

The article by Díaz-Rosas et al. presents an interesting examination of coccolithophore impacts on PIC standing stocks in relation to upwelling and the oxygen minimum off the coast of Chile. The authors compare coccolithophore based estimates of PIC and discrete PIC chemical measurements and show that only 50% of the PIC is sourced from coccolithophores in these waters. There is also a strong gradient in species contributions which align with the base of the euphotic zone and upper part of the oxygen minimum zone. The authors explore these observations in terms of the context of the fate of sinking organic carbon and how reduced PIC ballasting of sinking organic carbon could strengthen low oxygen conditions.

The article is well written and contains interesting observations and measurements, however several issues need to be further clarified. Firstly, the link between PIC production, ocean acidification (pH) and PIC standing stocks, sinking POC ballasting by PIC, and the oxygen minimum zone needs to be better explained in the introduction – at the moment there is no mention of PIC ballasting so that the link between pH conditions and O<sub>2</sub> concentrations is not clear. Secondly, a lot of material is found in the supplementary material rather than the main article and this makes following the article difficult – this is especially in the case where the authors compare their coccolithophore and PIC dynamics with environmental conditions. This is wholly in the supplementary material and if the authors consider this an important element of the paper, this statistical analysis should be in the main article. Lastly, can the authors confirm that their conversions from coccolith CaCO<sub>3</sub> (their Table S3) to the values reported in the article (mmol C m<sup>-3</sup>) took into account the molecular weight of CaCO<sub>3</sub> – for most of the article this appears to be so, but then there is a calculation in the discussion which gives very high PIC concentrations (Ln 339) and its not clear how these values were achieved.

**We thank Dr. Poulton for the supportive and careful comments and suggestions. We confirm that we checked the conversions and corrected an error in one part. We respond to the detailed comments below.**

## **SPECIFIC COMMENTS**

Ln 12, Consider whether the term ‘pools’ or quotas or standing stocks (as used elsewhere in the paper) would be better.

**We appreciate your concern about the consistency of pools, quotas, and standing stocks terminology and offer to clarify them according to the following criteria:**

**- “Pools” will be used to describe integrated or total quantities, such as the cumulative PIC across a specific depth range or area, which is essential for understanding large-scale patterns. For example: “Below the Zeu, the sharp decline in the numerical abundances of coccospheres ( $< 0.1 \times 10^5 \text{ L}^{-1}$ ) and detached coccoliths ( $< 10 \times 10^5 \text{ L}^{-1}$ ) decreased coccolithophore PIC pools to below  $1 \mu\text{g L}^{-1}$ .” or “These results support the conclusion that coccolithophores are important contributors to total PIC pools in this OMZ.”.**

- Quotas refer to per-cell metrics, such as PIC per coccolithophore cell, which provide insights into species-specific contributions to the PIC pools. For example, “The PIC quotas of individual coccoliths and coccospheres were estimated following Young and Ziveri (2000), and used to calculate coccolithophore-derived PIC pools (PICCocco) from abundances of coccospheres and detached coccoliths”

- The term “Standing stocks” will be used in the sense of organism stocks (such as fish stock assessments). Here it will be employed to capture instantaneous measurements of total coccolithophore abundances at specific depths or times, helping to characterize temporal and spatial variability. For example: “Standing stocks of larger taxa were only noticeable below the  $Z_{eu}$  (~50 m depth).” or “This could represent the maximum coccolithophore standing stocks and PIC pools attainable in the Southeast Pacific margin”.

Each of these terms corresponds to a distinct analytical perspective reported in the manuscript, and using only one term would oversimplify the multifaceted nature of our findings. We will correct the manuscript to use them consistently in the appropriate contexts. For example, the header of the first section of Results will be corrected to

**“3.1 Oceanographic conditions. Standing stocks of coccolithophores, pools of PIC and POC, and PIC:POC ratios”**

Lns 12-13, The links between ocean acidification, PIC concentrations and production, the efficient ballasting of sinking POC, and the impact of remineralisation depth need to be further expanded on in the first couple of lines of the abstract; the links are not obvious without further explanation.

Thank you for pointing this out. We updated the first lines of the Abstract as follow:

**“A predicted consequence of ocean acidification is to reduce coccolithophore-produced Particulate Inorganic Carbon (PIC) pools. PIC is thought to promote the sinking of Particulate Organic Carbon (POC) to deeper waters, potentially influencing the depth of organic matter remineralization and subsurface oxygen levels.”**

Ln 17, What do the authors mean by ‘countable coccoliths’? Do they mean detached coccoliths, or are they hinting at a portion of coccoliths that is not countable?

Thank you for pointing out the ambiguity of “countable coccoliths”. It refers specifically to coccospheres and coccoliths observed and enumerated under the microscope. This excludes any coccoliths that were too small, fragmented, or otherwise indistinguishable for reliable counting. The term does not imply the

existence of a portion of coccoliths that is inherently uncountable but rather reflects the limitations of our counting method. We will update the sentence as follows:

**“On average, about half of the PIC was attributed to reliably enumerated coccospheres and detached coccoliths, with diminished proportions observed below the euphotic depth.”**

Ln 27, What about the important contributions from other calcifying plankton? As the paper concludes that coccolithophore PIC is only around 50% of PIC standing stocks, would it not be good to expand on other potential sources of biological PIC?

**We appreciate the suggestion to expand on other potential sources of biological PIC. We fully agree that other calcifiers play significant roles in the biological PIC pool. However, in the Abstract and Introduction, we specifically limit the scope of our study to phytoplankton calcification, excluding foraminifera and pteropods zooplankton from our analysis. Nevertheless, in response to another comment, we will acknowledge the roles of other calcifying plankton in the revised discussion. For example “How much coccolithophores contribute to PIC remains an open question, as contributions from calcifying zooplankton (e.g., foraminifera, pteropods; Ziveri et al., 2023), lithogenic sources (Daniels et al., 2012), and processes like fragmentation and dissolution in the water column (e.g., Barrett et al., 2014; Subhas et al., 2022) complicate the relationship between PIC and coccolithophores” and “In addition to PIC production by other planktonic organisms, which would not be detected by the microscopy protocols used here,...”**

Ln 34, There is no mention of the ballast effect on POC sinking in the first paragraph of the introduction so that the link between reduced PIC production and the ‘favouring of the respiration of more organic material’ is not obvious. Suggest adding introduction of the ballast effect earlier in the introduction to make this link obvious.

**Thank you for your feedback. We will update the first part of the Introduction to explicitly describe the potential role of coccoliths in the sinking process or ‘ballast effect’ as follows:**

**“The Particulate Inorganic Carbon (PIC) pool, a significant component influencing marine C cycles and atmospheric reservoirs (Ridgwell and Zeebe, 2005), originates from various sources, including land transport to coastal margins (Cai, 2011) and biological processes such as phytoplankton calcification (Taylor and Brownlee, 2016). Coccolithophores, particularly the cosmopolitan species *Gephyrocapsa (Emiliana) huxleyi*, significantly contribute to PIC through coccolith production. These coccoliths are thought to promote the sinking of organic matter (i.e., the ‘ballast effect’), facilitating the export of particulate organic carbon (POC) to deeper waters (e.g., Klaas and Archer, 2002), thereby influencing carbon sequestration and nutrient cycling in the global pelagic ocean (Monteiro et al., 2016; Balch, 2018). In turn, changes in PIC dynamics driven by alterations in coccolithophore communities at different spatial and temporal scales feedback into the ocean-atmosphere system (Balch et al., 2016; Claxton et al., 2022).”**

Ln 43, Consider the use of the term ‘coccolithophore bloom’ in the context of the references given – Beaufort et al. (2008) is based in the subtropical S Pacific waters and did not observe what many would consider a ‘bloom’.

**Thank you for pointing this out. You’re right, and we will replace ‘bloom’ with ‘high-density events’. We will also revise its use throughout the manuscript.**

Ln 92 and 105, Can the authors please confirm that all the PIC concentrations presented in the paper are in  $\text{mmol C m}^{-3}$  and that their conversions from the values in Table S3 have taken into account the molecular weight of calcite? It’s not obvious that this is a problem or that a mistake has been made but a calculation in the discussion (Lns 337-339) gives much higher PIC concentrations than expected in units of  $\text{mmol C m}^{-3}$ .

**Thank you for your feedback. You are correct that there was an erroneous use of units, where  $\text{mmol C m}^{-3}$  corresponds to  $\mu\text{g C L}^{-1}$ . We will correct the units in the figures and tables. This adjustment refines the overall narrative, particularly in relation to Fig. 8 and Table 1, as it impacts the PIC and PIC:POC ratio comparisons with other studies or sampling sites. The updated findings will be incorporated accordingly.**

Ln 102, Fig. 1 – What are the solid lines on (b) and (c)? As these lines do not line up with the discrete samples, could the legend explain more?

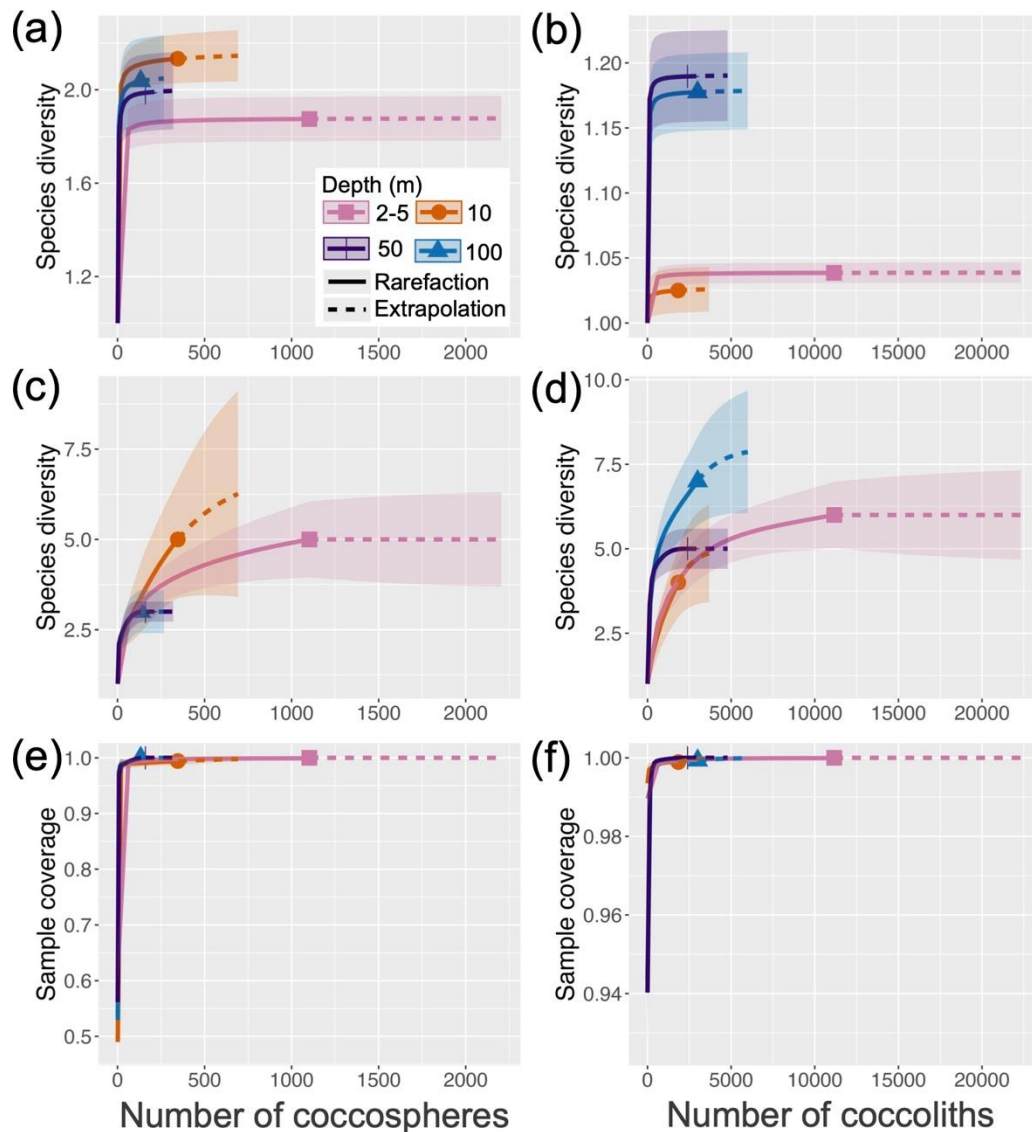
**Thank you for your feedback. We agree that these lines were not appropriately presented to highlight areas crossing the thresholds enclosing the OMZ core. For clarity, we will update the figure legend and ensure the OMZ core threshold is more clearly delineated by using thicker red lines for improved visualization. See response to Reviewer 1 where we include a corrected figure.**

Lns 112 and 118, Having given the proportion of volume counted from the filters for SEM and light microscope analysis (<20 mL or <2% of the volume filtered), it is a surprise that no comment is made on the potential impact this may have on the observations made – specifically the species diversity and the counts of large, often numerically rare species.

**Thank you for pointing this out. While SEM provides high-resolution imaging, its operational costs are substantial. To optimize resources, filters were scanned at lower magnifications (1000-1500x) with sufficient resolution to allow for post-scan zooming, enabling the detection of both coccospheres and detached coccoliths. For smaller detached coccoliths, we assumed their origin to be from the *G. parvula/ericsonii* assemblage, based on prior studies in the same area (e.g., see Beaufort et al., 2008; von Dassow et al., 2018). The dominance of *G. huxleyi* coccospheres in absolute coccosphere counts allowed us to reduce SEM image re-**

analysis for species/genus classification by approximately 25%, while maintaining the same effort for detached coccolith counts.

Please see our detailed comments to Reviewer 1 about these points. Also, we provide below rarefaction curves for different layers showing how we expect that we effectively captured rare species, especially when different samples are grouped by layers. As on average over 100 coccospheres were counted per sample, we should have been able to capture rare species in all but the most dilute (deepest) samples.



Rarefaction and extrapolation curves for late-spring 2015 and mid-summer 2018, showing species richness (a-b), the exponential of Shannon entropy (c-d), and sample completeness (e-f) for coccospheres and detached coccoliths observed at depth of 2-5, 10, 50 and 100 m. Each curve includes 95 % confidence intervals.

Ln 126, What is meant by a 'good fit'? The statistics of the relationship should be presented in the main article rather than the supplementary material.

**Thank you for pointing this out. This aligns closely with Referee #1's concern regarding the potential bias introduced by the use of different microscopic methods. To address this concern, we will present a revised version of Fig. S4 (see below), specifying the counting methods used on each axis (abbreviated as SEM and LM), along with a more detailed statistical analysis to assess the agreement between them.**

Ln 162, What is the oxycline? This needs to be defined somewhere as to what the authors consider either the absolute threshold, relative change or gradient of this feature.

**Thank you for your comment. We now define the OMZ core based 20  $\mu\text{mol kg}^{-1}$  threshold (see response to Reviewer 1). With this change, it should become clear that the oxycline is where vertical transition between the surface oxygen levels and this threshold is steepest, with the greatest relative change between 50-150  $\mu\text{mol kg}^{-1}$   $\text{O}_2$ .**

Ln 165, Are the authors referring to fluorescence or Chl-a peaks when discussing 'peaks of phytoplankton'? Please clarify.

**Thank you for your comment. We will include a threshold for higher fluorescence and Chl-a levels to clarify that we are referring to both proxies for phytoplankton biomass.**

Ln 166, In Fig. 2f the surface (<25 m) nitrate concentrations look much lower (<5  $\mu\text{M}$ ) than the 10  $\mu\text{M}$  quoted in this line. As 10  $\mu\text{M}$  is not generally considered 'low' in terms of biologically-limiting, this line should be changed to better reflect (biologically) 'low' concentrations (e.g., <2  $\mu\text{M}$ ).

**Thank you for your feedback. We will adjust the nitrate according to a biologically relevant threshold.**

Ln 176, Fig. 2 – ODV is notorious for problems with plotting discontinuous (discrete) data in that it often 'creates' patterns not supported by the data. The authors should review the patterns shown in the patchier data (e.g., fluorescence, Chl-a, PIC, coccospheres, coccoliths) as to whether they are confidence in the lateral (horizontal) patterns shown

where there is no data. The use of the colour scheme should also be reviewed as it is not possible to see the patterns discussed at low relative concentrations in the surface. Also, are the PIC concentrations shown in  $\text{mmol C m}^{-3}$ ?

**Thank you for your comment. We will revise the ODV sections to confirm the patterns shown. For fluorescence and Chl-a the colour scheme will be linearized to be consistent with other panels. The  $\text{PIC}_{\text{Cocco}}$  and  $\text{PIC}_{\text{Total}}$  unit will be corrected to  $\mu\text{g C L}^{-1}$  (see commentary above).**

Ln 191-192, What does 'over these abundance ranges, coccospheres and detached coccoliths varied in direct proportion' mean? Please report the statistics in the main paper rather than the supplementary material.

**Thank you for pointing this out. We will recreate the plot in R and include the relevant statistics in the main text. The updated sentence will be "Overall, coccosphere abundance tended to vary directly with detached coccolith abundances, although with a high scatter (Fig. S10;  $y = 13.27x + 6.74$ ,  $R^2=0.51$ )."**

Ln 195-196, What are figures in the supplementary material (Fig. S5-S6) referenced to support the decline below the euphotic zone in coccosphere and coccolith abundances rather than Fig. 2 in the main text?

**Thank you for the detailed review. We will include as Fig. 2m-n as well in an extended Fig. 2 (the new Fig. 2 is included in response to Reviewer 1).**

Ln 221, Do the authors mean 'coccoliths were estimated to account for 48% of the total PIC' or do they mean coccolithophores? It seems from Fig. 5c that the sum of coccospheres and coccoliths is about 50% of the total PIC concentrations rather than just coccoliths.

**Thank you for your feedback. We will clarify the sentence adding the coccospheres as the all-samples percentage as follows:**

**"On average, coccospheres plus coccoliths were estimated to account for 45-48 % of the  $\text{PIC}_{\text{Total}}$  (Fig. 5c)."**

Ln 237, Section 3.4 – All the examination of coccolithophore and PIC patterns with environmental conditions are in the supplementary material and not the main article. This should be changed as it makes it difficult to see this analysis as part of the main article. Further, Ln 249-250, what do the authors mean by 'phosphate [being] more limiting than nitrate'? Are the relatively high concentrations of nitrate and phosphate likely to be growth limiting to the cell densities of coccolithophores observed? Also, what about light availability – this doesn't seem to be a considered environmental condition in this section.

**Thank you for your feedback. As stated in the introduction, this study focuses on evaluating the relationship between PIC and coccolithophore-produced PIC with oxygen and carbonate chemistry conditions, as summarized in Fig. 7. However, to provide additional context, we included the analysis of these ancillary data in the supplementary material for readers interested in exploring this aspect further.**

**We acknowledge your concern that placing the examination of coccolithophore and PIC patterns with environmental conditions exclusively in the supplementary material may make it harder to see this analysis as part of the main article. In response, we will revise the manuscript to better integrate these findings into the main text while retaining detailed analyses in the supplementary material for thoroughness.**

**Regarding the Referee's concern about nitrate and phosphate limitation, we correct the sentence:**

**"Higher PIC<sub>Cocco</sub> showed some tendency to be related to lower phosphate above Z<sub>eu</sub> (Fig. S13f)"**

**Unfortunately, stations were sampled at different times of the day, so direct light levels are difficult to interpret. To address this limitation, we defined the euphotic zone depth using a Copernicus product and applied it to distinguish between samples collected above and below the euphotic zone (the CTD included a PAR sensor, and for mid-day the two depths correlated sufficiently for our purposes). However, this means that we do not feel confident in including light levels in this analysis.**

Ln 269-270, Could the higher *Gephyrocapsa* diversity observed in this study relative to the older studies referenced relate to the methods for species analysis in the different studies quoted (i.e. Hendricks et al., 2012, Venrick, 2012). Specifically, would they have been able to differentiate the weaker calcified species of the genus?

**Thank you for pointing this out. Of the two studies, Hendericks et al. (2012) employed methodologies similar to ours, combining cross-polarized light microscopy with scanning electron microscopy and a comparable sampling effort. In contrast, Venrick et al. (2012) utilized inverted microscopy and will therefore be excluded. Instead, we will include the study by Guerreiro et al. (2013), conducted off the coast of Portugal, which employed methodologies and sampling efforts comparable to those in our study.**

Ln 270, The phrasing of 'standing stocks of larger taxa were only noticeable below the euphotic zone' is rather vague and could be interpreted in two ways – either these larger species were absent in the upper ocean and only found at depth, or they made such a small contribution to the total community in the euphotic zone that they were not noticeable. Please rephrase to clarify the meaning.



**Thank you for pointing this out. We understand how the phrasing could lead to ambiguity and have revised the sentence for clarity:**

**“Standing stocks of larger taxa contributed minimally to the total community in the euphotic zone, becoming more prominent below this depth.”**

Ln 275, Do the authors mean PIC contributions from other calcifying phytoplankton (such as?) or other plankton (i.e. foraminifera, pteropods). Reference to the recent study by Ziveri et al. (2023) would seem to fit here.

**Thank you for pointing this out. We will incorporate the contribution from other planktonic groups to the PIC pool in the text as follows:**

**“How much coccolithophores contribute to PIC remains an open question, as contributions from calcifying zooplankton (e.g., foraminifera, pteropods; Ziveri et al., 2023), lithogenic sources (Daniels et al., 2012), and processes like fragmentation and dissolution in the water column (e.g., Barrett et al., 2014; Subhas et al., 2022) complicate the relationship between PIC and coccolithophores. These complexities challenge remote sensing algorithms (Balch and Mitchell, 2023) and the use of PIC as a paleoproxy indicator (Beaufort et al., 2011).”**

Ln 283, Daniels et al. (2012) was based in the Bay of Biscay, not coastal waters of Chile. This should be clarified in this use of the citation, currently it could be interpreted as supporting little resuspension of biogenic minerals or river discharge of lithogenic material in coastal waters of Chile. What evidence do the authors have of this negligible input?

**Thank you for your comment. We recognize that Daniels et al. (2012) is based in the Bay of Biscay, not the coastal waters of Chile. It is cited to refer to the Bay of Biscay “or springtime waters off the Bay of Biscay (Daniels et al., 2012)” or as an example of resuspended sediments as sources of PIC “lithogenic sources (Daniels et al., 2012),” Regarding the evidence for negligible input of resuspended biogenic minerals or river discharge of lithogenic material in the coastal waters of north-central Chile, we base this conclusion on the unique oceanographic and geological characteristics of the region. The relatively narrow shelf and the deep, steep topography of the Atacama Trench, located just offshore, prevent significant sediment resuspension from reaching the upper layers, thus limiting the contribution of lithogenic material to the coastal/shelf waters. Furthermore, available studies evaluating the resuspension of sediments and river discharges in coastal Chilean waters focus on shallower regions, often at the river mouth or in nearshore environments (i.e., where pollutants are presents), and not at the broader coastal/shelf scale studied here, where the dynamics differ. We will expand the argument as follows:**

**“In the northern Chilean coast, potential lithogenic PIC sources should be negligible. The exceptionally arid Atacama desert means fluvial inputs are negligible (Thiel et al. 2007) and the deep and steep topography of the Atacama Trench**

acts as a major depocenter effectively trapping sediments and limiting their resuspension into the upper water column (Xu et al., 2021). As sources of lithogenic PIC to these coastal waters are limited, we consider most PIC is likely biogenic. ”

Ln 299, What not put the satellite PIC images into the main article (e.g., as part of Fig. 1)?

**Thank you for your feedback. This concern was also raised by Referee #1, and we propose including this as the new Fig. 3 to enhance the context and address gaps in sampling coverage for PIC.**

Ln 304, What do the authors mean by ‘younger populations’ in the context used here? Do they mean in terms of stages of bloom growth and senescence when considering coccolith to coccosphere ratios?

**Thank you for your comment. We acknowledge that the term “younger populations” might be confusing without the presence of a bloom. In this context, we are referring to coccolithophore populations in early stages of development or in a more active physiological state, rather than specifically to bloom dynamics. We will moderate the statement to better align with the interpretations in these studies, emphasizing the active physiological state of the populations rather than specifically referring to bloom dynamics as follows:**

**“While these lower ratios might indicate populations in earlier stages of development or more active physiological states (Balch et al., 1991; Holligan et al., 1993b; Lessard et al., 2005), cell-attached coccoliths comprised 51-72% of the PIC<sub>Cocco</sub> quotas.”.**

Ln 306, The use of abbreviations (max.) here is confusing and unnecessary. Please use full word.

**Thank you for pointing this out. We will replace ‘max’ with ‘maximum’ throughout the text.**

Ln 310, Suggest adding ‘in culture’ to ‘inhibits the growth of many coccolithophore strains’ to emphasise that these are not field observations.

**Thank you for your feedback. We will incorporate this into the text accordingly.**

Ln 325, What about light availability – surely the 1% of surface irradiance at the base of the euphotic zone is an important limitation for growth?

**Thank you for pointing this out. We will update the text as follows:**

“At the base of the euphotic zone, in addition to low light impacting photosynthesis, O<sub>2</sub>-deficient, acidic subsurface waters show pH and carbonate saturation shift ( $\Omega$  calcite) to levels expected by 2100 (below 7.7 and 1.0, respectively) (Fig. 8; also see Vargas et al., 2021). The carbonate chemistry conditions may limit coccolithophore growth and calcification (Meyer and Riebesell, Müller et al. 2016, von Dassow et al. 2018, Kottmeier et al. 2022), and low O<sub>2</sub> conditions may also inhibit phytoplankton more generally (Wong et al. 2023). ”

Lns 337-339, The estimated PIC concentrations of 120 mmol C m<sup>-3</sup> from 400 x10<sup>6</sup> coccoliths L<sup>-1</sup> sounds too high. Can the authors check their calculations – using a PIC content of one *G. huxleyi* coccolith of 0.025 pmol C (their coccolith value; see Ln 156 and Table S3 converted from PIC to C) only leads to an estimate of 10 mmol C m<sup>-3</sup>. To get to 120 mmol C m<sup>-3</sup> the authors would need a coccolith C content of 0.3 pmol C coccolith<sup>-1</sup> (or 3.6 pg C coccolith<sup>-1</sup>), which is much higher than the (carbon values) given by Young and Ziveri (2000).

**Thank you for pointing this out. We have reviewed our calculations and identified that the originally reported value of 120 mmol C m<sup>-3</sup> was mistakenly expressed in the wrong units. The correct units should be  $\mu\text{g C L}^{-1}$ , not mmol C m<sup>-3</sup>. We will update the text to reflect the corrected units and ensure consistency throughout the manuscript.**

Ln 369, What depth is meant by ‘subsurface’ when discussing coccolithophore PIC and detached coccoliths? Euphotic zone or deeper?

**Thank you for pointing this out. This is below the euphotic zone. We will clarify this updating the text as follows:**

**“Below the euphotic zone, coccolithophore PIC is mostly due to detached coccoliths.”**

Ln 404-405, Is ‘the majority of PIC contributed by coccolithophores is dominated by *G. huxleyi* coccoliths shed during blooms’ a conclusion of the present study? What evidence do the authors present for ‘blooms’ preceding the observations presented?

**Thank you for pointing this out. We will replace ‘blooms’ by ‘high-density events’**

Ln 411, Please reconsider the use of ‘max.’ in the main text.

**Thank you for pointing this out. We will replace 'max' with 'maximum' throughout the text.**