



# 1 Hydrographic fronts shape productivity, nitrogen fixation, and 2 microbial community composition in the South Indian Ocean and the 3 Southern Ocean

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14 **Abstract.** Biogeochemical cycling of carbon (C) and nitrogen (N) in the ocean depends on both the composition and activity  
15 of underlying biological communities and on abiotic factors. The Southern Ocean is encircled by a series of strong currents  
16 and fronts, providing a barrier to microbial dispersion into adjacent oligotrophic gyres. Our study region straddles the boundary  
17 between the nutrient-rich Southern Ocean and the adjacent oligotrophic gyre of the South Indian Ocean, providing an ideal  
18 region to study changes in microbial productivity. Here, we measured the impact of C- and N- uptake on microbial community  
19 diversity, contextualized by hydrographic factors and local physico-chemical conditions across the Southern Ocean and South  
20 Indian Ocean. We observed that contrasting physico-chemical characteristics led to unique microbial diversity patterns, with  
21 significant correlations between microbial alpha diversity and primary productivity (PP). However, we detected no link  
22 between specific PP (PP normalized by chlorophyll *a* concentration) and microbial alpha and beta diversity. Prokaryotic alpha  
23 and beta diversity were correlated with biological N<sub>2</sub> fixation, itself a prokaryotic process, and we detected measurable N<sub>2</sub>  
24 fixation to 60° S. While regional water masses have distinct microbial genetic fingerprints in both the eukaryotic and  
25 prokaryotic fractions, PP and N<sub>2</sub> fixation vary more gradually and regionally. This suggests that microbial phylogenetic  
26 diversity is more strongly bounded by physical oceanographic features, while microbial activity responds more to chemical  
27 factors. We conclude that concomitant assessments of microbial diversity and activity is central in understanding the dynamics  
28 and complex responses of microorganisms to a changing ocean environment.



## 29 **1 Introduction**

30

31 The Southern Ocean (SO), and in particular its sub-Antarctic zone, is a major sink for atmospheric CO<sub>2</sub> (Constable et al. 2014).  
32 The SO is separated from the South Indian Ocean Gyre (ISSG) by the South Subtropical Convergence province (SSTC),  
33 comprising of the Subtropical Front (STF) and the Subantarctic Front (SAF). The SSTC is a zone of deep mixing and, thus,  
34 elevated nutrient concentrations (Longhurst, 2007). Further, the SSTC has been shown to act as a transition zone both  
35 numerically and taxonomically for dominant populations of marine bacterioplankton (Baltar et al., 2016).

36 In this dynamic context, a key driver of microbial productivity is nutrient availability, especially through tightly coupled carbon  
37 (C) and nitrogen (N) cycles. The constant availability of nutrients through vertical mixing in frontal zones, such as the STF,  
38 enhances primary productivity (Le Fèvre, 1987) and chlorophyll *a* (chl *a*) concentrations (Belkin and O'Reilly, 2009). Primary  
39 productivity (PP) and specific primary productivity ( $P^B$  = primary productivity per unit chl *a*) are reflected in the relative  
40 abundance of different phytoplankton size classes whose productivity are, in turn, stimulated by nutrient injections via  
41 shallowing of mixed layer depth (MLD) at the SO fronts (Strass et al., 2002); decreasing the possibility of N-limitation. N-  
42 limitation can also biologically be alleviated through N<sub>2</sub> fixation mediated by diazotrophs; significantly contributing to the N  
43 pool in oligotrophic regions (Tang et al., 2019). In high-latitude regions, biological N<sub>2</sub> fixation could potentially have a large  
44 impact on productivity (Sipler et al., 2017). However, large disagreements exist between models of high-latitude N<sub>2</sub> fixation  
45 and its coupling to microbial diversity due to sparse sampling in these regions (Tang et al., 2019).

46 Due to the dynamics of the region, conflicting observations, and climate-driven changes, resolving the coupling of microbial  
47 productivity and diversity is particularly important across the strong environmental gradients crossing the ISSG, through the  
48 SSTC into the SO. Indeed, climate variability has been shown to impact ocean productivity, and thus influences the provision  
49 of resources to sustain ocean life (Behrenfeld et al., 2006). To date, observations of climate change-related effects in this region  
50 of the SO have been synthesized only based on long-term nutrient concentration and physical (temperature and salinity)  
51 changes (Lo Monaco et al., 2010), however, these typically lack a microbial dimension. Microbial composition, activity, and  
52 C export may all be impacted by climate-driven changes in ocean dynamics (Evans et al., 2011) such as MLD shallowing,  
53 eddy formation, and poleward shifts of ocean fronts (Chapman et al., 2020). For a more holistic, ecosystem-based  
54 understanding of this region, concomitant assessments of 1) steady-state biogeochemical processes through rate measurements  
55 of key elements (such as C and N) and 2) the microbial diversity that underpins it are essential enhancements to such long-  
56 term investigations.

57 Here, we measure the impact of C- and N- uptake on microbial community diversity, alongside the effects of hydrography  
58 (e.g., dispersal limitation) and local physico-chemical conditions across the Southern Ocean and South Indian Ocean. We  
59 focused our investigation on surface communities, aiming to resolve horizontal, surface variation. We used our observation to  
60 assess whether the following relationships - previously observed in related systems - hold in our study region:



61 (1) *Microbial diversity increases with increasing primary productivity (PP)*. Previous work has claimed that more  
62 resources support higher species richness, until intermediate rates of PP (Fig.1; Vallina et al., 2014) within ocean  
63 provinces (Raes et al., 2018).

64 (2) *Frontal systems are discrete ecological transition zones between regions*: To provide perspectives on the findings  
65 of Baltar et al. (2016; see above). These systems often separate water masses with distinct trophic structures (e.g.  
66 Albuquerque et al., 2021).

67 (3) *Microbial alpha and beta diversity are impacted by N<sub>2</sub> fixation, itself correlated with the presence of other available*  
68 *sources of N and/or temperature*: To provide more evidence on the role of N<sub>2</sub> fixation to the N budget in high latitudes  
69 (see eg. Shiozaki et al., 2018; Sipler et al., 2017).

70 To our knowledge, there are no concomitant evaluations of how surface gradients, microbial activity, and community  
71 composition relate to one another in this region. Here, we provide perspectives on these key relationships across the Southern  
72 Indian Ocean Subtropical Gyre (ISSG), the Subtropical Front (STF), and Subantarctic Front (SAF), and the SO comprising  
73 the Polar Front (PF) and Antarctic Zone (AZ).

## 74 2. Materials and methods

### 75 2.1 Study region, background data and sample collection

76 Our study region ranged from La Réunion Island in the Southern Indian Ocean Gyre (ISSG) to south of the Kerguelen Islands  
77 in the Southern Ocean (56.5° S, 63.0° E; Fig. 1a) as part of a larger repeated “OISO” sampling program – (Metzl 1998;  
78 <https://doi.org/10.17600/17009700>). Samples were collected as part of the VT153/OISO27 (MD206) cruise onboard the R/V  
79 *Marion Dufresne* from 2017-01-06 to 2017-02-07. Physical and biogeochemical data, as well as metadata, were collected from  
80 a rosette equipped with Niskin bottles and a Conductivity Temperature Depth (CTD) (Seabird SBE32) equipped with a SBE43  
81 O<sub>2</sub> sensor and a Chelsea Aqua tracker fluorometer. OISO long-term data, starting in 1998, were used as a backdrop to our data  
82 collected in 2018 and allowed us to monitor changes in physical and chemical oceanographic properties over time  
83 (Supplementary A).

### 85 2.2 Province delineation after Longhurst

86 We identified three main clusters (i.e. ocean provinces) and five subclusters (i.e. water masses) on a temperature-salinity plot  
87 (Fig. 1b). As an overview, we used CTD depth profiles to validate the vertical extent of water masses in our samples (Fig.  
88 1c,d) and checked the horizontal extent of the identified clusters using remote sensing data of sea surface temperature (Fig.  
89 S2). Additionally, we checked the horizontal boundaries of these clusters for matches in strong chl *a* concentration gradients



90 as an approximate for biological component of ocean provinces, following the concept of Longhurst (2007). Satellite data were  
91 acquired from MODIS (<https://neo.sci.gsfc.nasa.gov/>), with images processed by NASA Earth Observations (NEO) in  
92 collaboration with Gene Feldman and Norman Kuring, NASA OceanColor Group (Fig. S3). We calculated the geodesic  
93 distance between sites from latitude/longitude coordinates using the geodist package in R (v0.0.4; Padgham et al., 2020).

## 94 2.2 Nutrient analysis

95 Dissolved inorganic nutrient concentrations, including phosphate ( $\text{PO}_4^{3-}$ ), silicate (Si), mono-nitrogen oxides ( $\text{NO}_x$ ), nitrite  
96 ( $\text{NO}_2^-$ ), and ammonium ( $\text{NH}_4^+$ ) were assayed on a QuAatro39 Continuous Segmented Flow Analyser (Seal Analytical)  
97 following widely used colorimetric methods (Armstrong, 1951; Murphy and Riley, 1962; Wood et al., 1967) with adaptations  
98 to particular needs for Seal Analytical QuAatro autoanalyzer.  $\text{NH}_4^+$  was measured using the fluorometric method of K erouel  
99 and Aminot (1997). Detection limits of these methods were  $0.1 \mu\text{mol L}^{-1}$  for  $\text{PO}_4^{3-}$ ,  $0.3 \mu\text{mol L}^{-1}$  for Si,  $0.03 \mu\text{mol L}^{-1}$  for  $\text{NO}_x$ ,  
100 and  $0.05 \mu\text{mol L}^{-1}$  for  $\text{NH}_4^+$ .

## 101 2.3 Dissolved inorganic nitrogen and carbon assimilation

102 At each CTD station, water samples to measure primary productivity (PP) and  $\text{N}_2$  fixation were taken from the underway flow-  
103 through system (intake at 7 m). As the ship was moving during sampling, the distance between samples of the same station  
104 can range up to ~15 km. Incubations were performed in acid-washed polycarbonate bottles on deck at ambient light conditions.  
105 All polycarbonate incubation bottles were rinsed prior to sampling with 10% HCl (3x), deionized  $\text{H}_2\text{O}$  (3x), and sampling  
106 water (2x). In order to obtain the natural abundance of particulate nitrogen (PN) and particulate organic carbon (POC), which  
107 we used as a t-zero value to calculate the assimilation rates, 4 L of water were filtered onto a 25 mm pre-combusted GF/F filter  
108 for each station.

109  $\text{N}_2$  fixation experiments were carried out in triplicate for each station. We used the combination of the bubble approach  
110 (Montoya et al., 1996) and the dissolution method (Mohr et al., 2010) proposed by Klawonn et al. (2015). 4.5 L bottles were  
111 filled up headspace-free. All incubations were initialized by adding a  $^{15}\text{N}_2$  gas bubble with a volume of 10 ml. We used  $^{15}\text{N}_2$   
112 labeled gas provided by Cambridge Isotope Laboratories (Tewksbury, MA). Bottles were gently rocked for 15 minutes. Finally,  
113 the remaining bubble was removed to avoid further equilibration between gas and the aqueous phase. After 24 h, a water  
114 subsample was transferred to a 12 ml exetainer<sup>®</sup> and preserved with 100  $\mu\text{L}$   $\text{HgCl}_2$  solution for later determination of exact  
115  $^{15}\text{N}$ - $^{15}\text{N}$  concentration in solution. Natural  $^{15}\text{N}_2$  was determined using Membrane Inlet Mass Spectrometry (MIMS; GAM200,  
116 IPI) for each station with an average enrichment of  $3.8 \pm 0.007$  atom %  $^{15}\text{N}_2$  (mean  $\pm$  SD;  $n=104$ ) Primary productivity was  
117 measured by adding  $\text{Na}^{13}\text{CO}_3$  at a final  $^{13}\text{C}$  concentration of  $200 \mu\text{mol L}^{-1}$ .

118 Incubation bottles were incubated on board at ambient sea surface temperature (SST; water intake at 7 m) using a continuous  
119 flow-through system. Temperature of both incubation bins was continuously measured. After 24 hours, the C and  $\text{N}_2$  fixation  
120 experiments were terminated by collecting the suspended particles from each bottle by gentle vacuum filtration through a 25



121 mm pre-combusted GF/F filter (<10 kPa). Filters were snap-frozen in liquid nitrogen and stored at -80° C while at sea. Filters  
122 with enriched (T24) and unenriched (T0) samples were acidified and dried overnight at 60° C. Analysis of <sup>15</sup>N and <sup>13</sup>C  
123 incorporated was carried out by the Isotopic Laboratory at the UC Davis, California campus, using an Elementar Vario EL  
124 Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

125 Carbon assimilation rates were calculated according to Knap et al. (1996), excluding the <sup>14</sup>C - <sup>12</sup>C conversion factor; and N<sub>2</sub>  
126 fixation was calculated according to Montoya et al. (1996), respectively. The minimum quantifiable rate was calculated  
127 according to Gradoville et al. (2017).

## 128 **2.4 Pigment analysis**

129 For pigment analyses, 4 L of seawater were filtered (< 10 kPa) on 47 mm Whatman GF/F filter and stored at -80° C until  
130 further analysis. High-Performance Liquid Chromatography (HPLC) was carried out as described in Kiliyas et al. (2013) with  
131 following modifications: 150 µL of the internal standard canthaxanthin was included to each sample. Samples were dissolved  
132 in 4 ml acetone and disrupted with glass beads using a Precellys 24 tissue homogenizer (Bertin Technologies, France) at 7200  
133 rpm for 20 seconds. Detection of the sample at 440 nm absorbance using an HPLC analyzer (VARI AN Microsorb- MV 100-  
134 3 C8). We used chl *a* concentration to estimate phytoplankton biomass. Pigment concentrations were calculated according to  
135 Kiliyas et al. (2013), and quality controlled according to Aiken et al. (2009) (Supplementary A).

136 HPLC output data were analyzed using diagnostic pigments for the different taxa, and phytoplankton functional types (PFTs)  
137 after Hirata et al. (2011) (Supplementary A, Table S2). This approach can be used to reveal dominant trends of the  
138 phytoplankton community and size structure at the regional and seasonal scales (Ras et al., 2008). Furthermore, diagnostic  
139 pigments were used to delineate three different size classes (pico-, nano-, and microplankton) according to Vidussi et al. (2001).  
140 The relative proportion of each phytoplankton size class (PSC) was calculated based on the linear regression model proposed  
141 by (Uitz et al. (2006). We investigated the patterns of PSCs with a second-order polynomial fit  
142 (S1\_code\_archive/pigment\_HPLC/diagnostic\_pigments.R L143:153).

## 143 **2.5 DNA analysis**

144 Two liters of seawater from the shipboard underway system from each station were filtered through a 0.22 µm Sterivex® filter  
145 cartridge for DNA isolation, snap-frozen in liquid nitrogen, and stored at -80 °C. DNA was extracted using a DNeasy® Plant  
146 Mini Kit (QIAGEN, Valencia, CA, USA, Catalog No./ID: 69106) following the manufacturer's instructions. Sterivex  
147 cartridges were gently cracked open and filters were removed and transferred into a new and sterile screw cap tube.  
148 Approximately 0.3 g of pre-combusted glass beads (diameter 0.1 mm; 11079101 Bio Spec Products) and 400 µL Buffer AP1  
149 were added to the filter, followed by a bead beating step using a Precellys 24 tissue homogenizer (Bertin Technologies, France)  
150 with two times at 5500 rpm for 20 seconds with two minutes on ice in between and a final beat beating step at 5000 rpm for  
151 15 seconds. DNA concentrations were quantified by the Quantus™ Fluorometer and normalized to 2 ng µL<sup>-1</sup>.



### 152 **2.5.1 Amplicon 16S and 18S rRNA gene PCR and sequencing**

153 Amplicons of the bacterial 16S rRNA gene and eukaryotic 18S rRNA gene (using primers from 27F–519R; Parada et al. 2016,  
154 TA-Reuk454FWD1 – TAReukREV3; Stoeck et al. 2010, respectively) were generated following standard protocols of  
155 amplicon library preparation (16S Metagenomic Sequencing Library Preparation, Illumina, Part # 15044223 Rev. B;  
156 Supplementary B). 16S and 18S rRNA gene PCR products were sequenced using 250-bp paired-end sequencing with a MiSeq  
157 Sequencer (Illumina) at the European Molecular Biology Laboratory (EMBL) in Heidelberg (Germany) and at the Leibniz  
158 Institute on Aging (FLI) in Jena (Germany), respectively.

### 159 **2.5.2 Amplicon sequence data analysis**

160 For both 16S rRNA gene and 18S rRNA gene amplicon sequences, we used the DADA2 R package, v1.15.1 (Callahan et al.,  
161 2016) to construct Amplicon Sequence Variant (ASV) tables by following steps: Prefiltering filterandtrim function with  
162 truncL=50 and default parameters (S1\_code\_archive/dada2). Primer sequences were cut using the Cutadapt software  
163 implementation (v1.18) in the DADA2 pipeline, removing a fixed number of bases matching the 16S forward (515F-Y, 19  
164 bp), reverse (926R, 20 bp), and the 18S forward (TA-Reuk454FWD1, 20 bp) and reverse (TAReukREV3, 21 bp) primers,  
165 respectively (S1\_code\_archive/dada2/dada2\_16S.R L88:104; S1\_code\_archive/dada2/dada2\_18S.R L92:104). Primer-  
166 trimmed fastq files were quality trimmed with a minimum sequence length of 50 bp, and checked by inspection of the average  
167 sequence length distribution (for both the 16S rRNA gene and 18S rRNA gene sequences). Samples within forward and reverse  
168 fastq files were dereplicated and merged with a minimum overlap of 20 bp. ASV tables were constructed and potential chimeras  
169 were identified de-novo and removed using the removeBimeraDenovo command. Sequencing statistics for removed reads and  
170 sequences in each step can be found in Table S3. Taxonomic assignment was performed using the SilvaNGS (v1.4; Quast et  
171 al. 2013) pipeline for 16S rRNA gene data with the similarity threshold set to 1. Reads were aligned using SINA v1.2.10  
172 (Pruesse et al., 2012), and classified using BLASTn (v2.2.30; Camacho et al. 2009) with the Silva database (v132) as a  
173 reference database (Supplementary C). For taxonomic assignment of 18S rRNA gene amplicons, we used the Plugin 'feature-  
174 classifier' (from package 'q2-feature-classifier', v2019.7.0) in QIIME2 (Bokulich et al., 2018) and the pr2 database (v4.12;  
175 Guillou et al. 2013). We removed ASVs annotated to mitochondria and chloroplasts from 16S rRNA gene ASV tables, and  
176 ASVs annotated as metazoans from 18S rRNA gene ASV tables, respectively (S1\_code\_archive/import/import\_16S.R L35:38;  
177 S1\_code\_archive/import/import\_18S.R L29). ASV tables of 16S rRNA gene amplicon (Table S4) and 18S rRNA gene  
178 amplicons (Table S5) were used for further statistical analyses.

### 179 **2.6 Ecological data and statistical analysis**

180 A combination of temperature, salinity, dissolved oxygen concentrations, and dissolved inorganic nutrient concentrations  
181 ( $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ , Si, and  $\text{PO}_4^{3-}$ ) were used to characterize the physical and biogeochemical environment of the study region.  
182 All statistical tests were performed in R version 3.6.3 (R Core Team, 2017). Statistical documentation, package citations and  
183 scripts are available in S1. Microbial alpha diversity was calculated with Hill numbers (Richness, Shannon entropy, Inverse



184 Simpson,  $q = 0 - 2$ ; Chao et al., 2014) using the iNEXT package v2.0.20 in R with confidence set to 0.95 and bootstrap = 100  
185 (S1\_code\_archive/alpha\_diversity). Accordingly, rarefaction curves are shown in Fig. S6. Pearson correlations between  
186 microbial richness ( $q=0$ ), inverse Simpson diversity ( $q=2$ ), environmental parameters, and biological rates were calculated and  
187 plotted (ggplot2) (Fig. S7). P-values were adjusted for multiple testing using Holm adjustment (Holm, 1979), and residuals  
188 checked for normal distribution (Fig. S8). For comparability and statistical downstream analyses, we performed the following  
189 transformations to the ASV table and the environmental metadata: To account for the compositionality of sequencing data (see  
190 Gloor et al. 2017), we performed a CLR-transformation for Redundancy Analysis (RDA). We used Hellinger transformation  
191 (*decostand()* function in *vegan*) of the ASV pseudocount data (minimum pseudocount per ASV cutoff = 3) for PERMANOVA  
192 analyses. Environmental data were z-scored for comparable metadata analysis (S1\_code\_archive/transformations). For  
193 multivariate analyses of microbial beta-diversity and environmental parameters, we performed redundancy analyses (RDA) of  
194 the CLR-transformed ASV tables (S1\_code\_archive/RDA). Differences of microbial beta-diversity (based on Hellinger  
195 transformed ASV tables), phytoplankton community composition (based on pigment concentrations), and water masses were  
196 tested with permutational ANOVA (PERMANOVA; Anderson, 2001) using the *adonis2()* function in *vegan* along with a beta  
197 dispersion test to evaluate the homogeneity of dispersion (Fig. S9). To investigate at where differences of environmental  
198 variables have an impact on microbial community dissimilarity, we performed a general dissimilarity model (GDM) of the  
199 community dissimilarity and environmental variables, and checked for the influence of geographic distance based on spline  
200 magnitude (*gdm* package; S1\_code\_archive/GDM).

201 As differences of microbial beta-diversity were significant in PERMANOVA analysis between provinces and water masses,  
202 we performed a similarity percentage (SIMPER) analysis in R using the *vegan* package to assess which ASVs contribute most  
203 to the observed variance of microbial community composition (Table S6; S1\_code\_archive/taxonomy\_analyses). To determine  
204 the number of ASVs shared between provinces (or unique to certain provinces), we transformed ASV pseudocount tables into  
205 binary tables and calculated shared and unique ASVs using the *upsetR* package in R (v.4, Conway et al. 2017;  
206 S1\_code\_archive/upsetR). We calculated the percentage of all within-sample observed ASVs within the merged samples of a  
207 province (Table S7).

## 208 3. Results

### 209 3.1 Delimitation of regional water masses

210 Through our analysis of temperature, salinity, oxygen and dissolved inorganic nutrient (N, P, Si) concentrations, we identified  
211 five distinct water masses, fronts, and frontal zones: the ISSG, STF, SAF, PFZ, and AZ, which broadly aligned with three  
212 oceanographic provinces (ISSG, SSTC and SO; Fig. 1a). Within the Southern Ocean (SO), we identified four water masses in  
213 our transect including the Antarctic Zone (AZ) and three distinct frontal systems: 1) The Polar Front (PF), 2) The Subantarctic  
214 Front (SAF), and 3) The Subtropical Front (STF; Fig. 1). In our analysis, stations 6, 7, and 9 were placed within the Polar



215 Front Zone (PFZ), between the SAF and PF. Due to the bathymetrically-driven convergence of the STF and SAF around  
216 Kerguelen island, we consider the SAF as part of the convergence zone between the SO and IO, the South Subtropical  
217 Convergence province (SSTC), rather than as a Southern Ocean frontal system. At 7 m depth, we noted clear shifts in  
218 temperature (SST), salinity, and dissolved inorganic nutrient ( $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , Si) concentrations when crossing the STF. The STF  
219 is described as a circumpolar frontal zone creating the boundary between our measurements of the warm (20-25 °C), saline  
220 (>35), and oligotrophic ( $\text{NO}_3^- < 0.03 \mu\text{M}$ ;  $\text{PO}_4^{3-} : 0.04 - 0.21 \mu\text{M}$ ) subtropical waters (STW) of the Indian South Subtropical  
221 Gyre (ISSG) and the cold (3-6 °C), macro-nutrient rich ( $\text{NO}_3^- : 19.2 - 24.9 \mu\text{M}$ ;  $\text{PO}_4^{3-} : 1.43 - 1.71 \mu\text{M}$ ) SO (Fig. 1, Fig. 2, Fig.  
222 S3). In the context of this study, STW and ISSG could be used interchangeably; we refer henceforth to ISSG.

### 223 3.2 Primary productivity (PP)

224 Maximum primary productivity (PP) within our dataset were measured near the Kerguelen plateau in the Polar Front Zone  
225 (PFZ) at Station 9 (3236.8 and 3553.3  $\mu\text{mol L}^{-1} \text{d}^{-1}$ , respectively) and Station E (2212.4 - 2688.1  $\mu\text{mol L}^{-1} \text{d}^{-1}$ , n = 6). Comparing  
226 all PP measurements across water masses, we found relatively high PP in other stations of the PFZ (Stations 6, 7; Fig. 3a;  
227 Table 1) and in the Subantarctic Front (SAF) (Stations 4, 15). Lowest PP (190.4 - 642.6  $\mu\text{mol L}^{-1} \text{d}^{-1}$ ) were measured at the  
228 stations in the Indian South Subtropical Gyre (ISSG). While stations in the ISSG showed very little variations within one  
229 station (e.g. 226.09 - 371.07, n = 6, Station 18), variation within SO stations was relatively high (e.g. 587.42 - 1875.58  $\mu\text{mol}$   
230  $\text{L}^{-1} \text{d}^{-1}$ , n = 6, Station 37; Table 1).

231 Overall, the variation of specific primary productivity ( $P^B$ ) did not show great variations between provinces, with maximum  
232 rates at station 11 (Table 1; Fig. 3b). We did not find a significant correlation between mixed layer depth and  $P^B$  (Pearson  
233 correlation;  $r = 0.21$ ,  $p = 0.47$ ,  $n = 12$ ).

### 234 3.3 $\text{N}_2$ fixation

235 Di-nitrogen ( $\text{N}_2$ ) fixation was above the minimum quantifiable rate (MQR) at all stations (Table 1).  $\text{N}_2$  fixation measurements  
236 did not show a clear temperature-dependent trend (Fig. 3), neither were they directly associated with low DIN values (Fig.  
237 S10).  $\text{N}_2$  fixation in the warm oligotrophic waters of the Indian South Subtropical Gyre (ISSG) was up to 7.93  $\text{nmol L}^{-1} \text{d}^{-1}$   
238 (Station 18; Fig. 3c; Table 1). Lowest  $\text{N}_2$  fixations were measured in the productive zone of the STF and SAF (0.24 - 2.01  
239  $\text{nmol L}^{-1} \text{d}^{-1}$ , n = 3). In the AZ,  $\text{N}_2$  fixation ranged between 0.89 and 1.97  $\text{nmol L}^{-1} \text{d}^{-1}$ . The variation between replicates was  
240 high, e.g. rates ranged between 0.9 to 7.9  $\text{nmol L}^{-1} \text{d}^{-1}$  at station 18 (Table 1). Across provinces, we did not find notable  
241 differences in  $\text{N}_2$  fixation.

### 242 3.4 Phytoplankton pigment analyses

243 Photosynthetic pigment concentrations showed a clear separation between the oligotrophic ISSG and the nutrient-rich SO (Fig.  
244 S5). Chlorophyll *a* concentrations were relatively low in the warmer water stations of the ISSG than in the SSTC and SO  
245 (Table 1). The relative proportion of phytoplankton biomass to the total organic matter was estimated by calculating the ratio



246 of PN : chl *a* and showed a strong increase in the ISSG (11.5 - 29.7 PN : chl *a*, n = 4) in comparison to the SSTC (2.7 - 7.2 PN  
247 : chl *a*, n = 3) and SO (2.8 - 15.3 PN : chl *a*, n = 6; Fig. S4).

248 The phytoplankton community composition was significantly and markedly different across provinces (PERMANOVA;  
249 Permutations = 999,  $R^2 = 0.76$ ,  $p < 0.001$ ; n = 14) and water masses (PERMANOVA; Permutations = 999,  $p = 0.002$ ;  $R^2 =$   
250  $0.81$ , n = 14). The pigment concentration of prokaryote-specific pigment zeaxanthin was high in the ISSG (0.03 - 0.06 mg m<sup>-3</sup>,  
251 n = 4; Fig. S5a). Zeaxanthin still occurred in the STF and SAF (0.03 - 0.04 mg m<sup>-3</sup>, n = 3), but disappeared in the SO (< 0.01  
252 mg m<sup>-3</sup>, n = 6). *Prochlorococcus* was distinctly identified through its diagnostic pigment divinyl chl *a*, and showed a relatively  
253 high pigment concentration in the ISSG (0.02 - 0.03 mg m<sup>-3</sup>, n = 4; Fig. S5a). We found concentrations of diatom-specific  
254 fucoxanthin (except station 18) ranging from 0.021 mg m<sup>-3</sup> in the ISSG (station 16) to 0.34 mg m<sup>-3</sup> in the SO (station 37; Fig.  
255 S5a). Across water masses, fucoxanthin concentration was slightly higher in the AZ (0.06 - 0.5 mg m<sup>-3</sup>, n = 4) than in all other  
256 water masses (0 - 0.13 mg m<sup>-3</sup>, n = 10).

257 The distribution of potential phytoplankton size classes (PSCs; pico- nano- and microplankton), calculated from diagnostic  
258 pigments (Supplementary A), showed a clear pattern over temperature variations (Fig. S5b). The pigment data suggested that  
259 picoplankton dominated warm water in the ISSG, picoplankton abundance sharply decreased (second-order polynomial fit  $R^2$   
260 = 0.98,  $p < 0.001$ , n = 14) at lower values of SST. Pigment data also suggested that microplankton showed a contrary trend to  
261 the relative fraction of picoplankton, having high abundance in cold-water and decreasing at higher values of SST, with a  
262 minimum at 20°C SST and a slight increase (14% microplankton of all phytoplankton size classes) towards 25°C SST (second-  
263 order polynomial fit  $R^2 = 0.77$ ,  $p < 0.001$ , n = 14). Nanoplankton showed a maximum at 12°C SST and decreased both towards  
264 warmer and colder waters (second-order polynomial fit,  $R^2 = 0.58$ ,  $p < 0.01$ , n = 14).

### 265 3.5 Eukaryotic planktonic community composition

266 For each station, except station 4, the V4 region of the small subunit ribosomal RNA gene (18S rRNA) was amplified and  
267 sequenced to determine the community composition of micro-, nano-, and pico-eukaryotes in all three oceanic provinces. We  
268 recovered a total of 2618 ASVs. After removing sequences annotated to metazoans, 2501 ASVs remained (4.4% of ASVs  
269 removed).

270 We found a strong correlation between both eukaryotic richness and diversity (Inverse Simpson Index) with SST (Pearson  
271 correlation,  $r = 0.85$ ,  $p < 0.001$  for Richness, and  $r = 0.82$ ,  $p = 0.001$  for Inv. Simpson, n = 12, respectively; Fig. S7a, S7c).  
272 Overall, eukaryotic diversity was negatively correlated with PP ( $r = -0.66$ ,  $p = 0.02$ , n = 12; Fig. S7e) and significantly and  
273 positively associated with N<sub>2</sub> fixation ( $r = 0.74$ ,  $p = 0.01$ , n = 12; Fig. S7g). However, a strong correlation between rate  
274 measurements (PP, N<sub>2</sub> fixation) and eukaryotic diversity was only apparent in the ISSG, and no significant across other  
275 provinces (Pearson correlation after removal of ISSG samples from dataset: PP  $r = 0.47$ ,  $p = 0.24$ , and N<sub>2</sub> fixation,  $r = -0.48$ ,  
276  $p = 0.23$ , n = 8, respectively).



277 Our RDA constrained 81% of the variance in the ASV table, with a p-value of 0.095 (Permutations = 999, n = 12). Sites were  
278 well separated between Longhurst provinces along the first two RDA axes (capturing 52.67% constrained variance, Fig 4a).  
279 Our PERMANOVA, which tested the province-based separation, produced moderate but significant results (Permutations =  
280 999,  $R^2 = 0.54$ ,  $p = 0.001$ ; n = 12). An additional PERMANOVA grouping sites by water masses produced similar results  
281 (Permutations = 999,  $R^2 = 0.67$ ,  $p = 0.001$ , n = 12; Fig. 4a). We found that more ASVs only occurred in one province, rather  
282 than in two or more provinces (Fig. 4e). Sites within the ISSG province were associated with SST and  $N_2$  fixation. Sites in the  
283 SSTC were associated with high  $NH_4^+$  concentrations. Sites belonging to the SO were associated with dissolved inorganic  
284 nutrients ( $NO_3^-$ ,  $PO_4^{3-}$ , Si), dissolved oxygen, and chl *a* concentrations as well as high PP. Linear relationships between beta  
285 diversity and rates were only weak for PP (PERMANOVA; Permutations = 999,  $R^2 = 0.27$ ,  $p = 0.004$ , n = 12) and both weak  
286 and insignificant between beta diversity and  $N_2$  fixation (PERMANOVA; Permutations = 999,  $R^2 = 0.13$ ,  $p = 0.14$ , n = 12).

287 Investigating whether and at which magnitude environmental parameters have an effect on microbial community dissimilarity,  
288 our general dissimilarity model (GDM) showed the expected curvilinear relationship between the predicted ecological distance  
289 and community dissimilarity (Fig. 4c I). Based on I-spline magnitudes of all tested environmental variables, geographic  
290 distance had little effect on community dissimilarity (Fig. S11a). Community dissimilarity changed most notably in response  
291 to variability in low magnitudes of PP (i.e. ISSG and STF; 17% of total community dissimilarity, n = 12) and plateaued with  
292 PP above  $1100 \mu\text{mol C L}^{-1} \text{d}^{-1}$  (Fig. 4c III). A community dissimilarity change occurred most notably when  $N_2$  fixation when  
293 rates were above  $2 \text{ nmol L}^{-1} \text{d}^{-1}$  (~ 19% of change in total community dissimilarity associated to changes in  $N_2$  fixation rates.  
294 Fig. 4c IV). Among all tested environmental parameters, our I-spline results showed that community dissimilarity increased  
295 most in response to variability in MLD and  $PO_4^{3-}$  concentrations (49% of change in total community dissimilarity associated  
296 to MLD variability, and 63% to  $PO_4^{3-}$  variability, respectively, n = 12; Fig. S11a).

297 Significant differences in community dissimilarity structure between Longhurst provinces were associated with high-  
298 pseudocount taxa, dominated by dinoflagellates (Dinophyceae) and diatoms (Bacillariophyta; SIMPER analysis; Table S6).  
299 The pseudocount of ASVs belonging to the phylum Ochrophyta (Bacillariophyta\_X) contributed to differences between ocean  
300 provinces (contributing to at least 9.51% of the differences in community dissimilarity between the SO and ISSG). Moreover,  
301 4.79% of the differences in community dissimilarity between the SO and the SSTC were associated with a higher ASV count  
302 of Bacillariophyta\_X ASVs in the SO. Further, we identified ten ASVs belonging to the phylum Dinophyceae contributing  
303 with 2.1% to the community dissimilarity structure between the SO and ISSG; and with 5.79% to the community dissimilarity  
304 structure between the SSTC and ISSG. This was further supported by relatively high concentrations of the photosynthetic  
305 pigments chl *c3* and peridinin (both indicative pigments for dinoflagellates) in the SO and SAF. We found a relatively high  
306 number of ASV94 and ASV23 (*Chloroparvula pacifica*) in the SSTC, contributing 3.07% to the community dissimilarity  
307 between the SSTC and the ISSG.



### 308 3.6 Prokaryotic community composition

309 From each of 14 stations, a fragment of the small subunit ribosomal RNA gene (16S rRNA) was amplified and sequenced to  
310 obtain insights into the diversity and community composition of prokaryotes. A total of 1308 ASVs was recovered from which  
311 we removed 267 ASVs annotated as chloroplasts and 68 ASVs annotated as mitochondria. Prokaryotic richness increased with  
312 increasing sea surface temperature (Pearson correlation:  $r = 0.65$ ,  $p$ -value = 0.03,  $n = 11$ ; Fig. S7a). Maximum alpha diversity  
313 (Inverse Simpson) estimate was found in the SAF (81.92, Station 15; Fig. S7d). Prokaryotic alpha diversity (Inverse Simpson)  
314 was positively (but not significantly) linked to primary productivity ( $r = 0.36$ ,  $p = 0.55$ ,  $n = 11$ ; Fig. S7f) but showed a  
315 significant negative correlation with  $N_2$  fixation ( $r = -0.7$ ,  $p = 0.05$ ,  $n = 11$ ; Fig. S7h).

316 Our RDA of the prokaryotic ASV table captured 90% of the total variance with a  $p$ -value of 0.06 (Permutations = 999,  $n =$   
317 11). Sites clustered into Longhurst provinces along the first two RDA axes (62.48% of variance constrained; Fig 4b). This was  
318 also shown in the PERMANOVA solution for Longhurst provinces (Permutations = 999,  $R^2 = 0.62$ ,  $p < 0.001$ ,  $n = 11$ ) and our  
319 PERMANOVA grouping into water masses (Permutations = 999,  $R^2 = 0.74$   $p < 0.001$ ,  $n = 11$ ; Fig. 4b). We found more ASVs  
320 occurring in either the ISSG or the SO provinces rather than across all provinces (Fig. 4f). Further, the ISSG and the SO shared  
321 the least ASVs (Fig. 4f). In the RDA, sites within the ISSG province were positively associated with SST and  $N_2$  fixation.  
322 Sites belonging to the SO were positively associated with dissolved inorganic nutrients ( $NO_3^-$ ,  $PO_4^{3-}$ , Si), dissolved oxygen,  
323 and chl  $a$  concentrations as well as high PP (Fig. 4b). The community composition within the SSTC (STF and SAF) was  
324 distinct from that of the ISSG and SO along the 2<sup>nd</sup> RDA axis (21.67% variance constrained) and positively associated with  
325  $NH_4^+$  concentrations (Fig. 4b). Linear relationships between beta diversity and rates were weak for PP (PERMANOVA;  
326 Permutations = 999,  $R^2 = 0.31$ ,  $p = 0.007$ ,  $n = 11$ ) and  $N_2$  fixation (PERMANOVA; Permutations = 999,  $R^2 = 0.2$ ,  $p = 0.05$ ,  
327  $n = 11$ ).

328 Investigating whether and at which magnitude environmental parameters have an effect on prokaryotic microbial community  
329 dissimilarity, our general dissimilarity model (GDM) showed the expected curvilinear relationship (Fig. 4d I). Based on I-  
330 spline magnitude, geographic distance had little effect on community dissimilarity. The largest magnitude in community  
331 dissimilarity could be observed between 190 - 1200  $\mu\text{mol C L}^{-1} \text{d}^{-1}$  (Fig. 4d III). Community dissimilarity changed most notably  
332 in response to variability in low magnitudes of  $N_2$  fixation and did not change in samples with highest average  $N_2$  fixation  
333 measurements (2.8  $\text{nmol L}^{-1} \text{d}^{-1}$  Station 3, and 4.0  $\text{nmol L}^{-1} \text{d}^{-1}$  Station 18, respectively). Largest magnitudes of community  
334 dissimilarity were associated with dissolved oxygen concentrations (Fig. S11b).

335 Taxonomically, based on analysis of the CLR-transformed ASV table, the prokaryotic community was dominated by  
336 Proteobacteria, Cyanobacteria, and Bacteroidetes, which are all typical clades for surface water samples (e.g. Biers et al.,  
337 2009). The greatest community differences occurred between stations of the Southern Ocean (SO) and the Indian South  
338 Subtropical Gyre (ISSG) provinces. Structure in community dissimilarity between the ISSG and SO were mostly associated  
339 with the number of Flavobacteriaceae (11.52% of total community dissimilarity, SIMPER analysis, Table S6) and



340 Alphaproteobacteria (5.69% of the total difference in community dissimilarity, SIMPER analysis, Table S6). The ISSG was  
341 characterized by a high number of Cyanobacteria and some Actinobacteria. The cyanobacterial fraction was dominated by  
342 *Prochlorococcus* and *Synechococcus*, respectively.

343 Within the class level, all stations were dominated by Alpha-, and Gammaproteobacteria, Bacteroidia, Oxyphotobacteria  
344 (Cyanobacteria), and Verrucomicrobia. Within the Alphaproteobacteria, we found a great dominance of ecotype I, II, and IV  
345 of SAR11 clade throughout all samples (Table S4). The relative number of pseudocounts of bacteria belonging to the phylum  
346 Bacteroidetes decreased towards warmer SST in the ISSG, with significant differences between the SO and ISSG (Welch two  
347 sample t-test  $t = 4.58$ ,  $p < 0.001$ ,  $n_1 = 341$ ,  $n_2 = 151$ ). The phylum Bacteroidetes was largely dominated by the order  
348 Flavobacteriales (90.98% of annotated ASVs). Cyanobacteria mainly occurred in the SSTC and in the ISSG, which were  
349 dominated by *Prochlorococcus* in the ISSG and *Synechococcus* in the SSTC, respectively. Cyanobacterial pseudocounts were  
350 significantly lower in the SO in comparison to the SSTC (Welch two sample t-test,  $t = -3.86$ ,  $p\text{-value} < 0.001$ ,  $n_1 = 17$ ,  $n_2 =$   
351  $31$ ) and to the ISSG (Welch two sample t-test,  $t = -4.74$ ,  $p < 0.001$ ,  $n_1 = 17$ ,  $n_2 = 45$ ). *Atelocyanobacteria* (UCYN-A) ASVs  
352 occurred in the SAF (Station 14) and ISSG (Station 2, 3).

#### 353 4. Discussion

354 Each water mass in our study had a distinct microbial fingerprint, including unique communities in frontal regions. We  
355 highlight clear relationships between microbial diversity, and primary productivity and  $N_2$  fixation (high linear and nonlinear  
356 covariability) in the South Indian Ocean Gyre (ISSG), the Southern Ocean (SO), and their frontal transition zone. Below, we  
357 discuss how this clear provincialism of microbial diversity is dis-connected from regional gradients in primary productivity  
358 (PP) and  $N_2$  fixation across our transect. This could suggest that microbial phylogenetic diversity is more strongly bounded by  
359 physical oceanographic boundaries, while microbial activity (and thus, perhaps, their functional diversity, not assessed here)  
360 responds more to chemical properties that changed more gradually between the low- and high-nutrient provinces we sampled.

##### 361 4.1 $N_2$ fixation and associated microbial diversity display distinct regional variations

362 Overall, our  $N_2$  fixation (up to  $4.4 \pm 2.5 \text{ nmol L}^{-1} \text{ d}^{-1}$ ) was comparable to  $N_2$  fixation measured by González et al. (2014) above  
363 the Kerguelen Plateau (up to  $10.27 \pm 7.5 \text{ nmol L}^{-1} \text{ d}^{-1}$ ) and showed a similar latitudinal trend as  $N_2$  fixation further east in the  
364 Indian Ocean, however, around 10-fold lower absolute rates ( $0.8 - 7$  vs  $34 - 113 \text{ nmol L}^{-1} \text{ d}^{-1}$ ; Raes et al. 2014). We note that  
365 the localized rates reported by González et al. (2014) are to date the only published  $N_2$  fixation measurements in this region,  
366 likely to be close to the annual maxima because of high irradiance, however, further investigations across seasonal changes  
367 within the study area are needed to confirm our observations. Our regional data are therefore important in closing the gaps in  
368  $N_2$  fixation measurements in the Southern Ocean, especially considering that large disagreements exist between models of  
369 high-latitude  $N_2$  fixation rates (Tang et al., 2019).



370 N<sub>2</sub> fixation measurements often show high basin-wide variability as well as high variability between samples at the same site,  
371 being sensitive to details of experimental design, incubation, and sea-state conditions (Mohr et al., 2010). In aggregate, these  
372 issues are best accounted for by calculating the minimum quantifiable rate (MQR; Gradoville et al., 2017). We observed high  
373 heterogeneity of biological samples taken from the underway flow-through-system 5 minutes apart (separated by ~15 km)  
374 within the same water mass. Similar variability in absolute measurements of N<sub>2</sub> fixation (2.6 - 10.3 nmol L<sup>-1</sup> d<sup>-1</sup> ± 7.5 nmol L<sup>-1</sup>  
375 d<sup>-1</sup>) were reported by González et al. (2014) close to our sampling site around Kerguelen Island. This could imply a  
376 submesoscale variability or influence of other unmeasured parameters.

377 As oligotrophic gyres extend and displace southwards under climate change, (Yang et al., 2020), the biogeochemical and  
378 physical characteristics of the SO are changing (Caldeira and Wickett, 2005; Swart et al., 2018), biological regional N<sub>2</sub> fixation  
379 might become an important N-source for productivity. Our data showed maximal N<sub>2</sub> fixation in the oligotrophic waters of the  
380 ISSG, however, notably, measurable N<sub>2</sub> fixation occurred well into the SO, to 56° S, suggesting that N<sub>2</sub> fixation contributes to  
381 the regional N pool, despite other available sources of N (Shiozaki et al., 2018; Sipler et al., 2017). Similarly, we found a  
382 negative N\* in the SO, which potentially indicates a P excess supporting N<sub>2</sub> fixation (Knapp, 2012). Noteworthy is a slight  
383 increase in N<sub>2</sub> fixation in the Antarctic Zone (AZ). High-latitude measurements in northern polar regions (Bering Sea) reached  
384 10 -11 nmol N L<sup>-1</sup> d<sup>-1</sup> (Shiozaki et al., 2017), substantially higher than our measurements of the SO (0.8 - 1.9 nmol N L<sup>-1</sup> d<sup>-1</sup>),  
385 potentially supported by the close proximity to the coast or other factors such as day length, seasonality, diazotroph community  
386 or trace metal concentrations.

387 Our results suggest that regional N<sub>2</sub> fixation was not limited by the presence of other sources of bioavailable N (Fig. S10), a  
388 conclusion also reached in a number of studies including culture experiments (Boatman et al., 2018; Eichner et al., 2014;  
389 Knapp, 2012), as well as in situ measurements in the South Pacific (Halm et al., 2012), off the coast of Chile and Peru with  
390 rates up to 190 μmol m<sup>-2</sup> d<sup>-2</sup> (Fernandez et al., 2011), and across the Eastern Indian Ocean (Raes et al., 2015). This evidence  
391 counters the hypothesis of Breitbarth et al. (2007) that N<sub>2</sub> fixation occurs only when other sources of N are limited. The  
392 contribution of N<sub>2</sub> fixation to the N-pool – and thus to productivity – varies strongly with ecosystem structure: In the SO,  
393 despite the local N<sub>2</sub>-fixation measurements, N<sub>2</sub> fixation remains likely a very minor contributor to the N required by the  
394 microbial community for primary productivity.

395 Our results also strongly suggest that prokaryotic community structure and composition (beta diversity) were strongly impacted  
396 by the presence of biological N<sub>2</sub> fixation, itself a prokaryotic process (Karl et al., 2002). For example, the N<sub>2</sub>-fixing  
397 *Atelocyanobacteria* (UCYN-A) occurred in the SAF and ISSG; however, to gain a clear insight into the community and N<sub>2</sub>  
398 fixation, the diazotrophic community would need to be further resolved by amplicon analysis of functional (*nifH*) genes (Luo  
399 et al., 2012) as shown in other high-latitude studies (Fernández-Méndez et al., 2016; Raes et al., 2020).



#### 400 4.2 Total and specific primary productivity differentially affect microbial diversity

401 We found PP was highest in the PFZ and decreased towards higher latitudes in the SO (Fig. 3a). Strass et al. (2002) showed  
402 that frontal maxima of PP are expected, and the observed decrease was probably due to Fe limitation in the SO (Blain et al.,  
403 2008). Primary productivity can also be limited by Si concentration and light availability when the mixed layer deepens (Boyd  
404 et al., 2000), but in our data Si concentrations were high in the surface water samples, and light levels were close to maximum in  
405 austral summer. The measured maximum PP above the Kerguelen Plateau (station E) was likely stimulated by Fe inputs (Blain  
406 et al., 2007).

407 Our results did not support prior observations that frontal regions (SAF and STF) supported higher specific primary  
408 productivity ( $P^B$ ) (as reported in the Antarctic Atlantic sector; Laubscher et al. 1993): While phytoplankton community  
409 composition, phytoplankton size distribution, and nutrient concentrations were strikingly different between the ISSG and SO,  
410 we found little difference in  $P^B$ , with some slightly lower values observed within the SSTC (Fig. 3b). Differences in  $P^B$  usually  
411 arise from physiological changes due to variabilities in irradiance (Geider, 1987), nutrient concentrations (Behrenfeld et al.,  
412 2008; Chalup and Laws, 1990), or differences in phytoplankton community structure, where cyanobacteria have the highest  
413 PP efficiency and diatoms the lowest (Talaber et al., 2018). Thus, our observations suggest that either 1) there is a lack of  
414 selective pressure on photosynthetic efficiency between provinces or 2) mechanisms driving  $P^B$  are different between  
415 provinces, and the sum of beneficial (e.g. increased nutrient concentrations in the SO) and detrimental mechanisms (e.g. low  
416 irradiance and photoinhibition through deep vertical mixing, reported from the ACC, Alderkamp et al., 2011) result in similar  
417  $P^B$ . The slight variation around the frontal system is hard to interpret, as the complex interplay between factors may result in  
418 stochasticity.

419 Primary productivity can be an important driver for (phylogenetic) microbial alpha diversity (Vallina et al., 2014) especially  
420 within ocean provinces (Raes et al., 2018). While our observational study only has a small number of samples within and  
421 between oceanic provinces ( $n = 12$ ,  $n_{ISSG} = 4$ ,  $n_{SSTC} = 3$ ,  $n_{SO} = 4$ ), it did suggest that further validation of this assumption is  
422 needed. We observed that PP changed gradually across the sampling region, and that local variability in PP was high between  
423 samples taken ~15 km apart within the SSTC and SO (Fig. 3a). These local variabilities can arise from complex physico-  
424 chemical interactions between the STF, SAF and SO (Mongin et al., 2008). Counter to Vallina et al. (2014) and Raes et al.  
425 (2018), we found a negative correlation between eukaryotic alpha diversity and PP within the ISSG. Further, we found no  
426 correlation between eukaryotic diversity and PP within the SSTC and SO and none between prokaryotic alpha diversity across  
427 all provinces (Fig. S7).

428 In terms of beta diversity, we observed a structuring effect of PP for both pigment-, 16S rRNA gene-, and 18S rRNA gene-  
429 derived diversity profiles (Fig. 4 a,b, Fig. S5). Pigment analysis revealed that photosynthetic prokaryotic diversity is strongly  
430 impacted by the relative abundance of *Prochlorococcus*, which does not generally occur in cold, high-latitude waters (>40°S/N;  
431 Fig. S5) (Partensky et al., 1999) and, if so, only in low abundance (reviewed in Wilkins et al. 2013). Our 16S rRNA gene



432 analyses confirm these observations showing that 1) picoplankton - and specifically *Prochlorococcus* - had relatively high  
433 proportions in the ISSG but very low in the SSTC, 2) *Synechococcus* dominated the Cyanobacterial fraction in the SSTC, and  
434 3) both *Prochlorococcus* and *Synechococcus*, were not detected in the SO (Table S4, Table S6). In the SSTC and SO,  
435 phytoplankton communities had high proportions of dinoflagellates (Dinophyceae) and diatoms (Bacillariophyta) (up to 74%  
436 of diatom diagnostic pigment concentrations), which are known as essential contributors to marine PP and microbial diversity  
437 (Malviya et al., 2016) and known to dominate the phytoplankton fraction within the Polar Frontal Zone (PFZ), especially as  
438 the blooming season progresses (Brown and Landry, 2001).

439 Further, our results show that phytoplankton community structure appears to be tightly coupled to the occurrence of specific  
440 heterotrophic organisms (Table S6), and thus may mediate an indirect effect of PP through microbial food webs (as also noted  
441 in, e.g., Sarmiento and Gasol 2012). For example, in areas of relatively high diatom concentrations, we found increased  
442 proportions of Flavobacteria. These bacteria specialize on successive decomposition of algal-derived organic matter (Teeling  
443 et al., 2012) and are known associates of diatoms (Pinhassi et al., 2004).

#### 444 **4.3 Implications for microbial regionality**

445 Microbial diversity was regionally constrained independent of geographical distance (GDM analysis), but was partitioned into  
446 ocean provinces as repeatedly described for other ocean basins such as the Pacific (Raes et al., 2018) and the Atlantic Ocean  
447 (Milici et al., 2016). This supports the classical concept of microbial biogeography (Martiny et al., 2006). Further, we found  
448 that microbial beta diversity was even better resolved by individual water masses, highlighting the importance of including  
449 oceanographic boundaries that limit cross-front dispersal (Hanson et al., 2012; Hernando-Morales et al., 2017; Wilkins et al.,  
450 2013a).

451 Our beta diversity analysis confirmed the findings by Baltar and Arístegui (2017) who found unique environmental sorting  
452 and/or selection of microbial populations in the SAF and STF. Further, we were able to link these communities to high  $\text{NH}_4$   
453 concentrations. This suggests high recycling of nitrogen sources within the microbial loop, and potentially favoring  
454 nitrification in this area (Sambrotto and Mace, 2000). We also found increased Dinoflagellate concentrations (PFT) which  
455 have been described to grow well under  $\text{NH}_4$  conditions (Townsend and Pettigrew, 1997). Despite our small sample size within  
456 the SAF and STF, we were able to detect these characteristics, supporting the call from Baltar et al. (2016) of better integrating  
457 frontal zones in our understanding of microbial biogeography.

458 Different trade-offs such as nutrient limitation and grazing can shape the microbial seascape (Acevedo-Trejos et al., 2018). In  
459 our study, the deviation between  $\text{PN} : \text{chl } a$  was large between the So and IO with high  $\text{PN} : \text{chl } a$  ratios in the ISSG (Fig. S4),  
460 which has been used as an indicator of a relatively high abundance of heterotrophic microbes and protists over autotrophic  
461 organisms (Crawford et al., 2015; Hager et al., 1984; Waite et al., 2007). This would suggest that grazers formed a higher



462 fraction of total biomass in the ISSG than in the SO. However, we did not measure zooplankton biomass or grazing rates, so  
463 this remains speculative.

## 464 **5. Conclusion & outlook**

465 Our study leads us to conclude that simultaneous assessment of microbial diversity, biogeochemical rates, and the physical  
466 partitioning of the ocean (provincialism) is central to the understanding of microbial oceanography.

467 Each water mass in our study had a distinct microbial fingerprint, including unique communities in frontal regions. Microbial  
468 alpha diversity and community dissimilarity correlated with biogeochemical rate measurements; however, mechanisms driving  
469 this association need further investigation through high-resolution sampling across spatial and temporal scales. Our results also  
470 indicate that high-latitude N<sub>2</sub> fixation could meaningfully contribute to the global and regional N-pool (as reported for Arctic  
471 N<sub>2</sub> fixation by Sipler et al., 2017), which may become especially significant as global stratification (and concomitant  
472 restrictions in deep water replenishment of nutrients) intensifies.

473 While our sampling is too limited to conclude the point, our observations that phylogenetic diversity is constrained by  
474 hydrographic properties and province boundaries, but biogeochemical rates and nutrient concentrations are changing more  
475 gradually suggests that trans-province functional redundancy is present despite strong biogeographic separation in  
476 phylogenetic terms. As an outlook, we therefore encourage examining both phylogenetic and functional diversity to assess  
477 how functional groups and guilds contribute to the major biogeochemical (C, N) cycles across provinces and other  
478 biogeographic regions. Coordinated studies across ocean provinces are key to establishing the baselines we need to monitor  
479 the rapidly changing properties of the Southern high-latitudes in the face of rising temperature, acidification, and perturbations  
480 in regional currents.

## 481 **Code availability**

482 All code is available under S1\_code\_archive.zip and additionally publically archived under  
483 [https://github.com/CoraHoerstmann/MD206\\_Microbes/releases/tag/v1.0](https://github.com/CoraHoerstmann/MD206_Microbes/releases/tag/v1.0)

## 484 **Data availability**

485 All HPLC data, environmental and rate measurement data, including PN, MIMS data, PP, N<sub>2</sub> fixation, and minimum  
486 quantification rate calculations are stored at the PANGAEA database (Hörstmann et al. 2018). All sequences are archived in  
487 the European Nucleotide Archive (primary accession: PRJEB29488).



488 **Author contribution**

489 CH did the post-voyage processing and analysis of all samples and wrote the manuscript. ER conducted the fieldwork, designed  
490 the experiments and contributed to data analysis and writing the manuscript. PLB contributed to data analysis, ecological  
491 interpretation, and writing the manuscript. CLM provided the historic physical and chemical data and contributed to the write-  
492 up. UJ helped with the DNA sequencing and writing the manuscript. AW contributed to design of the experiments, data  
493 analysis, and writing the manuscript.

494

495 **Conflict of interest statement**

496 No conflict of interest.

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505

506

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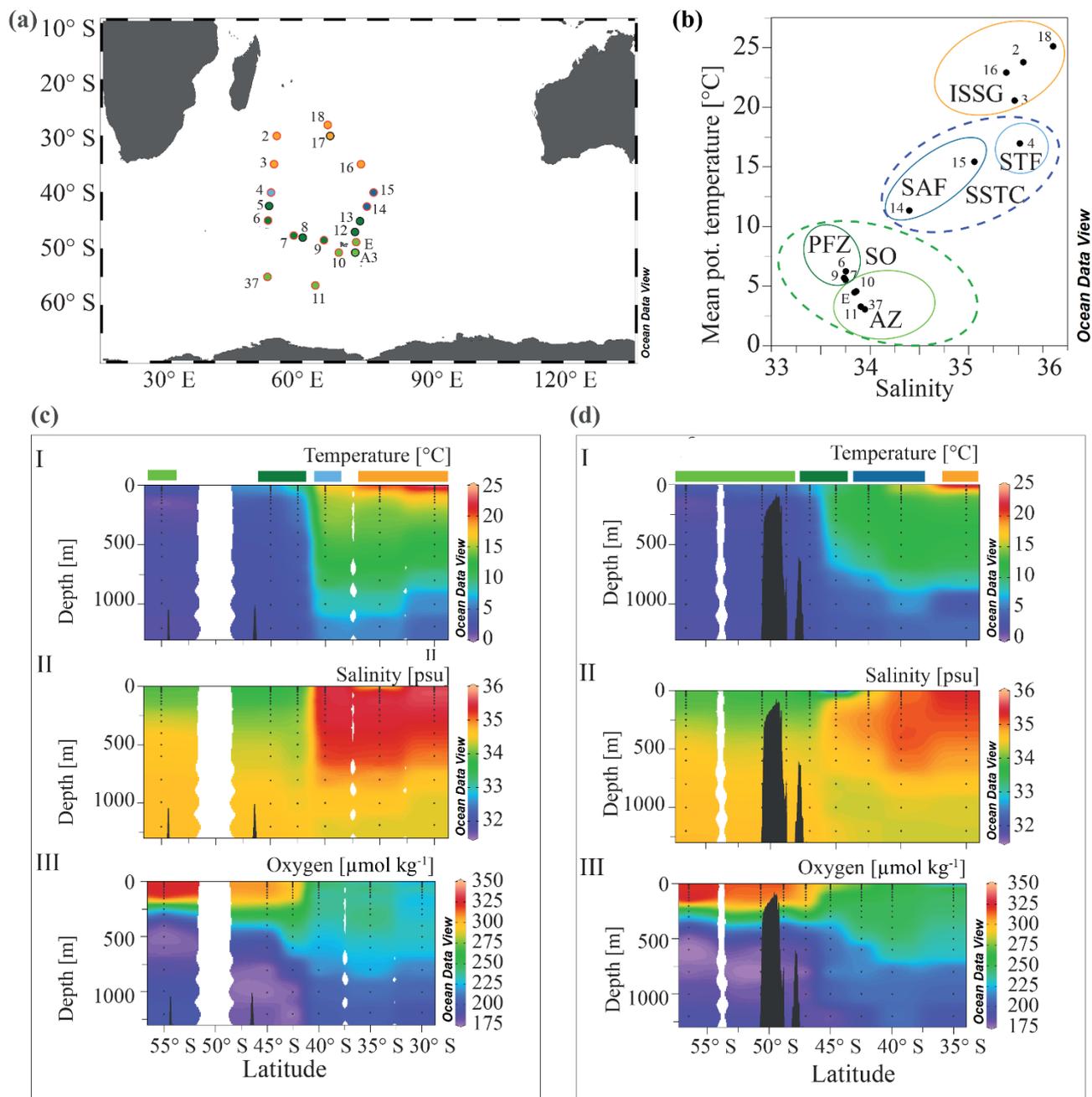
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758 **Figures**

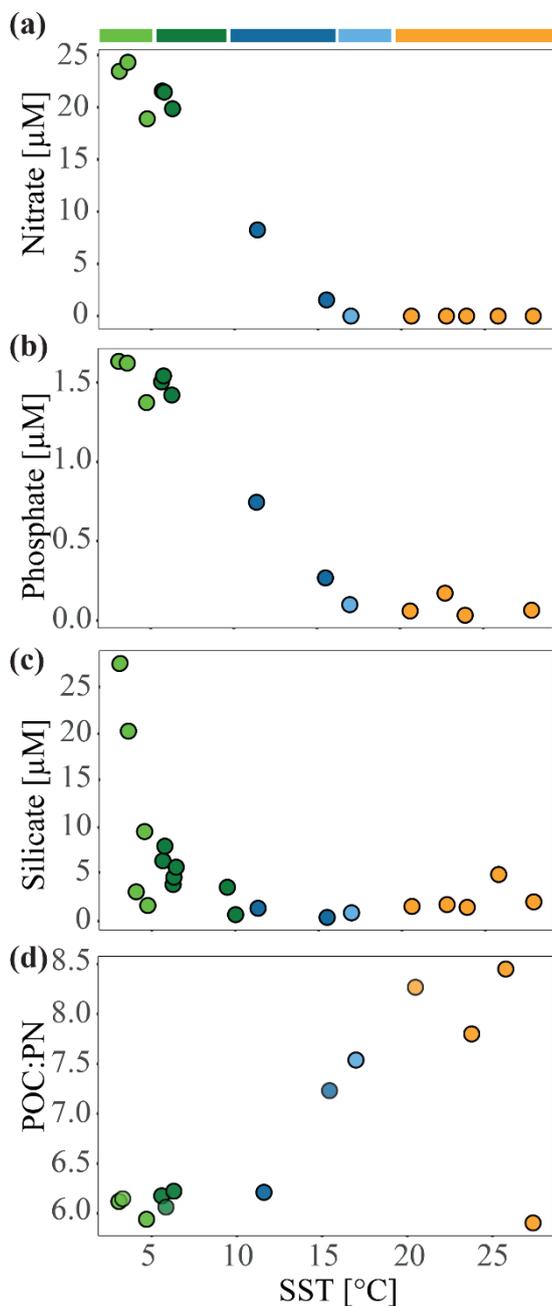


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 760 **Figure 1: (a) The MD206 transect and OISO stations. Stations are colored according to water masses and encircled by sampling**  
 761 **extent: black circles indicate stations where only CTD (conductivity, temperature, depth) data is provided, and stations encircled in**



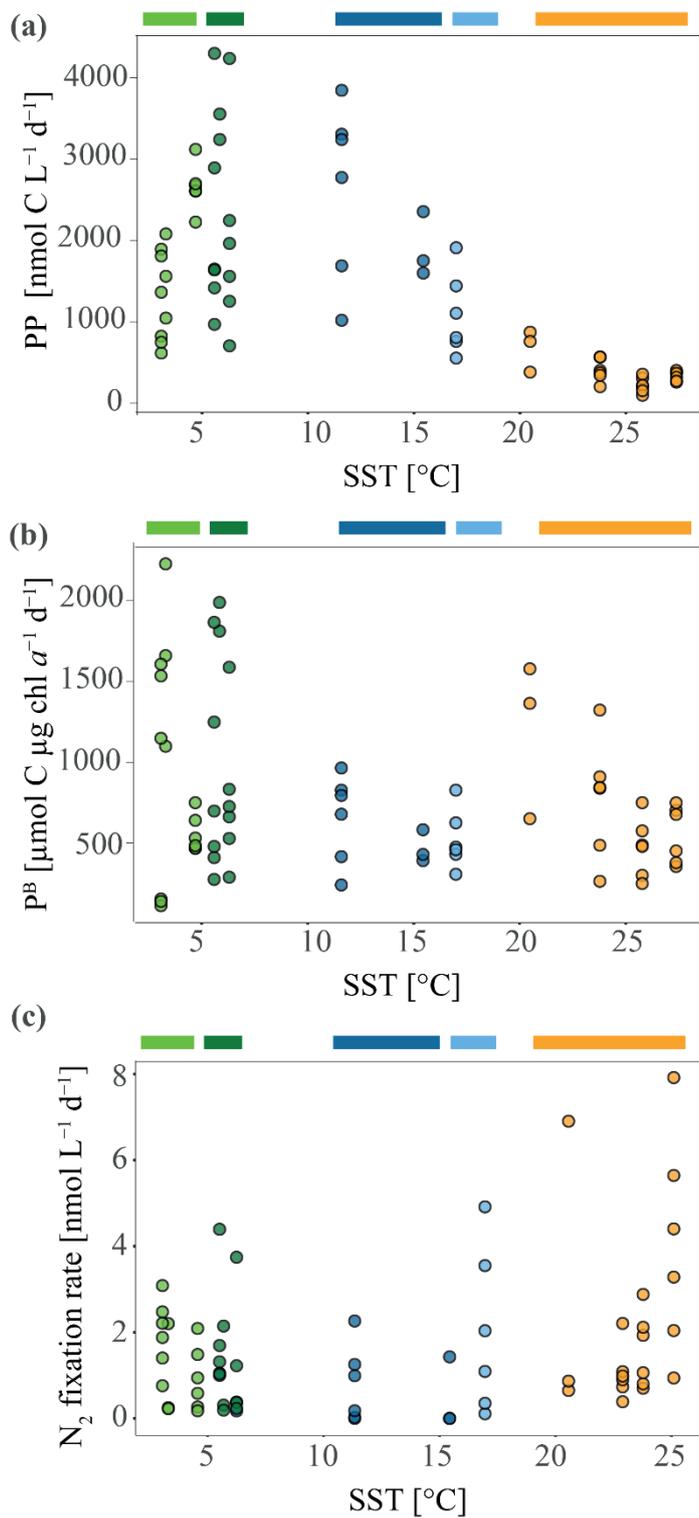
762 red denote where additional samples for C, N and community composition were taken. (b) A plot of potential temperature (in degrees  
763 Centigrade (°C) and salinity (in practical salinity units) using sea surface (7 m) data of the stations used in further microbial and  
764 C/N analyses. The yellow circle highlights the Indian Ocean gyre (ISSG), light blue circle the Subtropical Front (STF), blue circle  
765 the Subantarctic Front (SAF), dark green circle the Polar Front Zone (PFZ) and the light green circle indicates the Antarctic Zone  
766 (AZ), dashed lines indicate water masses clustered within ocean provinces: blue line marks the Subtropical Convergence province  
767 (SSTC) and green line the Southern Ocean (SO); (c) and (d) show depth profiles of temperature, oxygen and salinity along two  
768 transects of the OISO stations. Colored bars indicate water masses according to (b). (c) shows the western transect covering OISO  
769 stations 2, 3, 4, 5, 6 and 37 around  $53\pm 1^\circ\text{E}$  longitude; (d) shows the eastern transect of OISO stations 10,11,12,13,14,15,16 and E  
770 around  $68\pm 5^\circ\text{E}$ .

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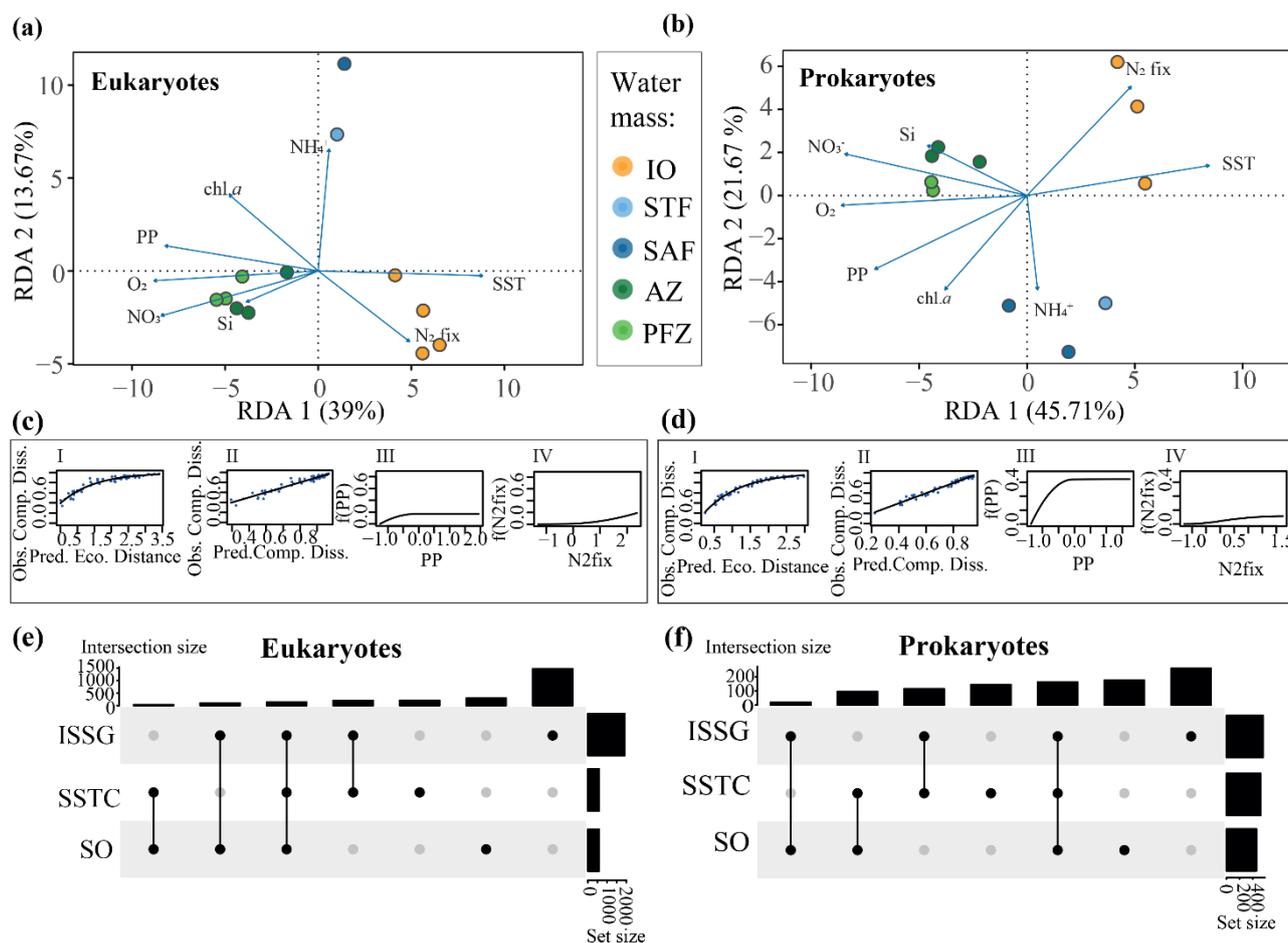
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773 **Figure 2: Nutrient concentrations ( $\mu\text{mol L}^{-1}$ ) and molar ratios of particulate organic carbon (POC) to particulate nitrogen (PN)**  
774 **during the MD206 expedition against sea surface temperature ( $^{\circ}\text{C}$ ): (a) nitrate, (b) phosphate, (c) silicate, (d) POC:PN ratio. Colored**  
775 **bars indicate water masses according to their sea surface temperature; yellow bar highlight the Indian Ocean gyre (ISSG), light blue**  
776 **bar the Subtropical Front (STF), blue bar the Subantarctic Front (SAF), light green bar the Polar Front Zone (PFZ) and dark green**  
777 **the Antarctic Zone (AZ).**





779 **Figure 3: Primary productivity (PP) and specific primary productivity (P<sup>B</sup>) measured during the MD206 cruise. (a) PP in micromole**  
 780 **per liter and day against sea surface temperature (SST) in °C. (b) P<sup>B</sup>, normalized by chl *a* concentration. (c) Nitrogen fixation rates**  
 781 **against sea surface temperature (SST) in degree centigrade measured during the MD206 cruise. Rates are shown in nanomol per**  
 782 **liter and day; Colored bars indicate water masses; yellow bar highlight the Indian Ocean gyre (ISSG), light blue bar the Subtropical**  
 783 **Front (STF), blue bar the Subantarctic Front (SAF), dark green bar the Polar Front Zone (PFZ) and light green bar marks Antarctic**  
 784 **Zone (AZ).**



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 786 **Figure 4: (a) Eukaryotic and (b) prokaryotic community structures of different water masses measured during the MD206 cruise.**  
 787 **Redundancy analysis (RDA) of 18S and 16S rRNA gene ASV tables as response variables and environmental metadata as**  
 788 **explanatory variables; environmental metadata are represented as arrows. Constrained Analyses were performed by water mass.**  
 789 **There were significant relationships between water masses and community dissimilarities (PERMANOVA, 999 permutations;  $p <$**   
 790  **$0.001$ ,  $R^2 = 0.67$  for eukaryotes and  $p < 0.001$ ,  $R^2 = 0.74$  for prokaryotes, respectively). Colors indicate major water masses according**  
 791 **to the legend; yellow bar highlights the Indian South Subtropical Gyre (ISSG), light blue bar the Subtropical Front (STF), blue bar**  
 792 **the Subantarctic Front (SAF), dark green bar the Polar Front Zone (PFZ) and light green marks the Antarctic Zone (AZ).**



793 Eukaryotic (c) and prokaryotic (d) general dissimilarity model (GDM) with (I) observed compositional dissimilarity against  
794 predicted ecological distance, calculated from temperature + dissolved oxygen +  $\text{NO}_3^-$  +  $\text{NH}_4^+$  + Si + chl *a* + PP +  $\text{N}_2$  fixation, (II)  
795 observed compositional dissimilarity against predicted compositional dissimilarity to test the model fit, and contribution of (III) PP  
796 and (IV)  $\text{N}_2$  fixation to community dissimilarity expressed as a function of the environmental parameter (f(PP) and f( $\text{N}_2$ fix),  
797 respectively). For all functional plots of environmental data of the GDM analysis see Fig. S11. Eukaryotic (e) and prokaryotic (f)  
798 UpSet plots of ASV intersections between Longhurst provinces. Analyses are based on binary tables (presence/ absence) and the  
799 sum of all ASVs found across samples within one province. Intersection size shows number of ASVs shared between provinces (black  
800 dots, associated) and ASVs only found in one province (only black dot). Set size shows number of ASVs found in a specific Longhurst  
801 province.

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**Table 1. Sampling stations visited during the MD206 cruise, including chlorophyll *a* concentrations, primary productivity (PP), specific primary productivity (P<sup>B</sup>), and N<sub>2</sub> fixation. Mixed layer depth (MLD) was calculated using  $\Delta d = 0.03 \text{ kg m}^{-3}$  compared to a surface reference depth of 7 m. NA indicates no data. Ranges and mean for sample replicates of N<sub>2</sub> fixation and PP are given (n = 3 for stations 3, 9, 11, 15; n = 6 for stations E, 37, 2, 4, 6, 7, 14, 16, 18). Colored bars indicate water masses; yellow bar highlight the Indian Ocean gyre (ISSG), light blue bar the Subtropical Front (STF), blue bar the Subantarctic Front (SAF), green bar the Polar Front Zone (PFZ), and dark green bar the Antarctic Zone (AZ)**

Station	Longitude [°E]	Latitude [°S]	MLD [m]	chl <i>a</i> [ $\mu\text{g L}^{-1}$ ]	Primary productivity (PP) [ $\mu\text{mol C L}^{-1} \text{ d}^{-1}$ ]	specific PP (P <sup>B</sup> ) [ $\mu\text{mol C } \mu\text{g chl } a^{-1} \text{ L}^{-1} \text{ d}^{-1}$ ]	N <sub>2</sub> fix [ $\text{nmol L}^{-1} \text{ d}^{-1}$ ]	MQR [ $\text{nmol L}^{-1} \text{ d}^{-1}$ ]
37	52.003	55.004	52.5	4.96	587.42 - 1875.58; 1185.59	118 - 1628; 795	0.76 - 3.09; 1.97	1.2
11	63.006	56.499	49.5	0.92	1020.91 - 2065.12; 1541.95	1115 - 2255; 1683	0.23 - 2.20; 0.89	1.2
10	68.421	50.667	88.2	NA	NA	NA	NA	NA
E	72.367	48.8	81.3	4.09	2212.37 - 3114.53; 2645.72	477 - 762; 567	0.18 - 2.09; 0.92	0.7
7	58.004	47.667	49.6	3.33	942.99 - 4305.26; 2129.45	283 - 1889; 843	1.0 - 4.39; 1.75	1.2
9	64.999	48.501	69.4	1.76	3236.8 - 3553.33; 3395.07	1834 - 2013; 1924	0.19 - 2.15; 0.88	0.8
6	52.102	45.000	41.7	2.28	676.44 - 4242.33; 1977.6	296 - 1609; 784	0.17 - 3.25; 0.93	0.9
14	74.884	42.499	30.8	3.93	994.1 - 3847.07; 2635.94	248 - 979; 665	0.0 - 2.26; 0.78	0.7
15	76.407	39.999	29.8	3.95	1579.92 - 2341.93; 1884.88	400 - 593; 477	0.0 - 1.43; 0.24	1.2
4	52.79	40.001	54.6	2.23	524.32 - 1876.67; 1069.21	315 - 841; 531	0.11 - 4.91; 2.01	3.5
3	53.499	35.000	15.9	0.53	350.33 - 845.86; 642.59	662 - 1599; 1215	0.65 - 6.91; 2.81	5.4
16	73.466	35.001	19.9	0.40	170.05 - 537.91; 378.28	271 - 1341; 790	0.39 - 2.21; 1.05	1.3
2	54.1	30.001	12.9	0.55	63.24 - 324.72; 190.38	257 - 762; 484	0.7 - 2.88; 1.58	2.6
18	65.832	28.0	16.9	0.49	226.09 - 371.07; 301.3755	364 - 762; 563	0.94 - 7.92; 4.04	5.0

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