

In this study the authors investigate the impact of upwelled low O₂/low pH waters on coccolithophore PIC quotas, coccolithophore PIC contribution to the total PIC pool and ultimately what impact this has on the flux of carbon to depth via the PIC:POC ratio in the oxygen minimum zone off central Chile. The general conclusion that this OMZ exhibits comparable PIC concentrations to selected open ocean areas but a greatly reduced PIC:POC ratio in the OMZ core is a nice observation that emerges from the study but the paper needs attention to clarify methodological ambiguities and the occasional lack of precision in reporting results.

We thank the Reviewer for the supportive comments and the very thoughtful and thorough suggestions and corrections that we respond to below.

Major comments

Methods: Aspects of the methodologies used need to be clarified. In particular, sections 2.3 and 2.4 are muddled presenting mixed LM and SEM methodologies and unclear reasoning. It is particularly unclear if bulk counting or species/genus level counting was followed consistently and the low number of SEM images examined (with a low magnification) may lead to underestimation of both coccospHERE and coccolith counts (see comment below regarding relationship in Figure S4b). Improved description of the methods used is required as this directly leads to ambiguity over how PIC_cocco was estimated (section 2.5) and presented (results). In particular the authors should improve description of the LM and SEM methods used, the apparent bias due to LM counting and the implications of the methodological bias on PIC quotas.

Thank you for highlighting this important point, which is further elaborated in the minor comments below and has been addressed in the relevant sections. We propose improving the corresponding Methods section to make clear that we mostly relied on LM for coccolithophore and coccolith counts, but the more expensive SEM was used in two separate ways. First, in some samples LM counts were not possible, so were replaced with SEM counts, and, to make sure the two methods were comparable, we counted a subset of samples with both LM and SEM and the two were statistically indistinguishable. Second, we used a higher resolution SEM analysis to evaluate in the taxonomic composition. This is important to understand which taxa are contributing to PIC pools. It allows us to get an idea of how estimations of coccolithophore-derived PIC might be improved with better taxonomic resolution.

We also specify more details of both methods as well as how they were used and compared. The proposed new section 2.3 makes this more clear:

2.3 Standing stocks of coccospHERES and detached coccoliths

For enumeration of coccospHERES and detached coccoliths, between 0.1 to 1.0 L of seawater (increasing with depth) were filtered onto 25 mm polycarbonate filters with a 0.8 μ m pore size, left to dry at room temperature in Petri dishes,

and stored with desiccant until microscopy analyses. Total coccospHERE counts were conducted on filter slide preparations with oil immersion, using cross-polarized light microscopy (Zeiss, Axioscope 5). The analysis of twenty fields of view at 400x magnification covered 5.1 mm² of the filter area, corresponding to a range of 1.9-16.3 mL of seawater analysed. For counts of total detached coccoliths, eleven fields of view per filter were screened (224 x 165 μ m per frame) at 630x magnification (oil immersion objective), covering 0.41 mm² of the filter area, corresponding to total volumes of 0.2-1.3 mL of seawater analysed. An issue arose where the filters from inshore-offshore 2015 sampling (20° S; Stations T1-T6) exhibited excessive brightness under cross-polarized light microscopy (LM), for which counts were made through scanning electron microscopy (SEM) analysis (Quanta FEG 250) as described in Díaz-Rosas et al. (2021). For the quantification of coccospHERE abundances (see equation in Díaz-Rosas et al., 2021), between 28-48 images taken at 800-1500x magnification were examined per filter, covering from 5 to 6 mm² of the filter area corresponding to a range of 2.1-18.4 mL of seawater analyzed. For total detached coccolith abundances, between 4-5 images were examined per filter, covering from 0.6 to 1.0 mm² of the filter area corresponding to a range of 0.2-2.8 mL of seawater analyzed. Layers of coccoliths detached from *G. huxleyi* (Fig. S3a-d) were added to the detached-coccolith counts. Collapsed coccospHERes were included when they remained mostly intact, but when more disintegrated could not be accurately counted, especially as they were often less reflective than intact coccospHERes and coccoliths (Fig. S3e-h). In a subset of samples, collapsed coccospHERes were estimated to contribute < 21 % (min. = 0 %, average = 7.1 %) of the total number of coccospHERes. As expected, the standard error of the means (among images) drops hyperbolically with the total number of coccospHERes or coccoliths counted, whether with LM or SEM (Fig. S4), but remains higher in SEM due to the smaller size of SEM images. To check for differences between counts obtained through cross-polarized light microscopy and SEM examination, five samples with varying coccolithophore abundances were analyzed with SEM as outlined above, revealing the slopes were highly linear with R² greater than 0.9, and were not significantly different from 1, while the intercepts were close to 0 (Fig. S5), allowing for counts from the two methods to be combined.

We also propose to make similar adjustments to the next section of Methods

“2.4 Diversity of coccospHERes and detached coccoliths

Identification of coccolithophores and detached coccoliths by light microscopy is sometimes limited, which might impact the estimation of coccolithophore-derived PIC (PIC_{Cocco}) to total PIC via the estimations of coccolith and coccospHERE PIC pools. To understand this effect, taxonomic classification by SEM was performed on samples from the LowpHOX 1 cruise (2015), focusing on samples from T1 to T6 as well as selected samples from L1 (at 5 and 25 m), L2 (at 5 and 50 m) and L3 (at 5 m)...”

Similar minor adjustments to the Results as well as figure legends can also help make these points clear.

We also propose to convert section 4.2 of the Discussion into a concise consideration of the types of uncertainties and how these are dealt with. This also makes clear that we focus the central interpretation on the manuscript on messages that are robust to these uncertainties. Despite the uncertainties, we can feel we can conclude that coccolithophores are important contributors to the surface and subsurface PIC pools in this OMZ area, and the conclusion that both layers of these waters show relatively low PIC and PIC:POC ratios.

“4.2 Potential uncertainties in PIC measurements

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. Additionally, PIC becomes increasingly difficult to measure as it becomes low. A classic method is to measure total particulate carbon before and after acidification to remove PIC, but this method is relatively insensitive and problematic when the PIC:POC ratio is low. Measuring PIC by the acid soluble Ca extracted from particulate matter is much more sensitive, yet it also must be corrected for Ca from seawater which is retained on the filter by organic matter even after gentle rinsing (the Na correction). These complexities challenge remote sensing algorithms (Balch and Mitchell, 2023) or the use of PIC as a paleoproxy indicator (Beaufort et al., 2011). This emphasizes the value of microscopy counts, which have been found to be effective in quantifying PIC due to coccolithophores (D’Amario et al., 2018; Guerreiro et al., 2021; Ziveri et al., 2023).

In the northern Chilean coast, potential lithogenic PIC sources should be negligible. The exceptionally arid Atacama desert means fluvial inputs are negligible () 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.

In addition to PIC production by other planktonic organisms, which would not be detected by the microscopy protocols used here, estimates of $\text{PIC}_{\text{Cocco}}$ by microscopy also include several sources of error which can cause underestimates of $\text{PIC}_{\text{Cocco}}$ relative to $\text{PIC}_{\text{Total}}$ due to difficulties in detection of smaller coccoliths, collapsed coccospores, and fragmented coccoliths. Other sources of error relate to taxonomic and phenotypic variability in conversion factors, PIC quotas per coccolith, and estimates of the number of coccoliths per coccospore. Young and Ziveri (2000) suggested that these considerations might result in up to 50% errors in the estimation of $\text{PIC}_{\text{Cocco}}$ using microscopic methods. Despite these important limitations, $\text{PIC}_{\text{Cocco}}$ accounted for nearly half of direct $\text{PIC}_{\text{Total}}$ measurements and $\text{PIC}_{\text{Cocco}}$ values calculated from coccospore and coccolith abundances were linearly correlated with chemical measurements of $\text{PIC}_{\text{Total}}$. These results emphasize the importance of coccolithophores as contributors to total PIC pools in this OMZ and also mean that using the two methods together improves confidence in the estimation of PIC pools in the OMZ system.

The patterns of PIC detected *in situ* correlated spatially with satellite PIC estimates but there were some quantitative differences. Our dataset revealed relatively higher coccolithophore-produced PIC during November and February at the border of the Southeast Pacific “PIC-data desert” (i.e., compared to the Atlantic Ocean; see Balch et al., 2018). In this context, the weekly and monthly satellite-PIC estimations, which did not exceed $10 \mu\text{g C L}^{-1}$ (average $\sim 2.4\text{--}3.6 \mu\text{g C L}^{-1}$; Fig. 3), were above the *in situ* PIC pools by a factor of 3–5. Satellite-PIC estimates have been reported to

overestimate the PIC_{Cocco} by a factor of 2-5 across the New Zealand and Drake Passage sectors of the Southern Ocean (Saavedra-Pellitero, 2024). It has been recently stressed that these optical PIC proxies need to be geographically adjusted (Balch and Mitchell, 2023), however contrasts between satellite and *in situ* estimates of PIC can also reflect the distinct spatial and temporal scales and resolutions of the measurements, which would be reduced by increased *in situ* coverage. Given these constraints, both types of *in situ* measurements from near surface waters aligned qualitatively well with satellite measures.”

POC data: Though PIC:POC ratios are a central aspect to this study the actual POC data is not presented. The absence of the POC data is a curious omission that is not explained and weakens the study. If at all possible this data should be included and not simply alluded to.

We agree that the POC data is a critical component of our study. We had not presented it directly before as this data is previously published. However, the reviewer has convinced us that it is necessary to include presenting the data in the main text. In the updated manuscript, we incorporate the POC data into the relevant sections to address this concern.

These data, as well as PIC and POC data from other regions used to calculate the PIC:POC ratios, are publicly available and have been appropriately referenced in the manuscript to ensure transparency and accessibility.

Results: Primarily section 3.1 (but see below). There is a sense that the description of where maximum values were found emphasises Transect T1-T6 and overlooks the broader spatial distributions of parameters along Transect L1-Hyd7, particularly for PIC and coccoliths. Movement of Figure S14 into the main text may help mitigate any uncertainty caused by the unfortunate gap in data along Transect T1-T6 by providing better spatial context and allowing the authors/readers to gauge the representativeness of the in-situ data (i.e. were key spatial features, such as regions with high PIC offshore, missed by the sampling?).

We responded to this valuable suggestion by moving the satellite PIC maps into a figure in the main text, a new Fig. 3. We will now explicitly note in the Results where enhanced coccolithophore stocks and PIC pools coincided in satellite and in situ data during the 2015 and 2018 sampling, and we will add a paragraph to the Discussion to this effect (see above for proposed expansion of Section 4.2).

We agree that including Fig. S14 in the main text enhances the context of sampling for PIC and coccolithophores by situating it within the broader spatial framework provided by satellite-derived PIC.

The new Fig. 3 of satellite imagery has been refined from the original S14, and we can add details on data source and processing in Methods Section 2.2.

“Lastly, we utilize the satellite PIC as proxy of coccolithophore standing stocks (Balch and Mitchell, 2023) to produce synoptic maps on the Southeast Pacific margin. To do this, the monthly and weekly PIC climatologies (November-December 2015 and January-February 2018) were obtained from the MODIS-Aqua mission (NASA Goddard Space

Flight Center, Ocean Ecology Laboratory, and Ocean Biology Processing Group, 2022) The data were then converted from mol CaCO₃ m⁻³ to µg C L⁻¹ by multiplying by 12010 and plotted using RStudio.

”

A description to be added in **Result Section 3.1 will explain how it addresses gaps in sampling coverage:**

“During the 2015 sampling period (Fig. 3a-d), satellite PIC concentrations exhibited notable spatial variability, with peaks ($> 10 \mu\text{g C L}^{-1}$) near 20° S effectively captured by sampling locations. However, a significant latitudinal gap was observed between 25° S and 30° S, where elevated offshore PIC concentrations were missed. In 2018 (Fig. 3e-h), PIC peaks at 20° S were again well-represented by sampling. South of 20° S (up to 24° S), a gap in coverage occurred as the sampling transect extended westward, away from the coastal band of elevated PIC concentrations. Likewise, the lowest satellite PIC corresponded to relatively low PIC in surface samples (Fig. 3). Overall, our discrete sampling captured snapshots of oceanographic processes along the Southeast Pacific. Notably, PIC levels remained relatively high off ~20° S (Fig. 3), highlighting this region as a potential hotspot for coccolithophore PIC production.”

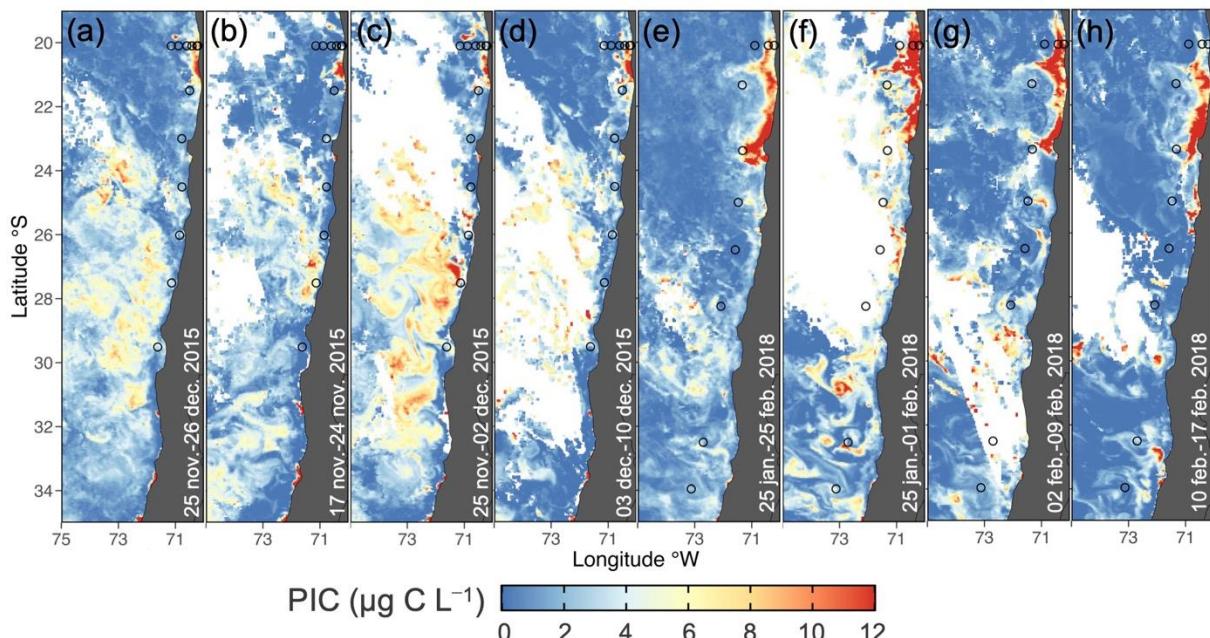


Figure 3. Monthly (a) and weekly (b-d) satellite-PIC climatologies during the LowpHOX 1 sampling (27-28 nov./05-09 dec. 2015; open circles), along with monthly (e) and weekly (f-h) satellite-PIC climatologies for the LowpHOX 2 sampling (30 ene./03-09 feb./12-13 feb. 2018; open circles).

Finally, this information will be incorporated into the Discussion, particularly in the last paragraph of the proposed expansion of section 4.2 above.

Minor comments

Page 1 Line 23: This statement appears at odds with the authors conclusion that upwelling of nutrients and generation of non-coccolithophore POC may be the cause of the low PIC:POC ratios. The link specifically to low O₂/low pH waters is therefore not quite correct.

We propose to replace these sentences of the Abstract to be clearer:

“Our findings are consistent with the prediction that the presence of a shallow OMZ in an upwelling region promotes POC production by phytoplankton other than PIC-producing coccolithophores through the injection of nutrient-rich but low pH water, decreasing PIC:POC ratios, and that the role of PIC in POC sedimentation might be decreased in such conditions. We highlight that comparing PIC in diverse upwelling conditions will be valuable to unravel how its role in POC fluxes may be affected by low pH and low O₂ conditions.”

P3 L79: Please specify the Copernicus product used for Kd490

Thank you for pointing this out. The Copernicus specific product citation will be included in the updated manuscript.

P3 L83: The definition of the OMZ core was not clear to me and use of the maximum O₂ concentration seems counterintuitive when defining the core of the oxygen minimum feature. Please check definition and expand how it was defined.

We appreciate the Referee’s comments and the opportunity to clarify the definition of the OMZ core in our study.

We can replace the actual percentage-based approach with 20 $\mu\text{mol kg}^{-1}$ fixed threshold, which frequently used. This results in a very minor update of the figures.

“Lastly, the OMZ core was defined as the water layer where oxygen concentrations fell below a threshold of 20 $\mu\text{mol kg}^{-1}$. The upper and lower boundaries delimiting the OMZ core (Fig. 1b) are consistent with those discussed by Vargas et al. (2021) during the same cruises.”

P3 L91: Please quantify the magnitude of the applied correction made to PIC measurements for Na residues. It is unclear if this is significant.

We appreciate the Referee’s comment regarding the correction for Na residues in PIC measurements. In our study, Ca from residual seawater was estimated from Na and subtracted. This calculation indicated that residual seawater contributed on average 29.1% \pm 25.8% of total Ca in LowpHOx 1 samples and on average 35.2% \pm 21.5% of total Ca in LowpHOx 2 samples.

It is important to note that, to the best of our knowledge, corrections for Na residues in PIC measurements have not been explicitly reported in the previous studies we compare to. Nevertheless, applying this correction aligns with the general practice in carbonate chemistry to mitigate contamination and improve the reliability of the results (e.g., procedures described in the GEOTRACES program and other inorganic carbon measurement guidelines).

As mentioned above, we offer above a modified section 4.2 of the Discussion where we consider all the different sources of uncertainty in PIC measurements, whether by chemical or microscopic estimates.

P3 L92: Typo, presumably this should be 'PIC concentrations' not 'calculations'

Yes, thank you. We will correct it in the revised manuscript.

P4 L94/95: The terms 'built' and 'building' seem inappropriate and the intention here is unclear. Please rephrase.

Thank you for pointing this out! We will rephrase it in the revised manuscript.

P4 L96: Direct reference to assessing the effect of this OMZ on POC concentrations suggests that the POC data are central to this study and therefore should be presented alongside the PIC data.

We propose to include this now.

P4 L97-99: What criteria were used to define the depth intervals used in this study? As the subsurface interval (5-100 m) presumably crosses the mixed layer there are strong gradients to consider in the distribution of both PIC and coccolithophore diversity which may be lost by the depth bins used.

The original depth bins used in the dataset were 0–5 m, 5–100 m, and 100–500 m. These finer intervals were initially designed to capture vertical gradients, including those within the mixed layer and deeper subsurface layers. However, now we re-binned the data from the global comparison into broader intervals of 0–100 m and 100–400 m. These new intervals were specifically chosen to be comparable with the “above” and “within” categories relative to the OMZ. We recognize that this approach may result in the loss of some fine-scale gradients, particularly in the mixed layer (which typically ranged between 50 and 100 m depth in the other publicly available datasets). Nevertheless, the re-binned depth intervals allow for more simple comparisons of broader ecological patterns across OMZ and non-OMZ regions. We will explicitly outline the bin choice criteria at the end of Section 2.2, as well as provide a discussion of the potential trade-offs involved in this binning approach and its implications for interpreting PIC, POC, and PIC:POC ratios as follows:

“These ratios were then categorized into two groups: above and within the OMZ core, to assess the influence of the OMZ on PIC and POC concentrations. These ratios were plotted against those reported for other open ocean or coastal margins (see Balch et al., 2018). To this end, PIC and POC data was obtained from the SEABASS (Werdell

et al., 2003) and BCO-DMO repositories (Balch, 2010), and PIC:POC ratios were binned for the well-mixed surface (0-100 m depth) and the stable sub-surface layer (100-400 m). These depth intervals were specifically chosen to be roughly comparable with the above and below categories relative to the OMZ, providing a consistent framework for direct comparisons of broader ecological patterns across OMZ and non-OMZ regions.”

Figure 1: The figure legend needs a better description of what the black and grey lines actually represent (the odd placement of the plot legend to the right of panel b was initially overlooked). The black and grey lines need to be better distinguished either by changing the line style or line thickness.

Thank you for pointing this out. We have updated the figure legend and ensure the threshold enclosing the OMZ core are most clearly delineated by using thicker white lines for improved visualization (see below).

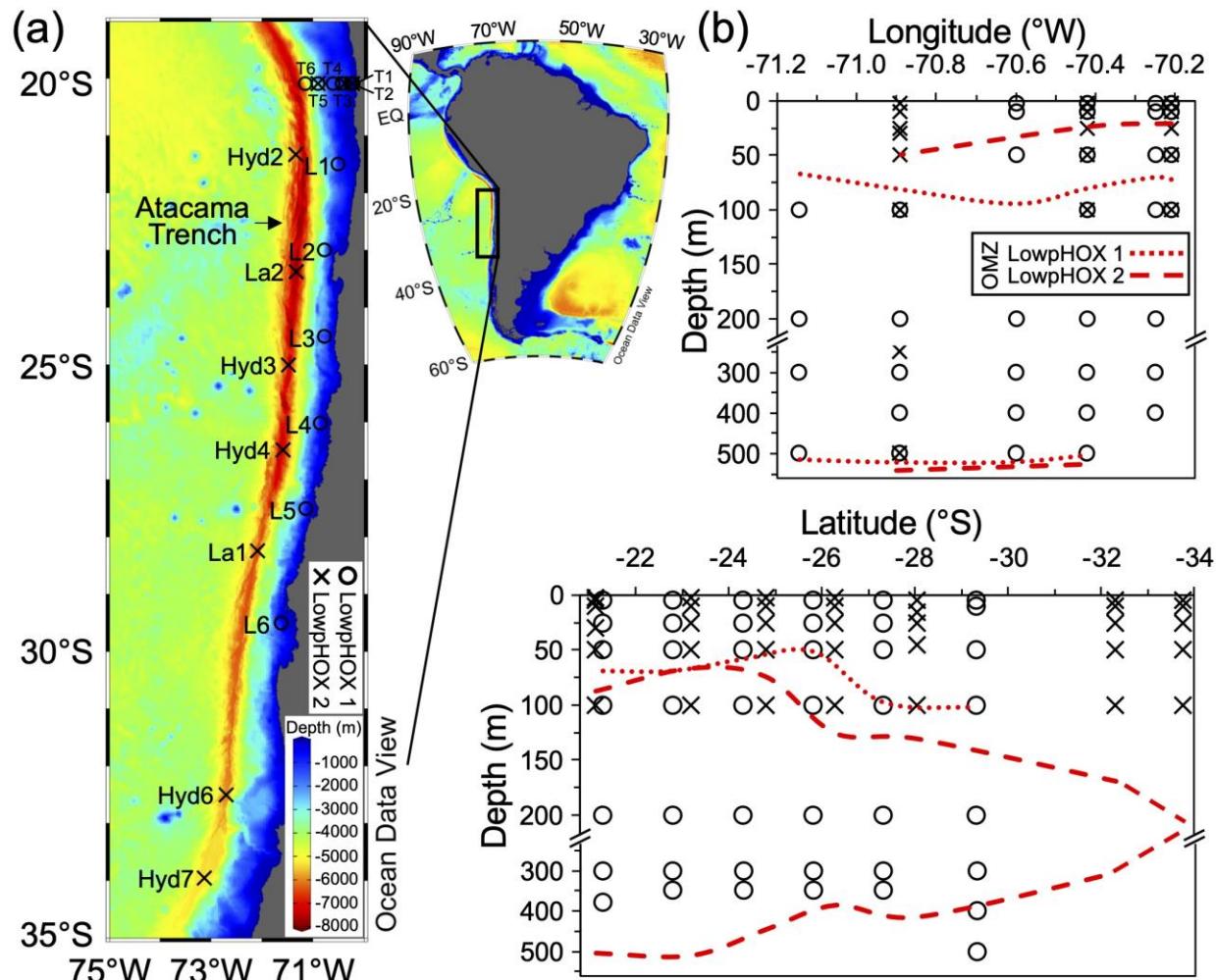


Figure 1: (a) Map of the Southeast Pacific margin showing the study site and stations sampled during late-spring 2015 (circles) and mid-summer 2018 (crosses). (b) Sampling depth coverage for coccolithophores, highlighting areas crossing the

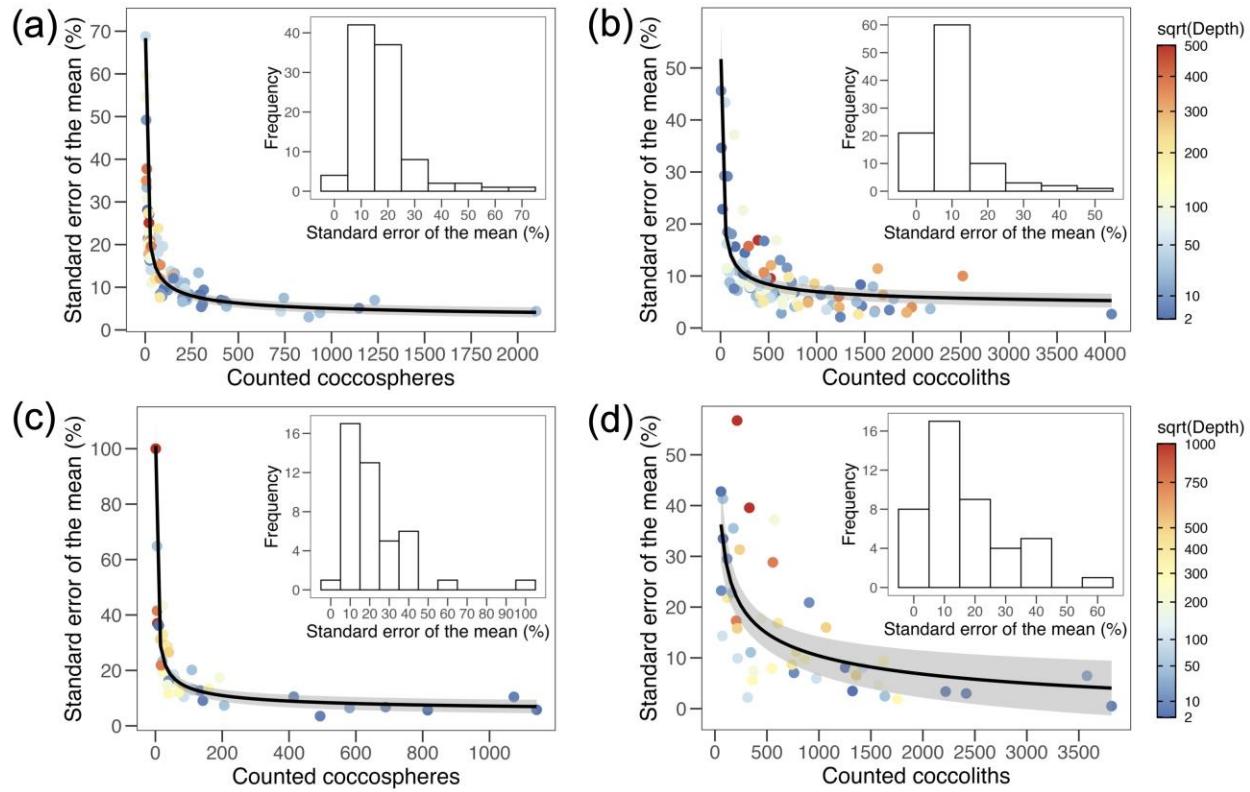
thresholds enclosing the OMZ core (dotted and dashed red lines). Map produced by Ocean Data View (Schlitzer, 2024), with bathymetry based on the GEBCO chart (GEBCO, 2023).

P5 L116/L126: Figure S4b implies a near constant bias exists between light microscopy and SEM methods when estimating coccolith abundances. As the identity of the two axes in Figure S4 are unclear I am presuming that the SEM counts are on the y-axis (and LM counts on the x-axis) in which case SEM coccolith counts are higher than LM counts, even though the general relationship between the two methods is linear. How significant is this bias and what does it mean for the results of this study? (particularly coccolith counts for stations T1-T6 given the reliance upon SEM; Line 116)

We fix Fig. S4 (now S5) so that the axes are labelled to make clear which are SEM counts, and which are light microscopy counts. We also now report the standard errors of the slopes and the intercepts. In all cases, the slopes were highly linear with R^2 greater than 0.9, and were not significantly different from 1, while the intercepts were close to 0. In the case of detached coccoliths, there was a significant difference in the intercept, but it was very minor and would not result in any significant changes to PIC estimates.

We relied on polarized light LM counts for almost all absolute counts, because one can cover much more of each sample for a lower cost in time as well as minimizing expensive SEM time. However, the comparison of LM to SEM counts was only necessary because a small number of samples could not be counted by LM, and we could only count with SEM, due to filters that were very bright in polarized light, for reasons we could not determine.

We also complement with a new analysis of the error from counting effort. For both LM and SEM, we compute the standard error of the mean (SE in %) among different fields of view vs the total number of coccospores or coccoliths counted (considering all fields). As expected, the SE drops hyperbolically with the total number of coccospores or coccoliths counted. The number of fields counted was constant at 20 and 11 for coccospores and detached coccoliths in LM and between 27-48 and 2-5 for coccospores and detached coccoliths in SEM depending on magnification, respectively, allowing us to estimate the expected error of counts within each sample, and we report this error as a percentage in the new Supplementary Figure S4, with actual Fig. S4 becoming S5.



Relationship between the counted coccospores and detached cocoliths with the standard error of the mean (SE in %) of counts performed using cross-polarized light microscopy (a-b) and scanning electron microscopy (c-d). Inset histograms show the frequency distribution of SE values. The solid black line represents the fitted hyperbolic curve, with shaded grey areas indicating the 95% confidence intervals. Counts and SEs showed a significant hyperbolic relationship for coccospores (a): equation; $R^2_{\text{adjusted}} = 0.89$; $p\text{-value}_{\text{slope, constant}} < 0.05$, and detached cocoliths obtained with cross-polarized light microscopy (b): equation; $R^2_{\text{adjusted}} = 0.59$; $p\text{-value}_{\text{slope, constant}} < 0.05$, as well as, coccospores (c): equation; $R^2_{\text{adjusted}} = 0.89$; $p\text{-value}_{\text{slope, constant}} < 0.05$, and detached cocoliths obtained with scanning electron microscopy (d): equation; $R^2_{\text{adjusted}} = 0.42$; $p\text{-value}_{\text{slope}} < 0.05$.

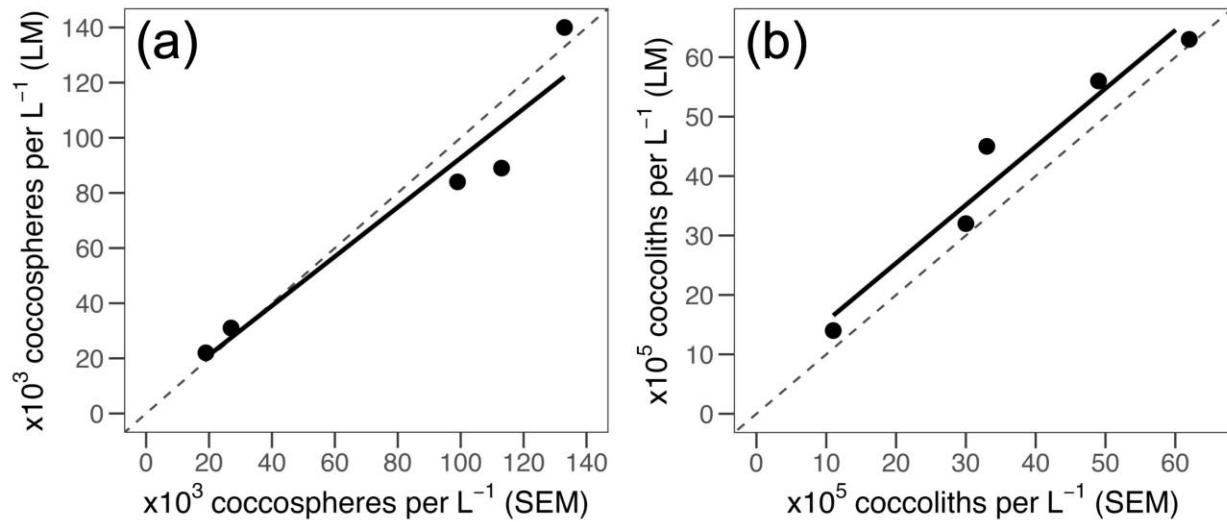


Figure S4. Linear relationships for coccospore counts (a) and detached coccoliths counts (b) obtained through scanning electron microscopy (SEM) and cross-polarized light microscopy (LM). The dotted line represents the 1:1 ratio, while the solid black line represents the lineal trend. SEM and LM approaches showed a significant correlation for coccospores (a): equation $Y=0.899(\pm 0.143)X+3.121(\pm 13.038)$; $R^2_{\text{adjusted}} = 0.91$; $p\text{-value}_{\text{slope}} < 0.05$, as well as for detached coccoliths counts (b): equation $Y=0.979(\pm 0.134)X+5.760(\pm 5.478)$; $R^2_{\text{adjusted}} = 0.93$; $p\text{-value}_{\text{slope}} < 0.05$.

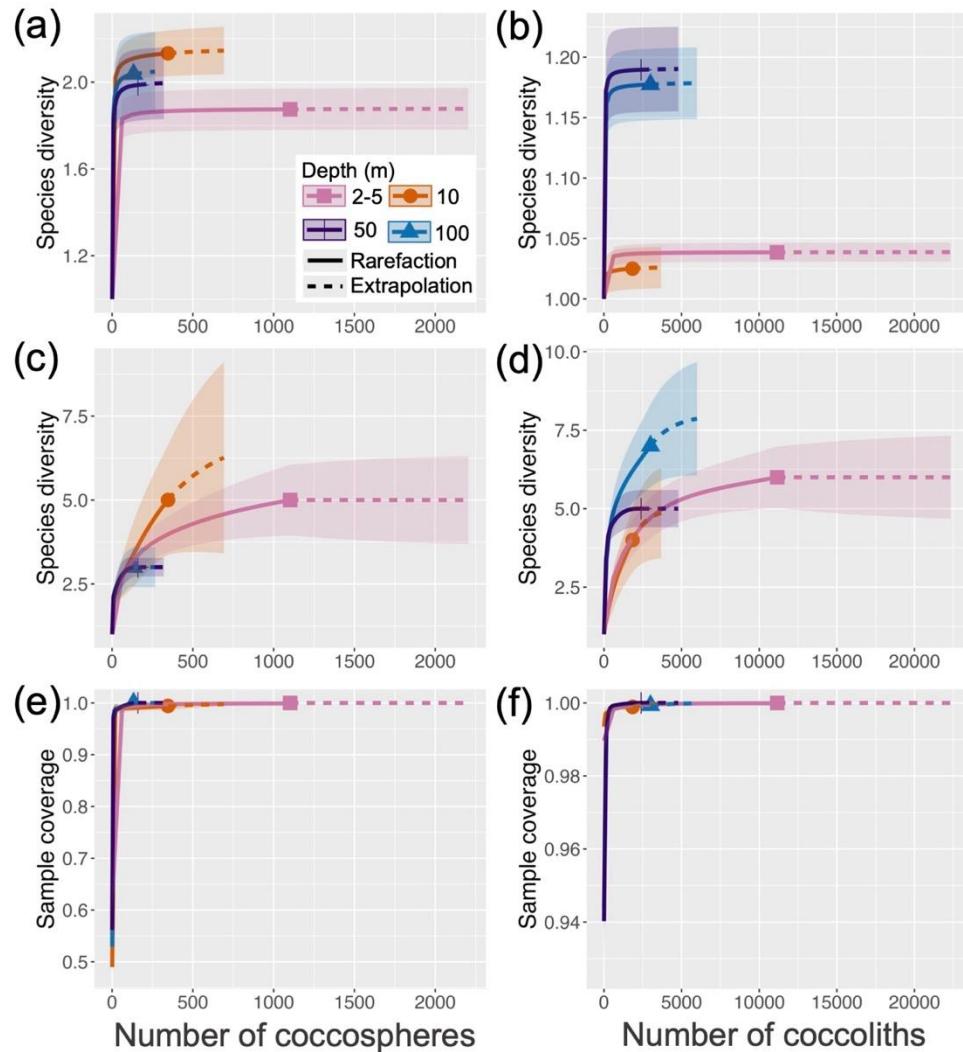
P5 L129-133: The approach used to estimate absolute abundances of species/genus coccospores and coccoliths from SEM images is a little unclear. Based on counts from a low number of images (magnification not reported) I do not understand the rational for multiplying the counts by the total coccospore or coccolith abundances to obtain total species counts particularly if there are biases between LM and SEM coccolith counts (mainly applicable to stations L1, L2 and L3). Should not the same approach as described in section 2.3 be used i.e. the equation in Diaz-Rosas et al 2021 thereby accounting for volumetric factors? I am concerned that there could be a scaling error here resulting from the use of mixed methodologies. Please clarify.

While SEM provides higher-resolution imaging, its operational costs are substantial, so total counts (for absolute abundances of total coccolithophores and coccoliths) are more efficiently made by LM. Therefore, the main use of SEM was to obtain relative abundances of the principal taxa with higher resolution, exactly the strategy followed in Diaz-Rosas et al. 2021 (again, a separate protocol for the SEM was used for counting a small number of samples where the filters were too bright under cross-polarized light to permit LM counts – we could not determine the source of that high brightness as they looked the same as other samples under SEM).

The principal difference with Diaz-Rosas et al. 2021, where we only analyzed epipelagic samples, was that here we analyzed mesopelagic samples, which are much more dilute in phytoplankton. Even with the maximum volumes of water we could filter before filters clogged, filters of deep samples had much sparser coccospores and coccoliths compared to surface samples. As a result, we had to

be much more careful with SEM time, and this obligated us to work to obtain a minimum sample coverage rather than a minimum number of coccospores analyzed for the SEM analysis. On average, 165 coccospores per sample were analyzed by SEM, but in some deeper samples, the numbers were much lower (the range was 1 to 1140 coccospores). When samples from different stations are grouped by depth layer, rarefaction analysis shows that we still had a decent view of total diversity from the subsurface layers. The main conclusions of this paper are based on estimating the pools of PIC and coccolithophore-derived PIC in the surface and subsurface layers of an OMZ region, because spatial variability in the subsurface waters is more difficult to sample for these reasons.

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; Díaz-Rosas et al. 2021). The dominance of *G. huxleyi* coccospores in absolute coccospores counts allowed us to reduce SEM image re-analysis for species/genus classification by approximately 25%, while maintaining the same effort for coccolith counts.



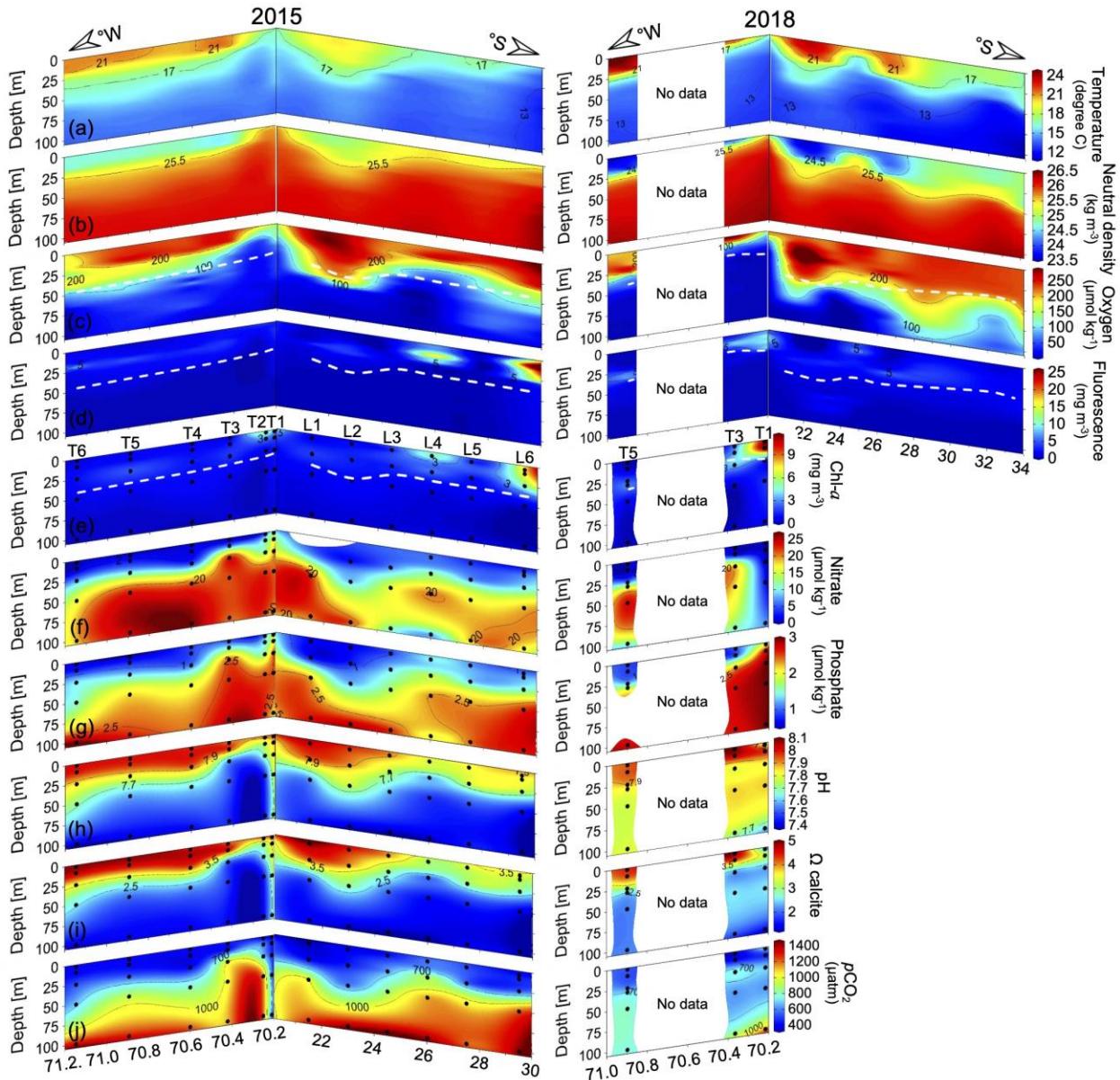
Rarefaction-extrapolation analysis for late-spring 2015 and mid-summer 2018, showing species richness (a-b), the exponential of Shannon entropy (c-d), and sample completeness (e-f) for coccospores and detached cocoliths observed at 2-5, 10, 50, and 100 m. Each curve includes 95 % confidence intervals.

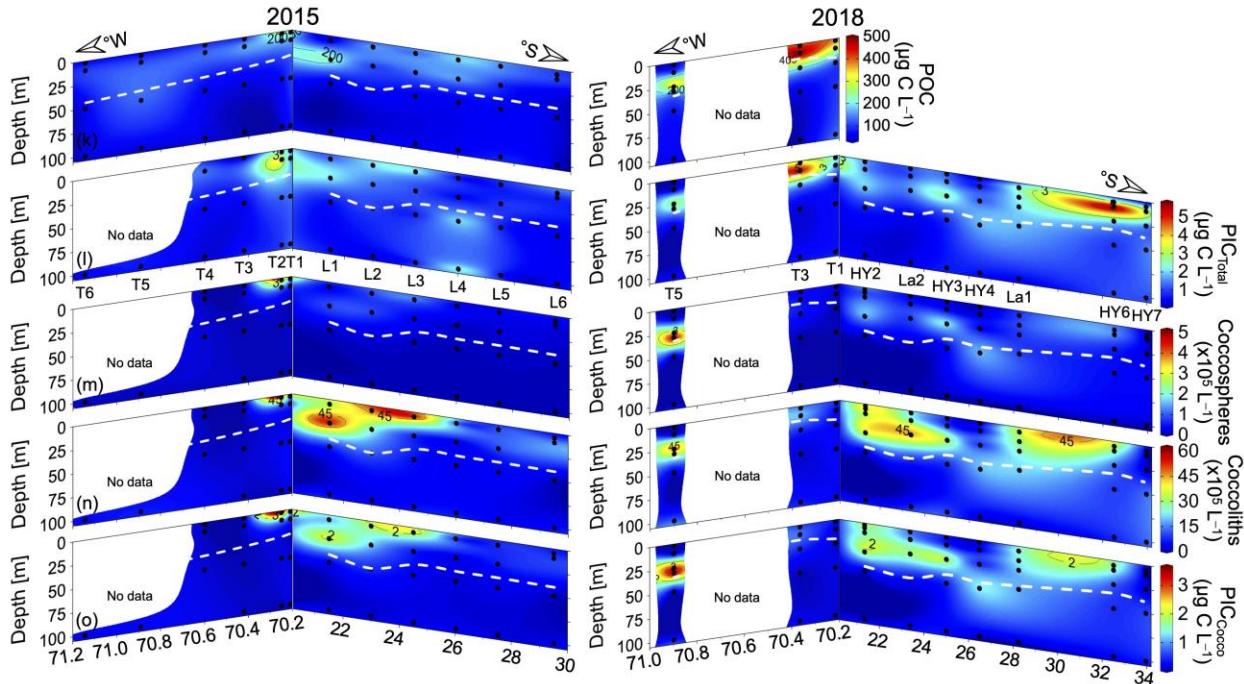
P5 L137: There is ambiguity in the methodology over whether species/genus level counts were obtained from SEM images and individual species/genus PIC quotas calculated or whether a size-class based approach was used with a mean size based conversion factor.

We can make more clear that the larger spatial and temporal study including samples from both 2015 and 2018 was based on LM images, whereas the focused approach only using 2015 samples used SEM to be able to better assess taxonomic composition and in particular whether the taxa contributing to PIC pools in the surface layer differed from the taxa contributing to PIC pools in the subsurface OMZ core.

Figure 2: This is a complex figure to understand which is made harder by the splitting of this figure across 2 pages. The separation of station names (along top of panel 2e) and the lat/lon (along bottom of panel 2n) is unfortunate and this information really needs to be present on both pages to help the reader. Other issues are the difficulty in seeing the white dashed line (euphotic depth) in all panels. Given the reliance upon POC data, why is there no POC section in Figure 2? Key contours or features cited in text should be more clearly visible.

Thank you for your feedback. To enhance visualization, we offer a modified figure (now Fig. 2, which extends across two pages) where we ensure that both parts include latitude/longitude coordinates and station labels. The euphotic zone is made more prominent by using white thicker lines, and additional panels for POC will be included in the second part to enable direct comparison with PIC. The contours will be simplified to those most discussed in the text. However, we are unable to adjust the contours line width, as the functionality does not appear to be fully supported by Ocean Data View (version 5.8.0).





P9 L184-196: There are several unclear statements here that can be clarified. For example i) L185 near surface waters <25m vs L192 surface waters 0-30m; ii) L187 highest PICtotal in 2018 was 5.3 mmol/m3 vs L189 highest PIC in 2018 was 5.86 mmol/m3; iii) apparent bias in emphasising max PICtotal in 2018 as being along Line T1-T6 when station Hyd6 had a higher concentration. Overall, this paragraph was a little muddled and unclear and lacking detail or precision in the reporting of results.

Thank you for your feedback. We will update the paragraph to clarify the depth ranges, address the discrepancy in the reported PIC_{Total} values, and highlight the peak in PIC_{Total} observed at station Hyd6 during 2018.

P10 L220: It is not clear where the value of 67% was derived from. I do not see this in Figure 5c?

We will correct the text to clarify that the detached coccolith fraction represents 63% of the total accounted PIC_{Total} within 100 m depth, based on the updated calculation (30 x 100 / 48 = 63%). The previously mentioned value of 67%, which referred to all samples, has been replaced with the value specific to the 100 m depth.

Figure 3: Panel b gives the impression of monospecific coccolith distributions at many sampled depths due to the approach used of grouping all liths <4um together. This is a limitation that needs to be briefly addressed in the discussion.

The dominance of the detached coccoliths pool by *G. huxleyi* was confirmed during the analysis of lower magnification SEM images and was also confirmed in selected higher magnification images. The inclusion of smaller coccoliths into the $<4\text{ }\mu\text{m}$ category reflects this observation, although their contribution is likely negligible, and the category could essentially be referred to as '*G. huxleyi*'. To address this more explicitly, we will add the following sentence to the Results Section *Diversity of coccospores and detached coccoliths*:

"Despite the presence of *G. parvula/ericsonii*, its small coccoliths were underrepresented, reinforcing the overwhelming prevalence of *G. huxleyi* in the samples"

Figure 4: Figure 4a arguably reproduces some of the data contoured in Figure 2k-n, and presented in Figure S5 so the figures could be simplified. Also, I understand $\text{PIC}_{\text{Cocco}}$ to be a bulk term representing the total contribution to PIC by both coccospores and coccolith PIC, thus it seems wrong to present the contribution of spheres and liths to $\text{PIC}_{\text{Cocco}}$ in two subplots with axes reaching 100% in both (fig 4b & 4c). How can both axes be correct when panel 4a indicates a changing contribution by both coccospores and liths to $\text{PIC}_{\text{Cocco}}$? (evident for station T1, T2 but an unclear contribution by coccospores for T3-T6).

We appreciate the Referee's concern regarding potential redundancy between the $\text{PIC}_{\text{Cocco}}$ values presented in Figures 4a, 2k-n, and S5, as well as the decomposition of the $\text{PIC}_{\text{Cocco}}$ pool in Figures 4b and 4c. First, it's important to note that the $\text{PIC}_{\text{Cocco}}$ values in Figures 4b and 4c are based on species/genus-level conversion factors, whereas the $\text{PIC}_{\text{Cocco}}$ values in Figures 2k-n and S5 (as well as related Figures 5, 6d and 7c) are derived using the *G. huxleyi* conversion factor as a maximum threshold. This distinction is outlined at the end of the Methods. Second, we clarify that Figures 4b and 4c presents the relative contributions of coccospores and detached coccoliths to the $\text{PIC}_{\text{Cocco}}$ pool, expressed as percentages of the total $\text{PIC}_{\text{Cocco}}$ pool. These percentages are calculated within the $\text{PIC}_{\text{Cocco}}$ fraction and do not represent absolute contributions to the $\text{PIC}_{\text{Total}}$ pool. We will update the figure caption to reflect this distinction:

"Figure 4: Estimated PIC masses from coccospores and detached coccoliths recorded in waters off Iquique (~ 20° S) during late-spring 2015. (a) Contribution of coccospores and detached coccoliths to the total $\text{PIC}_{\text{Cocco}}$ pool. (b) Taxonomic breakdown of the relative contribution of coccospores (b) and detached coccoliths to $\text{PIC}_{\text{Cocco}}$ quotas (c), expressed as percentages of the total $\text{PIC}_{\text{Cocco}}$ pool."

It is important to highlight that the figures in question are primarily aimed at describing the diversity of coccospores and coccoliths allocated to the total $\text{PIC}_{\text{Cocco}}$ pool. These figures provide essential context for understanding the taxonomic composition of the coccolithophore community and their contributions to PIC. However, to ensure consistency across the dataset, the PIC estimation was extended to all samples using the conversion factor of the most abundant species (*G. huxleyi*). While some samples have species/genus-level resolution, this generalized approach allowed for a robust estimation of the maximum potential PIC contribution.

P13 L235: Typo in legend of figure 5 (concentsdaration)

This will be correct in the updated manuscript.

P13 L238: The phrase 'marginally higher' is ambiguous without a quantified value or statistical support. Is the difference significant?

We can be more clear. There were no statistical differences between the coccospheres, detached coccoliths, PIC_{Total}, and PIC_{Cocco} across the 2015 and 2018 cruises although peaks are notable in 2018. We will revise the text as follows: "Coccospheres, detached coccoliths, PIC pools, and the estimated PIC_{Cocco} quotas were not statistically different ($p > 0.05$) between the 2015 and 2018 cruises, although there is a slight observable difference in the maximum values, with mid-summer 2018 showing higher peaks compared to late-spring 2015 (Fig. 6)."

P13 L245: Typo, panel 2c,g ? not 2h?

Thank you for your comment. We will double-check these panels references are accurate as per the panels shown.

P13 L249: Typo, panel 2e-f, not 2f-g?

We appreciate your careful attention. The missing "j" for pCO₂ panel will be add in the updated manuscript.

P15 L82: Can remove approximation by stating actual results (45-48%)

We appreciate the suggestion and agree that providing exact values can enhance clarity. However, since these values are explicitly detailed in the Results section, we believe that rounding to approximate percentages in the Discussion helps to convey a more concise and accessible message. This approach aligns with the purpose of the Discussion section, which is to synthesize the key findings.

P16 L303: Would be useful to state the ratios from Balch et al 1991, Holligan et al 1993b that were used in this comparison.

We agree. We will include a detached-coccolith-to-coccospHERE ratio of > 250, which encompasses values from both studies.

P16 L303/L306/L343/L411: No need to abbreviate maximum to max.

We will replace 'max' with 'maximum' throughout the text.

P16 L305: It is not clear where the stated values of cell-attached coccolith contribution to PIC_{Cocco} (51-72%) come from. Please clarify and highlight in the results.

Thank you for raising this point. To clarify, the stated values for the cell-attached coccolith contribution to PIC_{Cocco} were calculated from the same samples used for the detached-coccolith-to-coccospHERE ratio analysis mentioned in the preceding sentence. This represents the percentage of the total PIC_{Cocco} quota accounted for by coccospHERes. However, since this analysis provides an interpretative perspective rather than presenting raw observational data, we chose to include it in the Discussion rather than in Results.

To enhance clarity and directly address your concern, we will add "for these specific samples" in the Discussion to emphasize that these values correspond to the same samples used in the detached-coccolith-to-coccospHERE ratio calculations. This ensures transparency while maintaining the logical flow of the manuscript.

P17 L332: Missing appropriate references (for Calcite Belt, Bay of Biscay)

We will add the appropriate references for the studies conducted in the Calcite Belt and the Bay of Biscay.

P19 L373: From Figure 5c I do not see how the statement that up to two-thirds of the PIC_cococ quota comes from detached coccoliths can be correct? Please clarify

Thank you for your concern, which is similar to a previous point raised. The value provided represents an approximation of the percentage of the total accounted PIC_{Total} (48%) contributed by the detached coccolith fraction (30%) within the 100 m depth range. Thus, $30 \times 100 / 48 = 63\%$, which accounts approximately two-thirds of the total PIC_{Cocco}. We will clarify this in the updated manuscript.

P20 L388: The observation that the PIC:POC ratio (Figure 8c) is greatly reduced compared to other areas is intriguing despite the comparable PIC standing stocks (Figure 8b). Without more detail on the coincident POC dataset however it is difficult to rationalise this observation beyond the suggestion put forward by the authors that upwelling stimulates non-calcareous phytoplankton. For this reason, the authors should consider including the POC dataset in this study. It may be particularly important to ascertain the similarity or differences in POC concentrations between the various studies/sites used for comparison to validate the conclusions reached. Also, the PIC:POC results appear most comparable to results from the W. Arctic, which is not an upwelling zone. This point needs to be highlighted. What could be the cause of this similarity?

We appreciate your concern regarding the accuracy of the PIC:POC ratio analysis. During the preparation of the POC data for plotting, we identified an error in the PIC values for the OMZ dataset. Specifically, the PIC values were incorrectly expressed in mmol C m^{-3} instead of $\mu\text{g C L}^{-1}$, resulting in disproportionately high PIC levels. This issue has now been corrected, and the POC data have also been added. We present an updated version of Figure 8, which includes the corrected POC data, the newly added POC data, and revised binning of the external datasets (see below). The findings derived from Figure 8 will be updated in the respective sections of the manuscript.

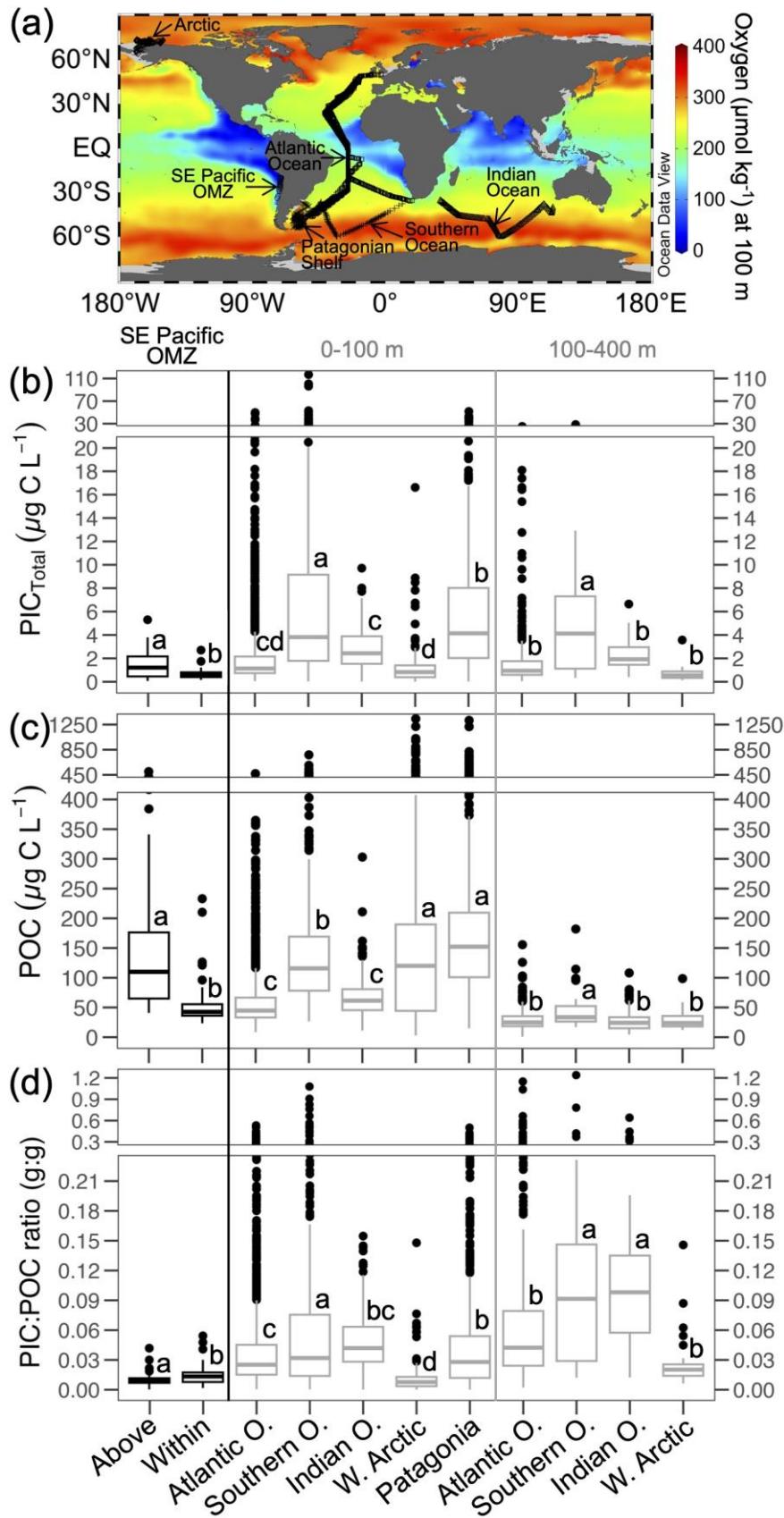


Figure 8: (a) Global map showing the annual oxygen content at 100 m depth and the sampling locations for (b) PIC_{Total}, (c) POC, and (d) PIC:POC ratios above and within the OMZ-core during late-spring 2015 and mid-summer 2018 in the SE Pacific (this study). Additional data represent the well-mixed surface (0-100 m) and the stable sub-surface layer (100-400 m) from other open ocean or coastal margin regions (data from Balch et al., 2018). The Atlantic Ocean dataset includes samples from six cruises (AMT17-22). One-way ANOVA results indicate significant differences ($p < 0.05$) in PIC_{Total}, POC, and PIC:POC ratios among SE Pacific OMZ-core depths, as well as across sample groups from the Atlantic Ocean, Southern Ocean, Indian Ocean, Western Arctic, and Patagonian Shelf (Atlantic). Tukey post-hoc comparisons are represented by lowercase letters above each boxplot. Only sample groups with more than 20 data points for both PIC and POC were included in the analysis. The map was generated using Ocean Data View (Schlitzer, 2024), with oxygen climatology based on the World Ocean Atlas 2018 (Boyer et al., 2018; García et al., 2018).

Regarding the comparable PIC:POC ratios, and the now observed low PIC and high POC levels between the Western Arctic and SE Pacific OMZ (Fig. 8), upwelling events in the Western Arctic significantly influence the distribution and composition of POC and PIC. These upwelling events, primarily driven by strong northeasterly winds, facilitate the vertical movement of nutrient-rich waters from deeper layers to the surface, particularly in regions like Barrow Canyon and the Chukchi Sea (see Li et al., 2022, and references therein). These events support high biological productivity, including diatom-dominated phytoplankton communities. At the same time, coccolithophore penetration of the Arctic is known to be limited (e.g. Winter et al. 2014). As a result, the low PIC and high POC levels observed in the Western Arctic are notably lower in PIC and higher in POC compared to other open ocean and coastal margin regions.

We propose to address this in the Discussion. Proposed text to a revised subsection “4.3 Surface variation in coccolithophores and PIC pools”:

“Although the periods sampled in this study were characterized by stratified summer water column conditions which may be conducive to coccolithophore growth (e.g., Matson et al., 2019), it is noteworthy that this zone, characterized by exceptionally low pH in sub-surface waters frequently brought to the surface by upwelling, does exhibit a tendency to lower PIC compared other ocean regions (Fig. 9a-b). The prominent exception was the Western Arctic Chukchi Sea, which also was reported to have lower PIC pools. Coccolithophores are known to have limited penetration of Arctic waters (e.g., Winter et al. 2014), and the Chukchi Sea is a site of high diatom productivity due to upwelling (Li et al. 2022). With the caveat that data from upwelling regions are still limited globally, these results are consistent with the prediction that the intrusion of nutrient-rich but low pH waters into the surface stimulates POC production from other phytoplankton (e.g., diatoms) while repressing coccolithophores. ”

Proposed text to a revised subsection “4.4 Subsurface variation in PIC and PIC:POC ratios”:

“Nevertheless, the PIC:POC ratios were significantly lower than those observed in other open-ocean and coastal sites in both surface and subsurface waters (Fig. 9d). The prominent exception was the Western Arctic Chukchi Sea, as discussed above. These findings, while emphasize that comparing diverse upwelling systems will be essential to resolving the role of coccolithophore PIC in POC fluxes and potential interactions with pH/low O₂ waters, are consistent with the prediction that PIC may play a lower role in POC fluxes in OMZ conditions.”

P22 L413: Conclusion 6 seems to contradict statements on P19 L379. On P19 is the statement that the PIC:POC ratio was significantly elevated in the OMZ core (due to a ballast effect), whereas on L413 the (relatively) low PIC:POC ratio of the OMZ core is highlighted for its difference to other coastal margin areas. Whilst the overall conclusion that this OMZ exhibits lower PIC:POC ratios compared to other locations is valid, it also seems that when examined in detail the core of this OMZ is associated with elevated PIC:POC ratios (Figure 8c), thus the broader significance of this could be addressed in section 4.4.

Thank you for your feedback. We will update Conclusion 6 to incorporate the new findings on PIC:POC ratios as detailed in the minor comment above.

Table S1: Are the units for PIC concentration incorrect? (i.e. uM not mM?)

Yes, thank you. We have corrected the units to $\mu\text{g C L}^{-1}$.

Figure S10: The SEM images have reproduced poorly in my copy. Maybe upload the SEM image files separately to allow greater accessibility. Missing word in Figure legend.

All scanning electron microscopy images used in this study have been stored in the Zenodo public repository. This dataset is referenced as:

Díaz-Rosas, F., Vargas, C. A., and von Dassow, P.: Scanning Electron Microscopy Datasets – Coccospores and detached coccoliths in waters off the Southeast Pacific margin, <https://doi.org/10.5281/zenodo.14048319>, 2024.

It is publicly available and so we consider it most efficient to share these images by that method. We also upload here separately a high resolution version specifically of what is now Fig. S11.

The legend has been corrected to:

“Figure S11. Example scanning electron microscopy images showing diverse coccolithophore and diatom assemblages in 2015 station T1 at 2 m depth (a), the dominance of the coccolithophore component by coccospores and detached-coccoliths of *G. huxleyi* during a diatom bloom in 2015 station L2 at 5 m depth (b), and example low biomass conditions in 2015 station L3 at 5 m depth (c). Each 800x frame corresponds to 0.2 mm².”

Figure S11: These LM images also reproduced poorly. Maybe upload the image files as well.

All cross-polarized light microscopy images shown in this figure have been stored in the Zenodo public repository. This dataset is referenced as:

Díaz-Rosas, F.: Cross-polarized light microscopy images – Coccospores and detached coccoliths in waters off the Southeast Pacific margin, <https://doi.org/10.5281/zenodo.14708540>, 2025.

It is publicly available. We also upload here separately a high resolution version specifically of what is now Fig. S12.

Figure S14: I find this figure to be a useful means of assessing the spatial variability of PIC during both cruise periods. I would encourage the authors to consider moving this figure into the main text as it provides useful context.

Thank you for the feedback, which has been addressed in the major comment above.