), should be taken with great caution and are (from our perspective) not worthwhile, especially considering the sample size and the unknown, approximately Late Holocene age.

AR: We included the cross-reference to Figure 10 in line 467. We specified that the first RDA axes in the caption of Figure 10 explain 84,3% of the constrained variance (not the total variance) to clarify the RDA outcome.

RC#1: Line 30 The content of this paragraph is correct but too descriptive. It should be placed in material and methods.

AC: We think that an overview of the Southern Ocean (SO) oceanography is relevant information for this study and the placement in the introduction is appropriate. We consider that the investigation is also important because of the special situation and location of the study area. We want to take this into account by placing this section in the Introduction. In our opinion, this has nothing to do with material and methods.

AR: -

RC#1: Line 46 Carbon is not respired. Please rephrase.

AC: We revised the sentence and made clear that Carbon Dioxide is respired (instead of Carbon).

AR: see AC

RC#1: Line 57 Please note that diatoms dominate in the subantarctic waters in terms of biomass. In fact, coccolithophores account for small fraction of the primary production in all the zonal systems of the Southern Ocean. In terms of cell numbers, it is likely that coccolithophores dominate even south of the polar front. While the content of this sentence is correct, it is somewhat misleading, so please, re-write clearly stating the role of each group. Moreover, some of the references do not report diatoms. Please find more appropriate references, for example:

Smith, H.E.K., Poulton, A.J., Garley, R., Hopkins, J., Lubelczyk, L.C., Drapeau, D.T., Rauschenberg, S., Twining, B.S., Bates, N.R., Balch, W.M., 2017. The influence of environmental variability on the biogeography of coccolithophores and diatoms in the Great Calcite Belt. Biogeosciences 14, 4905-4925.

AC: We are aware that diatoms dominate in the subantarctic waters and never questioned that. However, with our statement and the reference of the model results by Nissen et al. (2018), we only wanted to indicate that coccolithophores are also of (subordinate) importance in this region. We tried to clarify this better in the text and have now added the reference suggested by reviewer #1.

AR: We rewrote the sentences in lines 57-60

RC#1: Line 74 The poleward expansion of *E. huxleyi* in the Southern Ocean remains as a hypothesis that need to be proven. Please provide robust evidences (i.e. references) of leave it as a hypothesis.

AC: We attenuated the statement pointed to (lines 74-75): "Repeated sampling in the Australian sector of the SO over the past four decades suggests a dramatic range expansion of coccolithophores south of 60° S, dominated by the globally ubiquitous species *E. huxleyi* (Cubillos et al., 2007)".

In addition, we have added more references to provide more robust evidence (Winter et al, 2014; Charalampopoulou et al., 2016).

AR: see AC in lines 75 and 77

RC#1: Line 76 "Here, any ocean acidification effect appears outweighed by surface-ocean warming." This sentence is unclear, any effect on what? Please rephrase and also provide reference/s to support your statement.

AC: We acknowledge that this sentence was unclear, so we reworded it. We meant to state that in that specific region of the SO (referring still to Cubillos et al., 2007) temperature seems to be a more prominent factor affecting *E. huxleyi* morphotype distribution and coccolith mass than the car-

bonate chemistry, therefore suggesting that the influence of global warming will be stronger than ocean acidification in the future.

AR: see lines 77 - 79

RC#1: Line 82-83 "However, the number of coccolithophore studies in surface sediment in this area is very limited, and they mostly focus on assemblages." Please provide references.

AC: To our knowledge there are only three papers dealing with coccoliths in surface sediments of the Pacific and Atlantic sectors of the SO, which we have added to the text. These are:

Boeckel, B., Baumann, K.-H., Henrich, R., and Kinkel, H.: Coccolith distribution patterns in South Atlantic and Southern Ocean surface sediments in relation to environmental gradients, Deep Sea Research Part I: Oceanographic Research Papers, 53, 1073–1099, https://doi.org/10.1016/j.dsr.2005.11.006, 2006.

Findlay, C. S. and Giraudeau, J.: Movement of oceanic fronts south of Australia during the last 10 ka: interpretation of calcareous nannoplankton in surface sediments from the Southern Ocean, Marine Micropaleontology, 46, 431–444, https://doi.org/10.1016/S0377-8398(02)00084-1, 2002.

Saavedra-Pellitero, M. and Baumann, K.-H.: Comparison of living and surface sediment coccolithophore assemblages in the Pacific sector of the Southern Ocean, 61, 14, 2015a.

AR: see AC

RC#1: Line 91 It is unclear why authors analysed the coccolith mass of this species. Please clarify the purpose of this analysis.

AC: We analysed the coccolith mass of *E. huxleyi* because it is nowadays the dominant taxa in this region, as well in many other oceans, and an important driver of changes in the carbon cycle. Due to its relevance, this coccolithophore species, and not others, is considered in studies which analyse the sensitivity of their calcification parameters to present-day (e.g., Rigual Hernandez et al. 2020) or past (e.g., Baliestreri et al., 2021) shifting environmental conditions (e.g., Rigual Hernandez et al. 2020). Therefore, we consider that understanding mass variations in this taxon in the SO is of wide importance for the scientific community. We have now included this in the manuscript, stating that "Additionally, we evaluated the coccolith mass variations in the dominant taxa *E. huxleyi* within each different morphotype, an important information as part of the morphological diversity of *E. huxleyi* and their biogeography, which can serve as an estimate for CaCO3 export that may be affected by projected environmental change (e.g. Rigual-Hernandez et al., 2020)."

Balestrieri, C., Ziveri, P., Grelaud, M., Mortyn, P. G., & Agnini, C. (2021). Enhanced *E. huxleyi* carbonate counterpump as a positive feedback to increase deglacial pCO2sw in the Eastern Equatorial Pacific. *Quaternary Science Reviews*, 260, 106921.

AR: we added the mentioned edits to the sentence in lines 94 - 97

RC#1: Line 131 Delete "coccolithophores".

AC: That word was deleted from the text.

AR: see AC

RC#1: Line 131 If possible, include information about the model of the multicorer sampling device.

AC: Samples were retrieved with a 12-tubes Multicorer (MUC67; manufactures by Fa. Wuttke, Henstedt-Ulzburg, Germany), with an inner tube diameter of 6 cm and a length of 60 cm (Lamy et al., 2016). This information was added to the text.

AR: see lines 137 - 138

RC#1: Line 134 It is unclear if this dating was made on any of the samples analysed in this study or if it was undertaken in nearby samples. Please clarify.

AC: None of the samples we used had been dated so far. Since it was not possible for us to carry out our own datings, we relied on published age data, which give calibrated accelerator mass spectrometry (AMS) 14C ages for surface sediments in the work area of about 3 to 4.8 ka BP. The information is included in lines 131-135 and we wrote now the following: "Datings of adjacent near-surface sediments at the southern Chilean margin (Caniupán et al., 2011b) as well as south of the PF within the DP (Vorrath et al., 2019) give calibrated accelerator mass spectrometry (AMS) 14C ages of 2.91 – 3.06 ka BP and 4.83 ka BP respectively."

However, a newly published paper in which changes in ACC strength in the central Drake Passage were calculated for the last 140 kyr (Wu et al., 2021) using sediments of one of our stations (station PS97/085, in the Antarctic Zone). The calibrated AMS <sup>14</sup>C ages for the sediments from the piston core PS97/085-3 at 0.5 cm (= 56.5 cm composite depth) and for 20.5 cm (= 76.5 cm composite depth) were, 1.13 ka and 6.64 ka, respectively. The real sediment surface was partly missing in the piston core, so Wu et al. calculated a recent age for the trigger weight core, but even the surface of the piston core had an age which allows us to assume that the surface sediment is at least close to Recent. We therefore assume that our studied surface sediments represent relatively modern conditions.

Wu, S., Lembke-Jene, L., Lamy, F. *et al.* Orbital- and millennial-scale Antarctic Circumpolar Current variability in Drake Passage over the past 140,000 years. *Nat Commun* 12, 3948 (2021). <a href="https://doi.org/10.1038/s41467-021-24264-9">https://doi.org/10.1038/s41467-021-24264-9</a>

AR: We added the above informations accordingly, see line 139 and lines 141-145.

**RC#2:** Introduction, I. 91. The authors state that they will perform *huxleyi* coccolith mass variations evaluations in pre-industrial assemblages to compare with plankton data. It is not clear if this will be used for acidification effect evaluations? However the dating of the studied surface sediments is not very precise (see comments below), thus it cannot be used as a straightforward evidence for

evaluating current climatic/environmental impacts. Please clarify.

AC: We see the surface sediments - as common practice in the literature - as the accumulated mirror of the sedimentation of the last centuries or at least of the last few thousand years, depending on the sedimentation rate and influencing factors such as dilution or resuspension. Since it was not possible for us to carry out our own datings, we relied on published age data, which give calibrated accelerator mass spectrometry (AMS) <sup>14</sup>C ages for surface sediments in the work area of about 3 to 4.8 ka BP. As mentioned also to reviewer #1, a newly published paper in which changes in Antarctic Circumpolar Current strength in the central Drake Passage were calculated for the last 140 kyr (Wu et al., 2021) used sediments of one of our stations. The calibrated AMS <sup>14</sup>C ages for the sediments from the piston core PS97/085-3 at 0.5 cm (= 56.5 cm composite depth) and for 20.5 cm (= 76.5 cm composite depth) were 1.13 ka and 6.64 ka, respectively. The real sediment surface was partly missing in the piston core, so that they calculated a recent age for the trigger weight core, but even the surface of the piston core had a reasonable age which allows us to assume that the surface sediment is close to Recent.

We have added this information to our text and we hope to have removed the reviewer's scepticism regarding the sediment ages.

Wu, S., Lembke-Jene, L., Lamy, F. *et al.* Orbital- and millennial-scale Antarctic Circumpolar Current variability in Drake Passage over the past 140,000 years. *Nat Commun* 12, 3948 (2021). <a href="https://doi.org/10.1038/s41467-021-24264-9">https://doi.org/10.1038/s41467-021-24264-9</a>

AR: see also our answer to Reviewer 1 and lines 141- 146 in the revised manuscript

**RC#2:** Material and Methods, I. 132-135. The dating of the studied material is vague. Former AMS datings in near surface sediments of previous studies in the broader have been used and the authors conclude to ages "most likely mid to late Holocene". Do these datings come from the surface samples or from certain depths? Is there a possibility to estimate the age of the nearest of your surface samples to those of the former studies, by using an available sedimentation rate for the area? Please clarify.

AC: This point has been already addressed in the previous paragraph.

Also, please use Mid and Late Holocene, as these are official chronostratigraphic units.

AC: We changed this in our text accordingly.

AR: see AC

RC#1: Line 145 The sentence "were aimed to be counted" is not clear enough. Do authors mean that they actually counted at least 100 coccoliths or this target number was not always met?

AC: We actually aimed to count at a minimum of 300 coccoliths per sample, but counted at least 100 coccoliths in those samples with very low coccolith abundances. We clarified the sentence and add the respective sample names and a cross-reference to Table 2, where the total counted coccoliths is noted.

"A minimum of 300 coccoliths per sample was counted in transects across the filter area, except for eight relatively coccolith-poor samples south of the PF (083-1, 080-2, 042-1,044-1, 074-1, 048-1049-2, 052-3) and two in the SAZ (096-1, 094-1) in which at least 100 coccoliths were counted (see Table 2). All the sampling points were considered when plotting the number of coccoliths per gram of sediment, except for three samples with extremely low counts that were excluded in the plots of relative abundances. "

AR: see lines 154 - 156

RC#1: Line 166 Since one of the aims of the study is to compare results with modern living assemblages, why not using the classification of Savedra-Pellitero et al. (2019)?

AC: The one difference in the classification is that Saavedra-Pellitero et al. (2019) distinguished the *E. huxleyi* morphotypes in morphogroup B into types B (>=4 $\mu$ m), B/C (<4 $\mu$ m), C (<3.5 $\mu$ m) and O (with open central area or lamella in central area) in their studied water samples, while we did only classified them into types B/C (plated central area) and O (open central area, see Table 1) based on the central area feature. This grouping is based on the fact that we found no coherent characteristics in the studied surface sediment samples – other than size - that could help us to distinguish the studied *E. huxleyi* morphogroup B coccoliths into more than one morphotype. As it can be seen in Figure 6, the size range for morphotype B/C in this study lies between <3 $\mu$ m to >4 $\mu$ m with most coccoliths around 3.5 $\mu$ m. Similar size variability can be found within the coccoliths of one single coccosphere and "may originate from the variability in size of the OBPS during the cell growth/division cycle" (Beuvier et al. 2019).

AR: see AC

RC#1: Line 175 Does this mean that an average of 27 *E. huxleyi* coccoliths were analysed per sample? If this is correct, the dimensions of a some morphotypes in some samples are based on a very low number of specimens. Authors should provide statistical evidence that the sample size is representative of the different populations (i.e. morphotypes). Please provide a table indicating the number of coccoliths of each morphotype that were analysed in each sample.

AC: We guess that reviewer #1 simply divided the analysed 613 coccoliths of *E. huxleyi* by the number of samples. However the differentiation between the five *E. huxleyi* morphotypes within morphogroups A and B was done in additional counts (see line 164 f) of at least 100 coccoliths (AR: at least 50 coccoliths – correction from the Authors). We used the morphometric data measured with the Coccobiom2 Macro to describe the 5 morphotypes (and not 22 samples) in a second step. We therefore believe that we have a representative number of measurements per morphotype available for this classification (335 measurements of morphotype B/C, 134 measurements of morphotype O, 52 measurements of morphotypes A and A overcalcified merged together) The associated morphometric data, like all counting data, have been submitted to Pangea and is being currently curated. The data will be made publically available after publication.

AR: We controlled the number of pictures per morphotype and have to correct the stated numbers: We analysed 570 SEM pictures of E. Huxleyi for morphometry (instead of 613, because we excluded 43 pictures from further analysis because they included morphotype R, non-allocatable morphotypes or Gephyrocapsa – species). From these 570, 347 measurements belong to morphotype B/C, 156 measurements to morphotype O, 67 measurements to morphotypes A and A

overcalcified merged together (instead of 335, 134, 52, respectively. Latter numbers arose when excluding samples with less than 5 measurements of a morphotype, as done for the calculation of the length and mass distributions per sample – see Fig. 6 and 12). We apologize for the confusion. We changed the numbers in the revised MS accordingly and refer to the supplementary data that will be published at PANGAEA.

We also added the number of classified E. Huxleyi morphotypes per sample in Table 2. For the number of E.huxleyi SEM images analysed and the measurement results please refer to the corresponding dataset that we submitted to PANGAEA.

**RC#2:** 175, it is not clear how many specimens/morphotypes have been analyzed per sample. Is it at least 20 coccoliths?

AC: This question has also been raised by reviewer #1. We tried to measure at least 30 *E. huxleyi* per sample (some of the micrographs showed more than one specimen), but all the countings of the *E. huxleyi* morphotypes within morphogroups A and B were done in an additional count on top of the routine count (see line 164 f) of at least 100 coccoliths. The mentioned 613 SEM images were used to measure the specimens with the Coccobiom2 Macro and to morphologically describe the 5 morphotypes (335 measurements of morphotype B/C, 134 measurements of morphotype O, 52 measurements of morphotypes A and A overcalcified merged together).

AR: We included the additions as above, although we corrected the stated numbers of morphotypes to: (347 morphotype B/C, 156 morphotype O, 67 morphotypes A and A overcalcified together) in line 191. We also corrected the number of SEM images to 570 (from 613) in line 190

RC#1: Line 184 This explanation is not clear enough. Could you please explain how these constants with ranges were applied to the coccoliths measured in the sample?

AC: We did not apply the ranges in our mass calculations. When applying the maximum ranges of  $k_n$  and  $\beta$  both, the calculated masses are higher with up to approximately doubled maxima. Applying the minimum ranges do have a weaker effect. Overall, the total mass range lies between approximately 1 to 10pg, when considering the ranges of the two constants in the formula from Beuvier et al. (2019). However, since we want to focus on the biogeographical trend within our sample set and compare this trend to other studies in the Drake Passage area (Saavedra-Pellitero et al. 2019, Charalampopoulou et al. 2016, see figure 12), we think that this approach is appropriate.

We also realized that there is a typo in the caption of Table 3 – the unit for the mass measurements is of course pg and not  $\mu$ m, so we fixed it.

We also completed the calculation formulas. The number of segments n was calculated with n = p/w with p being the peripheral grid perimeter (central area perimeter) and w being the average width of the calcite crystals (also known as T-elements) that we set to  $0.12\mu m$  (see Beuvier et al 2019).

AR: Edits as mentioned above in caption of Table 3 (we also replaced the numbers in the method-column with the Authors names), in lines 197 – 199 and in equation (4).

RC#1: Line 195 The description provided by Poulton suggests that they lumped together B/C and O. In his figure 1c, the coccosphere looks like the B/C described here. Did you double-check this identification with him? Please verify.

AC: We did not double-check the identification with Poulton. However, the point is, that for morphotype O, the shape factor of 0.015 is leading to smaller masses in comparison to the shape factor of 0.02 (for A, and B/C), which makes sense because there is no calcite in the central area. If Poulton et al (2011) lumped O together with B/C, the "real" shape factor for O would be even smaller so the resulting mass would be even lighter. Additionally, it is possible and probable that some of the *E. huxleyi* coccoliths that we identified as morphotype O are originally morphotype B/C which lost their central area filling during and after sedimentation (but this is discussed in the manuscript, see line 518).

We also realized that there is typo in line 193, which has been fixed now (the shape factor for morphotype O is 0.015 and not 0.15).

AR: see line 207, and the discussion in line 518 is now in lines 547ff.

**RC#2:** 195, this is rather confusing, plankton samples may differentiate from the surface samples (e.g., due to dissolution as you state later on), so why identify type O a potential Type B/C that lost the thin plate when being in the sediment? Maybe clarify even at this stage that "type O" may not totally correspond to the certain morphotype recorded in the plankton, or give it a different, descriptive name that you will keep it all over the manuscript.

AC: Since one of our aims was to compare with the plankton data of Saavedra-Pellitero et al. 2019, we tried to differentiate as much as possible, as the former authors did. Although we could not distinguish sufficiently enough between the types B, B/C (which is an integration of B and C), and C, we could clearly distinguish a type O with open central area or lamella in central area. And from the description provided by Hagino et al. (2011), this is clearly Type O, and so we named it and kept it this way throughout the manuscript. We explained this in the text and in Table 1. Whether this is just a "potential Type B/C", as mentioned by the reviewer, or an own morphotype, and if this has the same or a different ecological adaptation than the other *E. huxleyi* types (in particular the B- and C-types) should actually be discussed in further investigations. In the end, we cannot rule out that these may be disguised, dissolution-related B / C types, but we believe most of them are real. Nevertheless, we tried to better clarify this in the text.

AR: see the comment to RC#1, edits in lines 211-212 and our discussion in line 547 ff.

RC#1: Line 199 replace "approache" by "approach"

AC: We replaced it.

AR: see AC

RC#1: Section 3.4 Authors should explain the objective of each of the statistical analyses and data transformations.

AC: We added more information in this section as suggested by reviewer #1: "Prior to any statistical analysis, we excluded three samples (PS97/077-1, PS97/079-1 and PS97/071-2) because of the very low number of coccoliths counted (< 40 per sample). We identified suitable ordination methods by applying detrended correspondence analysis (DCA) on the species relative abundance dataset. DCA resulted in a first axis length of 1 SD suggesting a short gradient for which linear ordination methods are more appropriate. The relative abundance data was standardized using Hellinger transformation, which is well suited for species abundance data, to make variability of the species abundances comparable and giving low weights to rare species (Legendre and Gallagher, 2001), using R package adespatial 0.3-8 (Dray et al., 2020). We chose the Hellinger distance measure because it is metric and performs well in linear ordination (Buttigieg and Ramette, 2014). To find groups of samples that are most similar to each other, average-linkage (UPGMA) hierarchical clustering was performed on the assemblage data with R function hclust (R Core Team, 2020) because it takes into account the average pair-wise distance between all members of clusters. The best number of clusters was suggested by the majority of 30 indices calculated with R package NbClust version 3.0 (Charrad et al., 2014). The significance of each cluster was assessed by multiscale bootstrap resampling with 10,000 replications using R package pyclust version 2.2-0 (Suzuki et al., 2019), to assess the stability of the clusters. We assessed relationships between environmental and biotic data using a transformation-based RDA (tb-RDA). We constrained our assemblage data (response, 16 species at 25 sites) to seven standardized environmental variables (explanatory): salinity, temperature, phosphate, and PAR at 10 m water depth; CO3 as representative variable for the carbonate system at surface sediment sample depth; the MLD and the respective surface sediment sample depth itself. The adjusted R2 was calculated, and the significance of the tb-RDA was tested at 9,999 permutations. Analysis was performed using R package vegan version 2.5-6 (decostand, rda, RsquareAdj, anova.cca, Oksanen et al., 2019). We determined the similarity between water column and fossil coccolithphore assemblages in this region by calculating the analogue distance of a subset of the sediment surface samples to the nearest plankton samples (Saavedra-Pellitero et al., 2019) using R package ggpalaeo version 0.0.0.9005 (Telford, 2019), see Figure A3. All figures were made with R package ggplot2 version 3.3.2 (Wickham, 2016) and modified with Inkscape version 1.0.2."

AR: see above mentioned edits in lines 233 - 251

New reference included: Buttigieg, P. L. and Ramette, A.: A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses, FEMS Microbiology Ecology, 90, 543–550, https://doi.org/10.1111/1574-6941.12437, 2014.

RC#1: Line 200 If the morphometric-based approach brings higher values than the birefringence-based method, why the analysis on the living assemblages in the Drake passage suggests higher values? Please explain better.

AC: Rigual Hernandez et al. (2020) indeed showed that the birefringence-based method gave higher coccolith mass estimates than the morphometric-based method. However, they also showed that this difference is negligible in small species, such as *E. huxleyi*, since it is within the margin of error. We can only speculate about the higher mass values in the plankton samples from Saavedra-Pellitero et al. (2019) in comparison to our mass data on *E. huxleyi* as well as the published masses from Charalampopoulou et al. (2016). Since the systematic uncertainty of the method in that plankton study is higher than in our study (due to the fact that calibration is not always done with a calcite wedge of known thickness), we consider that the measurements on the living assemblages in the Drake Passage are overestimated. Still, the trends in all three studies are comparable and this is what matters for our study (not the absolute values), as mentioned in the paper (line 530).

RC#1: Line 233 Please acknowledge the limitations of this comparison. Coccolithophore living communities may experience important seasonal changes through the year and, therefore the assemblages collected in a single water sample cannot be considered representative of an annual cycle.

AC: We fully agree with reviewer #1, but calculating the analogue distance allowed us to see how similar/different these communities are (quantitatively). In fact, Figure A3 shows that they are very different supporting reviewer's #1 statement.

AR: see AC

RC#1: Line 236 Include subheading

AC: We are not sure about this statement from the reviewer.

We wanted to include some information before section 4.1, so we did it right after 4. Perhaps reviewer #1 is suggesting a formatting issue. In that case, we are sure that the editorial team will point out the issue and they will help us to address it.

AR: -

RC#1: Line 262 Do you mean "at the deepest stations"?

AC: We meant at the stations below 3.1 km of depth, so we prefer to leave it as it is now.

AR: -

RC#1: Line 286 Many physical and chemical parameters that might influence coccolithophore community composition and abundance are known to decrease close to monotonically poleward across the Southern Ocean. These include temperature, salinity, alkalinity, pH and the saturation state of calcite. Thus, it is not easy to separate their possible influences on coccolithophore distributions. This problem has been previously noted by Charalampopoulou et al. (2016) in the Drake Passage. This should be taken into consideration when performing and interpreting the statistical analyses. Did authors consider this problem? And if so, how did you deal with it?

AC: This is an inherent problem in the SO, because of the strong gradients occurring there, which are specially marked in the Drake Passage. As reviewer #1 mentions, some of the variables co-vary with other dependent variables and the effect of one over any of the others is difficult to distinguish. However, the fact that two independent statistical analyses (hierarchical and multivariate) point towards the same factor (i.e. depth related to the calcium carbonate saturation state) makes to our interpretations robust. While parameters such as temperature, PAR, or salinity co-vary across the

gradient, depth of the samples is a variable that does not show a general poleward variation in the SO. Of course, we acknowledge that during their life cycle, coccolithophore assemblages were affected by these other physico-chemical parameters, but ultimately the main driver here was the depth of the samples.

AR: -

RC#1: Line 289 Please clarify how many specimens of *E. huxleyi* coccoliths were used to estimate the relative abundance. You can provide the average value including the minimum and maximum numbers for all samples.

AC: We provided additional information regarding the morphotype counts in the Material and Methods section (sect. 3.1):

AR: see mentioned edit in lines 185 – 188 and added absolute numbers of classified E. huxleyi numbers in Table 2.

We added percentage numbers as suggested by reviewer #1 in section 3.1 (those will also be available in an additional dataset submitted to PANGAEA). We also noticed an error in the relative abundances of morphotype B/C and corrected the numbers: in section 4.3

RC#1: Line 295 as authors state in the discussion it is very likely that at least a fraction of the type O coccoliths belong to B/C. Did you try to find correlations between this morphotype and any dissolution indicators (e.g. CEX)?

AC: In fact, we cannot tell for sure whether a fraction of the type O coccoliths belongs to B/C. That is the main reason why we did not explore further the relationship with the dissolution.

AR: -

RC#1: Line 300 Type O coccoliths display an almost identical size range than those of B/C. Could this be taken as an indication that both morphotypes belong to the same type/variety of *E. huxleyi*? What about their geographical distribution? Is there a relationship between abundance of type O and dissolution?

AC: This is a very interesting question similar to one of the points raised by reviewer #2. Although we could not distinguish sufficiently enough between the types B, B/C (which is an integration of B and C), and C, we could clearly distinguish a type O with open central area or lamella in central area. In the end, we cannot rule out that these may be disguised, dissolution-related B / C types, but we consider that most of them are real.

It is interesting to note, that *E. huxleyi* morphotype B/C dominates over morphotype O in plankton samples (Saavedra-Pellitero et al., 2019) but it does not in the presently studied surface sediments in the DP. This, together with the differences in the biogeographical distributions makes us speculate that this morphotype may be highly sensitive to lower calcite saturation state in depth

south of the Polar Front (see discussion in lines 579ff). Still, with our data we cannot be sure if <i>E. huxleyi</i> type O and B/C belong to the same type/variety of <i>E. huxleyi</i> or not.
AR: -
RC#1: Line 310 Coccolith mass values estimated here should be compared with previously published works in the Southern Ocean.
AC: Due to the fact that different methodologies have been used in the available studies (adding uncertainty), we prefer to stick to just comparing the trends, which are very similar (see previous comment regarding line 200).
AR: -
RC#1: Line 317 please be more precise and include a proportion or order of magnitude in the latitudinal comparison.
AC: A more precise description of the changes has been added to the text.
AR: We added the coccolith abundance ranges in line 343.
RC#1: Line 321 It makes sense that studies based on plankton samples are less diverse since they only capture a "moment" of the annual cycle while sediment samples must encompass hundreds of years. Also, the mentioned studies covered different latitudinal ranges. So, I wouldn't consider these results "surprising".
AC: This would be generally true but considering that we are in a low-diversity (sub-)polar area and that dissolution can notably diminish coccolithophore diversity in the surface sediments (Saavedra-Pellitero and Baumann 2015), it was indeed surprising for us. Therefore, we decided to keep this statement.
AR: -
RC#1: Line 328 "unusually high" compared to what?
AC: compared to other studies, which we have added now to the text (the same papers that we listed on the note to lines 82-83).
AR: see AC

RC#1: Line 346 This is somewhat confusing, the best way to assess algal biomass concentration in a region is to look at satellite chlorophyll-a in the region. High abundance of coccolithophores doesn't

necessarily imply high productivity since they only account for small fraction of the phytoplankton community. So please, rephrase.

AC: We have not mentioned algal biomass in the paper, but in any case, we meant coccolithophore productivity as stated in the following (line 349). We have clarified this in the text.

AR: see line 373

**RC#2:** 345-350, is it acceptable to correlate "Mid-Late Holocene" surface sediments with the present day surface waters conditions? This needs to be more documented, e.g., are there evidence (from sediment cores) about the paleoceanographic evolution of the area during the Holocene?

AC: We think this is a common practice, a fair assumption, and we consider it is fully acceptable, particularly when we have provided more publications which have dated a core-top sample used in our study, which were confirmed as of recent age. This information has been included in the revised version. The comparison of coccolith data from sediment traps with those of the underlying surface sediments (e.g., Sprengel et al., 2000; Skampa et al., 2019) shows that the data are similar in species fluxes, assemblages, etc., even if the surface sediments are usually not dated.

Sprengel, C., Baumann, K.-H., Hendricks, J., Henrich, R. & Neuer, S., 2002. Modern coccolithophore and carbonate sedimentation along a productivity gradient in the Canary Islands region: Seasonal export production and surface accumulation rates. Deep-Sea Research II, 49, 3577-3598.

Skampa, E., Triantaphyllou, M.V., Dimiza, M.D., Gogou, A., Malinverno, E., Stavrakakis, S., Panagiotopoulos, I.P., Parinos, C., Baumann, K.-H., 2019. Coupling plankton - sediment trap - surface sediment coccolithophore regime in the North Aegean Sea (NE Mediterranean). Marine Micropaleontology, <a href="https://doi.org/10.1016/j.marmicro.2019.03.001">https://doi.org/10.1016/j.marmicro.2019.03.001</a>

AR: We added the mentioned information about the surface sediment age in lines 141-145. See also our comments to RC#1 line 134 and RC#2 line 91.

RC#1: Line 367 Please cite Cubillos et al. (2007) paper here.

AC: We have added the suggested reference to the text.

AR: see AC

**RC#2:** 372-374. Overcalcified E. hux specimens (primary calcification) have been also found e.g., in the Aegean Sea, Mediterranean, during the winter season, strongly correlated with low temperatures. It would be useful to discuss the occurrence of these morphotypes in respect to what happens with them at different parts of the world ocean. Apparently your specimens cannot directly prove primary overcalcification as secondary calcite precipitation in the "Mid-Late Holocene" surface sediments cannot be excluded? In the same way that many "type O"'s might be originally type B/C

AC: We are relatively sure that we can exclude secondary calcite precipitation in the coccoliths in our samples, as we see no signs for this at all in our SEM based investigation and we also did not detect obvious carbonate dissolution in the same samples.

We suggested that some *E. huxleyi* O types might have been originally B/C type specimens, not many, which is possible because of the fragile nature of the central area platelet / lamella, especially in the southern water masses of the AZ and SZ+CZ. We are rather confident on our observations, especially regarding O type specimens and we tried to make this clear in the text.

We consider that comparing our morphotype coccolith results to other Southern Ocean locations (as we already did) makes more sense than comparing to data from the Mediterranean since it is a completely different setting.

AR: -

RC#1: Line 377 why shape? please clarify.

AC: We do not fully understand the question -- we mean the shape of a coccolith ... (big or small, more or less elements, the filling/shape of the central area, etc.)

AR: -

RC#1: Line 380 what does "quite common" mean? please be more precise.

AC: For us it means that it is often present (=common) in relatively high abundances. We reworded the sentence.

AR: see line 407

RC#1: Line 413 not only south the PF. Did any of these studies reported descriptions of the seasonal cycles of coccolithophores?

AC: Unfortunately not. The only "seasonal" studies come from sediment trap investigations (lines 364 and later), which are not located in the Drake Passage.

AR: -

RC#1: Line 418 What do you mean with "selectively enriched"? Are you suggesting selective dissolution? Please explain better.

AC: Yes, we believe that the larger species are selectively enriched due to the dissolution of the smaller and more fragile taxa. We tried to clarify this in the text.

AR: see line 446

RC#1: Line 519 This is an important point. Please note that even in the same coccosphere type B/C and Type O coccoliths can be observed. See Figure 3 of Cubillos et al. (2007) paper. Please discuss this point. Did authors compared the spatial distribution of B/C and "O" in terms of absolute abundances and relative abundances? is it similar between them?

AC: We actually do not see any differing types in Figure 3 of Cubillos et al. (2007). One can certainly intensely argue about this, but we definitely see differences in preservation of the coccoliths in Figure 3e. Still, all visible coccoliths can still be clearly assigned to one type in that specific coccosphere. We have also taken a close look at the plankton communities in the study area (Saavedra-Pellitero et al., 2019), have not observed any coccosphere with two clearly different morphotypes, and we are also not aware of any publication in which such a thing is described. We only showed the absolute abundances of the total assemblages (we called it total numbers, see Figure 3), and the relative abundances of the species as well as the relative proportions of the *E. huxleyi* morphotypes (see Figure 5). We did this because the changes in the absolute abundances would have resulted in very similar representations in the distributions, as can be seen from the comparison of the *E. huxleyi* types in Figure 5.

AR: -

RC#1: Line 521 Müller et. al. did not assess the response of type "O". Do you know any culture experiments with this morphotype? I find this statement quite speculative. The fact that type O displays slightly morphological differences with the rest of *E. huxleyi* morphotypes does not necessarily imply that they have different physiological responses. Please be more cautious.

AC: We do not know of any corresponding culture studies on type O and we also do not know whether the type was (ever) cultured. We agree with the reviewer that types B/C and O do not necessarily have to differ in their ecological adaptations, but Type A seems definitely different in its ecology, as pointed out in the literature.

AR: -

**RC#2:** 520-525, "type O" distribution is problematic as you also state. It is because what you have counted may not really represent original type O. Could you differentiate in a possible way, the real type O from the dissolved B/C? It would be useful to produce a figure similar to Fig. 11 with all Ehux morphotypes plotted with CEX, to see which of them are more related to dissolution.

AC: If it would be possible to differentiate them, we would have done so. However, that was not possible, and we just suggest that some of the *E. huxleyi* O type might be originally B/C types that are slightly dissolved and thus lost the central area platelet / lamella (but impossible to appreciate in SEM). We cannot rule out that some of them may belong to the B/C-type. We are, however, quite sure that morphologically most of them are actual O-types. And interestingly, while *E. huxleyi* morphotype B/C (including B, B/C and C) dominates over morphotype O in plankton samples (Saavedra-Pellitero et al., 2019) they do not in the studied surface sediments from the Drake Passage. This makes us speculate this morphotype may be highly sensitive to lower calcite saturation state at depth south of the Polar Front (see discussion in lines 579ff).

AR: in the revised MS this information is in lines 619ff.

RC#1: Line 525 Please compare your values and distributions with similar studies conducted in the Atlantic and Indian sectors of the Southern Ocean:

Horigome, M.T., Ziveri, P., Grelaud, M., Baumann, K.H., Marino, G., Mortyn, P.G., 2014. Environmental controls on the *Emiliania huxleyi* calcite mass. Biogeosciences 11, 2295-2308.

Rigual-Hernández, A.S., Sánchez-Santos, J.M., Eriksen, R., Moy, A.D., Sierro, F.J., Flores, J.A., Abrantes, F., Bostock, H., Nodder, S.D., González-Lanchas, A., Trull, T.W., 2020. Limited variability in the phytoplankton *Emiliania huxleyi* since the pre-industrial era in the Subantarctic Southern Ocean. Anthropocene, 100254.

AC: We have increased the discussion and compared our data to the mentioned studies.

AR: See lines 566 - 575

**RC#2:** 532-535, again a correlation of Holocene surface sediments with plankton assemblages must be treated with caution as the time interval in between them is not negligible. Also to show any agreement it would be useful to do a statistical correlation between all available mass values, taking into account the different methodologies.

AC: This comment is linked to remarks previously addressed. The newly published AMS <sup>14</sup>C ages surface sediments demonstrate, as the aforementioned published dates, that the surface sediments in the Drake Passage seem to be recent.

AR: see our replies to RC#2 to lines 345 - 350

**RC#2:** 538-539. The statement is rather vague, when projecting Mid-Late Holocene to the future. Will it be a primary coccolithophore feature? What about potential dissolution? May be additional correlation with potentially available sediment trap data of the broader area would be useful.

AC: See previous remarks to comment about the age of the sediments. We consider that hypothesizing about future coccolithophore response to anthropogenic warming, in terms of morphology or dissolution, is out of the scope of our study. There are multiple publications which include modelling work or are based on culture evidence, which are much more suited to make such predictions.

AR: -

RC#1: Line 586 Please clarify that pteropods are made of aragonite which is substantially more prone to dissolution than calcite. As it reads now, this statement is misleading for the reader.

AC: We have changed that accordingly. We believe that it is common knowledge for the reader that pteropods have an aragonite shell, and just wanted to show that more easily soluble carbonate organisms are already affected by increased ocean acidification.

RC#1: Line 586, second sentence. Authors cannot be completely sure that the dissolution in the sediments is necessarily enhanced by anthropogenic ocean acidification. Please rephrase being more cautious with your words.

AC: We have reworded that sentence to make our statement looser (even though we believe it is likely).

AR: see lines 616-618

## **Figures**

AR: We checked all figures for issues related with color vision deficiency with Cobis – Color Blindness Simulator. We adapted some colors so all features in the figures are visible for everyone.

RC#1: Please include the name of the fronts and zonal systems in the maps

AC: Done (note that the names of the fronts were already given).

AR: we included the names of the zonal systems in the map.

Additionally, we added information about the sources of the bathymetry and the summer sea ice extent in the caption

RC#1: Figure 10. Please discuss in detail the role of the most important environmental factors in the distribution of coccolithophore species in the sediments.

AC: We already discussed this aspect in detail above (see comment about lines 9-10 and 286).

#### **Tables**

AR: Table 2: We added the numbers of classified E. huxleyi morphotypes per sample to Table 2.

RC#1: Table 3. "All measurements in  $\mu$ m." Mass cannot be measured in micrometers, please correct. Also please include the name of the authors of the method in the column "methods", it would facilitate the interpretation of the table.

AC: That has been changed (see also comment on line 184). We did not notice that mistake and thank reviewer #1 for pointing this out. We have also added the author(s) of the method in the column "methods".

# Supplementary materials

RC#1: All coccolith counts and morphological measurements should be provided as

supplementary materials.

AC: All data has been uploaded in the world data center repository Pangaea and it will be available after publication.

### **List of Changes**

Line 47: carbon is respired as carbon dioxide

Line 57ff: We explained: The competitive interaction between coccolithophores and diatoms is also of particular relevance in the SO, with the former being prevalent north of the PF, while the second even though diatoms dominate subantarctic waters in terms of biomass. Nevertheless, small taxa, such as coccolithophores and small or lightly silicified diatoms, are known to dominate the protistan community in terms of numbers in the SAZ (e.g. de Salas et al., 2011), while diatoms are dominating in regions south of it (e.g., Saavedra-Pellitero and Baumann, 2015; Balch et al., 2016; Malinverno et al., 2016; Cárdenas et al., 2019, Smith et al., 2017).

Line 75: We attenuated the sentence

Line 76-77: We included references Winter et al. 2014, Charalampopoulou et al 2016

Line 79: We added: In this region temperature seems to be a more prominent factor affecting *E. huxleyi* 

morphotype distribution and coccolith mass than the carbonate chemistry, therefore suggesting that the influence of global warming on coccolithophores will be stronger than ocean acidification in the future

Line 88: We added references: (Boeckel et al., 2006; Findlay and Giraudeau, 2002; Saavedra-Pellitero and Baumann, 2015)

Line 97ff: We explained: Additionally, we evaluated the coccolith mass variations in the dominant taxa E. huxleyi within each different morphotype, which can serve as an estimate for CaCO 3 export that may be affected by projected environmental change (e.g. Rigual Hernández et al., 2020).

Figure 1 Caption: We added the reference for the shown bathymetric map and the summer sea ice extent; and noted how the areas between fronts are called.

Line 126: first occurrence of abbreviation SAZ - we indicated the full name

Line 134f: We added newly published information: Estimated Holocene sedimentation rates in the DP area are in the order of 3.5 cm/kyr to up to > 10 cm/kyr (Ho et al., 2012; Caniupán et al., 2011a; Wu et al., 2021).

Line 138f: We added information about the MUC

Line 142ff: Added newly published information about sedimenation rates: Additionally, Wu et al. (2021) calibrated AMS 14 C ages for the sediments from the piston core PS97/085-3 at 0.5 cm (= 56.5 cm composite depth) and for 20.5 cm (= 76.5 cm composite depth) with 1.13 ka and 6.64 ka, respectively. The real sediment surface was partly missing in the piston core, so Wu et al. (2021) calculated a recent age for the trigger weight core, but even the surface of the piston core had an age which allows us to assume that the surface sediment is at least close to recent times.

Line 158: reference to Table 2 (here added relevant numbers)

Line 186: Added info: In total, we classified between 53 and 115 E. huxleyi morphotypes in most samples in the SAZ and four samples in the AZ, while we could only classify between eleven and 48 E. huxleyi in eight samples from the AZ and SZ+CZ and in one sample in the SAZ (see Table 2).

Line 190 ff: corrected numbers: [...] analysed on 570 SEM micrographs of single flat-lying E. huxleyi coccoliths at a magnification of 10000x (347 morphotype B/C, 156 morphotype O, 67 morphotypes A and A overcalcified together).

Line 200 ff: Added relevant info: The number of segments (n) was calculated with p being the peripheral grid perimeter (central area perimeter) and w being the average width of the calcite crystals (also known as T-elements) that we set to  $0.12 \mu m$  (see Beuvier et al., 2019).

Equation 4: Added relevant info

Line 210: corrected number: [...] morphotype O (ks = 0.015) [...]

Line 212f: rewrote

Line 241ff: Added information on why we chose which statistical analysis

Table 2: added new column with number of classified E. huxleyi morphotypes per sample

Line 314 ff: added percentages to E. huxleyi morphotype numbers

Line 343: added total coccolith abundance numbers

Table 3: exchanged the equation numbers with the authors of the methods used

Line 355f: added references

Line 394: added reference

Line 407: rewrote

Line 446: added: [...] due to dissolution of the smaller and more fragile taxa [...]

Line 467: We included a cross-reference to Figure 10.

Figure 10 Caption: We specified that the first RDA axes in the caption of Figure 10 explain 84,3% of the constrained variance (not the total variance) to clarify the RDA outcome.

Line 545 (former 516): We removed the word "however"

Line 566: We compared our mass estimations with further literature:

In comparison to mass estimations based on light microscope images of surface sediment samples in other sectors of the SO, our results fall into a common range: slightly higher masses of E. huxleyi with an average of  $3.6 \pm 0.8$  pg but comparable ranges of 1.73 to 4.85 pg were reported from the Atlantic Sector in the area south of South Africa Horigome et al. (2014) by using an mass estimation method from Beaufort (2005). In the Indian sector south of Tasmania, Rigual-Hernández et al. (2020a) showed comparable mass estimates and a similar north to south trend based on C- Calcita measurements with an total average of  $2.65 \pm 1.2$  pg. Average E. huxleyi masses of approximately 2.5 to 2.8 pg were determined for the SAZ, which is slightly higher than the average of our findings. However, although the mass estimations were based on coccoliths whose morphotypes were not identified due to methodological reasons, Rigual-Hernández et al. (2020a) linked the decreasing mass trend to morphotype distribution across the ACC. With our mass estimates on identified morphotype coccoliths, we can support this interpretation and thus generally attribute changes in the masses across the fronts to a change in the morphotype assemblages.

Line 624: More details to discussion: Since pteropod shells consists of aragonite, a more soluble carbonate mineral than calcite, the decrease in their shell growth, extensive shell dissolution and malformations are the first evidence of ocean acidification (e.g., Bednaršek et al., 2012; Gardner et al., 2018). This loss in carbonate fossils in the geological record could be a consequence of the increase in anthropogenic acidification [...]

### We added the following references:

Amante, C.: ETOPO1 1 Arc-Minute Global Relief Model: Procedures, Data Sources and Analysis, https://doi.org/10.7289/V5C8276M, 2009.

Buttigieg, P. L. and Ramette, A.: A guide to statistical analysis in microbial ecology: a community-focused, living review of multivariate data analyses, FEMS Microbiology Ecology, 90, 543–550, <a href="https://doi.org/10.1111/1574-6941.12437">https://doi.org/10.1111/1574-6941.12437</a>, 2014.

de Salas, M. F., Eriksen, R., Davidson, A. T., and Wright, S. W.: Protistan Communities in the Australian Sector of the Sub-Antarctic Zone during SAZ-Sense, Deep Sea Research Part II: Topical Studies in Oceanography, 58, 2135–2149, https://doi.org/10.1016/j.dsr2.2011.05.032, 2011.

Fetterer, F., Knowles, K., Meier, W. N., Savoie, M., and Windnagel, A. K.: Sea Ice Index, Version 3. [Median\_extent\_S\_03\_1981- 2010\_polyline], https://doi.org/10.7265/N5K072F8, 2017, updated daily.

Horigome, M. T., Ziveri, P., Grelaud, M., Baumann, K.-H., Marino, G., and Mortyn, P. G.: Environmental Controls on the Emiliania Huxleyi Calcite Mass, Biogeosciences, 11, 2295–2308, https://doi.org/10.5194/bg-11-2295-2014, 2014

Rigual-Hernández, A. S., Sánchez-Santos, J. M., Eriksen, R., Moy, A. D., Sierro, F. J., Flores, J. A., Abrantes, F., Bostock, H., Nodder, S. D., González-Lanchas, A., and Trull, T. W.: Limited Variability in the Phytoplankton Emiliania Huxleyi since the Pre-Industrial Era in the Subantarctic Southern Ocean, Anthropocene, 31, 100 254, https://doi.org/10.1016/j.ancene.2020.100254, 2020a.

Wu, S., Lembke-Jene, L., Lamy, F., Arz, H. W., Nowaczyk, N., Xiao, W., Zhang, X., Hass, H. C., Titschack, J., Zheng, X., Liu, J., Dumm, L., Diekmann, B., Nürnberg, D., Tiedemann, R., and Kuhn, G.: Orbital- and Millennial-Scale Antarctic Circumpolar Current Variability in Drake Passage over the Past 140,000 Years, Nature Communications, 12, 3948, https://doi.org/10.1038/s41467-021-24264-9, 2021.