

1 **Biogeographic partitioning of Southern Ocean picoplankton revealed**  
2 **by metagenomics**

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21  
22 **Summary**

23 We performed a metagenomic survey (6.6 Gbp of 454 sequence data) of Southern Ocean  
24 picoplankton during the austral summer of 2007–2008, examining the genomic signatures of  
25 communities across a latitudinal transect from Hobart (44°S) to the Mertz Glacier,  
26 Antarctica (67°S). Operational taxonomic units (OTUs) of the SAR11 and SAR116 clades  
27 and the cyanobacterial genera *Prochlorococcus* and *Synechococcus* were strongly  
28 overrepresented north of the Polar Front (PF). Conversely, OTUs of the  
29 Gammaproteobacterial Sulfur Oxidizer-EOSA-1 (GSO-EOSA-1) complex, the phyla  
30 Bacteroidetes and Verrucomicrobia and order Rhodobacterales were characteristic of  
31 waters south of the PF. Functions enriched south of the PF included a range of transporters,  
32 sulphur reduction and histidine degradation to glutamate, while branched-chain amino acid  
33 transport, nucleic acid biosynthesis and methionine salvage were overrepresented north of  
34 the PF. The taxonomic and functional characteristics suggested a shift of primary  
35 production from cyanobacteria in the north to eukaryotic phytoplankton in the south, and  
36 reflected the different trophic statuses of the two regions. The study provides a new level of  
37 understanding about Southern Ocean microbial communities, describing the contrasting  
38 taxonomic and functional characteristics of microbial assemblages either side of the PF.

## 40 Introduction

41 The SO plays a critical role in sustaining marine life around the globe. Upwelling of nutrient-rich  
42 Circumpolar Deep Water (CDW) returns nutrients transported to the deep ocean by the sinking of  
43 organic matter (Rath *et al.*, 1998) and supports 75% of global ocean primary production north of  
44 30°S. Surface waters at high southern latitudes remain cold (< 3°C) year-round but undergo  
45 extreme seasonal variations in sea-ice cover, light levels and day length. Primary production and

47 biomass are high in summer and very low in winter. Bacteria are abundant in the SO despite the  
48 low temperatures and seasonal variability in productivity and are a major route for carbon flow  
49 (Hessen *et al.*, 2004).

50 The SO is composed of several zones separated by circumpolar fronts, the locations of which  
51 vary temporally and with longitude (Whitworth III, 1980; Orsi *et al.*, 1995; Sokolov and Rintoul,  
52 2002). The fronts separate regions with different physiochemical properties, such as density,  
53 salinity, temperature and nutrient concentrations (Sokolov and Rintoul, 2002). Hydrographic,  
54 bathythermographic and satellite altimetry data have been used to determine the frontal structure  
55 of the Antarctic Circumpolar Current (ACC) south of Australia (Sokolov and Rintoul 2002).  
56 From north to south, the major fronts are the Subtropical Front (STF), the Subantarctic Front  
57 (SAF), the Polar Front (PF) and the southern ACC front (SACCF). Each of these fronts consists  
58 of multiple branches (Sokolov and Rintoul, 2002; Sokolov and Rintoul, 2009b, a). The  
59 Subantarctic Zone (SAZ) lies between the STF and SAF, the Polar Frontal Zone (PFZ) lies  
60 between the SAF and the PF, and the Antarctic Zone (AZ) lies between the PF and the Antarctic  
61 continent.

62 The PF has been suggested to be a major biogeographical boundary in the distribution and  
63 abundance of both zooplankton (Chiba *et al.*, 2001; Hunt *et al.*, 2001; Esper and Zonneveld,  
64 2002; Ward *et al.*, 2003) and bacterioplankton (Selje *et al.*, 2004; Abell and Bowman, 2005;  
65 Giebel *et al.*, 2009; Weber and Deutsch, 2010). However, the microbial assemblages that  
66 characterize Antarctic waters are generally poorly understood, and their diversity and functional  
67 capacity are not well characterized (Murray and Grzyski, 2007). Large scale metagenome  
68 surveys have not previously been performed.

69 Recent anthropogenic climate change may be driving the warming and freshening of the  
70 ACC (Boning *et al.*, 2008) and shifting it and its fronts poleward (Fyfe and Saenko, 2005;

71 Biastoch *et al.*, 2009). A community-level understanding is required to effectively understand the  
72 main components and dynamics of the microbial food web in the SO and thereby predict the  
73 effects of a shifting ACC on the distribution and abundance of plankton. The oceanic changes  
74 may have global ecological significance as the SO performs many ecosystem functions, including  
75 significant sequestration of anthropogenic CO<sub>2</sub> (Sabine *et al.*, 2004; Mikaloff Fletcher *et al.*,  
76 2006) through both physiochemical processes and the “biological pump” of CO<sub>2</sub> fixation  
77 (Thomalla *et al.*, 2011).

78 In the austral summer of 2006 we initiated a metagenome program based on the sampling  
79 design of the Global Ocean Sampling expedition (Rusch *et al.*, 2007), aimed at providing a  
80 baseline to monitor microbial communities in the Australian section of the SO. To date we have  
81 sampled SO water from 73°E to 150°E and 44°S to 68°S at depths from the surface to ~ 3700 m.  
82 In this study, we present data for plankton assemblages passed through a 20 µm prefilter and  
83 captured onto sequential 0.1 µm, 0.8 µm and 3.0 µm filters, ~~representing surface communities~~  
84 ~~north and south of the PF.~~ This fractionation method allows deeper sequencing of the community  
85 to improve identification of low-abundance taxa, and provides additional context for interpreting  
86 sequence data, for example particle attachment and trophic status (Lauro *et al.*, 2011). The  
87 samples were collected in summer 2007/2008 on the SR3 transect (Sokolov and Rintoul, 2002) of  
88 the Climate of Antarctica and the Southern Ocean (CASO), and Collaborative East Antarctica  
89 Marine Census (CEAMARC) projects during the International Polar Year (IPY) program (Fig. 1).  
90 We assessed the taxonomic and functional profiles of microbial communities from either side of  
91 the PF, thereby contributing important new information about the microbial ecology of the SO  
92 and defining the microbial communities most influenced by the effects of the PF forming a  
93 biogeographical barrier.

94

## 95    **Results and discussion**

96

### 97    *Overview of taxonomic biogeography*

98

99    6.6 Gbp of 454 sequence data representing picoplankton in the size range 0.1 – 3.0 µm was  
100    obtained from 16 samples. After removal of low-quality reads, 454 sequencing yielded 157,507 to  
101    597,689 reads per sample (mean 354,399) of lengths ranging from 100 to 606 bp (mean 378). The  
102    proportion of reads in each sample which yielded matches to RefSeq ranged from 25% to 85%  
103    (mean 62%). The most abundant OTUs in each sample are given in Table 1 and a full list of OTU  
104    abundances in Table S2. It is important to note that the taxonomic assignments are confident  
105    assignments (*i.e.* above an E-value threshold of  $1.0 \times 10^{-3}$ ) that are limited to the sequences of  
106    organisms available in the database and identified by MINSPEC as part of the minimal species set.

107    ANOSIM was performed to test for statistically significant differences between the OTU  
108    profiles of the zones. The analysis showed that the zones harbor significantly different microbial  
109    communities ( $R = 0.451$ ,  $p < 0.004$ ). SIMPER was performed in order to identify the contribution  
110    of individual OTUs to the differences between the zones. The analysis found that no single OTU  
111    contributed more than 2.9% of variance (Fig. 2), and 74% of variance was contributed by OTUs  
112    with a contribution less than 1%. There was also a large difference in the contribution to variance  
113    of the three size fractions, with approximately 52% of all variance contributed by OTUs from 3.0  
114    µm fraction, 37% by the 0.8 µm fraction, and 9% by the 0.1 µm fraction (Table S3, supporting  
115    information).

116    Notably, OTUs within several taxonomic groups that had high contribution to variance  
117    covaried in their relative representation in the NZ and SZ. For example, Bacteroidetes and GSO-  
118    EOSA-1 representatives were on average more abundant in the SZ; while *Prochlorococcus* and

119 *Synechococcus* spp., SAR11 and SAR116 were on average more abundant in the NZ (Fig. 2).

120 Some groups, such as the Alteromonadales, had variable relative representation depending on size

121 fraction.

122

123 *GSO-EOSA-1*

124

125 The Gammaproteobacterial Sulfur Oxidizer-EOSA-1 (GSO-EOSA-1) cluster, represented in

126 RefSeq by the OTUs *Candidatus* Vesicomysocius okutanii strain HA and *Ca. Ruthia magnifica*

127 strain Cm. (*Calymene magnifica*) (Walsh *et al.*, 2009), made a large contribution to variance

128 between the NZ and SZ, with higher abundance in the SZ: relative abundances of GSO-EOSA-1

129 in the SZ were 5.2%, 3.4% and 0.25% in the 0.1, 0.8 and 3.0  $\mu\text{m}$  size fractions respectively,

130 compared to 1.1%, 0.84% and 0.30% in the NZ. The contribution to variance of this group was

131 highest in the 0.1  $\mu\text{m}$  size fraction, followed by 0.8  $\mu\text{m}$  and 3.0  $\mu\text{m}$  (Fig. 2). This pattern most

132 likely represents a small cell size and lack of association with particulate matter.

133 *Ca. R. magnifica* and *Ca. V. okutanii* are chemoautotrophic endosymbionts of deep-sea

134 bivalves (Kuwahara *et al.*, 2007; Newton *et al.*, 2007) and are thus unlikely to be present in open

135 ocean surface waters. However, GSO-EOSA-1 representative ARCTIC96BD-19 has recently

136 been reported at high abundance in Antarctic coastal waters (Ghiglione and Murray, 2011;

137 Grzymalski *et al.*, 2012). The majority of 16S rRNA genes from our metagenome with best BLASTN

138 matches to *Ca. R. magnifica* and *Ca. V. okutanii* clustered with ARCTIC96BD-19 in a neighbour-

139 joining phylogenetic tree (Fig. S1, supporting information), indicating this is the dominant GSO-

140 EOSA-1 representative. Single-cell genomic analysis of ARCTIC96BD-19 from global

141 mesopelagic waters indicates the lineage is probably mixotrophic, able to couple carbon fixation

142 to oxidation of reduced sulphur compounds as well as assimilate organic carbon (Swan *et al.*,

2011). GSO-EOSA-1 cytochrome C oxidase (CoxII) has been identified in a winter metaproteome of Antarctic Peninsula coastal waters, suggesting the capacity for aerobic respiration (Williams *et al.*, 2012)(Williams *et al.*, (2012)). We assembled our GSO-EOSA-1 affiliated reads and identified several contigs containing ORFs with high identity to aerobic respiration genes, including cytochrome C oxidase subunits, from *Ca. R. magnifica* and *Ca. V. okutanii* (Fig. S2, supporting information). Taken together, this evidence suggests the GSO-EOSA-1 representative in Antarctic coastal waters is a versatile chemolithoautotroph capable of aerobic respiration.

It has been proposed that during the winter months, chemolithoautotrophy is dominant over photoautotrophy as the major carbon fixation input in AZ waters due to the lack of available light, both from seasonal darkness and ice cover (Grzyski *et al.*, 2012). The high relative abundance of GSO-EOSA-1 we detected in SZ compared to NZ waters may therefore represent the remnants of an annual winter increase in population in the marginal ice zone which does not occur in the open ocean.

#### *Ammonia-oxidizing Crenarchaeota*

*Nitrosopumilus maritimus* SCM1 and *Cenarchaeum symbiosum* are chemolithoautotrophic, nitrifying members of the Marine Group I Crenarchaeota (MGI) (Preston *et al.*, 1996; Walker *et al.*, 2010) and are the only representatives in the reference database of the Ammonia Oxidizing Archaea (AOA). The contribution of OTUs of *C. symbiosum* to the AOA signature was low (Table S3, supporting information). As *C. symbiosum* is a sponge symbiont (Preston *et al.*, 1996) and given the poor representation of AOA in RefSeq, it is likely this OTU has attracted sequences originating from planktonic AOA and *C. symbiosum* itself is not present. AOA were moderate

167 contributors to variance between the NZ and SZ, and were overrepresented in the SZ in all size  
168 fractions (Fig. 2). As with the GSO-EOSA-1 cluster, MGI have been proposed to be abundant  
169 chemolithoautotrophs and therefore major drivers of winter carbon fixation in Antarctic coastal  
170 waters (Grzyski *et al.*, 2012; Williams *et al.*, 2012).

171 Sample 353 had a particularly high relative abundance of *N. maritimus* OTUs (7.5% of the  
172 0.1  $\mu\text{m}$  fraction; 0.8  $\mu\text{m}$ : 11%; 3.0  $\mu\text{m}$ : 12%). This sample was taken closer to the Antarctic  
173 continent (3.7 km) than any other, in relatively shallow (180 m) waters 17.6 km from the Mertz  
174 Glacier. The high abundance of ammonia oxidizers may reflect an input of ammonia from  
175 terrestrial sources (*e.g.* penguin guano), resuspension of benthic sediments in which MGI are  
176 abundant (Bowman and McCuaig, 2003) by near-shore turbulence and iceberg scouring.  
177 Breakdown of water column stratification has been previously suggested as a cause of increased  
178 AOA abundance in Antarctic coastal surface waters (Kalanetra *et al.*, 2009).

179

#### 180 *Cyanobacteria*

181

182 OTUs of the cyanobacterial genera *Prochlorococcus* and *Synechococcus* were overrepresented in  
183 the NZ in all size fractions (Fig. 3). The mean relative abundance of cyanobacteria in samples 367  
184 and 368, the two northernmost samples, was strikingly higher than the mean abundance across all  
185 other samples in the NZ. *Synechococcus* sp. CC9902 alone composed greater than 22% of the 0.8  
186  $\mu\text{m}$  fraction (Table S2, supporting information), consistent with *Synechococcus* species' average  
187 cell diameter of approximately 0.9  $\mu\text{m}$ . The high abundance of both cyanobacterial genera on the  
188 3.0  $\mu\text{m}$  fraction was unexpected given the cell diameter, although it has previously been reported  
189 (Lauro *et al.*, 2011)(Lauro *et al.*, 2011a) and attributed to aggregation (Lomas and Moran,  
190 2011)(Lomas, 2011 #712). Samples 367 and 368 were separated from the other samples north of

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191 the PF by the Subtropical Front (STF). While the STF was not a significant boundary on the  
192 assemblage level, it may mark a significant biogeographical boundary for these cyanobacteria.  
193 *Synechococcus* and *Prochlorococcus* together represent a large proportion of both phytoplankton  
194 abundance and carbon fixation in temperate and tropical waters, in many regions contributing  
195 more than half of total primary production (Liu *et al.*, 1997; Liu *et al.*, 1998; André *et al.*, 1999).  
196 The role of the STF in determining the latitudinal range of *Synechococcus* and *Prochlorococcus* is  
197 therefore important, as it will affect models of ocean productivity under changing climatic  
198 conditions, and warrants further investigation.

199 -  
200 Despite the high abundance of cyanobacteria north of the STZ, they were also a significant  
201 feature of the SAZ; for example, *Synechococcus* sp. CC9902 composed 3-5% of the 0.8 µm  
202 fraction in SAZ samples. Our results extend the latitudinal distribution of both *Prochlorococcus*  
203 and *Synechococcus* to include presence at very low abundance as far south as the Antarctic coast.  
204 *Prochlorococcus* have been reported to be restricted to tropical and subtropical waters within 40°  
205 of latitude (Partensky *et al.*, 1999), and to be a negligible (Ghiglione and Murray, 2011) or ~~an-~~  
206 undetectable ~~component~~ (Gryzyski *et al.* 2012) component of marine picoplankton in Antarctic  
207 waters. However, our findings are consistent with findings of a logarithmic relationship of  
208 cyanobacterial numbers with temperature, where cyanobacteria were found at concentrations of  
209  $10^3 - 10^4$  cells per litre even in the coldest waters, approximately four orders of magnitude less  
210 than in waters around Tasmania (Marchant *et al.*, 1987). Cyanophage proteins have also been  
211 detected in a metaproteomic analysis of Antarctic Peninsula coastal surface waters (Williams *et*  
212 *al.* 2012). The depth of shotgun metagenome sequence coverage of our samples is likely to have  
213 contributed to the detection of these cyanobacteria.

214

215 *SAR11 and SAR116 clades*

216

217 *Ca. Pelagibacter ubique* HTCC1062 is a good representative of total SAR11 abundance in our  
218 study, as it is a member of the SAR11 phylotype which is most abundant in SO waters 22(Brown  
219 *et al.*, 2012). *Ca. P. ubique* HTCC1062 was the most abundant OTU across all samples and  
220 fractions (NZ average: 62%, 25% and 24% of the 0.1  $\mu\text{m}$ , 0.8  $\mu\text{m}$  and 3.0  $\mu\text{m}$  fractions  
221 respectively; SZ: 59%, 22% and 18%) and one of the most significant contributors to variance  
222 between the NZ and SZ. The high abundance of SAR11 in the 0.1  $\mu\text{m}$  fraction is consistent with  
223 the small size of SAR11 cells (Rappé *et al.*, 2002). The higher representation in the NZ may  
224 reflect the competitiveness of SAR11 members in regions with low DOC concentrations due to  
225 low primary productivity (Giovannoni *et al.*, 2005; Alonso and Pernthaler, 2006), such as the  
226 high-nutrient, low-chlorophyll (HNLC) SAZ region of the SO. Overall, our findings are  
227 consistent with reports that SAR11 is ubiquitous in the world's oceans (*e.g.* (Mary *et al.*, 2006;  
228 Carlson *et al.*, 2009) and more abundant north of the ACC (Giebel *et al.*, 2009).

229 OTUs of *Ca. Puniceispirillum marinum* from the SAR116 clade were a moderate contributor  
230 to variance between the NZ and SZ with higher abundance in the NZ (Fig. 2). A genomic analysis  
231 reported *Ca. P. marinum* IMCC1322 to be a metabolic generalist with genes for aerobic CO  
232 fixation, C<sub>1</sub> metabolism and a *Ca. P. ubique*-like dimethylsulfoniopropionate (DMSP)  
233 demethylase, suggesting SAR116 and SAR11 occupy similar ecological niches (Oh *et al.*, 2010).  
234 In the Scotia Sea, SAR116 abundance (determined using fluorescence *in situ* hybridisation) was  
235 reported to be higher in more productive waters where SAR11 numbers were lower (Topping *et*  
236 *al.*, 2006). However, our analysis across an extended latitudinal transect indicates that overall  
237 SAR11 and SAR116 have similar biogeographic distributions.

238

239 *Bacteroidetes*

240

241 OTUs of the phylum Bacteroidetes, in particular members of the class Flavobacteria, were found  
242 to be abundant (NZ average: 1.2%, 5.0% and 6.9% of the 0.1  $\mu\text{m}$ , 0.8  $\mu\text{m}$  and 3.0  $\mu\text{m}$  fractions  
243 respectively; SZ: 2.3%, 9.8% and 9.1%) and significant contributors to variance between the NZ  
244 and SZ (Fig. 2). Flavobacteria have been previously reported to compose the majority of both  
245 Bacteroidetes (Murray and Grzyski, 2007) and total planktonic biomass (Abell and Bowman,  
246 2005) in the SO, as well as being abundant in sea ice (Brown and Bowman, 2001). As  
247 heterotrophic degraders of High Molecular Weight (HMW) compounds in the form of both  
248 Dissolved and Particulate Organic Matter (DOM and POM) (Kirchman, 2002), marine  
249 Flavobacteria are major components of marine aggregates (Rath *et al.*, 1998; Crump *et al.*, 1999;  
250 Zhang *et al.*, 2007). The higher abundance of Flavobacteria OTUs on the 0.8  $\mu\text{m}$  and 3.0  $\mu\text{m}$   
251 fractions indicates their association with particulate matter.

252 The higher abundance of OTUs of Flavobacteria in the SZ may reflect an input of cells from  
253 melting sea ice (Brown and Bowman, 2001), the higher rates of primary productivity in the south,  
254 and the role of the Flavobacteria as degraders of HMW DOM. Because deposition of marine  
255 snow is a major route for sequestration of fixed carbon in the ocean (*e.g.* Hessen *et al.* (2004)),  
256 the Flavobacteria that associate with this particulate matter represent a remineralizing shunt,  
257 which would decrease carbon sequestration by this route.

258

259 *Rhodobacterales*

260

261 Members of the order Rhodobacterales were abundant (NZ average: 1.2%, 10% and 5.5% of the  
262 0.1  $\mu\text{m}$ , 0.8  $\mu\text{m}$  and 3.0  $\mu\text{m}$  fractions respectively; SZ: 1.6%, 13% and 7.9%) and high

263 contributors to variance, overrepresented in the SZ on all size fractions. As several members of  
264 the Roseobacter clade have been shown to have symbiotic relationships with marine eukaryotic  
265 algae (Buchan and Moran, 2005; Wagner-Dobler and Biebl, 2006), and their abundance in the SO  
266 has previously been linked to phytoplankton blooms (West *et al.*, 2008; Obernosterer *et al.*,  
267 2011), it is likely that their overrepresentation in the SZ is related to the higher density of  
268 phytoplankton in the AZ.

269 OTUs of *Roseobacter denitrificans* Ochl14 and *Silicibacter pomeroyi* DSS-3 were  
270 consistently the most abundant Roseobacter clade representatives. *R. denitrificans* and *S.*  
271 *pomeroyi* fall within a subclade of Aerobic Anoxygenic Phototrophic (AAP) members of the  
272 *Roseobacter* clade (Swingley *et al.*, 2007). These species have diverse mixotrophic metabolisms,  
273 with genomic and experimental evidence of photoheterotrophic respiration of organic carbon,  
274 fixation of CO<sub>2</sub>, oxidation of CO, oxidation of reduced sulfur compounds, and utilization of the  
275 abundant marine osmolyte DMSP (King, 2003; Moran *et al.*, 2004; Wagner-Dobler and Biebl,  
276 2006; Swingley *et al.*, 2007; Brinkhoff *et al.*, 2008; Howard *et al.*, 2008). This metabolic diversity  
277 suggests a complex ecological role, particularly with respect to the capture and release of  
278 climatically active gases (CO<sub>2</sub>, CO, dimethylsulfide) involved in carbon and sulfur cycling.

279

## 280 *Alteromonadales*

281

282 Members of the gammaproteobacterial order Alteromonadales were large contributors to  
283 variance. Most OTUs were overrepresented in the SZ but some were overrepresented in the NZ  
284 on the 3.0 µm fraction (Fig. 2). ~~*Colwellia psychrerythrae* sp. 34H, type strain of *Colwellia*~~  
285 ~~*psychrerythrae*~~, was one of the most abundant alteromonads and exhibited this distribution (NZ  
286 average: 0.14%, 2.2% and 16% of the 0.1 µm, 0.8 µm and 3.0 µm fractions respectively; SZ:

0.52%, 5.1% and 10%). *C. psychrerythraea* 34H is a well-characterised psychrophile isolated from Arctic sediment (Myers, 2000), a lifestyle and environment consistent with its overrepresentation in the SZ on the 0.1  $\mu\text{m}$  and 0.8  $\mu\text{m}$  fractions. However, it is noteworthy that water temperatures at the NZ sample sites (2.9–14 °C) were better suited to *C. psychrerythraea* 34H's optimum growth temperature of 8 °C (Methé, 2005 #6) than those in the SZ (−1.8–0.7 °C). As *C. psychrerythraea* 34H has been shown to synthesise extracellular polysaccharides (Myers, 2000), we speculate that the higher abundance on the 3.0  $\mu\text{m}$  fraction in the NZ may represent aggregation under conditions of optimum growth. ~~*C. psychrerythraea* is a well characterised psychrophile isolated from Arctic sediment (Methé et al., 2005), a lifestyle and environment consistent with its overrepresentation in the SZ on the 0.1  $\mu\text{m}$  and 0.8  $\mu\text{m}$  fractions. We hypothesized that the 3.0  $\mu\text{m}$  fraction *C. psychrerythraea* hits may represent *Thalassomonas viridans*, a close relative of *Cohwellia* identified in temperate waters (Macián et al., 2001) but not represented in RefSeq. However, a comparison of reads with identity to *C. psychrerythraea* 16S rRNA to 16S sequences of *C. psychrerythraea*, *T. viridans* and other proteobacterial species did not support this hypothesis. The *C. psychrerythraea* OTU abundant in the NZ in 3.0  $\mu\text{m}$  fraction may therefore represent an uncharacterized relative or ecotype with a preference for warmer environments.~~

#### Verrucomicrobia

Two representatives of the phylum Verrucomicrobia, *Coralimargarita akajimensis* and *Akkermansia* sp. Muc-30, were moderate contributors to variance and overrepresented in the SZ (Fig. 2). Surprisingly given the small cell size of *C. akajimensis* (Yoon et al., 2007), contribution to variance increased with size fraction; a study in the North Sea reported a similar fractionation

311 pattern, and suggested marine Verrucomicrobia may be predominantly particle attached (Trull *et*  
312 *al.*, 2001). However, little else is known about the distribution and ecological roles of marine  
313 Verrucomicrobia (Trull *et al.*, 2001).

314

#### 315 *Overview of functional biogeography*

316

317 ANOSIM analysis of the samples' KEGG ortholog group and module profiles revealed that the

318 zones had significantly different functional potential (ortholog group:  $R = 0.642$ ,  $p < 0.001$ ;

319 module:  $R = 0.819871$ ,  $p < 0.001$ ). SIMPER was performed on the profiles in order to identify the

320 specific functional differences between the zones. No single ortholog group or module

321 contributed more than  $1.0122\%$  of the variance, indicating a complex and diverse pattern of

322 functional differences (Table S4 and Table S5, supporting information). There was a strong trend

323 for ortholog groups and modules with higher contributions to variance to be overrepresented in

324 the NZ in the 3.0  $\mu\text{m}$  fraction but the SZ in the smaller fractions, indicating that the functional

325 diversity of each zone was strongly segregated by size fraction.

326

#### 327 *Functional capacities distinguished by the PF*

328

329 A number of modules with transport functions (sn-glycerol 3-phosphate transport system,

330 dipeptide transport system, peptides/nickel transport system, simple sugar transport system, -

331 sulfonate/nitrate/taurine transport system) were overrepresented in the SZ (Table 2). As the

332 genomes of copiotrophic bacteria have evolved to have a higher number of narrow-specificity

333 transporters relative to oligotrophic genomes (Lauro *et al.*, 2009), these differences may reflect

334 the higher nutrient availability and thus a dominance of copiotrophs in the SZ. The taxonomic

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335 contributors to these modules were varied, although members of the Rhodobacterales were  
 336 prominent (Fig. 3). The glycine betaine/proline transport module was also overrepresented in the  
 337 SZ, though this probably reflects glycine betaine's role as an osmo- and cryoprotectant in the  
 338 colder SZ waters. This is supported by the major taxonomic contributor to this module, genus  
 339 *Psychromonas*, which comprises several psychrophiles.  
 340 ~~Two~~ One exceptions to this pattern ~~were~~ was the branched-chain amino acid transport system  
 341 ~~and multiple sugar transport~~ modules, ~~both of which were~~ overrepresented in the NZ. ~~The higher~~  
 342 ~~abundance of the multiple sugar module is consistent with the oligotrophic preference for broad-~~  
 343 ~~specificity transporters, while the relatively lower abundance of the branched-chain amino acid~~  
 344 ~~module.~~ This may reflect a higher availability of more labile dissolved free amino acids (DFAA)  
 345 and dipeptides in the SZ as byproducts of blooming eukaryotic phytoplankton. Additionally, as  
 346 the genera *Pelagibacter* and *Puniceispirillum*, were major contributors to this module's  
 347 overabundance in the NZ (Fig. 3). ~~phytoplankton,~~ this may reflect a generalised adaptation to  
 348 more oligotrophic environments.

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349 Biosynthesis pathways for all major nucleic acids (pyrimidine deoxyribonucleotide  
 350 biosynthesis, adenine nucleotide biosynthesis, guanine nucleotide biosynthesis) were consistently  
 351 high contributors to variance and overabundant in the NZ. This pattern seems inconsistent with  
 352 the more oligotrophic nature of the NZ, as oligotrophic cells generally have smaller genomes  
 353 (Lauro *et al.*, 2009) and slower growth rates than copiotrophs, and would therefore be expected to  
 354 require a lower rate of *de novo* nucleotide biosynthesis. A possible explanation for this is that SZ  
 355 cells have higher availability of extracellular DNA as a byproduct of decaying phytoplankton  
 356 (Lomas and Moran, 2011), which can be imported and salvaged for nucleic acids (Pop *et al.*,  
 357 2004) thus reducing the requirement for *de novo* synthesis. No single taxonomic group

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358 contributed a large fraction of the difference in this module (Fig. 3), suggesting this is a  
359 widespread adaptation.

360 The methionine salvage pathway module had a large contribution to variance between the  
361 zones and was overrepresented north of the PF. This may reflect the higher availability of  
362 dimethylsulphoniopropionate (DMSP) in the SZ as a byproduct of blooming eukaryotic algae.  
363 DMSP is a major carbon and sulfur source for marine microorganisms, and is commonly  
364 assimilated by bacteria through demethylation to methylmercaptopropionate (MMPA), followed  
365 by further catabolism to the climatically important dimethylsulfide or methanethiol (reviewed by  
366 Curson *et al.* (2011)). However, when DMSP is scarce, MMPA ~~can~~may be derived from  
367 methionine through the alternative methionine salvage pathway (Reisch *et al.*, 2011). The genus  
368 *Synechococcus*, a noted contributor to marine DMSP uptake and assimilation (Vila-Costa *et al.*,  
369 2006), was a very high contributor to the abundance of this module in the NZ (Fig. 3), suggesting  
370 *Synechococcus* species may use this route when DMSP is unavailable.

371 The sulfur reduction module was overrepresented in the SZ, and it is likely that this result is  
372 strongly driven by taxonomic differences— While the taxonomic breakdown indicated a large  
373 number of genera contributed to the difference, the gammaproteobacteria were the highest-  
374 contributing class (Fig. 3). This module also includes the assimilatory sulfate reduction pathway,  
375 which is widespread in marine bacteria, but is absent from SAR11, with known representatives  
376 reported to lack genes for assimilatory sulfate reduction (*cysDNCHIJ*) (Tripp *et al.*, 2008). The  
377 higher relative abundance of SAR11 in the NZ may therefore contribute- to the lower abundance  
378 of genes for assimilatory sulfate reduction in that zone.

379 The sulfur reduction module also included adenylylsulfate reductase (APS reductase,  
380 encoded by *aprAB*), an enzyme implicated in sulfite detoxification during heterotrophic growth  
381 on organosulfonates (Meyer and Kuever, 2007) (N.B. in recent KEGG releases, *aprA* is no longer

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382 included in this module). APS reductase is used during sulfur oxidation by autotrophic GSO-  
383 EOSA-1 (Walsh *et al.*, 2009). Also, *Roseobacter* clade bacteria are involved in the decomposition  
384 of abundant organic sulfur compounds (*e.g.*, DMSP, organosulfonates), and hence have been  
385 accorded an important role in marine sulfur cycling (Moran *et al.*, 2007). ~~*Roseobacters* and GSO-~~  
386 ~~*EOSA-1* were both among the highest contributors to variance and overrepresented in the SZ,~~  
387 ~~suggesting they contributed to the overabundance of this module.~~

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388 The photosystem I and II modules were overrepresented in the NZ. As the SZ has on average  
389 a higher chlorophyll concentration than the NZ (Moore and Abbott, 2000), this pattern was not  
390 expected. The KEGG module for eukaryotic ribosomes was also unexpectedly slightly  
391 overrepresented in the NZ. Underrepresentation of plastid sequence in the KEGG database may  
392 have contributed to a systematic bias against eukaryotic genes. As our filtration approach used a  
393 20 µm prefilter, cyanobacteria may have been enriched relative to large phototrophic algae. These  
394 findings highlight complexities presently associated with interpreting data for marine eukaryotes.  
395 The photosystem II module was overrepresented in the NZ. Given the underrepresentation of  
396 cyanobacterial OTUs in the SZ, this may reflect a dominance of primary production by eukaryotic  
397 algae south of the PF and cyanobacteria to the north. Decomposition of the taxonomic affiliations  
398 of ortholog groups contributing to this module found OTUs of *Synechococcus* and  
399 *Prochlorococcus* spp. to be major contributors to the difference (Fig. 3). Variation in the  
400 photosystem I module, which was marginally overrepresented in the SZ, could largely be  
401 attributed to diatoms and other eukaryotic photosynthesisers (Fig. 3), again supporting a  
402 dominance of eukaryotic phytoplankton in SZ primary production. Diatoms have previously been  
403 reported at higher abundance south of the PF, and their distribution is likely to be linked to the  
404 higher concentration of dissolved silica in that region (Trull *et al.*, 2001). As both eukaryotic  
405 photosynthesisers and cyanobacteria would be expected to encode both complete photosystems,

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406 the differences in module abundance probably represent the degree of similarity between the  
407 photosystem I and II genes in the KEGG database and those found in the sampled environments.

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408 The histidine degradation to glutamate module, which comprises four ortholog groups  
409 mediating the degradation of histidine to glutamate via N-formiminoglutamate, was  
410 overrepresented in the SZ. The histidine biosynthesis module was also overrepresented in the SZ.  
411 While the concentration of dissolved histidine in the SO is generally low (Kawahata and Ishizuka,  
412 2000), blooming eukaryotic phytoplankton (which are more prevalent in the SZ) may deplete  
413 nitrate while releasing DFAA. As DFAA become available, they are used by bacteria to sense the  
414 decaying bloom. Histidine may therefore act as a proxy for DFAA to regulate the expression of  
415 bacterial aminopeptidases, which are involved in lysing diatoms (Bidle and Azam, 2001). The  
416 class Bacteroidetes, while a small contributor to the histidine biosynthesis module in the SZ, was  
417 a large contributor to histidine degradation (Fig. 3), supporting an association with  
418 phytoplanktonic bloom products. It is also possible that uptake and degradation of histidine to  
419 glutamate (which generates ammonia as a by-product) may function as a limited nitrogen source.

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420 ~~Two different KEGG modules representing the NADH dehydrogenase complex (Complex I~~  
421 ~~of the electron transport chain) were very high contributors to variance, but were overrepresented~~  
422 ~~in different zones. KEGG module M00145 (“Complex I (NADH dehydrogenase), NADH~~  
423 ~~dehydrogenase I/diaphorase subunit of the bidirectional hydrogenase”) was significantly~~  
424 ~~overrepresented in the NZ, while module M00142 (“Complex I (NADH dehydrogenase), NADH~~  
425 ~~dehydrogenase I”) was overrepresented in the SZ. The distinction between these modules in the~~  
426 ~~KEGG orthology is taxonomic: M00145 contains NADH dehydrogenases associated with~~  
427 ~~oxidative phosphorylation in cyanobacteria and chloroplasts, while M00142 comprises the~~  
428 ~~mitochondrial equivalents. The distribution of these modules therefore reflects the taxonomic~~

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429 differences between the zones, with cyanobacteria overrepresented in the NZ and eukaryotic  
430 plankton in the SZ.

**Comment [w4]:** This section added little to the analysis

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#### 432 *Biogeographic role of the PF*

433

434 Our results show that there are major taxonomic and functional differences across the PF. The  
435 differences in functional potential between the NZ and SZ reflect both their taxonomic profiles  
436 and fundamental trophic and ecological differences. In particular, they provide genomic support  
437 that the NZ is more oligotrophic than the SZ (Pollard *et al.*, 2002; Giovannoni *et al.*, 2005;  
438 Alonso and Pernthaler, 2006; Lauro *et al.*, 2009), and are consistent with the observation that  
439 primary production is higher south of the PF (Strutton *et al.*, 2000; Williams *et al.*, 2010). Our  
440 findings extend previous work in defining the PF as a strong biogeographic boundary which  
441 shapes not only the composition, but also the functional capacity of microbial communities in the  
442 SO.

443 A possible alternative hypothesis for the observed separation is that the samples are  
444 partitioned by the continental margin, as all but one of the SZ samples were taken in waters over  
445 the Antarctic continental shelf and slope in the vicinity of the Mertz glacier polynya. However,  
446 ANOSIM analysis of an alternative grouping of the samples into “polynya” and “open ocean” had  
447 poorer support ( $R = 0.309$ ,  $p < 0.01$ ) than the grouping based on the PF. Additional taxonomic  
448 profiles for samples taken from the region south of the PF in other seasons (austral summers  
449 06/07, 08/09) and in other sectors of the SO (70-115 °E) also supported the PF as the major  
450 discriminator (data not shown). Taken together, this evidence strongly supports the hypothesis  
451 that the PF is a major biogeographical boundary in the SO independent of a latitudinal gradient or  
452 of the effect of the continental margin and Mertz polynya.

453        These results do not exclude the possibility that other major SO fronts, particularly the STF  
454        and SAF, are also significant biogeographic boundaries, as has been reported in some previous  
455        reports for specific taxonomic groups (*e.g.* Abell and Bowman (2005)). While the sampling  
456        resolution in this study was not sufficient to resolve the effects of other fronts, there are some  
457        indications in the data of further structure within the zones. The two samples north of the STF had  
458        significantly larger cyanobacterial populations than the remaining NZ samples (see discussion of  
459        *Prochlorococcus* and *Synechococcus*, above). Future sampling across these fronts at higher  
460        resolution will provide the data necessary to investigate finer biogeographic patterns.

461        The nature and function of microbial communities in the SO are of global significance  
462        because of the large oceanic expanse that is involved and the importance of the carbon fixation  
463        and nutrient cycling that occurs there. Knowledge of these communities and their biogeographic  
464        drivers has relevance for understanding and predicting the long-term effects of environmental  
465        change in the region. Our findings provide a basis for predicting how climate change-driven shifts  
466        in the SO may affect microbial communities; in particular, the effects of changes in the nature and  
467        location of the ACC on the ecosystem functions of SO picoplankton.

468

## 469    **Experimental procedures**

470

### 471    *Sampling and DNA sequencing*

472

473        A volume of ~ 500 L per sample was collected by sequential size fractionation through a 20 µm  
474        prefilter directly onto 3.0, 0.8 and 0.1 µm pore sized 293 mm polyethersulfone membrane filters,  
475        and cryogenically preserved (Rusch *et al.*, 2007; Ng *et al.*, 2010). DNA extraction (Rusch *et al.*,  
476        2007) and pyrosequencing on GS20 FLX Titanium (Roche, Branford, CT, USA) was performed

477 at the J. Craig Venter Institute in Rockville, MD, USA as described previously (Lauro *et al.*,  
478 2011). Duplicate reads and reads with many pyrosequencing errors were removed as described  
479 previously (Lauro *et al.*, 2011).

480

#### 481 *Grouping of samples by oceanographic zone*

482

483 A range of data were recorded on board the RSV *Aurora Australis*, including position, sampling  
484 and water column depth, ocean temperature, salinity and fluorescence, and meteorological data  
485 (Table S1, supporting information). These were used to locate the PFZ based on a surface  
486 temperature gradient  $\sim 1.35^{\circ}\text{C}$  across a distance of 45–65 km, placing the PF at approximately  
487  $-59.70^{\circ}$ , consistent with previous descriptions (Moore *et al.*, 1999; Sokolov and Rintoul, 2002).  
488 Samples were accordingly grouped into “North” (NZ) and “South” (SZ) zones (Table S1,  
489 supporting information). The NZ represents waters from Subtropical, Subantarctic and Polar  
490 Frontal Zones, while the SZ represents the AZ.

491

#### 492 *Comparison to RefSeq database*

493

494 A subset of the RefSeq microbial (bacteria and archaea) genome database (release 41, retrieved  
495 May 31 2010 from <ftp://ftp.ncbi.nih.gov/refseq/release/>) was prepared by excluding sequences  
496 with the words “shotgun”, “contig”, “partial”, “end” or “part” in their headers (Angly *et al.*,  
497 2009). Because this database was not expected to contain representative genomes for every  
498 species present, Operational Taxonomic Units (OTUs) in this study are defined by the best  
499 species match in this database, and may for example represent congeners.

500 The metagenomic reads from each sample were compared against this database using  
501 TBLASTX, with default parameters except for: E-value threshold [-e]  $1.0 \times 10^{-3}$ ; cost to open gap  
502 [-G] 11; cost to extend gap [-E] 1; masking of query sequence [-F] m S (SEG masking for lookup  
503 table only).

504

#### 505 *Identification of minimal species sets*

506

507 A computational method to minimise false OTU identifications and increase the accuracy of OTU  
508 abundance estimates (MINSPEC) was developed and implemented in PERL. Following the approach  
509 of Ye and Doak (2009) to the parsimonious reconstruction of biochemical pathways (MINPATH),  
510 MINSPEC computes the smallest set of OTUs sufficient to explain a set of observed high-quality  
511 hits against RefSeq (or any other sequence database). The minimal set computation is framed as a  
512 linear programming problem and solved with the GNU Linear Programming Kit (GLPK) tool  
513 “GLPK linear programming/MIP solver” (GLPSOL) (Free Software Foundation, Boston). This  
514 approach eliminates many of the spurious OTU identifications which result from reads with  
515 strong identity to more than one OTU. The “minimal species set” is liable to exclude some low-  
516 abundance OTUs, but gives more faithful abundance estimates and eliminates many false  
517 positives.

518 To validate this approach and estimate error rates, an assemblage of hypothetical taxa was  
519 simulated with varying degrees of overlapping genomic identity and a logarithmic rank-  
520 abundance curve. A simulated metagenomic sampling and BLAST search was performed on this  
521 set, and the results processed with MINSPEC. Over multiple replications, MINSPEC was consistently  
522 able to identify the true set and reject spurious identifications, with false positive and negative  
523 rates smaller than 5% (excluding unsampled rare taxa).

524 The outputs of all TBLASTX searches against RefSeq were processed by MINSPEC, and hits not  
525 belonging to the minimal sets were removed.

526

#### 527 *OTU abundance and variance between zones*

528

529 The relative OTU abundances for each sample were determined using the PERL script Genome  
530 relative Abundance and Average Size (GAAS) (Angly *et al.*, 2009). Briefly, GAAS estimates the  
531 relative abundance of OTUs from the number and quality of BLAST hits to each species, taking  
532 into account differences in genome size. GAAS was run with the default settings. To normalise for  
533 reads which did not yield acceptable hits, the relative abundances for each sample were scaled by  
534 that sample's effective BLAST hit rate. An OTU profile was generated for each sample by  
535 encoding the scaled relative abundance of each OTU from each size fraction as a separate  
536 variable.

537 To test the hypothesis that the oceanic zones harbour significantly different communities,  
538 one-way Analysis Of SIMilarities (ANOSIM) with 999 permutations was performed on a  
539 standardised, log-transformed Bray-Curtis resemblance matrix of OTU profiles with PRIMER 6.  
540 SIMilarity PERcentages (SIMPER) analysis was performed with PRIMER 6 to identify the  
541 contribution of individual OTUs to differences between the zones. All statistical procedures using  
542 PRIMER 6 were performed as described by Clarke and Warwick (2001).

543

#### 544 *Assembly of GSO-EOSA-1 contigs*

545 To investigate the physiological potential of the GSO-EOSA representative identified in the SZ,  
546 reads with identity to *Ca. R. magnifica* and/or *Ca. V. okutanii* were assembled using the Celera  
547 WGS Assembler v6.1 (Myers, 2000)(Huson *et al.*, 2001)(Pop *et al.*, 2004). 242 large (> 2 kbp)

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548 | contigs were used for ORF prediction by MetaGene (Noguchi *et al.*, 2006), and predicted ORFs  
549 | compared against the NCBI nr database with blastx (E-value threshold [-e]  $1.0 \times 10^{-3}$ ). The  
550 | resulting annotated contigs were manually analysed for genes of interest.  
551 |

#### 552 | *Comparison to KEGG database*

553 |

554 | In order to identify functional differences between the zones, the set of metagenomic reads from  
555 | each sample was compared against the Kyoto Encyclopedia of Genes and Genomes (KEGG)  
556 | GENES database (retrieved July 2 2010 from <ftp://ftp.genome.jp/pub/kegg/genes/fasta/genes.pep>)  
557 | with BLASTX, with default parameters except for: maximum number of database sequence  
558 | alignments [-b] 10; E-value threshold [-e] ~~1.0~~  $\times 10^{-3}$ ; gap opening penalty [-G] 11; gap  
559 | extension penalty [-E] 1; masking of query sequence [-F] m S (SEG masking for lookup table  
560 | only).  
561 |

#### 562 | *Analysis of functional potential*

563 |

564 | Genes identified by BLASTX were aggregated to KEGG ortholog groups according to the KEGG  
565 | Orthology schema (<ftp://ftp.genome.jp/pub/kegg/genes/ko>, retrieved Mar 29 2011), and ortholog  
566 | group abundances calculated for each sample. Following (Coleman and Chisholm, 2010), a read  
567 | was considered a hit to a given ortholog group if the top three hits for that read (or all hits if fewer  
568 | than three total hits) were to genes from the same ortholog group, and had bit scores  $> 40$ . If the  
569 | bit score difference between any two top hits was greater than 30, only the hits above this  
570 | difference were considered.



571 Ortholog group counts were then used to calculate the abundance of KEGG modules.  
572 Because many ortholog groups are members of more than one module, the abundance ( $a_m$ ) of  
573 each module  $m$  was calculated as

$$a_m = \sum_{K=1}^n \frac{C_K}{M_K}$$

574 where  $n$  is the number of ortholog groups  $K$  belonging to module  $m$ ,  $C_K$  is the number of hits to  
575 ortholog group  $K$ , and  $M_K$  is the total number of modules to which  $K$  belongs. To account for  
576 differences in sequencing depth between samples, module abundances were scaled to 500,000  
577 reads per sample. To test the hypothesis that the NZ and SZ harbour significantly different  
578 functional potential, one-way ANOSIM with 999 permutations was performed as above on a  
579 standardised, log-transformed Bray-Curtis distance resemblance matrix of the module and  
580 ortholog group profiles. A functional profile was generated for each sample by summing the  
581 scaled abundances of each module from all size fractions, and SIMPER performed as above to  
582 identify modules which contributed highly to the variation in functional potential between the two  
583 zones. Modules with a high contribution to variance or otherwise of interest were then linked to  
584 taxonomy (“taxonomic decomposition”) by noting the genus of the organism associated with  
585 each gene in the KEGG GENES database and thus calculating the relative contribution of each  
586 genus to each module’s abundance. This allowed us to putatively assign functional contributions  
587 to genera which were not identified in our taxonomic analysis, as the database included gene  
588 sequences for organisms for which a full genome was not available.

589 ~~A functional profile was generated for each sample by summing the scaled abundances of each~~  
590 ~~module from all size fractions.~~

591 ~~To test the hypothesis that the NZ and SZ harbour significantly different functional potential,~~  
592 ~~one-way ANOSIM with 999 permutations was performed as above on a standardised, log-~~

593 ~~transformed Bray-Curtis distance resemblance matrix of the module and ortholog group profiles~~  
594 ~~To identify which modules contributed significantly to the variation in functional potential~~  
595 ~~between the two zones, SIMPER was performed as above.~~

596  
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598  
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604

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818  
819 **Supporting information**

820

821 Additional Supporting Information may be found in the online version of this article:

822

823 **Fig. S1.** Neighbour-joining tree of GSO-EOSA-1-like 16S rRNA gene sequences from the SO.  
824 Sequence labeled in black text are reads from our metagenomic dataset; in red text are 16S rRNA  
825 gene sequences from Gammaproteobacterial Sulphur Oxidisers (GSO) and other  
826 gammoproteobacteria from Genbank. The tree was constructed using ARB (Ludwig et al. 2004).

827

828 **Table S1.** Full sample information including physiochemical parameters.

829

830 **Table S2.** Relative abundances of all OTUs in all samples from all size fractions. Size fraction is  
831 given in OTU name, e.g. the column “Mycoplasma genitalium strain G37-08” represents the  
832 relative abundance of the *Mycoplasma genitalium* G37 OTU in the 0.8 µm size fraction.

833

834 **Table S3.** Contributions of individual OTUs to variance between the North and South zones.  
835 Size fraction is given in OTU name, e.g. the row “Mycoplasma genitalium strain G37-08”  
836 represents the contribution to variance of the *Mycoplasma genitalium* G37 OTU in the 0.8 µm  
837 size fraction.

838

839 **Table S4.** Genes related to aerobic respiration annotated in scaffolds from assemblies of GSO-  
840 EOSA-1 affiliated reads. Assembly of reads with identity to *Ruthia magnifica* or *Ca.*  
841 *Vesicomysocius okutanii* was performed with WGS-ASSEMBLER (Celera, Alameda) and ORFs



842 predicted with METAGENE (Noguchi et al. 2006). ORFs were annotated with BLASTN against the  
843 NCBI nr database (E-value threshold  $1.0 \times 10^{-3}$ ). Only selected matches relevant to aerobic  
844 respiration are shown.

845

846 **Table S5.** Contributions of KEGG ortholog groups to variance between the North and South  
847 zones. Size fraction is given in ortholog group name, e.g. the row “DNA polymerase III subunit  
848 alpha [EC:2.7.7.7]-08” represents the contribution to variance of the DNA polymerase III subunit  
849 alpha ortholog group in the 0.8 µm size fraction.

850

851 **Table S6.** Contributions of KEGG modules to variance between the North and South zones.

852

853 **Figure legends**

854

855 **Fig. 1.** Sites of samples used in this study. Area depicted is in the Australian sector of the  
856 Southern Ocean. Samples are from the North zone (triangles) and South zone (squares). The  
857 dashed line gives the approximate position of the Polar Front, associated with a major core of the  
858 Antarctic Circumpolar Current (ACC), at the time of the voyage.

859

860 **Fig. 2.** Contribution of OTUs to variance between North and South, and differential abundance  
861 of OTUs from each size fraction between the two zones. Each coloured (red or blue) rectangle  
862 represents an OTU identified through analysis of BLAST matches between SO metagenome data  
863 and the RefSeq database. The area of each rectangle as a proportion of the total plot area  
864 corresponds to that OTU’s contribution to the total variance between the two zones. The colour of  
865 each rectangle corresponds to difference in relative abundance of that OTU between the zones,

866 with blue indicating a higher relative abundance south of the PF, and red a higher abundance  
867 north of the PF. OTUs from clades or taxonomic ranks of interest have been grouped, with labels  
868 in bold and groups separated by gray lines. Groups and OTUs with a low contribution to variance  
869 which were not grouped are unlabeled. OTUs from each size fraction have also been grouped,  
870 with labels in black outline and size fractions separated by thick black lines. The total  
871 contribution to variance of each size fraction is given as a percentage. Full data are given in Table  
872 S3, supporting information.

873  
874 **Fig. 3. Decomposition of KEGG modules of interest to contributing classes, orders or genera. The**  
875 left side of each stack (S) indicates the proportion of the module abundance contributed by each  
876 class, order or genus in the South Zone, while the right side (N) represents the North Zone. As the  
877 contributions are relative and represent unitless module abundances, no axis is given and  
878 proportions are not comparable between modules. Contributing classes, orders or genera are  
879 arranged in descending order of the difference in the relative contributions between the zones.  
880 Only the eight highest contributors for each module are shown, with the remainder collapsed into  
881 the “Other” group. The taxonomic ranks to which each module was decomposed are as follows:  
882 sn-glycerol 3-phosphate transport, peptide-nickel transport, simple sugar transport and  
883 sulfonate/nitrate/taurine transport were decomposed to order; glycine betaine/proline transport  
884 and branched-chain amino acid transport to genus; pyrimidine deoxyribonucleotide biosynthesis,  
885 adenine nucleotide biosynthesis and guanine nucleotide biosynthesis to order; methionine salvage  
886 to genus; sulphur reduction to class; photosystem I and photosystem II to genus; histidine  
887 degradation to glutamate and histidine biosynthesis to class.

889 **Table 1.** Average OTU abundances for each size fraction and zone

OTU	North Zone			South Zone		
	0.1 $\mu\text{m}$	0.8 $\mu\text{m}$	3.0 $\mu\text{m}$	0.1 $\mu\text{m}$	0.8 $\mu\text{m}$	3.0 $\mu\text{m}$
<i>Pelagibacter ubique</i> HTCC1062	61.8	25.0	23.9	58.9	22.4	17.6
<i>Synechococcus</i> sp. CC9902	0.11	9.84	4.97	0.00	0.00	0.10
<i>Roseobacter</i> sp. OCh114	0.31	2.93	1.59	0.45	3.99	2.66
<i>Synechococcus</i> sp. CC9311	0.03	4.62	4.41	0.00	0.00	0.03
<i>Ruthia magnifica</i> str. Cm ( <i>Calyptogenia magnifica</i> )	0.67	0.65	0.55	2.99	2.62	1.03
<i>Silicibacter pomeroyi</i> DSS-3	0.26	2.29	1.15	0.31	2.51	1.58
<i>Gramella forsetii</i> strain KT0803	0.24	1.21	1.75	0.50	2.35	1.89

<i>Candidatus</i> Puniceispirillum marinum IMCC1322	0.64	2.08	1.27	0.36	1.38	0.71
<i>Robiginitalea biformata</i> strain HTCC2501	0.28	1.10	1.30	0.47	1.88	1.40
<i>Flavobacterium psychrophilum</i> strain JIP02/86	0.17	0.84	1.22	0.43	1.96	1.60
<i>Silicibacter</i> sp. TM1040	0.23	1.65	0.87	0.27	1.80	1.23
<i>Candidatus</i> Vesicomysocius okutanii strain HA	0.46	0.46	0.21	1.97	1.81	0.22
<i>Jannaschia</i> sp. DFL-12	0.18	1.38	0.74	0.24	1.69	0.80
<i>Zunongwangia profunda</i> strain SM-A87	0.15	0.75	1.06	0.30	1.41	1.20
<i>Colwellia</i> sp. 34H	0.02	0.36	2.74	0.05	0.51	1.04

<i>Pseudoalteromonas atlantica</i> strain T6c	0.01	0.48	1.99	0.02	0.41	1.13
<i>Jannaschiana</i> sp. CCS1	0.12	0.93	0.48	0.17	1.23	0.82
<i>Nitrosopumilus maritimus</i> SCM1	0.02	0.01	0.01	1.08	1.31	1.21
<i>Coralimargarita akajimensis</i> strain DSM 45221	0.04	0.08	0.12	0.12	1.54	1.68
<i>Flavobacterium johnsoniae</i> strain UW101	0.09	0.42	0.61	0.20	0.94	0.86

890  
891

892 \* OTU identifications and abundances based on GAAS and MINSPEC analysis of BLAST matches  
893 between the SO metagenomic dataset and RefSeq. This table includes the twenty overall most  
894 abundant OTUs. A complete of all OTU abundances for all samples and size fractions is available  
895 in the supporting information (Table S2). Abundances are relative and expressed as percentages.

896

897 **Table 2.** KEGG modules which contribute highly to variance between the NZ and SZ. A  
898 complete list of modules which contribute to variance is given in the supporting information  
899 (Table S6S).

KEGG module	Average abundance (standardised and log transformed)		Contribution to variance (%)
	North Zone	South Zone	
<del>Photosystem II</del> Photosystem-H	<del>0.57</del> 0.6	<del>0.42</del> 0.44	<del>2.21</del> 2.18
<del>Complex I (NADH dehydrogenase), NADH dehydrogenase I/diaphorase subunit of the bidirectional hydrogenase</del> Complex I (NADH dehydrogenase); NADH dehydrogenase I/diaphorase subunit of the bidirectional hydrogenase	<del>0.24</del> 0.26	<del>0.01</del> 0.01	<del>1.84</del> 1.83
<del>Photosystem I</del> Photosystem-I	<del>0.34</del> 0.36	<del>0.43</del> 0.3	<del>1.74</del> 1.2
<del>Pyrimidine deoxyribonucleotide biosynthesis, CDP/CTP =&gt; dCDP/dCTP,dTDP/dTTP</del> Pyrimidine deoxyribonucleotide biosynthesis; CDP/CTP => dCDP/dCTP;dTDP/dTTP	<del>0.66</del> 0.68	<del>0.51</del> 0.53	<del>1.16</del> 1.15
<del>Histidine degradation, histidine =&gt; N-formiminoglutamate =&gt; glutamate</del> sn-Glycerol 3-phosphate transport system	<del>0.31</del> 0.17	<del>0.42</del> 0.31	<del>1.14</del> 1.13
<del>Methionine salvage pathway</del> Methionine salvage pathway	<del>0.43</del> 0.43	<del>0.29</del> 0.29	<del>1.14</del> 1.1
<del>sn-Glycerol 3-phosphate transport system</del> Histidine degradation; histidine => N-formiminoglutamate =>	<del>0.16</del> 0.33	<del>0.29</del> 0.44	<del>1.11</del> 1.08

glutamate			
<u>Complex I (NADH dehydrogenase), NADH dehydrogenase I; Aminoacyl-tRNA biosynthesis; prokaryotes</u>	<u>1.050.08</u>	<u>1.080.14</u>	<u>1.061.07</u>
<u>Branched-chain amino acid transport system; Complex I (NADH dehydrogenase); NADH dehydrogenase I</u>	<u>0.831.07</u>	<u>0.791.1</u>	<u>0.961.04</u>
<u>Dipeptide transport system; Glycine betaine/proline transport system</u>	<u>0.020.55</u>	<u>0.140.67</u>	<u>0.951.02</u>
<u>Adenine nucleotide biosynthesis, IMP =&gt; ADP/dADP; ATP/dATP; Branched-chain amino acid transport system</u>	<u>0.740.86</u>	<u>0.620.82</u>	<u>0.950.98</u>
<u>Glycine betaine/proline transport system; Adenine nucleotide biosynthesis; IMP =&gt; ADP/dADP; ATP/dATP</u>	<u>0.560.77</u>	<u>0.660.64</u>	<u>0.940.97</u>
<u>Sulfur reduction, sulfate =&gt; H<sub>2</sub>S; Dipeptide transport system</u>	<u>0.440.02</u>	<u>0.540.15</u>	<u>0.910.96</u>
<u>Simple sugar transport system; Ribosome; eukaryotes</u>	<u>0.390.29</u>	<u>0.460.27</u>	<u>0.90.91</u>
<u>Peptides/nickel transport system</u>	<u>0.98</u>	<u>0.99</u>	<u>0.89</u>

900

901 The following figures are presented in the order Figure 1-2.