

## **EGUSPHERE-2025-5902**

### **Point-by-point response to reviewers' comments**

#### **New insights into the primary production and the structure of the phytoplankton community in the South Indian Ocean using size fractionation experiments**

Valentin Deteix, Céline Ridame, Céline Dimier,  
Claire Lo Monaco, Aline Tribollet, Frédéric Planchon

*EGUSPHERE-2025-5902 Research article*

#### **Reviewer #1 – Andres Gutiérrez Rodríguez**

We would like first to thank the reviewers for their relevant comments and suggestions which helped us to improve our manuscript. Reviewer comments are in black font, and responses are in blue font. Figures that have been modified in the revised manuscript were added to this document. Below are the authors' point-by-point responses to Andres Gutiérrez Rodríguez's comments and questions.

#### **General comments:**

Deteix et al preprint's investigates spatial variability of phytoplankton community structure and net primary productivity across different zones and regions of the Indian Ocean using size fractionated net primary production and size-fractionated pigment-based community composition. This is a powerful approach rarely used, in general and specifically in the Indian Ocean, which together with the adopted vertically-resolved strategy generates a novel dataset with potential to shed light on the relationship between phytoplankton community composition and net primary production. Yet, I find at times that the preprint does not fully exploit the potential of the data and lacks discussion of certain aspects, such as growth rates and C:Chla and reported size and taxonomic dependent differences, that are critical for the interpretation of the data and conclusions about the linkage between Chla biomass and NPP. While similar data from the Indian Ocean are scarce or absent (see Landry et al. 2022), studies containing bulk community, size- and taxon-specific data on Chla biomass, NPP and growth rates conducted in analogous oceanographic zones and biogeochemical conditions in other ocean basins are missing and improve the discussion.

The authors would like to thank Andres Gutiérrez Rodríguez for his insightful feedback, relevant suggestions and time spent reviewing this manuscript. All comments were carefully considered, and the issues raised have been addressed We believe that these revisions have significantly improved the clarity of the manuscript.

#### **Specific comments:**

1. The preprint builds on the analysis of the relationship between size-fractionated and taxon-specific Chla partition to assess the influence of phytoplankton size structure and composition on NPP. Yet, C:Chla and growth rates, and their size and taxonomic dependence can alter this relationship and

the conclusions inferred. The influence of differences in C:Chla and intrinsic growth rates between phytoplankton size and taxonomic groups needs to be discussed with regards to the links between primary production and phytoplankton (Chla) biomass structure. Some studies investigating this bulk and group-specific variability that could help improve the discussion in this regard are listed below Strom et al. 1991; Landry et al. 1993; Arin et al. 2002; Behrenfeld et al. 2005; Sathyendranath et al. 2009; Latasa et al. 2014; Jakobsen and Markager 2016; Liefer et al. 2018; Marañón et al. 2021; Landry et al 2022; Gutierrez-Rodríguez et al. 2023; Yingling et al., 2025.

Thank you for this comment and your relevant insight. We have modified the paragraph, in section 4.3, by adding several references and by emphasizing the importance of considering C:TChla ratios and growth rates in future studies when investigating the relationships between NPP and phytoplankton size structure and composition. The following additions and modifications (in bold) have now been included in the manuscript:

“Therefore, our findings contribute to a better understanding of the role of phytoplankton community size structure in modulating primary production in the **South Indian Ocean**, highlighting that NPP was influenced by the phytoplankton size structure and was not necessarily driven by a single dominant phytoplankton group within a given zone. **Nevertheless, the relationship between NPP and phytoplankton TChla biomass size structure should be interpreted with caution, as it is influenced by ecological and physiological factors such as the carbon to TChla ratio (C:TChla) and the growth rates. For instance, C:TChla ratio in phytoplankton varies with temperature, irradiance and the degree of nutrient limitation, being the lowest under high temperature, low irradiance and nutrient-replete conditions (Geider, 1987; Geider et al., 1997; Jakobsen and Markager, 2016; Landry et al., 2022). Moreover, the C:TChla ratio depends on cell size and taxonomy, with larger cells having higher C:TChla ratio than smaller cells (e.g. Geider, 1987; Yingling et al., 2025). Consequently, observed correlations between size-fractionated TChla biomass and NPP may be partly influenced by differences in the C:TChla ratio among size classes and taxa. Similarly, growth rate displays taxonomic dependence, with diatoms, cryptophytes and chlorophytes exhibiting higher rates than dinoflagellates, haptophytes and pelagophytes in the Southern Ocean (e.g. Latasa et al., 2014; Gutiérrez-Rodríguez et al., 2023). As a result, high growth rates can lead to elevated NPP even when TChla is low, while slow-growing taxa may accumulate TChla without contributing proportionally to NPP (Behrenfeld et al., 2005). Consequently, additional field studies using the size fractionation approach combined with measurements of C:TChla ratios and growth rates across size classes and phytoplankton groups are needed to improve our understanding on the influence of phytoplankton biomass size structure on NPP, especially in the SAZ and STZ where NPP and phytoplankton data remain sparse.**”

2. Results presented in figures and tables refer to stations, regions and zones (Figure 3 and 5) or zones (Tables 2 and 3) but results described throughout the text mostly refer to specific stations instead. Figure 2, for instance, presents size-fractionated Chla biomass and NPP profiles for individual stations representative of different zones while the rest of stations are included as supplementary material. It is not obvious for the reader how representative these are or how marked the differences between zones are. I think showing the mean +/- error profiles estimated for stations of each zone would be useful to investigate the differences in the biomass and productivity structure in each zone, while leaving the individual stations profiles for supplementary material. Similarly, the starting sentence of the discussion “At several offshore stations in the STZ (O16), SAZ (O14), PFZ (O6, O7, O9) and AZ (O11), NPPTChlaMICRO peaked below the SML while NPPTChlaNANO and NPPTChla PICO were minimal

(Fig. 2k-o; Fig. A3)”. It is not clear how representative this feature is of the region/zone. I think the message would be more clearly conveyed if the focus is placed on zones, pointing then to specific stations to describe intra-zone or intra-region variability. This would also facilitate comparison with other studies and identification of shared patterns or differences with analogous zones and biogeochemical conditions in other basins.

Thank you for this comment. We have revised figure 2 following your recommendations, by plotting the mean +/- standard deviation profiles estimated for stations within each hydrological zone. We have also modified the associated result text (section 3.2.1) and in discussion (section 4.1.1) as well as the starting sentence of the discussion cited above, to better reflect the description for each zone. The profiles for individual stations are provided in the appendix.

Section 3.2.1:

“The **mean** TChla profiles of the total fraction (TChla<sub>TOTAL</sub>) and the size classes (TChla<sub>PICO</sub>, TChla<sub>NANO</sub>, TChla<sub>MICRO</sub>) are presented in Fig. 2a-e **for each hydrological zone**, and in Fig. A1 (Appendix A) for all stations. **Across all zones**, the **depth of SCM (Z<sub>SCM</sub>)** of TChla<sub>TOTAL</sub> occurred between 60 and 100 m and was usually below the Z<sub>SML</sub>, except in **the KER region** where the SCM was located above the Z<sub>SML</sub>. **For all zones**, the Z<sub>SCM</sub> was similar for the total fraction and the size classes, **except in the KER region where the Z<sub>SCM</sub> for TChla<sub>MICRO</sub> peaked around 40 m while the Z<sub>SCM</sub> of TChla<sub>NANO</sub> was deeper (between 60 and 80 m)**. Despite vertical variations in TChla, the TChla size structure – *i.e.* the relative contributions of each size class to TChla<sub>TOTAL</sub> – remained unchanged with depth for all **zones (not shown)**.

As for TChla, the **mean** NPP profiles of the total fraction (NPP<sub>TOTAL</sub>) and the size classes (NPP<sub>PICO</sub>, NPP<sub>NANO</sub>, NPP<sub>MICRO</sub>) are presented in Fig. 2f-j **for each zone** and displayed in Fig. A2 (Appendix A) for all stations. Here, the subsurface NPP maximums were not as marked as the SCM. Moreover, the subsurface NPP maximums coincided with SCM in the STZ **and PFZ**. Contrary to TChla, the NPP size structure – *i.e.* the relative contributions of each size class to NPP<sub>TOTAL</sub> – was heterogeneous with depth (**not shown**). In the STZ, surface NPP was evenly distributed in each size class, while subsurface NPP maximum was dominated by one size class. It is noteworthy that **in the STZ**, subsurface NPP maximums at O2 and O3 were mainly dominated by nanophytoplankton while that **at O16** was surprisingly dominated by microphytoplankton (**Fig. A2**). In the SAZ, PFZ and AZ, surface NPP was mainly supported by nano- and microphytoplankton, while subsurface NPP maximum was dominated by microphytoplankton. In the KER region, NPP was mainly dominated by microphytoplankton. By normalizing NPP to TChla, **we calculated NPP<sup>TChla</sup> (in mgC mgTChla<sup>-1</sup> d<sup>-1</sup>)** which can reflect photosynthesis efficiency under given environmental conditions (*e.g.* light/nutrient availability; Cermeño et al., 2005). NPP<sup>TChla</sup><sub>TOTAL</sub> was maximal **in the first 50 m** at all **zones** – except at O3 and O6 where it peaked below the SML – and decreased with depth (Fig. 2k-o; Fig. A3). Interestingly when considering the size classes, NPP<sup>TChla</sup><sub>MICRO</sub> often peaked **at depth across all zones and coincided with minima in NPP<sup>TChla</sup><sub>NANO</sub> and NPP<sup>TChla</sup><sub>PICO</sub>, except in the STZ.**”

Section 4.1.1: “**In all zones within the study area, except in the STZ, NPP<sup>TChla</sup><sub>MICRO</sub> peaked at depth and coincided with minima in NPP<sup>TChla</sup><sub>NANO</sub> and NPP<sup>TChla</sup><sub>PICO</sub> (Fig. 2k-o).**”

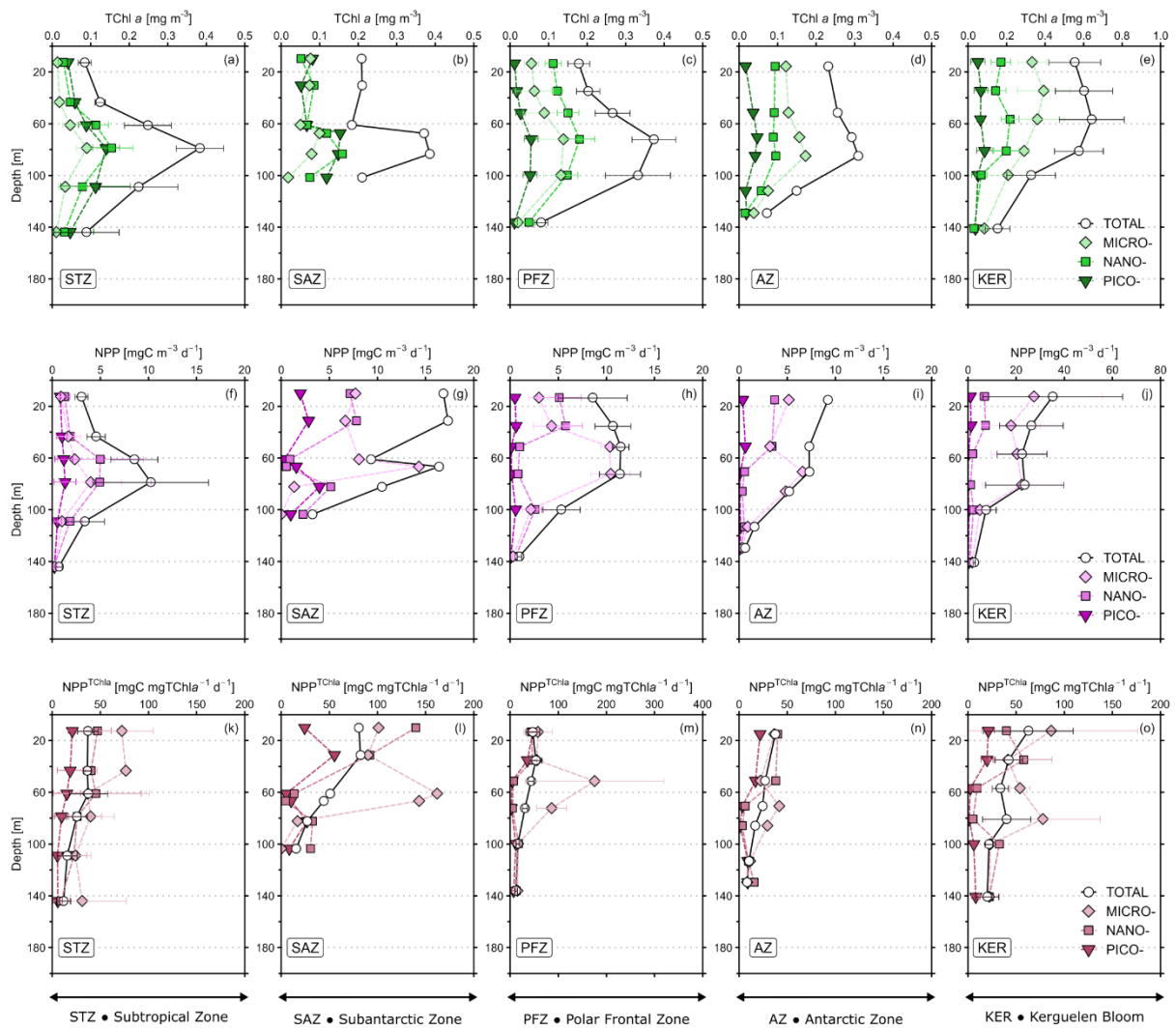


Figure 2: Mean vertical profiles of (a-e) total chlorophyll *a* (TChla), (f-j) net primary production, (k-o) TChla-normalised primary production ( $NPP^{TChla}$ ) for the five hydrographic zones in the study area: Subtropical zone (STZ: a, f, k, n=3), Subantarctic zone (SAZ: b, g, l, n=1), Polar Frontal zone (PFZ: c, h, m, n=3), Antarctic zone (AZ: d, i, n, n=2) and Kerguelen bloom (KER: e, j, o, n=3). Values are mean  $\pm$  SD (or mean value only when  $n < 3$ ). Note the differences in scale for KER for TChla and NPP, and in PFZ for  $NPP^{TChla}$ . All the profiles for every station are presented in Appendix A: Fig. A1 for TChla, Fig. A2 for NPP and Fig. A3 for  $PP^{TChla}$ .

3. In the discussion section 4.1.2. Global feature of phytoplankton biomass and productivity size structure across the study area – The authors indicate that their results of microphytoplankton being the dominant contributor to NPP while nano and micro dominated the TChla biomass are in agreement with previous studies. But they only refer to Froneman et al. 2001. Relevant studies investigating phytoplankton structure, growth and productivity across contrasting zones and regions are not mentioned. Examples of such studies include Boyd et al. 1999 JGR; Bradford and Grieve et al. 1999; and McKay et al. 2005 conducted in ST, SA and AZ of the SW Pacific. I would also refer to studies conducted around islands, plateaux and bathymetric features such as the Kerguelen, Crozet, Campbell plateaux (e.g. Irion et al. 2020 or Gutierrez-Rodriguez et al. 2020) with relevant data obtained in analogous productive and oligotrophic (HNLC and HNLC-LSi) conditions that could be useful to broaden the discussion.

Thank you for this comment. Our goal of section 4.1.2 is to discuss the size structures features of phytoplankton biomass and productivity across the study area, by averaging our values to provide a synoptic view. At the time of the writing, we had identified only the study by Froneman et al. (2001) which also investigated during the austral summer, size-fractionated phytoplankton biomass and productivity across similar zones in the Atlantic sector, allowing a comparison in a synoptic view. To strengthen our comparison with additional studies, we have now included the study by Mishra et al. (2020), who found similar results in TChla size structure in the South Indian Ocean, during the austral summer, using phytoplankton functional pigments tools to the bulk fraction. The studies cited by Andres Gutiérrez Rodríguez focused on specific zones or regions, which would fit better in section 4.1.3, where we compare our results at the scale of individual zones and regions with the literature. Note that we do not cite Bradford-Grieve et al. 1999 or Irion et al. 2020 in the following additions, because Irion et al. 2020 do not provide size fractionated data, and Bradford-Grieve et al. 1999 conducted their study during austral winter and spring, which is not directly comparable to our austral summer observations.

The following additions and modifications (in bold) are now included in the manuscript:

Section 4.1.2: “Across the study area, microphytoplankton was the main contributor of NPP<sub>TOTAL</sub> ( $56 \pm 12\%$ ) while the main contributors to TChla<sub>TOTAL</sub> were nano- ( $40 \pm 11\%$ ) and micro- ( $37 \pm 18\%$ ). **At the scale of the study area, our results in TChla size structure are similar with previous studies conducted during the austral summer, in the South Indian Ocean using phytoplankton functional pigments approaches to the bulk fraction (Mishra et al., 2020), and in the South Atlantic and the Atlantic sector of the Southern Ocean from size-fractionation approaches (Froneman et al., 2001).**

Section 4.1.3: “In our study, the TChla and NPP size structures in the PFZ, AZ and KER were mainly sustained by nano- (TChla:  $40 \pm 12\%$ ; NPP:  $30 \pm 12\%$ ) and microphytoplankton (TChla:  $47 \pm 14\%$ ; NPP:  $66 \pm 13\%$ ), which were consistent with previous studies conducted in the Atlantic and Indian sectors of the Southern Ocean, **encompassing both HNLC and HN-LSi-LC low-productivity waters as well as high-productivity waters near the Crozet and Kerguelen Islands (Froneman et al., 2001, 2004; Seeyave et al., 2007; Uitz et al., 2009).** By contrast, **the TChla size structure in the SAZ was mainly dominated by pico- (41%) and nanophytoplankton (36%), consistent with observations from other sectors of the SAZ in the Atlantic (Froneman et al., 2001) and western Pacific sectors (Boyd et al., 1999; McKay et al., 2005; Gutiérrez-Rodríguez et al., 2020).** Similarly, the TChla size structure in the STZ was mainly driven by the pico- ( $43 \pm 10\%$ ) and nanophytoplankton ( $40 \pm 10\%$ ).”

4. I am concerned with the Spearman rank correlation approach adopted to explore the role of each size fraction and phytoplankton group in driving the NPP variability, particularly with regards to potential spurious correlation and covariation in phytoplankton groups's Chla biomass. I wonder whether multiple regression (e.g.  $(NPP\_SF \sim \text{group1} + \text{group2}, \dots + \text{environmental variables})$  or the use of partial least squares could help handle these respective issues.

Thank you for pointing that observation. As indicated in Materials and Methods section, not all volumetric datasets met the assumptions of normality and homoscedasticity, consequently the choice of statistical tools available to explore the role of each size fraction and phytoplankton chemotaxonomic group in driving NPP variability was limited. Moreover, we wanted to explore this aspect for each zone in our study, which further limits the possible options for statistical analyses.

Multiple regression proposed by Andres Gutiérrez Rodríguez may also fall in some limitations in this context:

- It requires normality and homoscedasticity assumptions to be met, which is not the case for our dataset;
- It requires a sample size of  $n > 20$  which is not compatible with our approach when exploring for each zone with  $6 < n < 18$ ;
- It requires to avoid multicollinearity: this might be the case when computing the size-fractionated phytoplankton group biomass as multiple predictors – although phytoplankton biomass data could be transformed to improve the test.

Nevertheless, we consider that the PCA used in our manuscript remains an appropriate and relevant tool for our dataset and enables to provide a different perspective on the issue, with results complementary to Spearman's correlation analysis.

5. Pigment size-fractionated results. I found interesting and striking the significant contribution of picoplanktonic groups such as Syn, Prochl or green algae to the micro-size Chl a biomass as well as the dominant contribution of haptophytes in the picophytoplankton fraction, which yielded almost 3-fold higher contribution than Chlorophytes, which tend to co-dominate in similar open ocean waters (see SAWS region in Figure 7 of Nunes et al. 2019 mentioned in the preprint. Given the importance of these results, I recommend the authors to expand the discussion around these points and potential methodological bias. Given the scarcity of this type of data the study by Rodríguez et al., 2006, although from a different region and conditions, could add to this discussion.

Thank you for this comment. We have expanded the discussion to incorporate the points raised by Andres Gutiérrez Rodríguez, including potential methodological biases, that were not sufficiently addressed in the initial manuscript. The following additions and modifications (in bold) are now included in section 4.1.2 of the revised manuscript. Please note that the additions also include one from the second reviewer's comments.

“To our knowledge, **Rodríguez et al. (2006) and Nunes et al. (2019) are the only two studies that have applied** these approaches – with CHEMTAX algorithm and two size classes ( $< 3 \mu\text{m}$  and  $> 3 \mu\text{m}$ ) – to investigate phytoplankton communities in the **northwestern Iberian basin and in the surface Atlantic Ocean, respectively. Consistent with these studies, our results highlight** that each phytoplankton chemotaxonomic group was not strictly associated **with** one specific size class (Fig. 4). These results underline the **limitations** of phytoplankton functional type approaches used to estimate phytoplankton size structure from bulk measurements (e.g. Uitz et al., 2006; Hirata et al., 2011). For instance, diatoms and dinoflagellates, which are commonly associated with the microphytoplankton in **such** approaches, were also distributed in the pico- and nanophytoplankton **size classes** (Fig. 4). **This likely reflects the presence of nanoplanktonic dinoflagellate genera such as *Amphidinium*, *Gymnodinium*, *Protoperidinium* and *Prorocentrum* which have been reported in the Indian sector of the Southern Ocean (Georges et al., 2014; Hörstmann et al., 2021; Sreerag et al., 2023). Additionally, pico- and nanoplanktonic diatom genera such as *Minidiscus* and *Fragilariopsis*, as well as holidophytes, a eukaryotic picophytoplankton group genetically very close to diatoms and sharing a similar pigments composition (Guillou et al., 1999), have previously been observed in the South Indian and South Atlantic Oceans (Hinz et al., 2012; Leblanc et al., 2018; Nunes et al., 2019; Deteix et al., 2024). Also, the presence of haptophytes in the picophytoplankton could be attributed to some coccolithophore genera such as small *Emiliana*, and to other genera such as *Chrysochromulina* and *Phaeocystis* (Poulton et al., 2007; Hinz et al., 2012; Patil et al., 2017; Hörstmann et al., 2021). Notably, Nunes et al. (2019) have shown that phytoplankton functional types approaches **based on** bulk measurements predicted a high **contribution** of nano- and**

microphytoplankton in the Atlantic sector of the Southern Ocean, while the size fractionation approach indicated the dominance of picophytoplankton.

Furthermore, **our findings revealed that *Prochlorococcus*, *Synechococcus* and chlorophytes** were mainly distributed in the picophytoplankton, but were also detected in the nano- and microphytoplankton (Fig. 4). This result, also reported in previous studies (Rodríguez et al., 2006; Nunes et al., 2019), can be explained by the size fractionation methodology, as the 3 µm and 20 µm pore sizes may retain a part of these organisms **due to aggregation and/or adhesion**. In addition, **the attribution of pigments like zeaxanthin – associated with *Synechococcus* in this study – to larger size classes may be influenced by the presence of this pigment in nanophytoplankton (e.g. UCYN-B; UCYN-A in symbiosis within nanophytoplankton haptophytes) and microphytoplankton (e.g. *Trichodesmium* spp.; diatom diazotroph associations) previously detected in the STZ of the South Indian Ocean (Metzl et al., 2022; Chowdhury et al., 2024) or in some diatoms under high irradiance (Lohr and Wilhelm, 1999).** **Thus, our result underline the importance of interpreting phytoplankton size structure data in the context of methodological constraints. The presence of picophytoplankton groups in larger size classes and the dominance of haptophytes in the picophytoplankton underscore the need for complementary validation using microscopy, flow cytometry or molecular techniques.”**

6. It is unclear why in the 4.2 intrazonal variability Discussion section subsections include the Subtropical Zone and the Polar Frontal Zone but the Kerguelen bloom region instead of the Antarctic Zone, comprising both the KER and HNLC and HNLC-LSi regions as in Table 1.

Thank you for this comment. We have updated the section 4.2.3, by adding a paragraph on the intrazonal variability of phytoplankton biomass and productivity in the Antarctic Zone and the Kerguelen Bloom area. The following addition has now been included in section 4.2.3 of the revised manuscript:

#### 4.2.3 The Antarctic Zone and the Kerguelen bloom area

The HN-LSi-LC station O10, located southwest of the Kerguelen plateau, and the KER stations (A3, E and O12), exhibited similar NPP size structure, with a dominance of the microphytoplankton ( $75\% \pm 10\%$ ), although  $NPP_{TOTAL}$  was approximately 2.5 times higher at the KER stations (Fig. 3b, Table S3). Similarly, the TChl*a* size structure at O10 and the KER stations was similar, with a dominance of microphytoplankton ( $59\% \pm 2\%$ ) dominated by diatoms, although  $TChl*a*_{TOTAL}$  was approximately 1.6 times higher at the KER stations (Fig. 3a and 5d, Table S3). No major differences were observed in the chemotaxonomic biomass structure – except a slightly higher contribution of pelagophytes in the TChl*a*<sub>MICRO</sub> at O10 compared to the KER stations (Fig. 5d). In contrast, the offshore HNLC station O11 displayed distinct size structures relative to the other stations. Both  $NPP_{TOTAL}$  and  $TChl*a*_{TOTAL}$  were dominated by nanophytoplankton (mainly haptophytes and diatoms) with respective contributions of 50% and 57%. In addition, both  $NPP_{TOTAL}$  and  $TChl*a*_{TOTAL}$  at O11 were lower than those measured at station O10. Our results are consistent with the study by Uitz et al. (2009), conducted during the austral summer, which reported a dominance of microphytoplankton (mainly diatoms) in the Fe-fertilized waters of the Kerguelen Plateau and an increasing contribution of nanophytoplankton at offshore HNLC stations.

**We now compare the southeastern and northeastern blooms in KER, at stations A3 and O12, respectively.** The highest integrated TChl<sub>a</sub> and NPP at A3 and O12 reflected the well-documented natural Fe fertilization. Despite sharing similar TChl<sub>a</sub> and NPP size structures, integrated NPP displayed variability, as integrated NPP<sub>TOTAL</sub> at A3 was 70% lower compared to O12 (Fig. 3)."

7. Lines 449 – 455. If I understand correctly the authors here cite Schlüter et al 2011 to point that opportunistic taxa belonging to nano and microphytoplankton are adapted to exploit excess nutrients in a way consistent with size-fractionated trends observed in richer AZ and KER regions. There have been recent studies pointing in this direction (e.g. Leblanc et al. 2018) or even that certain picophytoplankton specific groups such as prasinophytes (Barber and Hiscock 2006; Irion et al. 2020; Gutierrez-Rodriguez et al. 2023) often respond also to improved nutrient conditions in HNLC open ocean waters.

Thank you for this comment and insight. The following addition (in bold) is now included in the manuscript in section 4.1.3. Please note that the additions also include one from the second reviewer's comments.

"Phytoplankton chemotaxonomic groups biomass also varied in association with changes in TChl<sub>a</sub> size structure. Cyanobacteria, pelagophytes and chlorophytes mainly sustained picophytoplankton in the STZ – typical for LNLC areas with low mixing – while diatoms, haptophytes and dinoflagellates mostly sustained nano- and microphytoplankton in the PFZ, AZ and KER region – typical in areas where these opportunistic taxa are particularly well suited to take advantage of excess nutrient (Fig. 5 and 6) (Schlüter et al., 2011; Leblanc et al., 2018). **Possible species that may account for much of the biomass of these phytoplankton chemotaxonomic groups include: *Synechococcus* and *Prochlorococcus* for cyanobacteria; *Pelagomonas*, *Micromonas* for pelagophytes; *Chloroparvula*, *Chloropicon* for chlorophytes; *Chaetoceros*, *Corethron*, *Coscinodiscus*, *Eucampia*, *Fragilariopsis*, *Thalassiosira* for diatoms; *Gephyrocapsa*, *Chrysochromulina*, *Phaeocystis* for haptophytes; and, *Amphidinium*, *Gymnodinium*, *Prorocentrum* for dinoflagellates (Armand et al., 2008; Lasbleiz et al., 2016; Patil et al., 2017; Hörstmann et al., 2021; Irion et al., 2020; Sreerag et al., 2023, 2025; Thyssen et al. 2024).** Recent studies have nevertheless underlined that some eukaryotic picophytoplankton groups, such as prasinophytes – belonging to the green algae lineage within chlorophytes – can also benefit from enhanced nutrient conditions (e.g. ammonium; Irion et al., 2020) or deep-mixing and low-light regimes in HNLC open ocean waters (Gutiérrez-Rodríguez et al., 2023)."

8. Section 4.2.1. Here the authors invoke the passage of a cyclonic eddy to explain increased productivity observed in ST O3 compared to O2 without concomitant shift in community composition. Similar results have been reported in the NPSG (Landry et al. 2008; Beatty et al. 2025) in more recent and complementary studies that I find relevant for this discussion point.

Thank you for this comment and additional references. The following additions and modifications (in bold) are now included in section 4.2.1 of the manuscript:

"Our NPP fluxes are **similar to** previous studies, reporting a 20–100% increase in integrated NPP<sub>TOTAL</sub> in cyclonic eddies compared to non-eddy areas in the Bay of Bengal (Prasanna Kumar et al., 2007; Sarma et al., 2020), in the South Indian Ocean (Dalabehara and Sarma, 2021) **and in the subtropical North Pacific Ocean (Landry et al., 2008)**. Moreover, Sarma et al. (2020) reported no significant differences in the NPP size structure between cyclonic eddy and non-eddy areas, which

supports our findings. About TChla, there is a lack of previous studies focusing on eddies in the Indian Ocean to compare with our dataset. Nevertheless, Vaillancourt et al. (2003) reported a similar 28% increase of integrated TChla in cyclonic eddy compared to non-eddy areas in the subtropical North Pacific Ocean. **In addition, Beatty et al., (2025) reported, in the latter region, based on amplicon sequencing data, that protistan community composition showed no response to eddy forcing in the water column, which is consistent with our results from phytoplankton chemotaxonomic group biomass.**”

9. I found the discussion point developed in the paragraph starting in line 402 of particular relevance and suggest including it as part of the abstract.

Thank you for this comment. The following addition (in bold) is now included in the abstract:

“In the Subtropical and Subantarctic zones, NPP and TChla were sustained by pico- and nanophytoplankton with a diversified community (cyanobacteria, haptophytes, chlorophytes, pelagophytes). Conversely in the Polar Frontal and Antarctic zones, NPP and TChla were dominated by nano- and microphytoplankton with a less diversified community (diatoms, haptophytes). **The coupling of pigment-based chemotaxonomy with size fractionation reveals new insights into the size-specific distribution of phytoplankton chemotaxonomic groups, challenging traditional functional type approaches on the bulk fraction and highlighting the presence of key groups such as diatoms and haptophytes across all three size classes.** Our results also underline the intra-zonal variability of NPP and TChla through bottom-up processes, such as cyclonic eddy in the Subtropical zone or Si-depleted water mass intrusion in the Polar Frontal zone.”

#### **Minor comments:**

Line 114 – Please clarify what “shaken vigorously” means as it may affect protistan grazers to a greater extent than their phytoplankton prey and bias the NPP estimates.

After the addition of the  $^{13}\text{C}$  tracer to each bottle, we ensured that the seawater was thoroughly homogenized with  $^{13}\text{C}$  by flipping the bottle upside down, shaking it and repeating this operation twice. We agree that the term “shaken vigorously” can appear rough according to your comment; in this way, we have replaced it with “thoroughly homogenized”.

Line 378 – Please indicate these stations belong to different zones.

The three stations cited were associated with their respective zone in brackets as follow (addition in bold): “However, haptophytes were the main contributor to TChla<sub>MICRO</sub> at O14 (**SAZ**) and O7 (**PFZ**), where NPP<sup>TChla</sup><sub>MICRO</sub> peaked.”

Line 401 – Please indicate “bulk Chla biomass” here and throughout the document when referring to Chla-based phytoplankton biomass.

This has been taken into account.

Line 406 – See Rodríguez et al. 2006 as another example of the size-fractionated pigment analysis approach.

Thank you, this reference was added to the discussion.

<b>Reviewer #2 – anonymous reviewer</b>
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We would like first to thank the reviewers for their relevant comments and suggestions which helped us to improve our manuscript. Reviewer comments are in black font, and responses are in blue font. Figures that have been modified in the revised manuscript were added to this document. Below are the authors' point-by-point responses to the reviewer's comments and questions.

**General comments:**

In this manuscript, Valentin Deteix and colleagues analyze the size structure of phytoplankton biomass and primary production in the Southern Indian Ocean during the austral summer of 2023. The authors partitioned the phytoplankton community into three size classes: picophytoplankton (< 3  $\mu\text{m}$ ), nanophytoplankton (3–20  $\mu\text{m}$ ), and microphytoplankton (> 20  $\mu\text{m}$ ). They then estimated the contribution of each size class to total Chlorophyll-a (TChl-a) and net primary production (NPP) across all stations analyzed. Furthermore, the authors estimated the contribution of major phytoplankton groups to Chl-a within each size class. Their findings indicate that TChla was primarily controlled by the nano- and microphytoplankton size classes, while integrated NPP was dominated by the microphytoplankton size class. Regarding phytoplankton group contributions to total Chl-a, the data revealed a latitudinal gradient consistent with previous reports in the Southern Ocean. These data are particularly interesting because phytoplankton studies often focus on specific groups rather than on the entire phytoplankton community. Moreover, the study area is an undersampled region of the global ocean, and therefore, the presented results are valuable and deserve to be published.

Overall, the manuscript is well-presented and reasonably well-written, the methodology is appropriate, and the figures are of good quality. However, several points require improvement. In general, the authors differentiate phytoplankton by size and major groups but scarcely mention the phytoplankton species or genera present in the region. Although their methodological approach does not allow for species-level identification, comparing their results with previously published information on the species present in the study area could aid in some of their interpretations.

Thank you for your comment. Following your recommendations, we have updated the discussion in several sections, by including phytoplankton genera that have previously been observed in the study area – and in other sectors of the Southern Ocean – and that are consistent with our results based on size-fractionated phytoplankton chemotaxonomic groups biomass.

Additionally, the description of the results, which often refers to stations numerically, is sometimes difficult to follow. It would be helpful to refer to the zonal systems where they are located,

as this would improve the readability of the text. Below, I include a series of more specific points that the authors should consider for the next version of the manuscript.

Thank you for your comment. We have specified the hydrographic regions associated with the stations in the study area throughout the manuscript.

### Specific comments:

Line 16. Could the authors specify the regions analyzed during the experiment?

The sentence has been rephrased to specify the biogeochemical regions analyzed in this study (modifications in bold):

“As part of the South Indian Ocean CARBON fluxes from the surface to the mesopelagic twilight zone (SOCARB) project, the phytoplankton biomass and net primary production (NPP), along with the biomass of phytoplankton chemotaxonomic groups, were assessed **during late austral summer 2023 in contrasting biogeochemical areas: the oligotrophic subtropical waters of the South Indian Ocean, High Nutrient Low Chlorophyll (HNLC) waters, and the highly productive waters in the vicinity of Kerguelen Islands in the Southern Ocean.**”

Line 40. The term "irrelevant" is not the most appropriate here. Maybe "insufficient" or "too general"? Please rephrase.

The term “irrelevant” has been replaced with “insufficient”.

Line 49. Please replace the comma with "and" before dissolved inorganic phosphorus.

The comma before dissolved inorganic phosphorus has been replaced with “and”.

Line 58. What about silicate concentrations in the AZ?

Surface dissolved silicon concentrations in the AZ are usually  $> 20 \mu\text{mol.L}^{-1}$  (Sarmiento et al., 2004). The following addition (in bold) is now included in the manuscript:

“The Polar Frontal Zone (PFZ), between the PF and the Subantarctic Front (SAF), and the Subantarctic Zone (SAZ), between the SAF and the STF, display high NOX and DIP concentrations but low dissolved silicon (DSi) concentrations (usually  $< 5 \mu\text{mol L}^{-1}$ ), **in contrast to HNLC waters of the AZ (usually  $> 20 \mu\text{mol.L}^{-1}$ )**, resulting in High Nutrient Low Silicon Low Chlorophyll (HN-LSi-LC) conditions (Nelson et al., 2001; Sarmiento et al., 2004).”

Line 60. "SIO". Please reduce the use of acronyms such as this one. It will facilitate understanding of the text.

The acronym “SIO” has been removed from the manuscript.

Line 65. "Euphotic layer". Do the authors mean deeper layers of the euphotic zone, or subsurface layers of the photic zone? Please rephrase, as the upper surface layer (mentioned in the previous sentence) also belongs to the euphotic layer.

By "Euphotic layer", we mean the productive layer, which includes both subsurface and deeper layers of the euphotic zone. We have replaced the previous term "upper surface layer" with "surface layer", to avoid any confusion with the generic term "upper layer" and to stress the point that most previous data on phytoplankton biomass, composition and productivity in this area were sampled at ~10 m depth only.

Line 69. Please add "and" before Heard and McDonald Islands.

This has been added.

Line 74. Please provide background information about the differing roles of the different phytoplankton groups and size classes in the biological pump. So far, it is not clear in the text why it is important to differentiate between phytoplankton size classes.

In the initial manuscript, we provided some context in the first paragraph of the introduction section about the different roles of phytoplankton size structure in the biological pump, without going into extensive detail. To underline the importance of distinguishing between phytoplankton size classes, the following addition (in bold) has now been included in the first paragraph of the introduction:

"More specifically, the taxonomic composition and the size structure of phytoplankton communities can affect significantly the intensity and fate of NPP by controlling the photosynthetic CO<sub>2</sub> uptake efficiency (*e.g.* Cermeño et al., 2005), the transfer of NPP through either microbial trophic pathway or higher trophic levels (*e.g.* Marañón, 2009) and the carbon export and sequestration in the deep ocean (*e.g.* Guidi et al., 2009). **For instance, phytoplankton communities dominated by large cells are expected to contribute greatly to organic carbon export through their faster sinking velocity rates and more efficient transfer towards higher trophic levels compared to phytoplankton communities dominated by smaller cells (Legendre and Le Fèvre 1989; Wassmann 1998). Also, particulate organic carbon export may be enhanced when phytoplankton communities are dominated by biomineralizing organisms, as mineral ballast increases particle sinking rates (Armstrong et al., 2001; Klaas and Archer, 2002).**"

Line 79. "The long-term monitoring of oceanic CO<sub>2</sub> parameters in the SIO". Please provide references.

The following reference has been added in the manuscript and in the references section: Metzl, N., and Lo Monaco, C.: OISO-Océan Indien Service d'Observation, <https://doi.org/10.18142/228>, 1998.

Line 92. Could the authors relate these regimes to the stations shown in Figure 1? This could be done either here or later in this section.

Because Figure 1 provides an overview of surface chlorophyll *a* without surface nutrient data, these regimes cannot be directly related to the stations at this stage. The attribution of stations to these regimes is presented later, at the beginning of the Results section (section 3.1 and table 1).

Figure 1. Please identify the islands on the map.

The figure 1 has been updated following your recommendations.

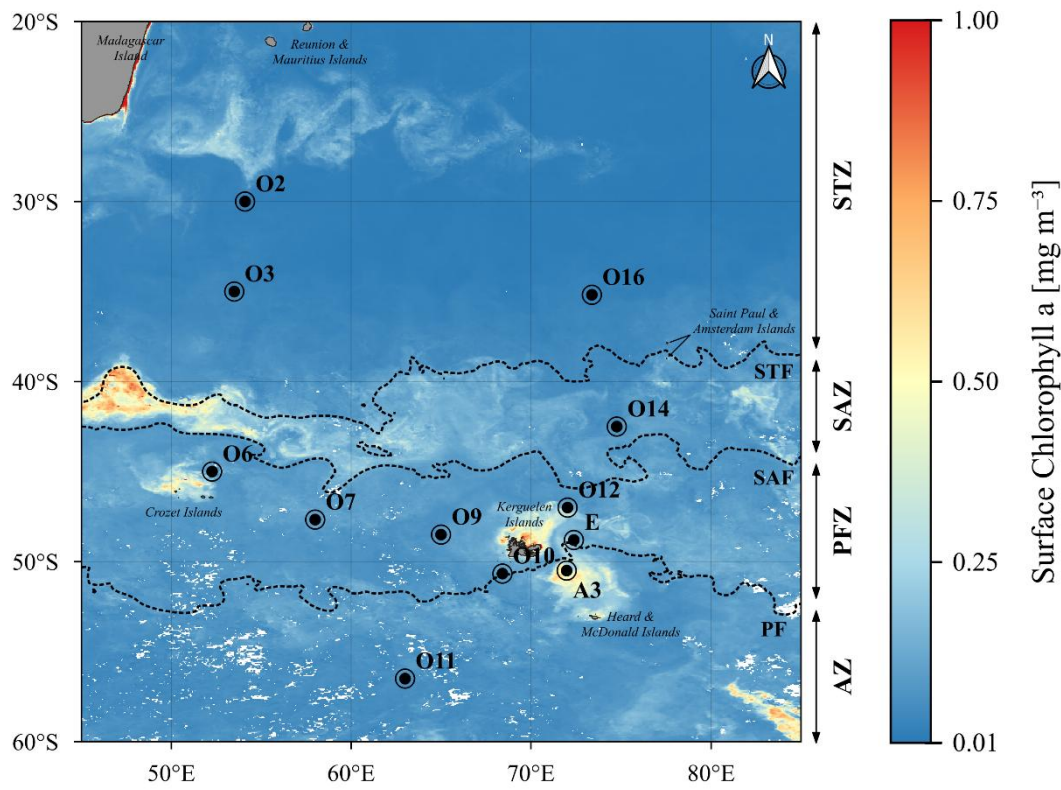


Figure 1: Map of the OISO33-SOCARB study area showing the location of the stations from this study, overlying the satellite-derived surface chlorophyll a concentration averaged over February 2023 (MODIS L3 product). The dotted lines indicate the positions of the main fronts determined from satellite-derived surface temperature averaged over February 2023 (CMEMS L4 product): STF, Subtropical Front (18°C); SAF, Subantarctic Front (13°C); PF, Polar Front (4.5°C).

Lines 99-103. So, this means that different depths were sampled at each station? Please clarify and provide specific depths somewhere in the text or as supplementary material.

That is correct. The specific depths are shown in the vertical profiles for each station displayed in Appendix A. Also, all data associated with this study – including the specific depths sampled for each station – will be available in the section “Data Availability” section through a DOI on the SEANOE database.

Line 200. Please write SML in full the first time it is mentioned in the text.

This has been taken into account.

Line 201. This information is very interesting, but it would be useful for the non-specialized reader if it were explained in further detail why values lower than the Redfield ratio are interpreted as N limitation. Please also extend your explanation to all the statements related to nutrients in this paragraph.

The paragraph, describing the distinct biogeochemical regions at the stations within the study area, has been revised to provide clarification on the relationship between the NO<sub>x</sub>/DIP and DSi/NO<sub>x</sub> ratios and associated nutrient limitations, while remaining concise. The terms “optimal” and “suboptimal” have been replaced to avoid any potential confusion. Modifications are indicated in bold:

“The study area can be further subdivided into distinct biogeochemical regions, with contrasting surface TChl<sub>a</sub> and nutrient concentrations in the surface mixed layer (SML) (Table 2). In the STZ, stations O2, O3 and O16 exhibited LNLC conditions, with very low surface TChl<sub>a</sub>. The NO<sub>x</sub>/DIP ratios in the SML were notably lower than the Redfield ratio of 16/1 (Redfield, 1958), indicating **a relative deficiency of NO<sub>x</sub> with respect to DIP for phytoplankton nutritional requirements, and thus suggesting** a potential NO<sub>x</sub> limitation of the phytoplankton activity (Geisen et al., 2022). Station O11 in the AZ featured HNLC conditions, with low surface TChl<sub>a</sub> despite high macronutrient concentrations. The NO<sub>x</sub>/DIP and DSi/NO<sub>x</sub> ratios in the SML were close to **the Redfield and Brzezinski ratios** (Si/N for diatoms =  $1.12 \pm 0.33$ , Brzezinski 1985), **indicating that NO<sub>x</sub>, DIP and DSi were not limiting, and thus** suggesting a potential micronutrient limitation (Geisen et al., 2022). Stations O6, O7, O9 **in the PFZ** and O10 **in the AZ** shared similar features with O11 (AZ) but exhibited lower surface DSi concentrations, leading to DSi/NO<sub>x</sub> ratios **notably lower than the Brzezinski ratio**. These stations exhibited HN-LSi-LC conditions, indicating a potential (co-)limitation by Si (Pondaven et al., 2000). Station O14 stood out from the latter HN-LSi-LC stations, exhibiting lower NO<sub>x</sub>, DIP and DSi concentrations in the SML along with NO<sub>x</sub>/DIP and DSi/NO<sub>x</sub> ratios **below the Redfield and Brzezinski ratios**. Stations O12, E and A3, located in the naturally Fe-fertilized Kerguelen bloom (Blain et al., 2008; Qu erou e et al., 2015), exhibited the highest surface TChl<sub>a</sub> and **a DSi/NO<sub>x</sub> ratio in the SML lower than the Brzezinski ratio, indicating a potential (co-)limitation by Si** (Geisen et al., 2022). These stations were grouped into a region hereafter referred to as “Kerguelen bloom” (KER), which differed from the offshore stations in the PFZ (O6, O7, O9) and AZ (O11, O10) (Table 2).”

Line 209. What values are suboptimal and why? Please explain in further detail.

The terms “optimal” and “suboptimal” have been removed to avoid any confusion. Please refer to the previous comment for the revised paragraph, with modifications in bold.

Lines 247-249. "By normalizing NPP to TChl<sub>a</sub> (NPPTChl<sub>a</sub>, mgC mgTChl<sub>a</sub><sup>-1</sup> d<sup>-1</sup>) which can reflect photosynthesis efficiency under given environmental conditions (e.g. light/nutrient availability; Cerme no et al., 2005), NPPTChl<sub>a</sub> TOTAL was maximal in the SML at all stations, except at O3 and O6 where it peaked below the SML, and decreased with depth (Fig. 2k-o; Fig. A3 in Appendix A)." Please rephrase this sentence.

The sentence has been rephrased to separate the computation and purpose of the parameter NPP<sup>TChl<sub>a</sub></sup>, from the results. Modifications are indicated in bold.

“By normalizing NPP to TChl<sub>a</sub>, **we calculated NPP<sup>TChl<sub>a</sub></sup> (in mgC mgTChl<sub>a</sub><sup>-1</sup> d<sup>-1</sup>)** which can reflect photosynthesis efficiency under given environmental conditions (e.g. light/nutrient availability; Cerme no et al., 2005). NPP<sup>TChl<sub>a</sub></sup><sub>TOTAL</sub> was maximal **in the first 50 m at all zones, [...]**”

Line 309. This is interesting because dinoflagellates are often relatively large. Do the authors have an idea of the species present in the study area? Could you refer to the existing bibliography to hypothesize possible components of this group of organisms within the study area?

The literature on the diversity and distribution of peridinin-containing dinoflagellates in this region remains scarce. We found some studies reporting some dinoflagellate genera potentially in the nano- size range such as *Amphidinium* (size range < 10-100  $\mu\text{m}$ ), *Gymnodinium* (size range < 5-200  $\mu\text{m}$ ), *Protoberidinium* or *Prorocentrum* (size range 15- 200  $\mu\text{m}$ ) in the South Indian Ocean (Georges et al., 2014; Hörstmann et al., 2021; Sreerag et al., 2023). The following addition (in bold) has now been included in discussion, section 4.1.2, to explain the latter results:

Section 4.1.2: “For instance, diatoms and dinoflagellates, which are commonly associated with the microphytoplankton in **such** approaches, were also distributed in the pico- and nanophytoplankton size classes (Fig. 4). **This likely reflects the presence of nanoplanktonic dinoflagellate genera such as *Amphidinium*, *Gymnodinium*, *Protoberidinium* and *Prorocentrum* which have been reported in the Indian sector of the Southern Ocean (Georges et al., 2014; Hörstmann et al., 2021; Sreerag et al., 2023). Additionally, pico- and nanoplanktonic diatom genera such as *Minidiscus* and *Fragilariopsis*, as well as bolidophytes, a eukaryotic picophytoplankton group genetically very close to diatoms and sharing a similar pigments composition (Guillou et al., 1999), have previously been observed in the South Indian and South Atlantic Oceans (Hinz et al., 2012; Leblanc et al., 2018; Nunes et al., 2019; Deteix et al., 2024). Also, the presence of haptophytes in the picophytoplankton could be attributed to some coccolithophore genera such as small *Emiliana*, and to other genera such as *Chrysochromulina* and *Phaeocystis* (Poulton et al., 2007; Hinz et al., 2012; Patil et al., 2017; Hörstmann et al., 2021). Notably, Nunes et al. (2019) have shown that phytoplankton functional types approaches based on bulk measurements predicted a high contribution of nano- and microphytoplankton in the Atlantic sector of the Southern Ocean, while the size fractionation approach indicated the dominance of picophytoplankton.”**

Lines 311-312. "As expected, cyanobacteria (*Prochlorococcus* and *Synechococcus*) biomass was mainly distributed in the picophytoplankton, but also surprisingly detected in the nano- and microphytoplankton." Could the authors provide a possible explanation for these results? Was sieving not effective? Were they attached to larger phytoplankton? Did they form colonies? Please provide possible explanations in the discussion section.

Thank you for this comment. The sentence you cited in lines 311-312 was discussed in section 4.1.2. We stated that it is possible that the 3  $\mu\text{m}$  and 20  $\mu\text{m}$  pore sizes from the filter cartridges may retain a part of picophytoplankton < 3  $\mu\text{m}$  such as *Prochlorococcus* and *Synechococcus*. The following modifications (in bold) are now included in the manuscript:

Section 4.1.2: “Furthermore, **our findings revealed that *Prochlorococcus*, *Synechococcus* and chlorophytes** were mainly distributed in the picophytoplankton, but were also detected in the nano- and microphytoplankton (Fig. 4). This result, also reported in previous studies (**Rodríguez et al., 2006; Nunes et al., 2019**), can be explained by the size fractionation methodology, as the 3  $\mu\text{m}$  and 20  $\mu\text{m}$  pore sizes may retain a part of these organisms **due to aggregation and/or adhesion**. In addition, **the attribution of pigments like zeaxanthin – associated with *Synechococcus* in this study – to larger size classes may be influenced by the presence of this pigment in nanophytoplankton (e.g. UCYN-B; UCYN-A in symbiosis within nanophytoplankton haptophytes) and microphytoplankton (e.g.**

*Trichodesmium* spp.; diatom diazotroph associations) previously detected in the STZ of the South Indian Ocean (Metzl et al., 2022; Chowdhury et al., 2024) or in some diatoms under high irradiance (Lohr and Wilhelm, 1999).-Thus, our result underline the importance of interpreting phytoplankton size structure data in the context of methodological constraints. The presence of picophytoplankton groups in larger size classes and the dominance of haptophytes in the picophytoplankton underscore the need for complementary validation using microscopy, flow cytometry or molecular techniques.”

Line 369. The title is vague; please rephrase.

The title of section 4.1 has been rephrased as follow: “**Analyzing the interplay between phytoplankton biomass and productivity in relation to size structure**”

Line 378. Could you please associate these stations with environments/zonal systems?

The stations cited were associated with their respective zone in parentheses as follow (addition in bold): “However, haptophytes were the main contributor to TChl<sub>a</sub><sub>MICRO</sub> at O14 (**SAZ**) and O7 (**PFZ**), where NPP<sup>TChl<sub>a</sub></sup><sub>MICRO</sub> peaked.”

Line 390. The term "global" is misleading; please rephrase.

The terms “global features” from the title of section 4.1.2. have been replaced by “overall patterns”.

Line 392. I would suggest using the term "similar" instead of "are in agreement," as the regions are not directly comparable. The Atlantic and Indian oceans exhibit different physical and chemical distributions, which hamper direct comparisons between regions.

Thank you for this comment. The term “are in agreement with” has been replaced by “are similar with” following your suggestion.

Lines 417-420. The relationships between pigments and phytoplankton groups should be presented in the Materials and Methods.

Thank you for this comment. We have added a new table in section 2.4 of the Materials and Methods, specifying the assignment of pigments to the corresponding phytoplankton chemotaxonomic groups in the phytoclass algorithm.

**Table 1: Phytoplankton chemotaxonomic groups and associated pigments computed with phytoclass in this study.**

Phytoplankton group	Pigments used for phytoclass in this study
Diatom	Chl <sub>a</sub> ; Fuco
Haptophytes	Chl <sub>a</sub> ; But-fuco; Fuco; Hex-fuco
Cryptophytes	Chl <sub>a</sub> ; Allo

Dinoflagellates	Chla; Peri
Chlorophytes	Chla; Chlb; Lut; Neo; Viola; Zea
Pelagophytes	Chla; But-fuco; Fuco
<i>Synechococcus</i>	Chla; Zea
<i>Prochlorococcus</i>	DVChla

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Line 419. Please, do not capitalize "diatoms."

This has been corrected for every phytoplankton group (except *Prochlorococcus* and *Synechococcus* as they refer to genera).

Line 421. "Inter-zonal spatial variability of phytoplankton biomass..." The title of this section is unnecessarily complex. Why not simply "Geographical distribution of phytoplankton biomass"?

The title for section 4.1.3 has been corrected following your suggestion (modifications in bold):  
**“Geographical distribution of phytoplankton biomass and productivity size structures in relation with environmental factors”**

Line 424. Please provide references documenting similar changes in the size and/or composition of phytoplankton assemblages between the STZ and the Southern Ocean.

Thank you for this comment. We have provided references documenting similar changes in the size structure of phytoplankton biomass and productivity between the STZ and the Southern Ocean. The following modifications (in bold) are now included in the manuscript:

Section 4.1.3: “The size structures of integrated biomass and primary production clearly shifted between the oligotrophic subtropical waters and the Southern Ocean waters, **consistent with previous studies conducted in the Indian and Atlantic sectors of the Southern Ocean (Froneman et al., 2001; Mishra et al., 2017, 2020).**”

Lines 425-427. This part of the discussion is unclear. The authors list the main factors controlling the spatial distribution of phytoplankton, but in the second sentence, other parameters are addressed. I would suggest that the authors commence with their findings, then compare results with studies in the literature that found similar results, then with studies that do not match (if such studies exist), and then reach a conclusion based on all the evidence presented.

Thank you for this comment. We have revised the PCA to better take into account the relevant environmental variables with ecological significance. Note that with the corrected PCA does not alter the main results and the conclusions. We have also revised the corresponding text in the Material and Methods and the Discussion following your recommendations. The following additions and modifications (in bold) have now been included in the manuscript:

Section 2.6: “Principal component analysis (PCA) was performed on the volumetric dataset (n = 72) to **explore the relationships between** environmental parameters (explanatory variables) **and net primary production as well as** phytoplankton chemotaxonomic groups **biomass** (supplementary

descriptors). The initial explanatory variables were potential temperature, salinity,  $\sigma$ , dissolved oxygen, PAR, DIC, NO<sub>x</sub>, DIP, DSi, Z<sub>SML</sub> and Z<sub>EL0.01%</sub>. Prior to the analysis, explanatory variables and supplementary descriptors were standardized (vegan::deconstand() function). Furthermore, collinearity among explanatory variables was assessed using a Spearman correlogram (Fig. S2). Potential temperature was strongly correlated with  $\sigma$  ( $\rho = -0.98$ ), dissolved oxygen ( $\rho = -0.92$ ) and DIC ( $\rho = -0.92$ ); among these variables, potential temperature was retained, as it is a key driver of water mass structure and biological activity. NO<sub>x</sub> and DIP were also highly correlated ( $\rho = 0.98$ ), and only NO<sub>x</sub> was retained. After this selection, potential temperature and salinity displayed a variance inflation factor (VIF) > 20 (vegan::vif.cca() function); salinity was discarded in favour of temperature. Final explanatory variables were potential temperature, PAR, NO<sub>x</sub>, DSi, Z<sub>SML</sub> and Z<sub>EL0.01%</sub>. All variables displayed VIF values < 10, except for NO<sub>x</sub> (14).

All statistical analyses were conducted in the programming environment R 4.4.2 (R Core Team 2024). The package tidyverse (v2.0.0; Wickham et al., 2019) was used for data manipulation; oce (v1.8.3; Kelley & Richards 2024) for trapezoidal integration computations; stats (v4.4.2; R Core Team 2024), rstatix (v0.7.2; Kassambara 2023) and corrplot (v0.95; Wei and Simko 2024) for statistical analyses; FactoMineR (v2.12; Lê et al. 2008) and vegan (v2.6.10; Oksanen et al. 2025) for multivariate analyses.”

Section 4.1.3: “Result from the multivariate analysis showed that potential temperature (theta) and NO<sub>x</sub> concentration (and DIP due to its strong correlation with NO<sub>x</sub>, see section 2.6) were the major factors driving the spatial variability between the different zones (Fig. 6). Indeed, temperature and NO<sub>x</sub> concentration are recognized as key factors to shape phytoplankton biomass and productivity size structures, with picophytoplankton usually prevalent in warm and oligotrophic waters (Marañón, 2009; Hörstmann et al., 2021; Berthelot et al., 2025).”

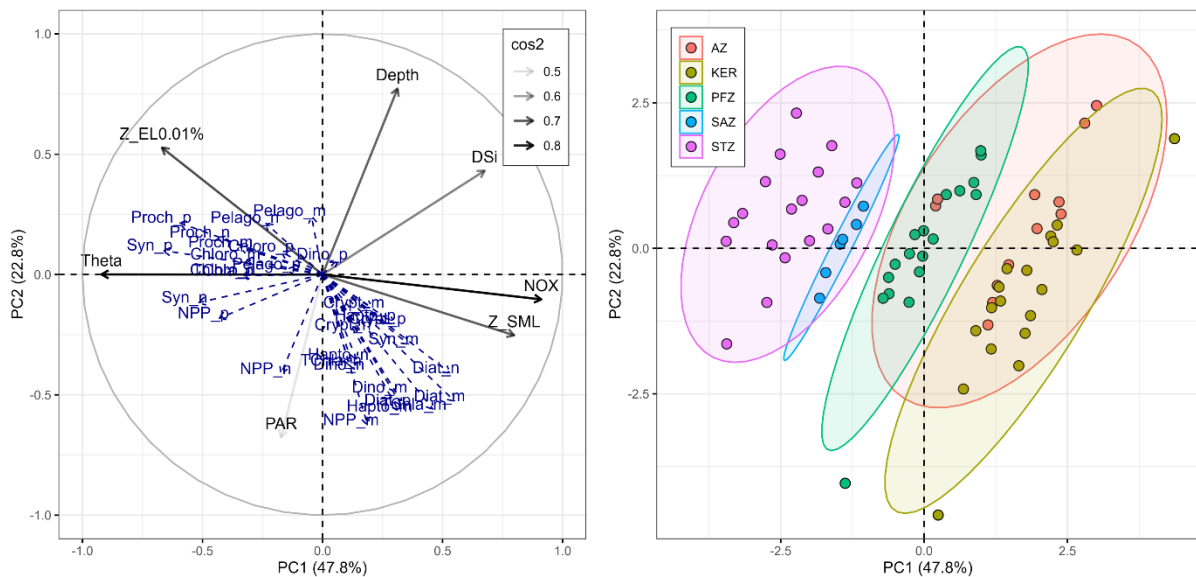


Figure 1: Principal Component Analysis illustrating the relationships between explanatory variables and supplementary descriptors across the global study area. The first principal component (PC1) axis explains 47.8% of the variance and the second principal component (PC2) axis explains 22.8% of the variance. On the left panel, the black arrows indicate the explanatory variables (environmental factors) with their transparency defined by their  $\cos^2$ : the better the variables are well represented by the principal components, the higher the  $\cos^2$ . The blue arrows show the supplementary descriptors: NPP, TChla and

phytoplankton chemotaxonomic groups biomass in each size class. On the right panel, the colour of each point represents the zone of each sample (n = 72).

Line 426. Is salinity an important factor determining phytoplankton distribution in the study area, or is it just a spurious correlation? Please provide references that support the influence of salinity on the distribution of phytoplankton groups in environments similar to those analyzed here.

Thank you for this comment. Indeed, temperature and NO<sub>x</sub> concentrations are the most important factors determining phytoplankton distribution in our study area. Temperature and salinity were significantly correlated ( $\rho = 0.45$ ,  $p < 0.05$ ), and this correlation was not spurious. As mentioned in the previous comment: “potential temperature and salinity displayed a variance inflation factor  $> 20$ ; salinity was discarded in favour of temperature”. Consequently, we removed the term “salinity” from the sentence.

Line 449. "Phytoplankton chemotaxonomic groups biomass". How similar or different are the STZ of the Atlantic and Indian oceans in terms of physical and chemical parameter distributions? This should be mentioned somewhere in the text, as comparisons with the Atlantic sector are frequent.

The following addition (in bold) has now been included in the manuscript:

“Nevertheless, our results **were similar to those of Froneman et al. (2001) in the South Atlantic STZ, which reported a TChla size structure driven by pico- ( $49 \pm 10\%$ ) and nanophytoplankton ( $39 \pm 6\%$ ), but differed from other studies conducted in the northern and southern subtropical Atlantic**, where picophytoplankton accounted for 60–75% of TChla<sub>TOTAL</sub> (Marañón et al., 2001; Morán et al., 2004). **As temperature and NO<sub>x</sub> concentrations were similar between our study and the latter, the differences in TChla size structure between the Atlantic and the Indian basins may be attributed to factors** such as regional-scale hydrodynamics and/or atmospheric inputs (Marañón, 2009).”

Line 452. It would be interesting for the reader to mention some possible species that account for much of the biomass of the phytoplankton groups addressed in the discussion. This information would strengthen the interpretations. For example, there are several studies by Shramik Patil et al. that documented coccolithophore species distributions in this region, and there must be papers documenting diatom assemblages as well. Also, it is interesting that diatoms account for a substantial fraction of the nanoplankton. Diatom species are often larger than 20 micrometers, so the number of species smaller than 20 micrometers must be quite limited. The authors should revise the available bibliography in the study region to put their results into a broader context.

Thank you for this comment. Complementary to the updates made in section 4.1.2 following a previous comment from the anonymous reviewer (indicating line 309), we have now mentioned some possible genera that may account for a large part of the biomass of the phytoplankton groups addressed in section 4.1.3. References including studies by Shramik Patil *et al.* have been added to support these possible taxa. The following addition has now been included in the manuscript:

Section 4.1.3: “Possible species that may account for much of the biomass of these phytoplankton chemotaxonomic groups include: *Synechococcus* and *Prochlorococcus* for cyanobacteria; *Pelagomonas*, *Micromonas* for pelagophytes; *Chloroparvula*, *Chloropicon* for chlorophytes;

*Chaetoceros*, *Corethron*, *Coscinodiscus*, *Eucampia*, *Fragilariopsis*, *Thalassiosira* for diatoms; *Gephyrocapsa*, *Chrysochromulina*, *Phaeocystis* for haptophytes; and, *Amphidinium*, *Gymnodinium*, *Protoperidinium*, *Prorocentrum* for dinoflagellates (Armand et al., 2008; Lasbleiz et al., 2016; Patil et al., 2017; Irion et al., 2020; Hörstmann et al., 2021; Sreerag et al., 2023, 2025; Thyssen et al. 2024).”

Lines 481-482. Please explain in further detail the findings of Lamont and Barlow mentioned here.

The sentence has been completed to include the findings of Lamont and Barlow (2017). The following addition (in bold) has now been included in the manuscript:

“These hydrographic and biogeochemical features are consistent with previous observations of cyclonic eddies in the Mozambique Channel and Basin, **which are characterized by a shallower  $Z_{SML}$  and nitracline, and a deeper euphotic zone** (Lamont and Barlow, 2017).”

Line 482. It is the first time the authors refer to NPP fluxes; please explain the units when presenting this parameter either in the Introduction or Materials and Methods.

In marine biogeochemistry, the net primary production (NPP) represents the net flux of organic carbon synthesized by phytoplankton through photosynthesis. As defined in this manuscript, NPP is expressed in units of mass per unit volume (or per unit area for integrated data) per unit time, which is characteristic of a flux. For volumetric data, units are  $\text{mgC m}^{-3} \text{d}^{-1}$ ; for integrated data units are  $\text{mgC m}^{-2} \text{d}^{-1}$ .

To facilitate the reading with a vocabulary coherent throughout the manuscript, the following sentences have been included in the Material and Methods section:

- Section 2.2: “Volumetric NPP is expressed as a flux in  $\text{mgC m}^{-3} \text{d}^{-1}$ .”
- Section 2.3: “Volumetric pigment concentrations are expressed as stocks in  $\text{mg m}^{-3}$ .”
- Section 2.6: “Integrated NPP are expressed thereafter in  $\text{mgC m}^{-2} \text{d}^{-1}$ , while integrated pigment concentrations and integrated biomass of phytoplankton chemotaxonomic groups are expressed in  $\text{mg m}^{-2}$ .”

Line 511. Which value range is considered suboptimal and why?

According to the previous reviewer’s comments addressed in section 3.1, the term “suboptimal” has been removed to avoid any confusion. The following addition (in bold) has now been included in the manuscript: “First, A3 and O12 exhibited  $\text{DSi/NO}_x$  ratios in the SML **lower than the Brzezinski ratio** (Table 1), [...]”

Line 512. "Si availability hypothesis" — shouldn't this be "Si limitation" instead?

“The Si availability hypothesis” refers to one of the potential factors which could explain the variability in integrated NPP in iron-fertilized areas, listed in the previous sentence. For a better clarity, the following modification (in bold) has now been included in the manuscript: “[...], indicating a potential Si limitation; **this indicates that Si availability does not explain the observed difference in  $\text{NPP}_{\text{TOTAL}}$** .”

Line 512. Please replace "noticeable shift" with "noticeable difference."

The term “noticeable shift” has been replaced with “noticeable difference”.

Lines 517-518. Please rephrase this sentence, as you indicate the purpose of the ratio twice.

From this sentence, we removed “as an indicator of the mixing processes in the water column (Moline, 1998)” to avoid repetition in the next sentence. The sentences are now as follow:

“Third, to investigate the light-mixing regime, we computed the ratio of the diadinoxanthin (DD) and diatoxanthin (DT) concentrations to TChla ((DD+DT):TChla). Although DD and DT have limited chemotaxonomic values, they have a photoprotective role, with concentrations that respond rapidly to changes in irradiance (Demers et al., 1991). Because most phytoplankton contain these pigments, the (DD+DT):TChla ratio provides useful information on the vertical mixing rates in the water column along with the light regime (Moline, 1998).”

Line 567. Please do not capitalize "chlorophytes, dinoflagellates and cryptophytes."

This has been corrected for all phytoplankton groups throughout the manuscript (except for *Prochlorococcus* and *Synechococcus* as they refer to genera).

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**Additional minor changes have also been made in addition to those requested by the reviewers, as detailed below:**

- Section 2.3: Typo error in pigment listing has been corrected: prasinoxanthin has been replaced by lutein. The following modification (in bold) has now been added to the manuscript: “In this study, the following eleven accessory pigments were further used to study the TChla biomass of the phytoplankton chemotaxonomic groups: fucoxanthin (Fuco), peridinin (Peri), 19'-hexanoyloxyfucoxanthin (Hex-fuco), 19'-butanoyloxyfucoxanthin (But-fuco), alloxanthin (Allo), chlorophyll *b* (Chlb), zeaxanthin (Zea), neoxanthin (Neo), **lutein (Lut)**, violaxanthin (Viola), and DVChla.”

- Table 1:

- Surface TChla at O2: ~~0.07 mg m<sup>-3</sup>~~ → **0.08 mg m<sup>-3</sup>**
- Surface TChla at O12: ~~0.71 mg m<sup>-3</sup>~~ → **0.59 mg m<sup>-3</sup>**

- Table S2 (in supplement): NPP values have been correctly reordered according to their respective station.

-Figure S5 (in supplement): ~~mg·m<sup>-2</sup>~~ → **mg m<sup>-3</sup>**

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## EGUSPHERE-2025-5902

### Point-by-point response to reviewers' technical comments

#### New insights into the primary production and the structure of the phytoplankton community in the South Indian Ocean using size fractionation experiments

Valentin Deteix, Céline Ridame, Céline Dimier,  
Claire Lo Monaco, Aline Tribollet, Frédéric Planchon

*EGUSPHERE-2025-5902 Research article*

We would like first to thank the reviewers for their time and continued engagement with our manuscript through this review process. Reviewer comments are in black font, and responses are in blue font. Figures that have been modified in the revised manuscript were added to this document. Below are the authors' point-by-point responses to minor and technical comments from Andres Gutiérrez Rodríguez and anonymous reviewer #2.

#### Reviewer #1 – Andres Gutiérrez Rodríguez

The authors have addressed adequately my comments making the corresponding changes in the manuscript or explaining clearly why, when they have not. I only have a couple minor comments detailed below that I would like to be taken care of. Other than that, I think this is a good and interesting study that contributes significantly to our understanding of marine phytoplankton ecology and biogeochemistry in the South Indian Ocean and recommend its publication. Congratulations to the authors for the study.

Thank you for this general comment and the kind recommendation for publication. The minor comments raised by Andres Gutiérrez Rodríguez were addressed below and taken care of.

Line 268 – "...O16 was surprisingly dominated by microphytoplankton (Fig. A2)". It is not clear why this is particularly noteworthy or surprising. I suggest explaining this further or remove.

Thank you for this comment. We revised the sentence and removed the previous eye-catching terms such as "noteworthy" and "surprisingly", as it is not discussed further in the manuscript. The following modifications (in bold) are now included in section 3.2.1 of the manuscript:

"In the STZ, surface NPP was evenly distributed in each size class, while subsurface NPP maximum was dominated by **nanophytoplankton at O2 and O3, and by microphytoplankton at O16** (Fig. A2). In the SAZ, PFZ and AZ, surface NPP was mainly supported by nano- and microphytoplankton, while subsurface NPP maximum was dominated by microphytoplankton."

Line 554 – “Similarly, The TChla size structure at O10 and ,...was similar, with dominance of microphytoplankton ( $59\% \pm 2\%$ ) dominated by diatoms,...”. I would revise this sentence as the use of similarly/similar and dominance/dominated sounds redundant.

Thank you for this comment. The following modifications (in bold) are now included in section 4.2.3 of the manuscript:

“**Likewise**, the TChla size structure at O10 and the KER stations was similar, with a dominance of microphytoplankton ( $59\% \pm 2\%$ ) **mainly sustained** by diatoms, although TChla<sub>TOTAL</sub> was approximately 1.6 times higher at the KER stations (Fig. 3a and 5d, Table S3).

**Reviewer #2 – anonymous reviewer**

In my opinion, the paper is now suitable for publication in Biogeosciences, with one minor modification: the island names in Figure 1 are presented in a font size that will likely become illegible upon printing/publication.

Thank you for this comment and the kind recommendation for publication. The figure 1 has been updated following your recommendations: the font of island names in Figure 1, initially presented in italic font, was changed to normal font.

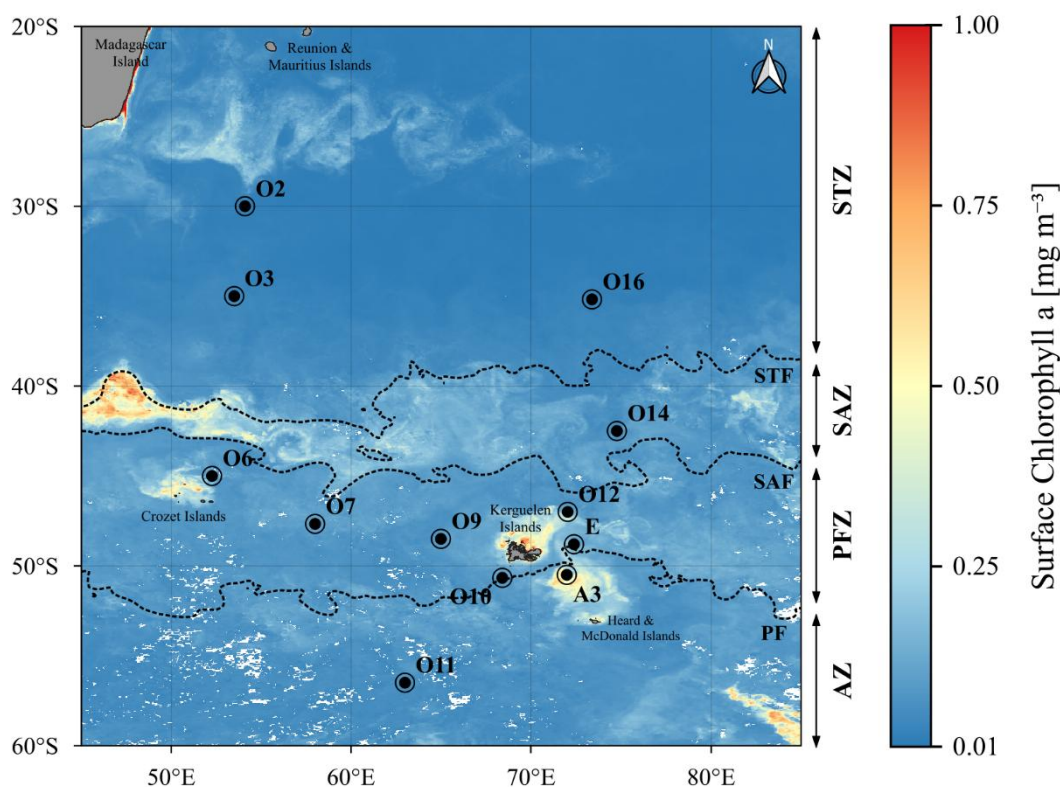


Figure 1: Map of the OISO33-SOCARB study area showing the location of the stations from this study, overlying the satellite-derived surface chlorophyll a concentration averaged over February 2023

(MODIS L3 product). The dotted lines indicate the positions of the main fronts determined from satellite-derived surface temperature averaged over February 2023 (CMEMS L4 product): STF, Subtropical Front (18°C); SAF, Subantarctic Front (13°C); PF, Polar Front (4.5°C).

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**Additional minor changes have also been made in addition to those requested by the reviewers, as detailed below:**

- Data availability section: the following modification (in bold) is now included in the manuscript:

**“All size-fractionated phytoplankton NPP, biomass and pigments data, either volumetric or integrated over the euphotic layer 0.01%, are available in the SEANOE database *via* the following address: <https://www.seanoe.org/data/01034/114546/>.”**

- Acknowledgements section: the following addition (in bold) is now included in the manuscript:

“The authors would like to thank the captain C. Souffre and the crew of the R/V *Marion Dufresne II* for their expertise and assistance on board; Fanny Kaczmar for managing clean laboratory and trace metal clean procedures before the cruise; the OISO-33 on board team for nutrients and DIC sampling, and the first DIC analyses on board; Jonathan Fin for DIC analyses at the SNAPO-CO<sub>2</sub> analytical platform (LOCEAN-IPSL); Magloire Mandeng-Yogo and Fethiye Cetin for IR-MS analyses at the Alysés analytical platform (IRD-SU); Eva Delcamp for pigments analyses at the SAPIGH analytical platform (IMEV); the IMAGO analytical platform (IRD) for nutrients analyses. We also thank Nicolas Metzl for his relevant comments on the draft manuscript. **Finally, the authors would like to thank Andres Gutiérrez Rodríguez and an anonymous reviewer for providing constructive and relevant comments that helped to improve this manuscript.** This work was part of the PhD’s degree research of V. Deteix.”