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1 **SAR11 clade microdiversity and activity during the early spring blooms**  
2 **off Kerguelen Island, Southern Ocean**

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15 *Running Title:* SAR11 microdiversity in the Southern Ocean

16 *Keywords:* iron fertilization, SAR11 clade, ecotypes, bacterial community composition, Kerguelen

17 Plateau

18

19 ***Originality-Significance Statement***

20 The present results expand current knowledge on the most abundant marine bacteria, the SAR11 clade,  
21 to the high nutrient low chlorophyll waters of the Southern Ocean.

22 This report describes:

- 23 - SAR11 clade major contribution to the microbial community and carbon cycle, at the onset of  
24 the spring phytoplankton bloom induced by natural iron fertilization
- 25 - SAR11 clade microdiversity in a mosaic of phytoplankton blooms at different early bloom  
26 stages

27 The spatio-temporal partitioning of some of the SAR11 subclades revealed in this study suggests a  
28 niche specificity and periodic selection of the different SAR11 ecotypes in the Southern Ocean. Despite  
29 these changes in microdiversity the SAR11 population remained stable in abundance and activity  
30 across different bloom sites. These are significant observations to further understand the ecology of the  
31 SAR11 clade and its impact on biogeochemical cycles in the rapidly changing Southern Ocean.

32

33

34 **Abstract**

35 The ecology of the SAR11 clade, the most abundant bacterial group in the ocean, has been intensively  
36 studied in temperate and tropical regions, but its distribution remains largely unexplored in the  
37 Southern Ocean. Through amplicon sequencing of the 16S rRNA genes, we assessed the contribution  
38 of the SAR11 clade to bacterial community composition in the naturally iron fertilized region off  
39 Kerguelen Island. We investigated the upper 300 m at seven sites located in early spring phytoplankton  
40 blooms and at one High Nutrient Low Chlorophyll site. Despite pronounced vertical patterns of the  
41 bacterioplankton assemblages, the SAR11 clade had high relative abundances at all depths and sites,  
42 averaging 40% ( $\pm 15\%$ ) of the total community relative abundance. Micro-autoradiography combined  
43 with CARD-FISH further revealed that the clade had an overall stable contribution (45-60% in surface  
44 waters) to bacterial biomass production (determined by  $^3\text{H}$ -leucine incorporation) during different early  
45 bloom stages. The spatio-temporal partitioning of some of the SAR11 subclades suggests a niche  
46 specificity and periodic selection of different subclades in response to the fluctuating extreme  
47 conditions of the Southern Ocean. These observations improve our understanding of the ecology of the  
48 SAR11 clade and its implications in biogeochemical cycles in the rapidly changing Southern Ocean.

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51

## 52 **Introduction**

53           The SAR11 clade of the Alphaproteobacteria is one of the most abundant bacterioplankton in  
54 marine ecosystems (Morris *et al.*, 2002; Carlson *et al.*, 2009; Eiler *et al.*, 2009) representing 25% or  
55 more of the total bacterial cells in seawater worldwide (Giebel *et al.*, 2009; Brown *et al.*, 2012;  
56 Sunagawa *et al.*, 2015; Ortmann and Santos, 2016). Since its first discovery (Giovannoni *et al.*, 1990),  
57 the clade's spatial, vertical and seasonal patterns and links to ecosystem variables have been  
58 extensively studied and described (e.g. Carlson *et al.*, 2009; Eiler *et al.*, 2009; Brown *et al.*, 2012;  
59 Morris *et al.*, 2012; Vergin *et al.*, 2013; Salter *et al.*, 2014; Thrash *et al.*, 2014; Ortmann and Santos,  
60 2016). Conserved traits such as small cell size, small streamlined genomes, simplified regulatory  
61 systems, or efficient energy acquisition strategies seem to make members of this clade steady  
62 competitors that thrive in the minimal conditions provided by oligotrophic marine waters, and likely  
63 explain the clade's remarkable success throughout the world's oceans (reviewed in Giovannoni, 2017).  
64 Their ubiquitous abundances and specialized, atypical carbon substrate utilization profiles make  
65 SAR11 significant contributors to fluxes of carbon and other nutrients in the ocean (Giovannoni, 2017  
66 and references therein). Beyond the existence of core characteristics shared by most members of the  
67 clade, detailed examination of SAR11 microdiversity has revealed several phylogenetic subclades that  
68 are consistently associated with specific environmental conditions (e.g. Field *et al.*, 1997; Carlson *et*  
69 *al.*, 2009; Vergin *et al.*, 2013). These subclades seem to represent ecologically coherent populations or  
70 ecotypes. Subclade-specific traits and metabolic needs have been identified and likely play an  
71 important role in the ecological niche partitioning observed among the subclades (Grote *et al.*, 2012;  
72 Thrash *et al.*, 2014; Tsementzi *et al.*, 2016; Haro-Moreno *et al.*, 2020).

73 There are, however, fewer studies on the distribution and ecology of SAR11 subclades in the Southern  
74 Ocean (Brown *et al.*, 2012; Liu *et al.*, 2019; Haro-Moreno *et al.*, 2020; Sow *et al.*, 2022). The Southern  
75 Ocean is the largest High Nutrient Low Chlorophyll (HNLC) region in the world, a result of the low  
76 concentrations of the essential element iron (Fe) which limits primary production in surface waters  
77 (Blain *et al.*, 2007; Pollard *et al.*, 2009). Concentrations of dissolved organic carbon (DOC) in Southern  
78 Ocean surface waters are among the lowest of the global ocean (about 50  $\mu\text{M}$ ; Hansell *et al.* 2010),  
79 which in turns limits heterotrophic bacterial activity. Bacterial growth can also be co-limited by both Fe  
80 and DOC (Church *et al.*, 2000; Obernosterer *et al.*, 2015). Thus, in this environment, metabolic  
81 interactions shaping microbial communities are particularly complex, as diverse phytoplankton and  
82 heterotrophic bacterial taxa compete for Fe (Fourquez *et al.*, 2016) while also relying on each other for  
83 key resources such as labile organic carbon and vitamins (Bertrand *et al.*, 2007). Members of the  
84 SAR11 clade are highly adapted to oligotrophic waters in which nutrient concentrations are low, but  
85 they also have atypical metabolic requirements driven by genomic streamlining that hint at a strong  
86 dependency on metabolites synthesized by co-occurring microbes. They have also been shown to be  
87 dominant members of the microbial communities in other HNLC regions such as the Subarctic Pacific  
88 and Equatorial Pacific (e.g. Jing *et al.*, 2013; West *et al.*, 2016). For these reasons, the Southern Ocean  
89 provides an interesting environmental framework to investigate the activity and diversity of SAR11 and  
90 can contribute unique insight into the ecology of this marine bacterial clade.

91 Previously published work showed that in the Kerguelen area of the Southern Ocean, SAR11  
92 populations can be abundant members of bacterial communities. The SAR11 clade was most successful  
93 in HNLC waters independent of season (West *et al.*, 2008; Landa *et al.*, 2016; Hernandez-Magana *et*  
94 *al.*, 2021) where they also actively contribute to bacterial carbon uptake and cycling (Obernosterer *et*

95 *al.*, 2011; Fourquez *et al.*, 2016; Sun *et al.*, 2021). By contrast, in the naturally Fe fertilized waters off  
96 the Kerguelen plateau (Blain *et al.*, 2007) the relative abundance of SAR11 revealed a pronounced  
97 seasonal pattern, starting with high relative abundances in the early bloom stage, followed by a drastic  
98 decrease after the spring phytoplankton bloom and an increase towards late summer (Liu *et al.*, 2020).  
99 Whether or not different sublineages were found in HNLC waters and in the blooms or throughout the  
100 water column has implications for understanding the lifestyle and specific traits of SAR11 bacteria in  
101 these environments. In the present report, we follow up on the aforementioned studies and leverage the  
102 rich existing sample set to provide a detailed analysis of SAR11 activity, sublineage diversity and  
103 distribution patterns in this mosaic of phytoplankton blooms induced by natural Fe fertilization. Our  
104 results indicate that SAR11 populations remained abundant and active throughout the water column.  
105 Microdiversity analysis detected members from most known SAR11 sublineages and revealed distinct  
106 community composition at depth and across bloom stations.

107  
108

## 109 **Results and discussion**

110 The samples for the present study were collected in naturally Fe-fertilized and in HNLC waters  
111 off Kerguelen Island in early spring during the KEOPS2 cruise (Fig.S1). Concentrations of Chl *a*,  
112 bacterial abundance and bacterial heterotrophic production were overall higher in naturally Fe-  
113 fertilized mixed and intermediate waters as compared to the HNLC station R-2 (Table S1 and  
114 references within). However, among the Fe-fertilized sites, considerable variability in these biological  
115 parameters were observed (Table S1), reflecting spatial and temporal variability in the blooms  
116 development (Lasbleiz *et al.*, 2016) . By contrast, the major inorganic nutrients N and P, and DOC  
117 were similar across sites and characteristic for this region (Blain *et al.*, 2015; Tremblay *et al.*, 2015). In  
118 the present report, samples from eight stations and four sampled depths (20 to 300 m) were sorted into  
119 three water layers named mixed layer, intermediate layer and deep layer (Table S1), identified based on  
120 oceanographic parameters (Park *et al.*, 2014).

### 121 **Figure 1**

122 We assessed the contribution of SAR11 to bacterial community using 16S rDNA amplicon  
123 sequencing at all stations and depth layers (Fig. 1). Additionally, we used CARD-FISH for 3 distinct  
124 Fe-fertilized bloom stations (A3.2; F-L and E-5) and the HNLC site R-2 (Table 1). Sequencing analysis  
125 showed that OTUs of the SAR11 clade represented on average  $41\pm 13\%$  (and up to 62% in intermediate  
126 waters) of total sequences and were dominant at all stations down to 300 m regardless of the bloom  
127 regimes (Fig. 1). This observation expands our initial observations of generally high SAR11 relative  
128 abundances in surface waters (20 m) (Landa *et al.*, 2016),. The contribution of the SAR11 clade to bulk  
129 bacterial abundance based on CARD-FISH counts varied between  $44.3\pm 4\%$  in HNLC waters and  
130  $33\pm 10\%$  at the three Fe-fertilized bloom stations A3.2, F-L and E-5, in the mixed and intermediate



131 layers (Table 1). Both microscopic and molecular methods provided comparable values that are in line  
132 with previous studies showing SAR11 abundances between 20 and 55% of the total bacterial  
133 communities in the same study area (West *et al.*, 2008; Obernosterer *et al.*, 2011; Hernandez-Magana  
134 *et al.*, 2021) and in other regions of the Southern Ocean (Giebel *et al.*, 2009; Tada *et al.*, 2013). Both  
135 methods were generally in good agreement for most samples, however discrepancies between the two  
136 were observed in the deep-water samples (Table 1). The decrease in SAR11 relative abundances at 300  
137 m compared to the mixed layer values was more pronounced for the CARD-FISH data than for the  
138 sequencing data. One limitation of the CARD-FISH approach is that cells with a low rRNA content are  
139 not always detected, while the PCR step required for 16S rRNA gene sequencing targets live, dormant  
140 or even dead cells. Another possible explanation for the observed differences between the two methods  
141 could be the existence of distinct deep SAR11 clades missed by the set of probes used in our study. This  
142 latter was likely not the case as our probes appeared to target all the subclades present.

143

### **Table 1**

144 The success of SAR11 as the most abundant marine bacterial group suggests that they play an  
145 important role in organic matter fluxes. Here, micro-autoradiography combined with CARD-FISH  
146 showed that 11-84% of SAR11 cells were active in the upper 80 m (Table 1) and this fraction was  
147 generally lower below 150m. The average  $48.2 \pm 15.6\%$  of total active cells as SAR11 in the upper  
148 150 m, is similar to that observed previously in other, warmer oceanic regions, such as the North  
149 Atlantic and Mediterranean Sea where SAR11 contributed to 30-50% of the leucine-incorporating  
150 community (Malmstrom *et al.*, 2004; Malmstrom *et al.*, 2005; Laghdass *et al.*, 2012) and higher than  
151 observed in other regions of the Southern Ocean (Straza *et al.*, 2010; Tada *et al.*, 2013). The  
152 contribution of SAR11 to bulk leucine incorporation was slightly lower than expected from their

153 contribution to abundance (Figure 2), a trend often observed in other regions (e.g., Elifantz *et al.*, 2005;  
154 Alonso-Saez and Gasol, 2007; Alonso-Saez *et al.*, 2008, Straza *et al.*, 2010). A possible explanation is  
155 that SAR11 cells grow slower than other bacterial groups, and thus do not incorporate as much leucine  
156 as faster growing taxa. Interestingly and despite this seemingly moderate activity level of the SAR11  
157 group, the taxon accounted for most of the leucine incorporating cell population, particularly in upper  
158 layers (Table 1). Overall, our data indicate that SAR11 are important contributors to carbon cycling  
159 across the studied region at this time of the year as a result of high abundances and sustained activity  
160 levels in various bloom conditions.

161

## Figure 2

162 The present and previous studies (Giebel *et al.*, 2009) highlight SAR11 to be major community  
163 members in non-productive Southern Ocean waters. Despite the differences in the environmental  
164 setting among stations (Table S1, Lasbleiz *et al.*, 2016) the contribution of SAR11 to the total cells and  
165 to active cells appeared to be relatively constant. This is likely due to their successful adaptation to  
166 oligotrophic waters and to their capacity to take up efficiently organic substrates, among those some  
167 phytoplankton derived metabolites, such as very labile volatile molecules (Sun *et al.*, 2011; Moore *et*  
168 *al.*, 2020, 2022), while other taxa may favor high molecular weight DOM utilization (Malmstrom *et al.*,  
169 2005) as reported during the present cruise (Fourquez *et al.*, 2016; Landa *et al.*, 2018). So far, no  
170 known siderophores or heme uptake genes have been observed in SAR11 genomes (Hogle *et al.*, 2016),  
171 suggesting that the success of SAR11 in these Fe-limited waters could further be due to other specific  
172 strategies related to uptake, storage and utilization of this limiting micronutrient (Beier *et al.*, 2015;  
173 Debeljak *et al.*, 2019; Sun *et al.*, 2021). Pelagibacteraceae utilize predominantly inorganic Fe ( $\text{Fe}^{3+}$ ,  
174 Hopkinson and Barbeau 2012; Debeljak *et al.*, 2019); their metabolic activity is likely to be sustained

175 by the seasonally high concentrations of dissolved Fe prior to the phytoplankton bloom development  
176 (Qu  rou   *et al.*, 2015). Using MICRO-CARD-FISH, it was indeed shown that SAR11 made up 25% of  
177 the community taking up Fe in surface waters at the sites investigated during the same cruise (Fourquez  
178 *et al.*, 2016).

### 179 **Figure 3**

180 SAR11 subclades distribution over the studied area and dynamics during the onset of the spring  
181 bloom was further resolved to phylogenetic subclades partitioning. Overall, 47 OTUs were closely  
182 related to Pelagibacterales at 99% identity (covering 64310 reads, Fig. 3). The phylogenetic  
183 relationship between these OTUs and previously identified SAR11 subclasses (e.g. Field *et al.*, 1997;  
184 Carlson *et al.*, 2009; Vergin *et al.*, 2013) showed that OTUs observed in this study separated between  
185 six different known subclades. Most of our reads (80±6%) belonged to subclade Ia, which is the most  
186 abundant and most studied SAR11 ecotype in the ocean (e.g. Giovannoni, 2017; Delmont *et al.*, 2019).  
187 This cluster could be further separated in three subgroups (Fig. 3). The most abundant OTU in our  
188 dataset (KEOPS-6089, Fig. 3) represented 15% of the total relative abundance of all bacterial OTUs  
189 across all samples (on average 80% of all SAR11) and clustered in the Ia.1 group, with the first  
190 cultivated member of the clade: Pelagibacter HTCC1062 (Rapp   *et al.*, 2002). Ia. is the only subclade  
191 with Fe regulatory mechanisms for adaptation to Fe limitation (Smith *et al.*, 2010; Gr  te *et al.*, 2012).  
192 Subclade Ia is also adapted to respond to phytoplankton derived one-carbon and volatile organic  
193 compounds (Sun *et al.*, 2011; Halsey *et al.*, 2017; Moore *et al.*, 2020, 2022). Its capacity to efficiently  
194 utilize inorganic Fe and organic substances, or its low requirements of each to maintain cellular  
195 activity, may explain the success of this SAR11 Ia.1 clade in the region.

### 196 **Figure 4**

197 The SAR11 clades distribution as a function of depth showed that specific OTUs were more  
198 represented in different depth layers (Fig. 4). More specifically, subclades Ia, IIIa and IV were  
199 relatively more abundant in mixed and intermediate layers, while subclade Ib was more abundant in the  
200 deep layers (Fig. 4.A). Subclade Ib has also been reported in epi- and bathypelagic waters in the Red  
201 Sea (Jimenez-Infante *et al.*, 2017). At the Atlantic time series site BATS, subclade Ib is usually found  
202 in mixed water in late spring and early summer, while Ic is found in deeper water (Vergin *et al.*, 2013;  
203 Trash *et al.*, 2014). Here, subclade Ic also increased in deeper waters, which may be related to specific  
204 adaptation to nutrient availability (Tsementzi *et al.*, 2016; Ruiz-Perez *et al.*, 2021). Subgroup Ia.3 was  
205 more abundant in the deep layers, while Ia.1 and Ia.2 had higher relative abundances in mixed and  
206 intermediate layers (Fig. 4.B). Ia.1 has been observed in colder surface coastal waters (Rappe *et al.*,  
207 2002; Brown *et al.*, 2012; Grote *et al.*, 2012), and in other regions of the Southern Ocean (Haro-  
208 Moreno *et al.*, 2020). Ia.3 has been reported in surface gyre and tropical waters (Brown *et al.*, 2012;  
209 Grote *et al.*, 2012; Delmont *et al.*, 2019). Nevertheless, Ia.3 has also been observed in deep water of the  
210 Red Sea (Ngugi and Stingl, 2012).

211

## Figure5

212 The microdiversity of SAR11 subclades appeared more dynamic in surface waters (20m, Fig.  
213 5), similar to the shift in overall surface bacterial community composition at the sites characterized by  
214 different early bloom stages (Landa *et al.*, 2016). The different subclades showed evident patterns  
215 throughout the water column, with a shift in lineage identity and abundances at 300 m, compared to  
216 more similar mixed and intermediate layers (Fig. 4 and 5). This pronounced layer difference in  
217 microdiversity might be linked to the specific water masses circulation in the Southern Ocean. This

218 could also explain the general differences in microdiversity at station F-L, which is closest to the Polar  
219 Front and more influenced by Indian Ocean warmer waters.

220 SAR11 microdiversity also exhibited specific patterns linked to the bloom progression (Fig. 5).  
221 For instance, subclade IV was most abundant in more advanced bloom stage stations (A3.2, E4W and  
222 FL) where it probably benefited from specific relationships with phytoplankton cells (e.g. Becker *et al.*,  
223 2019; Tucker *et al.*, 2021), such as public good secondary metabolites and volatile organic compounds  
224 produced by the phytoplankton (e.g. Giovannoni, 2017). Conversely, subclades Ic and IIa seemed most  
225 abundant in the non-bloom stations (R-2, HNLC station and E3, Fig. 5) with low organic carbon  
226 concentration. The bi-polar distribution of subclade IIa in surface waters has been previously reported  
227 (Kraemer *et al.*, 2019); its adaptation to cold waters may explain its relatively stable distribution in less  
228 productive stations across the water column in the study area. Subclades Ia and Ib did not seem to  
229 respond to bloom stages (Fig. 5), which showcases their ability to maintain their metabolism regardless  
230 of conditions. Ia.2 appeared to be more abundant in the HNLC waters; this subgroup was the second  
231 most abundant in the region and did not cluster with published reference sequences (Fig. 3), suggesting  
232 that this group was locally adapted to less productive and Fe-limited waters of the Southern Ocean.

233

234 In conclusion, Pelagibacterales are highly adapted to the cold, organic carbon- and iron-limited  
235 waters of the Southern Ocean, which is consistent with the clade's notorious ability to efficiently  
236 harvest limiting resources. The spatio-temporal partitioning of some of the SAR11 subclades revealed  
237 in this study followed observations made on niche specificity and periodic selection in other oceanic  
238 regions.

239 Nevertheless, the contribution of SAR11 to leucine incorporation was relatively stable across sites  
240 despite the variations in microdiversity, suggesting that subclades have a redundant impact on the  
241 carbon cycle or that one stable clade Ia1 was responsible for the overall activity. Investigating the  
242 metabolic potential of these SAR11 subclades are key to better understand the underlying mechanisms  
243 for their spatial distributions, and to further understand their evolution and ecological adaptation to the  
244 extreme conditions of the Southern Ocean, where they contribute substantially to the bacterial biomass  
245 and production and probably to other microbially mediated fluxes.

246

247

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259

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**Table 1:** SAR11 clade contribution to bacterial abundance and bacterial leucine incorporation. Station R-2 represents the HNLC site while A3-2, F-L and E-5 represent Fe-fertilized sites at various bloom development stages (see Figure S1 and Table S1). Data are from this study unless specified, with \* indicating surface data previously published in Fourquez *et al.* (2016). n.a.: not available. The percentage of active SAR11 was assessed by leucine uptake through Micro-CARD-FISH. The details of the method are described in (Fourquez *et al.*, 2016). Briefly, 10 mL of seawater samples were incubated with radiolabeled leucine for 6-8h, fixed with paraformaldehyde and filtered onto 0.2  $\mu\text{m}$  polycarbonate filters. The abundance of SAR11 was determined with catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) with the probes SAR11-152R, SAR11-441R, SAR11-542R and SAR11-732R (Morris *et al.*, 2002). The micro-autoradiography development with photographic emulsion was exposed for 2-3d. After development the proportion of substrate active SAR11 were determined as the proportion of probe positive cell with silver grains.

Station	Depth (m)	BA <sup>(1)</sup> (x10 <sup>5</sup> cells ml <sup>-1</sup> )	% of BA cells as SAR11 <sup>(2)</sup>	SAR11 abundance (x 10 <sup>4</sup> cells ml <sup>-1</sup> )	% of active SAR11 <sup>(3)</sup> cells	% of total active cells as SAR11	% relative abundance of SAR11 OTUs <sup>(4)</sup>
R-2	20	2.30	49 ± 7*	11.2	51 ± 9*	60 ± 5	47
R-2	60	2.95	42 ± 4	12.4	38 ± 5	50 ± 3	n.a.
R-2	150	2.87	42 ± 2	12.1	42 ± 3	65 ± 6	14
R-2	300	1.30	19 ± 0	2.47	15 ± 3	23 ± 4	13
A3-2	20	2.70	35 ± 12*	9.45	12 ± 3*	52 ± 10	36
A3-2	80	3.53	43 ± 1	15.2	84 ± 3	58 ± 2	39

A3-2	160	3.47	34 ± 1	11.2	58 ± 9	21 ± 6	41
A3-2	300	1.90	20 ± 3	3.80	30 ± 6	28 ± 4	24
F-L	20	6.06	46 ± 6*	27.9	37 ± 8*	55 ± 5	31
F-L	70	6.48	34 ± 2	22.0	24 ± 4	50 ± 5	41
F-L	150	2.24	11 ± 14	2.46	3 ± 3	13 ± 8	34
F-L	300	1.81	5 ± 9	0.91	0 ± 1	0	19
E-5	20	4.60	34 ± 5*	15.6	60 ± 9*	45 ± 7	n.a.
E-5	80	4.56	36 ± 6	16.4	11 ± 4	58 ± 7	11
E-5	150	3.57	27 ± 3	9.64	25 ± 7	51 ± 3	42
E-5	300	2.20	8 ± 2	1.76	1 ± 1	4 ± 2	22

<sup>(1)</sup>BA: Bacterial abundance determined by flow cytometry (data as published in Christaki *et al.*, 2014)

<sup>(2)</sup> Positive cells hybridized with SAR11 clade probes (\*: marks 20 m depth data published in Fourquez *et al.*, 2016)

<sup>(3)</sup>Micro-autoradiography positive cells showing leucine incorporation (\*: marks 20 m depth data published in Fourquez *et al.*, 2016)

<sup>(4)</sup>Based on 16s rDNA sequencing (the community-level analysis of the 16S sequencing data from 20m-depth samples can be found in Landa *et al.*, 2016)

## Figure legends

**Figure 1:** Relative abundance (%) of the main bacterial taxonomic groups over the three depth layers. A total of 31 samples from eight different stations were analyzed for bacterial community composition by 454 pyrosequencing of the V1-V3 regions of the 16S rRNA gene. Each bar shows the average contribution of the specified groups across: 12, 11, 8 samples for the mixed, intermediate and deep layer respectively. Filtration, extraction, sequencing procedures and denoising of the sequences are described in [Landa et al. \(2016\)](#). Clean reads were subsequently processed using the Quantitative Insight Into Microbial Ecology pipeline (QIIME v1.7; [\(Caporaso et al., 2010b\)](#)). Reads were clustered into (OTUs) at 99% pairwise identity using Uclust and representative sequences from each bacterial OTU were aligned to Greengenes reference alignment using PyNAST ([Caporaso et al., 2010a](#)). All singletons and operational taxonomic units (OTUs) present in only one sample were removed. Taxonomy assignments were made using the Ribosomal Database Project (RDP) classifier ([Wang et al., 2007](#)) against the database Greengene 13\_8 ([McDonald et al., 2012](#)) and SILVA 128 ([Quast et al., 2013](#)). The data were deposited in the Sequence Read Archive (SRA) database under accession number SRP041580.

**Figure 2:** Contribution of SAR11 clade to total <sup>3</sup>H-leucine incorporating cells versus contribution of SAR11 clade to total bacterial abundance. The solid line indicates a 1:1 relationship.

**Figure 3:** Phylogenetic relationships of SAR11 OTUs: Maximum likelihood tree of OTUs closely related to SAR11 clade. Only SAR11 OTUs representing more than 0.1% of the total SAR11 reads are included. Reference sequences from previously published SAR11 subclades

identifications are indicated in blue italic. Bootstrap values (n=1000) are indicated at nodes; scale bar represents changes per positions. SAR11 maximum likelihood tree was computed with Mega7 ([Tamura et al., 2013](#)).

**Figure 4:** Layer distribution of SAR11 subclades (A) and subclades Ia (B): % relative abundance of SAR11 related OTUs representing more than 0.1 % of all SAR11 OTUs. Average of SAR11 relative abundance across all stations are shown, with error bars representing standard deviation between stations.

**Figure 5:** Vertical distribution of SAR11 subclades under different bloom conditions (integrated weight average of SAR11 relative abundance to total community, note different z scales, Grey line represent the limit of the mixed layer depth).

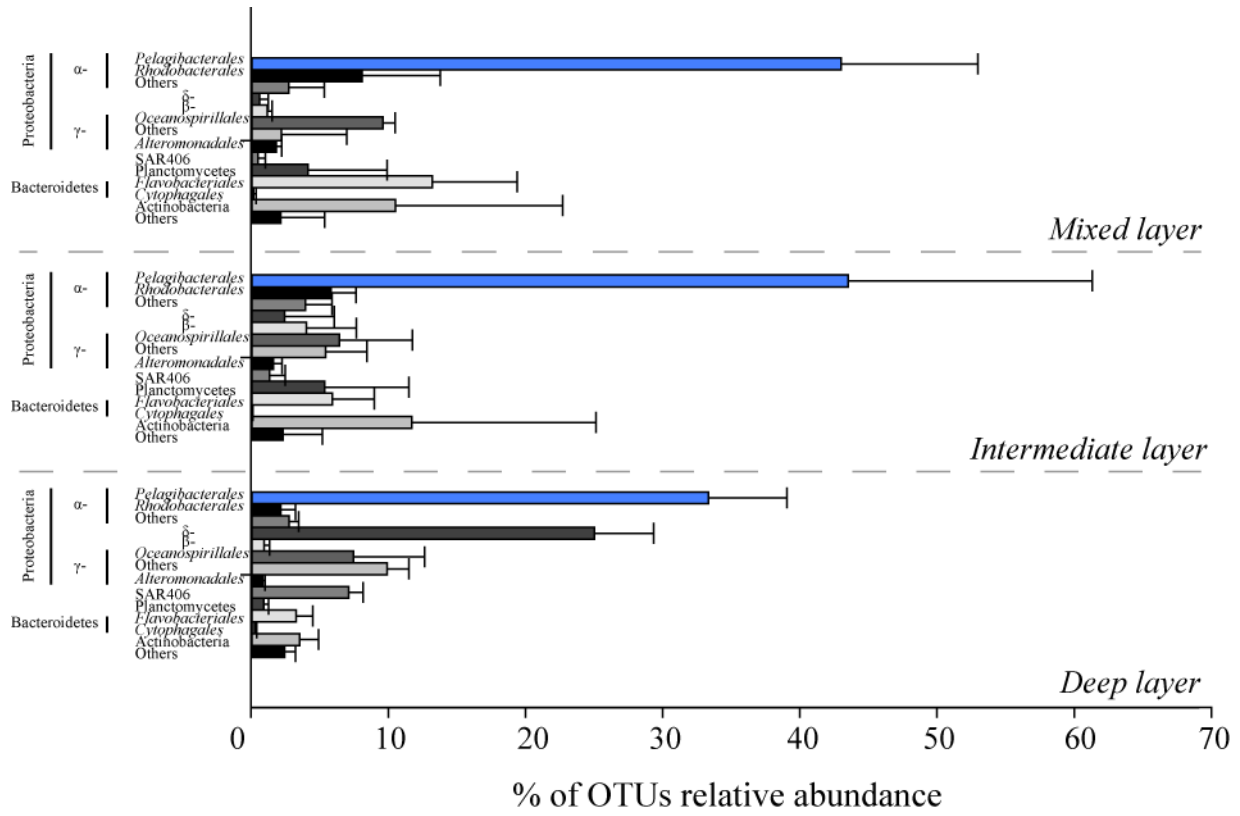


Figure 1

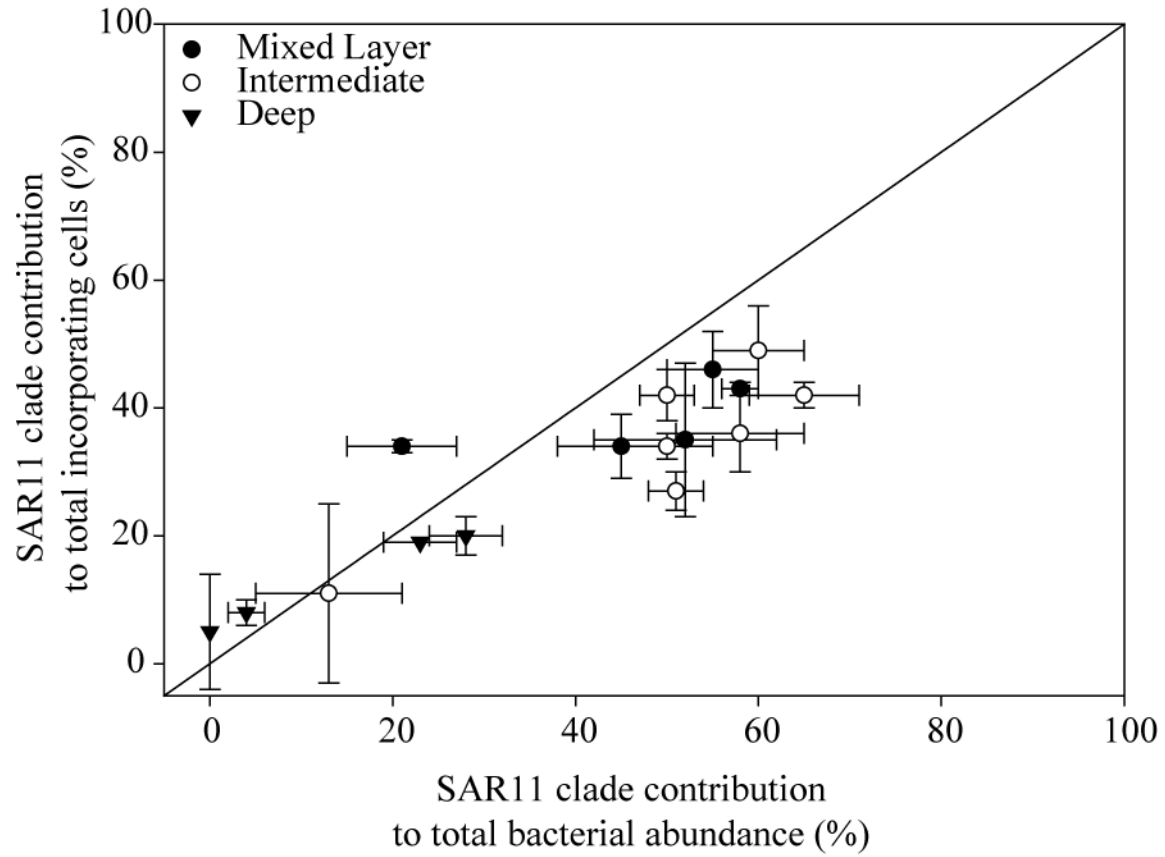


Figure 2

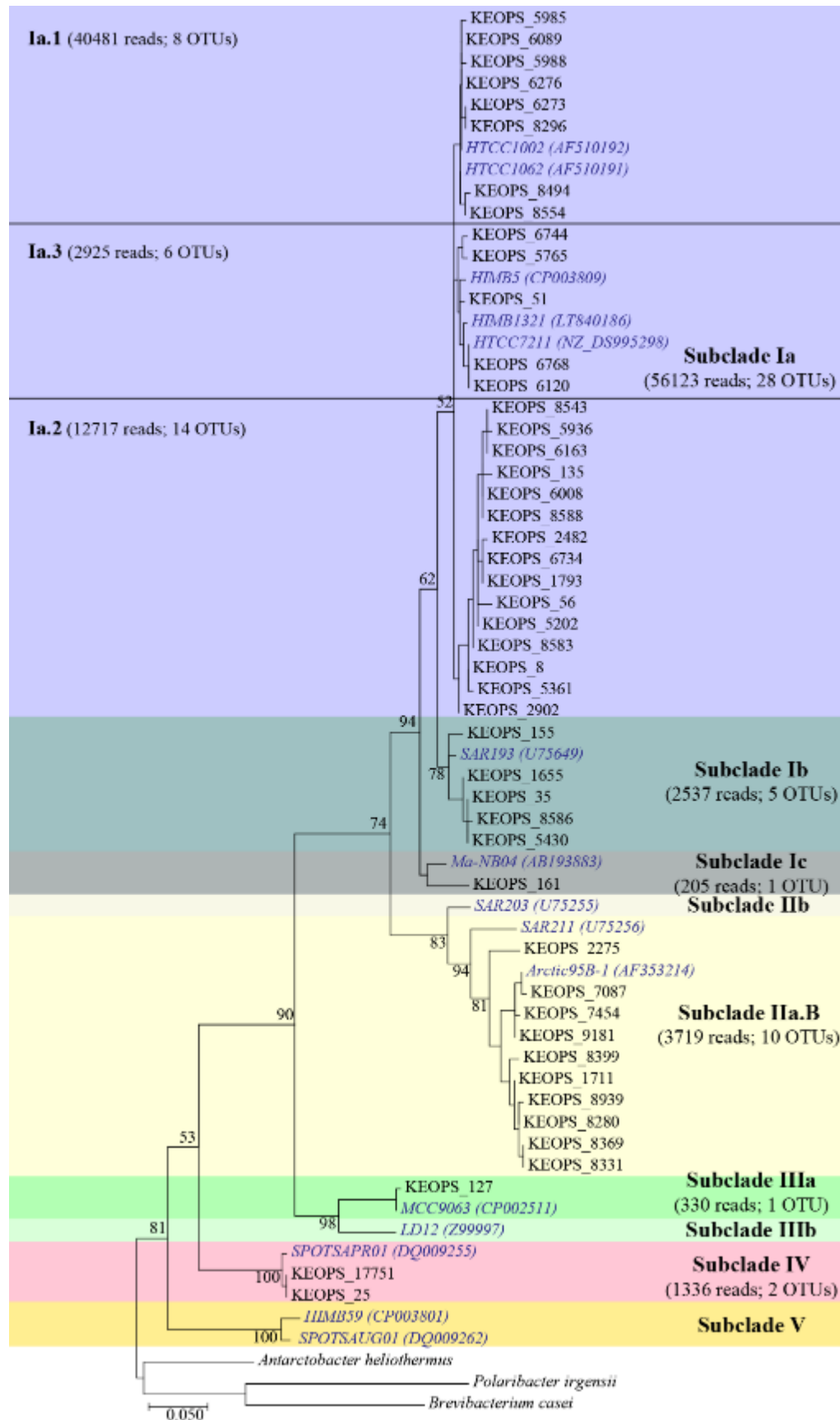


Figure 3

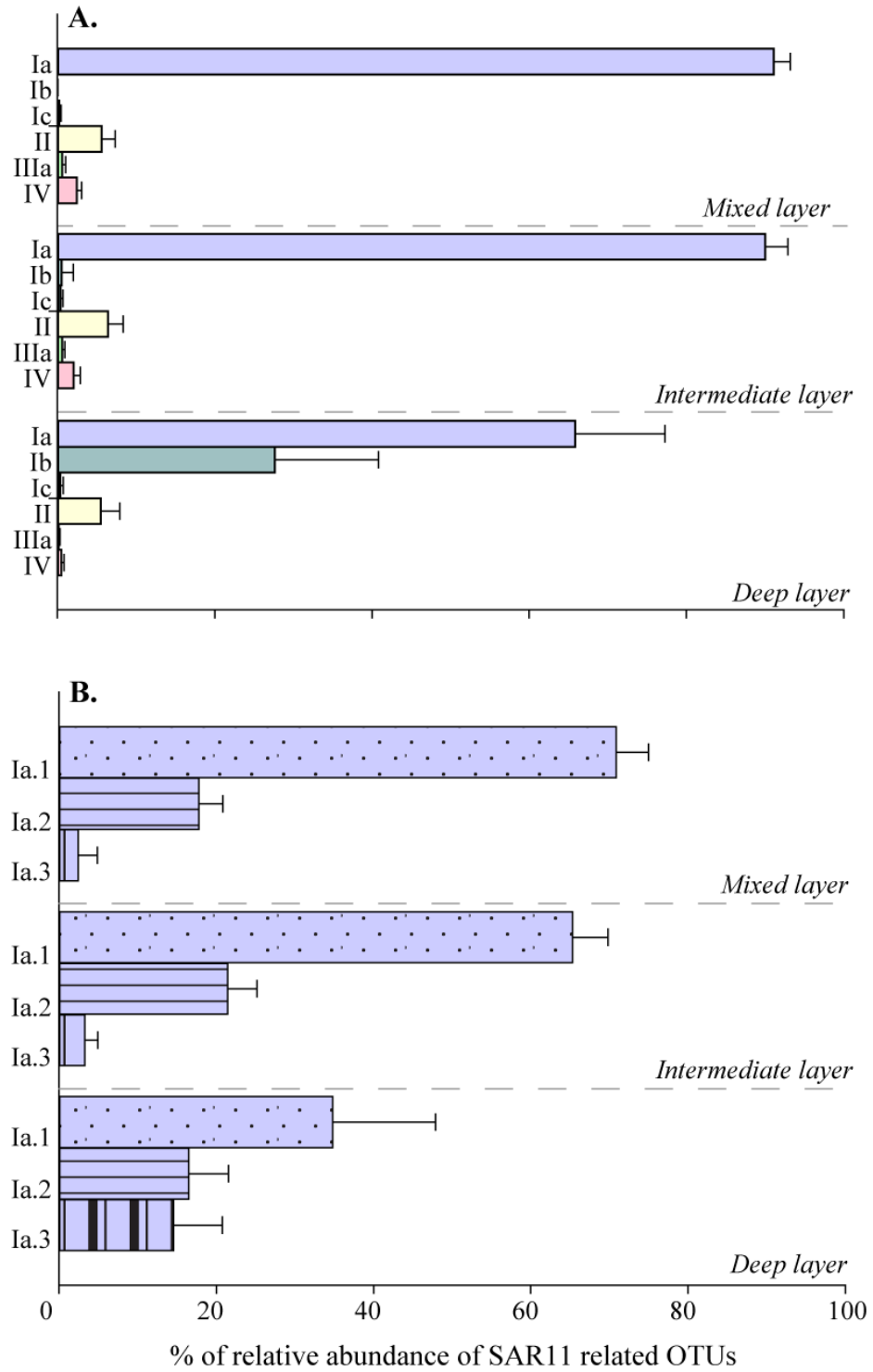


Figure 4



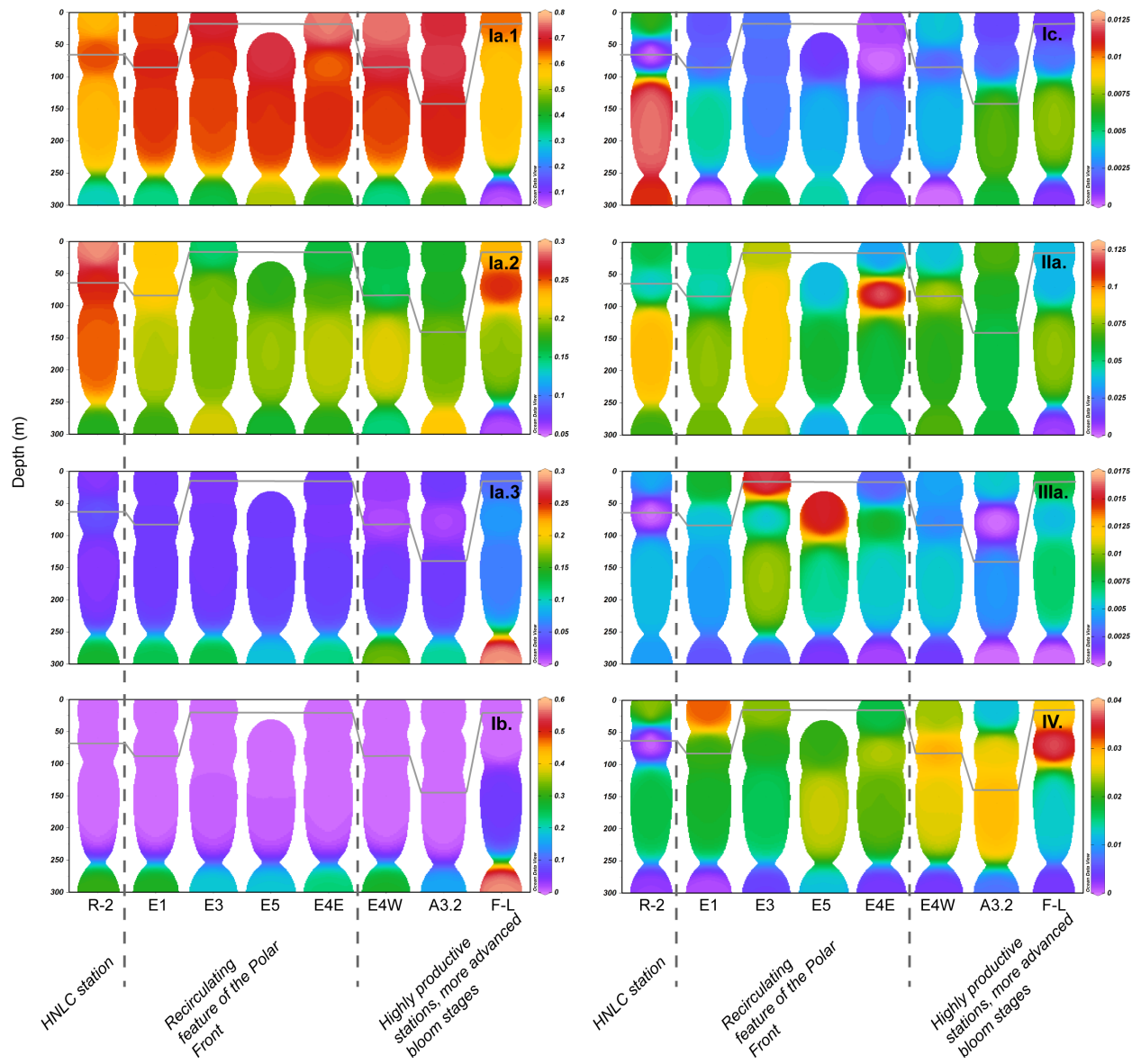


Figure 5

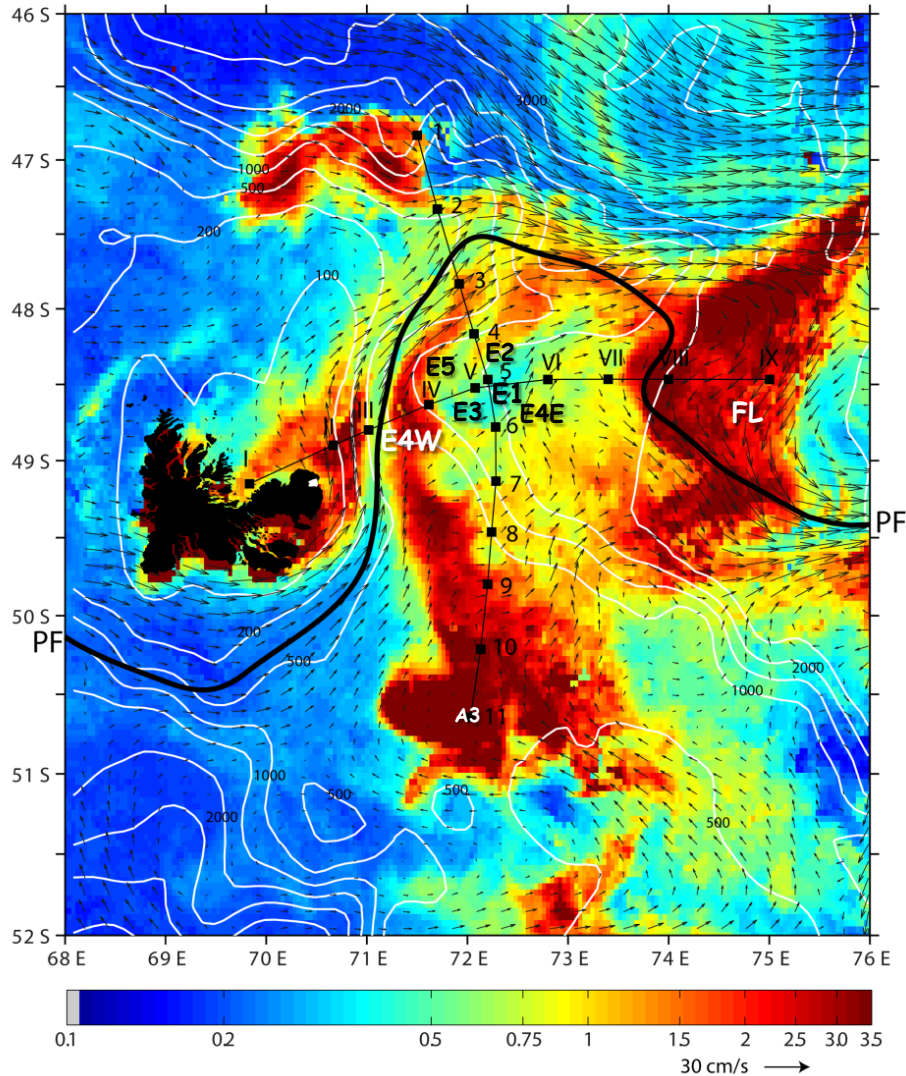
## Supplementary Table and Figures

**Table S1:** Depth layer classification, environmental and bacterial parameters for the sampled stations. (n.a.: not available, HNLC: High nutrients low chlorophyll station). The wind mixed layer depth (MLD) as determined based on a difference in sigma of 0.02 to the surface value, as for instance described in de Boyer Montégut *et al.* (2004). The samples were then attributed to one of the three categories: surface (MLD), intermediate (transition zone between MLD and winter water) and deep (winter water) based on the physical properties as published in Park *et al.* (2014). The sampling was conducted during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study 2) cruise from October to November 2011 on board the R/V Marion Dufresne in the Kerguelen region (FigS1 and see Fig. 1 in Landa *et al.*, 2016). A total of seven stations (A3.2, E stations and F-L) were sampled in the naturally iron-fertilized regions east of the Kerguelen Islands and a reference station (R-2) was sampled in high nutrient low chlorophyll waters (HNLC) located west of the islands. E1, E3, E4-E and E5 were sampled temporally in a quasi-Lagrangian manner. For each station four depths were sampled according to CTD profiles.

Station group	Station	Depth (m)	layer	Chl. <i>a</i> ( $\mu\text{g L}^{-1}$ )*	Bacterial abundance ( $\times 10^5$ cells $\text{mL}^{-1}$ )*	Bacterial production ( $\text{ng C L}^{-1} \text{h}^{-1}$ )*
HNLC	R-2	20	Mixed layer	0.32	2.29	2.08
		60	Mixed layer	0.27	2.95	2.77
		150	Intermediate	0.07	2.87	0.88
		300	Deep	-	1.30	0.63
Polar front plume	F-L	20	Mixed layer	5.12	6.06	64.6
		70	Intermediate	0.34	6.48	6.82
		150	Intermediate	0.04	2.24	1.49
		300	Deep	-	1.81	0.23
Kerguelen Plateau	E4W	30	Mixed layer	1.40	6.04	30.16
		80	Mixed layer	1.22	5.96	24.83

		150	Intermediate	0.22	3.15	4.34
		300	Deep	-	1.76	0.31
	<b>A3.2</b>	20	Mixed layer	1.65	2.70	20.15
		80	Mixed layer	2.12	3.53	20.63
		160	Mixed layer	2.30	3.47	22.43
		300	Deep	-	1.90	1.09
Recirculation feature	<b>E1</b>	20	Mixed layer	1.00	4.33	15.98
		80	Mixed layer	0.85	4.26	14.73
		150	Intermediate	0.60	3.83	9.78
		300	Deep	-	1.34	0.25
	<b>E3</b>	20	Mixed layer	0.69	5.06	23.65
		70	Intermediate	0.42	4.93	16.6
		150	Intermediate	0.50	4.18	9.02
		300	Deep	-	1.81	0.48
	<b>E4E</b>	30	Mixed layer	1.09	5.63	39.65
		80	Intermediate	0.39	5.28	10.97
		150	Intermediate	0.19	3.18	5.06
		300	Deep	-	1.67	0.17
	<b>E5</b>	20	Mixed layer	1.21	4.60	28.27
		80	Intermediate	0.92	4.56	26.43
		150	Intermediate	0.20	3.57	3.3
		300	Deep	-	2.20	0.15

\*data from Christaki et al. 2014



**Supplementary Figure S1:** KEOPS2 study area. The sampling was conducted during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study 2) cruise from October to November 2011 on board the R/V Marion Dufresne in the Kerguelen region (FigS1 and see Fig. 1 in Landa et al., 2016). A total of seven stations (A3.2, E stations and F-L) were sampled in the naturally iron-fertilized regions east of the Kerguelen Islands and a reference station (R-2) was sampled in high nutrient low chlorophyll waters (HNLC) located west of the islands. For each station four depths were sampled according to CTD profiles.

Chl *a* (color scale), surface velocity fields (arrows), the polar front (PF, black line), and the position of the different stations: The Chl *a* rich stations: A3, on the Kerguelen plateau; F-L and E-4W north and south of the polar front; and “E” stations sampled in a quasi-Lagrangian manner (E-1, E-2, E-3, E-4E, and E-5) within a complex meander south of the polar front. The reference HNLC station (R-2) is not shown as it is out of the area of the map (66.692743 E longitude,

50.38954 N latitude). Map is courtesy of Y. Park and colleagues. To note: the chlorophyll content represented on the map corresponds to the last week of the KEOPS2 cruise.

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