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1     **Seasonal shifts in Fe-acquisition strategies in Southern Ocean microbial communities**  
2                     **revealed by metagenomics and autonomous sampling**

3  
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13  
14    **Key words:** marine prokaryotes, iron and organic carbon acquisition, metagenomics, MAGs,  
15    Southern Ocean, autonomous sampler

16  
17    **Competing of interests**

18    The authors declare no competing interests.

19 **Originality Significance statement**

20 The trace element iron is key for microbial metabolism, as a large number of enzymes  
21 involved in carbon-related pathways require iron as a co-factor. The acquisition of both  
22 elements is therefore essential for a cell to thrive in a given environment. Despite this key  
23 role of iron, the pathways used by diverse prokaryotes to access the different chemical forms  
24 of iron remains poorly understood. Understanding the iron acquisition strategies is of  
25 particular importance in marine environments where this trace element is present in growth-  
26 limiting concentrations. We report here high-resolution seasonal observations from a  
27 naturally iron-fertilized region in the Southern Ocean. Applying environmental  
28 metagenomics we identified different iron uptake strategies in marine prokaryotic  
29 communities and pronounced seasonal changes as an acclimation to varying iron and organic  
30 carbon regimes. Using metagenome-assembled genomes we further deciphered the Fe-related  
31 gene repertoire of individual taxa assigned to abundant prokaryotic groups. Our  
32 study provides novel insights to iron-related ecological strategies of diverse marine  
33 prokaryotes and how the acquisition of this element is linked to organic carbon.

34 **Abstract**

35 Iron (Fe) governs the cycling of organic carbon in large parts of the Southern Ocean. The  
36 strategies of diverse microbes to acquire the different chemical forms of Fe under seasonally  
37 changing organic carbon regimes remain, however, poorly understood. Here, we report high-  
38 resolution seasonal metagenomic observations from the region off Kerguelen Island (Indian  
39 Sector of the Southern Ocean) where natural Fe-fertilization induces consecutive spring and  
40 summer phytoplankton blooms. Our data illustrate pronounced, but distinct seasonal patterns  
41 in the abundance of genes implicated in the transport of different forms of Fe and organic  
42 substrates, of siderophore biosynthesis and carbohydrate active enzymes (CAZymes). The  
43 seasonal dynamics suggest a temporal decoupling in the prokaryotic requirements of Fe and  
44 organic carbon during the spring phytoplankton bloom and a concerted access to these  
45 resources after the summer bloom. Taxonomic assignments revealed differences in the  
46 prokaryotic groups harboring genes of a given Fe-related category and pronounced seasonal  
47 successions were observed. Using MAGs we could decipher the respective Fe- and organic  
48 substrate-related genes of individual taxa assigned to abundant groups. The ecological  
49 strategies related to Fe-acquisition provide insights on how this element could shape  
50 microbial community composition with potential implications on organic matter  
51 transformations in the Southern Ocean.

## 52 **Introduction**

53 Organic carbon and iron (Fe) are essential elements for heterotrophic prokaryotes and from a  
54 metabolic point of view the two elements are intimately linked. Organic substrates provide  
55 building units for the cell and carbon metabolism is a source of energy, but a large number of  
56 enzymes involved in carbon-related pathways require Fe as a cofactor. The acquisition of  
57 both elements is therefore essential for a cell to thrive in a given environment. The  
58 concentration of marine organic matter varies on spatial and temporal scales and a large  
59 diversity of substrates with highly variable degrees of bioavailability make up this pool  
60 (Moran *et al.*, 2016). The concentration of inorganic nutrients can regulate the utilization of  
61 labile forms of organic matter, and Fe could be a rate-limiting micro-nutrient in High  
62 Nutrient Low Chlorophyll (HNLC) regions (Obernosterer *et al.*, 2015).

63 The marine Fe pool is made up by various dissolved and particulate forms originating from  
64 biological, lithogenic or authigenic sources (Tagliabue *et al.*, 2017), but the availability of  
65 these different forms of Fe to prokaryotes remains poorly investigated (Debeljak *et al.*, 2021;  
66 Hogle *et al.*, 2022; Manck *et al.*, 2022). The presence of multiple chemical forms of Fe is  
67 both a challenge and an opportunity for prokaryotes which have evolved a large panel of  
68 uptake strategies. Fe requirements and resource acquisition mechanisms vary among taxa  
69 (Hopkinson and Barbeau, 2012). *Roseobacter* genomes contain genes for multiple Fe uptake  
70 mechanisms, while SAR11 has a reduced set of transporters (Roe *et al.*, 2013; Hogle *et al.*,  
71 2016). Siderophore biosynthesis appears to be restricted to a few prokaryotic groups, and  
72 many of these belong to *Gammaproteobacteria* (Hopkinson and Barbeau, 2012; Payne *et al.*,  
73 2016; Manck *et al.*, 2022). By contrast, transporters of siderophore-bound Fe are more  
74 widespread (Hopkinson and Barbeau, 2012; Debeljak *et al.*, 2021; Hogle *et al.*, 2022), a  
75 differentiation that can lead to potential cross feeding (Cordero *et al.*, 2012). The prevalence  
76 of specific Fe uptake strategies was shown to be linked to Fe concentrations across large

77 spatial scales (Toulza *et al.*, 2012). Together, this results in a complex interplay between the  
78 genomic and physiological properties of individual microbes and their interaction among  
79 each other and with their micro-environment.

80 Responses of microbial communities from HNLC waters to additions of Fe and organic  
81 carbon in short-term bottle incubation experiments largely depend on location and season  
82 (Obernosterer *et al.*, 2015). While these experiments provide evidence that Fe and organic  
83 carbon can constrain prokaryotic growth, they do not inform on potential acclimation of  
84 microbes to changes in the supply of these elements. The aim of the present study was to  
85 better understand the link between microbial Fe uptake strategies, organic matter supply and  
86 microbial diversity in the Southern Ocean. Our specific objectives were firstly to determine  
87 the seasonal dynamics in the functional repertoire of Fe- and organic substrate acquisition  
88 genes and secondly to identify the microbial community members harboring these genes  
89 under varying organic matter supply. We carried out our study in the region off Kerguelen  
90 Island, a suitable environment because the intense spring and summer phytoplankton blooms  
91 induced by natural Fe fertilization (Blain *et al.*, 2007) result in pronounced seasonal patterns  
92 related to Fe and organic carbon availability. To address these objectives in an ocean region  
93 that is difficult to access due to logistic constraints, we used an autonomous in situ sampler  
94 providing high-resolution observations over an entire seasonal cycle.

## 95 **Experimental Procedures**

### 96 ***Sample collection***

97 We used 12 seawater samples collected with a Remote Autonomous Sampler (RAS-500®,  
98 Mac Lane) from 25 October 2016 to 24 February 2017 in surface waters (40 m) of the  
99 Kerguelen plateau in the Indian sector of the Southern Ocean (Station A3, 527 m overall  
100 depth) (Figure 1). The technical aspects of the RAS and its mooring, and the collection of  
101 seawater are described in detail in Blain et al. (Blain *et al.*, 2021). Briefly, three samples (500  
102 mL each) were collected at each time point; for inorganic nutrient analyses, one sample was  
103 filtered *in situ* through an 0.8 µm polycarbonate filter (PC, 47 mm diameter, Nuclepore,  
104 Whatman, Sigma-Aldrich, St Louis, MO), and for phytoplankton and prokaryotic diversity  
105 (Liu *et al.*, 2020; Blain *et al.*, 2021), two samples remained unfiltered. Fixatives (mercuric  
106 chloride or glutaraldehyde) were added to the sample bags prior to sampling. Upon recovery  
107 of the RAS, seawater for metagenomic analyses (fixed with mercuric chloride) was filtered  
108 sequentially through 0.8 and 0.2 µm PC filters (47 mm diameter, Nuclepore, Whatman,  
109 Sigma-Aldrich, St Louis, MO) and both filters were kept at -80 °C until DNA extraction.  
110 Only the DNA extracted from the 0.2 µm filters were used for metagenomic analyses.  
111 Temperature and salinity were obtained from sensors mounted on the RAS.

### 112 ***DNA extraction and metagenome sequencing***

113 Total DNA was extracted from 0.2 µm PC filters using the DNeasy PowerWater Kit (Qiagen)  
114 according to the manufacturer's instructions with a few modifications. Each filter was cut into  
115 small pieces with the scissors, and added solution PW1 and incubated at 65°C for 10 min to  
116 promote cell lysis, the complete details of DNA extraction are given in the study by Liu et al.  
117 (Liu *et al.*, 2020). DNA concentration was measured using quantus fluorometer (Promega)  
118 with QuantiFluor® Double stranded DNA (dsDNA) system. Metagenome samples (n=12)  
119 were sequenced with an Illumina NovaSeq 6000 system using 2 × 150 bp chemistry at

120 FASTERIS SA, Inc. (Switzerland), and yielded 59-83 Mio pairs of metagenomic reads per  
121 sample, corresponding to a total of 263.8 GB (Table S1). The data sets are available in the  
122 European Nucleotide Archive (ENA) repository at <https://www.ebi.ac.uk/ena> under the  
123 project ID PRJEB56376.

### 124 ***Metagenome assembly***

125 The raw reads were checked with FastQC v0.11.9 and processed with Trimmomatic (v 0.32)  
126 (Bolger *et al.*, 2014). The short reads that passed the quality control (QC) were individually  
127 assembled by MEGAHIT v1.2.9 (Li *et al.*, 2015) (parameters used --presets meta-large). We  
128 also used metaSPAdes v3.14.1 (Nurk *et al.*, 2017) (parameters used -k 21, 33, 55, 77, 99, 127)  
129 to assemble short reads for comparison. MetaQuast with default parameters was used to  
130 evaluate metagenomic assembly (Mikheenko *et al.*, 2016). For assembly using MEGAHIT,  
131 the total number of contigs varies between 0.1 and 1.6 Mio with an average length of 600 bp  
132 (Table S2). For the assembly using metaSPAdes, the total number of contigs varies between  
133 0.3 and 1.8 Mio with an average length of 500 bp (Table S2). Due to the length of N50 we  
134 decided to continue the analysis from the MEGAHIT assembly results.

### 135 ***Metagenomic analysis***

136 ORFs (open reading frames) were predicted using Prodigal v2.6.3 (Hyatt *et al.*, 2010)  
137 (parameters used -p meta) (Table S3). CD-HIT v4.8.1 (Li and Godzik, 2006) (parameters  
138 used -c 1 -aS 1 -g 1) was used to construct a non-redundant protein database pooling the  
139 twelve samples (10 984 076 proteins). Based on the non-redundant protein sequence number,  
140 we constructed the non-redundant gene sequence set. Salmon v1.4.0 (Patro *et al.*, 2017) was  
141 used for read recruitment of the short reads to the non-redundant gene sequences collection,  
142 and for the quantification of each gene in each sample (parameters used -incompatPrior 0.0 --  
143 seqBias --gcBias --biasSpeedSamp 5 -validateMappings). The quantified protein occurrences



144 for each sample were normalized as genes per kilobase million (GPM) using the following  
145 formula:

$$146 \quad GPM = 10^6 \times \frac{\text{total reads mapped to genes/gene length in bp}}{\text{sum}(\text{total reads mapped to genes/gene length in bp})}$$

147 This value can be applied to the metagenomes to remove the effect of library size and gene  
148 length, and GPM are thus comparable among samples.

149 To detect genes that are involved in Fe-related pathways, we used FeGenie (Garber *et al.*,  
150 2020) based on individual contig files for alignment and annotation (parameters used --meta -  
151 t 8 --norm). FeGenie is a bioinformatics tool to annotate iron-related genes by combining a  
152 Hidden Markov Model (HMM) and BLAST. HMM is a probabilistic model used for gene  
153 finding of sequencing data. The authors of FeGenie have constructed their own database of  
154 HMMs for Fe related genes by obtaining HMMs from Pfam/TIGRFAMS or by manually  
155 constructing HMMs. The HMMs were calibrated through queries against the NCBI's  
156 database. Bit score cutoffs that optimally delineate between true and false positives among  
157 hits from the non-redundant protein database were identified by manually inspecting each  
158 hmm search result. In the present study we used this comprehensive library to identify  
159 proteins that are related to Fe transport and storage, and siderophore biosynthesis and  
160 transport (Table S4). The genes identified from the present dataset passed the recommended  
161 bit score cutoffs. Different pathways can share homologous genes, as is the case for example  
162 in siderophore biosynthesis. HMMs developed in FeGenie are sensitive to the entirety of each  
163 gene family. In the metagenomes used here, not all domains of a given siderophore pathway  
164 were detected at each time point (see Fig. S3). This is most likely due to the overall lower  
165 GPMs of siderophore biosynthesis genes as compared to other Fe-related genes, and thus a  
166 limit of detection at our sequencing depth. In order to obtain GPM for the identified FeGenie  
167 proteins, each protein sequence related to the Fe pathway in each sample was retrieved in

168 fasta format and mapped against the non-redundant protein database using DIAMOND  
169 blastp. Proteins at 100% similarity were retrieved with the according GPM.  
170 For the taxonomic affiliation of Fe-related genes, sequences were obtained using Centrifuge  
171 v1.0.4 (Kim *et al.*, 2016) by searching against the GTDB database (Parks *et al.*, 2022) and the  
172 alignments with highest score were kept. We then calculated the relative contribution of a  
173 prokaryotic group assigned to a gene of interest. The limitation of this approach is that  
174 taxonomy is assigned based on relatively short sequencing length of the individual genes; for  
175 this reason, we considered mainly the family level and used metagenome assembled genomes  
176 for a more resolute description. Therefore, the GPM for each Fe-related gene in each  
177 sample were summed separately (corresponding to *a*), the GPM per taxa associated with each  
178 Fe-related gene in each sample were summed (corresponding to *b*), and the relative  
179 contribution of the prokaryotic group was calculated as  $b/a$ .  
180 Organic substrate transporter annotation was carried out based on the non-redundant protein  
181 sequences set against TCDB (Saier, 2006) using DIAMOND blastp (parameters used --more-  
182 sensitive -e 1e-5), and amino acid similarity of  $\geq 70\%$  was chosen as threshold for further  
183 analysis. CAZymes were annotated based on the non-redundant protein sequences set using  
184 hmmsearch against the dbCAN database (e value  $< 1 \times 10^{-10}$ , coverage  $> 0.3$ ) (Zhang *et al.*,  
185 2018). The domain with the highest coverage was selected for sequences overlapping  
186 multiple CAZymes domains. The phylogenetic affiliation of organic substrate transporters  
187 and CAZymes sequences and the relative contribution of prokaryotic groups was determined  
188 using the same method as described above.  
189 Visualization of data was performed using the packages ggplot2 in the R v4.0.3 and  
190 SigmaPlot v14.5 software. The heatmap was generated using the pheatmap package in the R  
191 v 4.0.3, with Euclidean distance as the distance function.  
192 ***Analysis of Metagenome Assembled Genomes (MAG)***

193 One MAG assigned to each SAR11 and *Nitrospiraceae*, and two MAGs assigned each to  
194 *Polaribacter* and *Rhodobacteraceae* were downloaded from data previously published by  
195 Sun et al. (Sun *et al.*, 2021). The seawater samples from which the MAGs were constructed  
196 were collected from 3 sites in the Kerguelen region including the study site of the RAS  
197 deployment. After sequencing, metagenomes were assembled and binned using the methods  
198 described by Sun et al. (Sun *et al.*, 2021). MAGs were reassessed using CheckM (v1.1.2)  
199 (Parks *et al.*, 2015) for completeness, and the completeness of the 6 MAGs used in the  
200 present study ranged from 55-94% (Table S5). The ANI values between MAGs, calculated  
201 using the OrthoANIu algorithm (Yoon *et al.*, 2017), were below 95%. ORFs of MAGs were  
202 predicted using Prodigal v2.6.3 (Hyatt *et al.*, 2010) (parameters used -p meta) and functional  
203 annotation of MAGs was carried out using the same method as above. To estimate the  
204 abundance of MAGs and their Fe- and organic substrate transporter and CAZymes-related  
205 genes across the twelve metagenomes, we first used Bowtie2 v2.4.4 (Langmead and  
206 Salzberg, 2012) with default parameters to recruit short reads from the twelve metagenomes  
207 and samtools (Li *et al.*, 2009) was used to convert the resulting SAM files into sorted and  
208 indexed BAM files. We used anvi'o v7.1 (Eren *et al.*, 2015) to profile short metagenomic  
209 reads aligned to MAGs to estimate coverage statistics per metagenome. Briefly, we first used  
210 the program 'anvi-gen-contigs-database' turning MAGs into a contigs-db, then used the  
211 program 'anvi-profile' to process the BAM file and generate individual anvi'o profile  
212 database, and used the program 'anvi-merge' to merge these individual profiles into a final  
213 merged profile database which stored the coverage statistics of each MAGs in the 12  
214 metagenomes. Finally, the program 'anvi-summarize' generated mean coverage values of  
215 each MAG and each gene within them across the twelve metagenomes and was visualized  
216 by the program 'anvi-interactive' in 'gene mode'.

## 217 **Results**

### 218 ***Environmental context***

219 The site of the RAS deployment is located southeast of Kerguelen Island in the Indian Sector  
220 of the Southern Ocean (Figure 1). This naturally Fe-fertilized region has been extensively  
221 studied during previous oceanographic cruises focusing on distinct time periods, that are  
222 early spring, mid and late summer (projects KEOPS1&2 and MOBYDICK). The deployment  
223 of the RAS from 25 October 2016 to 24 February 2017 has for the first time provided a full  
224 seasonal picture of the physical context (Pellichero *et al.*, 2020), inorganic nutrient  
225 concentrations and characteristics of the consecutive phytoplankton blooms and their carbon  
226 and trace element export (Blain *et al.*, 2021, 2022). Briefly, the spring phytoplankton bloom  
227 started in late October and peaked by mid-November and the summer bloom occurred in  
228 early January (Figure S1A). Diatoms dominated both blooms but varied considerably in their  
229 composition between seasons (Blain *et al.*, 2021). Surface water temperature steadily  
230 increased from 1.70°C to 4.17°C over the course of the sampling period. Concentrations of  
231 nitrate (range 21.95 to 27.69 µM) and silicic acid (range 2.45-8.24 µM) decreased during the  
232 observation period, while ammonium concentrations (range 0.3 to 2 µM) reached a maximum  
233 in late December (Figure S1B) (Blain *et al.*, 2021).

### 234 ***Seasonal trends in Fe and C transporters***

235 In total 28 662 Fe-related genes were annotated in the FeGenie database, 66 005 genes related  
236 to substrate transport in the TCDB database and 136 460 genes related to enzymatic activity  
237 in the CAZymes database. Fe-related genes exhibited lowest normalized gene abundances  
238 (genes per kilobase million, GPM) (10-600 GPM), followed by organic carbon membrane  
239 transporters (600 to 1400 GPM) and CAZymes (7000 to 12000 GPM).  
240 Normalized gene abundances (GPM) of each of these categories revealed pronounced  
241 seasonal patterns (Figure 2). Transporters of Fe ions ( $\text{Fe}^{3+}$ ,  $\text{Fe}^{2+}$ ) (Figure 2A), siderophores

242 and heme (Figure 2B) had the highest GPM after the blooms. Increases in GPM occurred  
243 rapidly after the spring bloom and with a time lag of about one month after the summer  
244 bloom. Siderophore biosynthesis genes had one peak during the spring bloom, and a second  
245 peak after the summer bloom in parallel with various Fe transporters (Figure 2B). Organic  
246 substrate transporters revealed the highest GPM during the spring bloom and a smaller peak  
247 was observed one month after the summer bloom (Figure 2C). Organic substrate transporters  
248 increased also during the final spring bloom decline, following the seasonal peak in  
249 CAZymes.

250 The most abundant transporters of Fe<sup>3+</sup> ions were *fbpABC*, *futA1/A2* and *fbpB-futB*, and the  
251 dominant Fe<sup>2+</sup> ion transporter genes were *feoAB* and *efeUOB* (Figure 3A) (Table S4).

252 Siderophore uptake genes were dominated by the *exbB-exbD-tonB* complex and among the  
253 genes identified for the transport of specific siderophores were those of pyoverdinin (*fpv*),  
254 aerobactin (*hat*), ferric enterobactin (*pir*), ferripyochelin (*fpt*), and vibrioferrin (*pvu*) (Figure  
255 3B). We further identified four siderophore biosynthesis clusters related to pyochelin (*pch*),  
256 pyoverdinin (*pvd*), rhizobactin (*rhb*) and vanchrobactin (*vab*) (Figures S2 and S3). Among  
257 other Fe-related genes identified, a seasonal trend driven by the two phytoplankton blooms  
258 was also detectable for Fe regulation (*fur*, *dtxR*, *fecR*), while genes for iron storage (*ftn*)  
259 remained stable over time (Figure S2). Transporters of organic substrates were dominated by  
260 those for amino acids and proteins, followed by sugars, glycerol and organic acids, and  
261 transporters for vitamins were also present throughout the season (Figure 3C). During the  
262 sampling period, 379 families of CAZymes were identified, among which 191 glycoside  
263 hydrolase (GH) families, 82 glycosyltransferase (GT) families, 41 polysaccharide lyase (PL)  
264 families, 34 carbohydrate-binding module (CBM) class families, 16 carbohydrate esterase  
265 (CE) families and 13 auxiliary activities (AA) families (Figure 3D).

266 To obtain a more detailed picture of the seasonal patterns of genes involved in Fe and organic

267 substrate acquisition, we determined the similarity in the temporal trend among individual  
268 genes (Figure 4). This analysis highlights 4 periods. The first period, corresponding to the  
269 spring bloom (6 Nov and 17 Nov), was characterized by seasonally highest GPM of genes  
270 coding for siderophore synthesis (e.g., Pyochelin (*pch*), Pyoverdin (*pvd*), Vanchrobactin  
271 (*vab*)) and transport (e.g., catecholate receptor (*piu*), ferripyochelin (*fpt*), Vibriobactin  
272 (*viuB*)), concurrently with several organic substance transporters, such as amino acids,  
273 sugars, spermidine/putrescine, and sulfonates. During the second period, that corresponds to  
274 the immediately following decline of the spring bloom (28 Nov), concurrent peaks in  
275 transporters of Fe<sup>3+</sup> ions (*fbp*, *fut*), heme (*hmu*) and siderophores (e.g., Vibrioferrin (*pvu*),  
276 Aerobactin (*hat*), Pyoverdin (*fpv*)) were observed. This period further revealed high GPM of  
277 several CAZymes (GT, CE, GH). The third period extends from the final declining phase of  
278 the spring bloom to the summer bloom (9, 20 and 31 Dec) and was characterized by  
279 enhanced GPM of transporters of Fe<sup>3+</sup> ions (*fbpB-futB*, *futA1/A2*, *yfe*). The fourth period is  
280 represented by two sampling dates one month after the summer bloom (22 Jan and 2 Feb).  
281 Siderophore synthesis genes present already during the first phase re-emerged (e.g., *Pch*, *Pvd*,  
282 *Vab*) in combination with a new group of siderophore synthesis (e.g., Rhizobactin (*rhb*)) and  
283 transport (Ferric Enterobactin (*pir*), Legiobactin (*lbt*)) genes. During the fourth period,  
284 transporters of Fe peaked in parallel with those of organic compounds and siderophore  
285 synthesis genes, a pattern that contrasted our observations during the spring bloom.

### 286 ***Linking function to taxonomy at the family level***

287 These temporal changes in the functional repertoire were paralleled by shifts in the  
288 prokaryotic community composition (Liu *et al.*, 2020) raising the question of the link  
289 between microbial diversity and Fe and organic substrate acquisition. To investigate whether  
290 the genes of interest were harbored by specific prokaryotic groups we assigned the taxonomy  
291 of the individual genes belonging to the different gene categories. We illustrate here the

292 taxonomic assignments of 4 genes, each representing the transport of a distinct form of Fe  
293 and each characterized by a marked seasonal trend; these are transport of Fe<sup>3+</sup> ions (*fbp*) and  
294 Fe<sup>2+</sup> ions (*feo*), of heme (*hmu*) and the siderophore ferric enterobactin receptor (*pir*) (Figure  
295 5). The taxonomic assignments of other Fe-related genes are illustrated in Figure S4. The  
296 transporter *fbp* (Fe<sup>3+</sup> ions) was assigned to several prokaryotic groups and their respective  
297 relative contributions changed considerably over time (Figure 5). During the spring bloom  
298 (period 1) when GPM were low, transport of Fe<sup>3+</sup> ions was assigned to *Pseudomonadaceae*,  
299 *Pelagibacteraceae* and *Rhodobacteraceae*, while *Nitrospiraceae* dominated the taxonomic  
300 assignments (75 % of all *fbp* genes) when GPM peaked during the bloom decline (period 2).  
301 *Rhodobacteraceae* (in particular the genus *Amylibacter*, Figure S5) were the most abundant  
302 contributors to *fbp* (19-51%) during the remaining season and *Pseudomonadaceae* had an  
303 increasing share towards the end of the season (4-35%). A contrasting picture was obtained  
304 for *feo* (transport of Fe<sup>2+</sup> ions) that was assigned to mainly two groups, *Sphingomonadaceae*  
305 in early spring (period 1) (28-39%) and *Flavobacteriaceae* throughout the remaining season  
306 (16-89%). *Flavobacteriaceae* were dominated by the genus *Polaribacter* (Figure S5) during  
307 the spring bloom decline (period 2). Heme uptake (*hmu*) revealed a seasonal abundance  
308 pattern similar to that of *fbp*, but had distinct taxonomic assignments. *Porticoccaceae* (7-  
309 43%), *Flavobacteriaceae* (2-76%) and *Nostocaceae* (2-14%) were the main contributors to  
310 heme uptake throughout the season. During the bloom decline (phase 2) when *hmu* peaked  
311 *Polaribacter irgensii* (*Flavobacteriaceae*) had a dominant contribution, and *Cellulophaga*,  
312 *hydrogenovibrio* and *Kordia* (*Flavobacteriaceae*) (Figure S5) prevailed over the remaining  
313 season. The ferric enterobactin receptor *pir* had a seasonal abundance pattern different to that  
314 of heme, with peaks during period 1 and 4, but overall similar taxonomic assignments at the  
315 family level. *Flavobacteriaceae* (6-66%), with substantial contributions of *Polaribacter*,  
316 *Pseudomonadaceae* and *Porticoccaceae* (1-13%) were the dominant contributors to *pir*, and

317 *Sphingomonadaceae* (0-15%) and *Cellvibrionaceae* (0-30%) had seasonally variable  
318 contributions. The taxonomic assignments of genes encoding for organic substrate  
319 transporters and CAZymes revealed overall small differences in the prokaryotic groups  
320 contributing to each category, but marked temporal changes in their relative contributions  
321 were observed, in particular during period 2 (Figures S6 and S7).

### 322 ***Genome-resolved functional potential***

323 To obtain insights on the functional potential for the acquisition of both Fe and organic  
324 substrates, we used previously published metagenome assembled genomes (MAGs) obtained  
325 from samples collected in the Kerguelen region, including our study site (Sun *et al.*, 2021).  
326 We choose MAGs belonging to SAR11, *Nitrincolaceae*, *Rhodobacteraceae*, and the genus  
327 *Polaribacter* (*Flavobacteriaceae*) (Figure 6 and Table S5). These MAGs belong to  
328 prokaryotic groups with substantial contributions to the different gene categories (Figure 5  
329 and Figures S5-S7). Further, ASVs belonging to each of these groups were shown to be  
330 abundant and have pronounced seasonal relative abundance patterns, based on 16S rRNA  
331 gene sequencing from the same RAS deployment (Liu *et al.*, 2020). Mapping our  
332 metagenomic short reads to these MAGs revealed that they largely varied in their respective  
333 coverages and had each a distinct seasonal trend. SAR11 MAG133 had slightly higher  
334 coverage in early spring as compared to the remaining season. By contrast, *Nitrincolaceae*  
335 MAG115 revealed one sharp peak during period 2. The 2 *Rhodobacteraceae* MAGs had both  
336 higher coverage during the transition between the spring and summer blooms (period 3). By  
337 contrast, the 2 *Polaribacter* MAGs differed in their overall coverage and their seasonal  
338 dynamics. The inventories of the Fe- and organic substrate-acquisition genes provided some  
339 insights on the metabolic versatility of these MAGs. SAR11 MAG133 harbored a reduced set  
340 of Fe (*fut*, *fbpB-futB*, Fe<sup>3+</sup> ions) and organic substrate (sugars and amino acids) transporters.  
341 In *Nitrincolaceae* MAG115 we identified transporters for siderophore-bound Fe (*exbB-exbD*



342 complex and *fpv* for pyoverdinin) and for several organic substrates (sugars, amino acids,  
343 peptides) and vitamins. Both *Rhodobacteraceae* MAGs harbored transporters for Fe<sup>3+</sup> ions  
344 (*yfe*, *fbp*), but appear to differ in their potential of organic substrate acquisition. We identified  
345 transporters of several substrates in *Rhodobacteraceae* MAG20 while only amino acid  
346 transporters were detected in *Rhodobacteraceae* MAG52. Among the MAGs considered here,  
347 *Polaribacter* MAG88 had the most diverse Fe-gene inventory, including heme and  
348 bacterioferritin, absent from MAG64. The 2 *Polaribacter* MAGs harbored both transporters  
349 of Fe<sup>2+</sup> ions (*feo*), proteins and carboxylic acids.

## 350 **Discussion**

351 We observed pronounced seasonal patterns in the prevalence of genes for the transport of Fe  
352 and organic substrates by prokaryotic communities in the Southern Ocean. The acquisition of  
353 Fe was temporally decoupled from that of organic carbon over the course of the spring  
354 phytoplankton bloom, while a concerted access to both elements occurred after the summer  
355 bloom. These dynamics on the community level likely result from seasonal changes in the  
356 requirements of Fe and organic carbon and the functional capabilities to acquire the different  
357 chemical forms in which these elements are present by diverse microbial taxa. Using  
358 metagenome assembled genomes, we illustrate differences in the Fe- and organic substrate  
359 acquisition repertoires among abundant prokaryotic taxa. These observations combined with  
360 marked seasonal changes in microbial community composition (Liu *et al.*, 2020) provide new  
361 insights on the potential ecological niches of prokaryotes in the Fe- and organic carbon-  
362 constrained Southern Ocean.

### 363 ***Seasonal microbial response to phytoplankton blooms induced by natural iron fertilization***

364 Natural Fe-fertilization in the region off Kerguelen Island leads to annually occurring  
365 phytoplankton blooms that impact ecosystem structure and functioning (Blain *et al.*, 2007).  
366 Heterotrophic prokaryotes respond markedly to these blooms in terms of growth, biomass  
367 production and respiration and thereby contribute to the processing of a substantial fraction of  
368 primary production during different seasons (Christaki *et al.*, 2021). The microbial  
369 communities present and active in these naturally Fe-fertilized waters vary over the course of  
370 the bloom (Liu *et al.*, 2020) and are distinct to communities in surrounding HNLC waters  
371 during different seasons (West *et al.*, 2008; Landa *et al.*, 2016; Hernandez-Magana *et al.*,  
372 2021). Differences among prokaryotic taxa in Fe requirements and acquisition strategies as  
373 well as in the metabolic potential for the uptake of diverse phytoplankton-derived organic  
374 substrates could be one underlying mechanism (Teeling *et al.*, 2012; Debeljak *et al.*, 2019).

375 Niche differentiation in relation to Fe and organic carbon was recently described on a spatial  
376 scale (Sun *et al.*, 2021), but a temporal perspective is thus far lacking.

### 377 ***Microbial strategies and Fe sources during the spring bloom***

378 The prevalence of organic substrate transporters during the spring bloom (period 1) points to  
379 a community that rapidly responds to the supply of labile phytoplankton-derived substrates.

380 Organic carbon has been identified as a growth-limiting resource for the winter community in  
381 the Kerguelen region (Obernosterer *et al.*, 2015; Landa *et al.*, 2016). Our data suggest that  
382 amino acids, organic acids, sugars and sulfonates are the most prominent substances utilized,  
383 confirmed by metatranscriptomics and -proteomics data at the same study site (Debeljak *et*  
384 *al.*, unpublished) and as reported previously from phytoplankton blooms in other marine  
385 environments (Teeling *et al.*, 2012; T. B. Francis *et al.*, 2021). The seasonal pattern of organic  
386 substrate transporters was, however, decoupled from that of Fe-transporters during the spring  
387 bloom, raising the question of how prokaryotes meet their Fe-requirements.

388 Dissolved Fe concentrations are high in surface waters in early spring (0.16 nM) (Qu erou  *et*  
389 *al.*, 2015), but heterotrophic prokaryotes compete with fast growing small phytoplankton for  
390 this Fe source (Fourquez *et al.*, 2015). As reported for diverse members of this clade (Liu *et*  
391 *al.*, 2020; Dinasquet *et al.*, 2022) SAR11 MAG133 was abundant in early spring. The small  
392 streamlined genomes of SAR11 (Giovannoni, 2017) have reduced Fe-uptake repertoires  
393 (Hogle *et al.*, 2016), as observed also for SAR11 MAG133 (Fe<sup>3+</sup> ion transporters *fut*, *fbpB*-  
394 *futB*). Using MICRO-CARD-FISH, SAR11 accounted for about 25% of community Fe  
395 uptake, while the contribution to leucine uptake was 50% in early spring in fertilized and  
396 HNLC waters (Fourquez *et al.*, 2016), indicating an efficient organic substrate utilization  
397 even under conditions when access to Fe is constrained. Low Fe requirements and an  
398 efficient transport of Fe and organic substrates could be a strategy of SAR11 to take  
399 advantage of the pool of labile substrates supplied by phytoplankton.

400 Lithogenic particles present a potentially important source of Fe in early spring above the  
401 Kerguelen plateau (Blain *et al.*, 2022). This Fe source is geochemically complex and needs to  
402 be rendered biologically available via dissolution as for example through the binding to high  
403 affinity ligands (Kalinowski *et al.*, 2000). Among the strongest binding ligands are  
404 catecholate-type siderophores and particle-associated prokaryotes were shown to express the  
405 genes implicated in the respective biosynthesis pathways (Debeljak *et al.*, 2021). The  
406 prevalence of a catecholate-type siderophore receptor gene (*piu*) during spring indicates that  
407 this Fe source could in part account for the prokaryotic demand. Taxonomic assignments  
408 revealed that *Pseudomonadaceae* were the most prominent group harboring this receptor, an  
409 observation that supports previous findings on the role of this group in siderophore  
410 biosynthesis and uptake (Schalk *et al.*, 2020). An alternative strategy could be the utilization  
411 of an internal reservoir, such as Fe stored in specific proteins (bacterioferritin, Ftn) (Andrews  
412 *et al.*, 2003). This capacity was shown for strains belonging to *Pseudoalteromonas* (Mazzotta  
413 *et al.*, 2020) and several prokaryotic groups, in particular *Flavobacteriaceae*, *Haliaceae* and  
414 *Altermonadaceae* contributed to the expression of the respective genes above the Kerguelen  
415 plateau in early spring (Debeljak *et al.*, 2019). We identified the gene coding for ferritin in  
416 *Polaribacter* MAG88, which had increased coverage during spring, in contrast to  
417 *Polaribacter* MAG64 that lacked this gene and revealed enhanced coverage later in the  
418 season. These observations illustrate potential niche differentiation among closely related  
419 taxa. The utilization of internal Fe stored as ferritin by some taxa could be a possible  
420 mechanism for the apparent temporal decoupling in requirements of organic substrates and  
421 Fe.

#### 422 ***Fe acquisition strategies during the spring bloom decline***

423 Microbial gene inventories related to Fe and organic carbon transport drastically changed  
424 during the decline of the spring bloom (period 2). The prevalence of all CAZyme families

425 indicates the presence of a chemically different pool of organic matter and the need to cleave  
426 polysaccharides to access organic carbon (Teeling *et al.*, 2012). The synchronized  
427 enhancement in the GPM of different types of Fe-transporters, including those for Fe<sup>3+</sup> and  
428 Fe<sup>2+</sup> ions, Fe bound to siderophores (Vibrioferrin (*pvu*), Aerobactin (*hat*), Pyoverdin (*fpv*))  
429 and heme suggest an increased prokaryotic Fe demand in response to the shift in the organic  
430 carbon regime during the phytoplankton bloom decline. In contrast to early spring,  
431 remineralization is a key process providing different sources of Fe to the system. Our  
432 observations allow us to provide insights into possible Fe and organic carbon related  
433 strategies of three bacterial groups with distinct abundance patterns.

434 *Nitrincolaceae*, represented by MAG115, rapidly responded to the bloom decline. The  
435 marked seasonal pattern of MAG115, reaching highest coverages of all MAGs considered  
436 here, matched the relative abundances of the respective ASVs as determined from 16S rRNA  
437 gene sequences (Liu *et al.*, 2020). *Nitrincolaceae* dominated the taxonomic assignments of  
438 transporters of Fe<sup>3+</sup> ions (*fbp*) during the bloom decline and MAG115 harbored several  
439 siderophore uptake genes, such as pyoverdine (*fpv*), a siderophore containing catecholate and  
440 hydroxamate groups. MAG115 also contained a large repertoire of organic substrate  
441 transporters indicating its capacity in the uptake of various labile compounds (B. Francis *et*  
442 *al.*, 2021). The rapid response to a pulsed supply of organic carbon by taking advantage of its  
443 potential to utilize different forms of Fe suggests a copiotrophic metabolic strategy, and idea  
444 that is further supported by the concurrent expression both at the transcriptomic and  
445 proteomic level of genes related to Fe- and organic carbon transport by *Nitrincolaceae*  
446 MAG115 (Debeljak *et al.*, unpublished).

447 *Polaribacter* MAG88 (*Flavobacteriaceae*) also responded to the bloom decline, but its  
448 coverage remained comparatively low. This MAG harbored a large repertoire of transporters  
449 for organic substrates and Fe, and among the latter we identified the heme transporter *hmu*.

450 Heme is a porphyrin-bound source of Fe that can account for a large fraction of cellular Fe  
451 concentrations in phytoplankton and can become a source to heterotrophs when cells are  
452 destroyed such as by viral lysis or grazing (Honey *et al.*, 2013). Utilization of heme was  
453 demonstrated experimentally for strains belonging to the *Roseobacter* clade (Roe *et al.*, 2013;  
454 Hogle *et al.*, 2017), but many other abundant microbial groups appear to lack heme  
455 transporters (Hogle *et al.*, 2017). In the present study, *Flavobacteriaceae* had a substantial  
456 share of the taxonomic assignments of heme transporters (*hmu*), in particular during the  
457 bloom decline. Our data suggest that members belonging to *Flavobacteriaceae* could use this  
458 source of Fe, to account for its requirements during the degradation of phytoplankton derived  
459 organic substances. This potential niche specialization could play an important role in large  
460 particles where access to this source of Fe is facilitated.

461 Both *Roseobacter* MAGs had increased coverage during the transition between the spring  
462 decline and summer bloom, matching observations of members of this clade at the ASVs  
463 level (Liu *et al.*, 2020). While functional Fe-profiles were overall similar for both MAGs,  
464 MAG20 appears to have a larger organic substrate repertoire than MAG52. An analysis of  
465 *Roseobacter* genomes revealed the presence of multiple and diverse pathways for the  
466 acquisition of inorganic and organically bound Fe and several copies of ABC transporters  
467 (Hogle *et al.*, 2016). This indicates that this group can make use of a variety of chemical  
468 forms of Fe, an idea that is supported by observations on the transcriptomic (Debeljak *et al.*,  
469 2019, 2021) level. This versatility with respect to Fe transport let us hypothesize that  
470 specialization of organic substrate utilization could be the underlying mechanism for the  
471 similar seasonal patterns of the two *Roseobacter* MAGs.

#### 472 ***Parallel Fe and C acquisition following the productive season***

473 Despite the smaller magnitude of the summer phytoplankton bloom as compared to spring,  
474 the community functional repertoire markedly changed with a time lag of about 1 month,

475 contrasting the rapid response to the spring bloom. The late summer period is characterized  
476 by slightly higher concentrations of dissolved organic carbon and seasonally highest  
477 prokaryotic abundance (Christaki *et al.*, 2021; Hernandez-Magana *et al.*, 2021) and  
478 remineralization presents the main source of Fe (Blain *et al.*, 2008; Sarthou *et al.*, 2008).  
479 These environmental conditions could be favorable for the energetically costly biosynthesis  
480 of siderophores, supported by our observation of the diverse assemblage of genes for the  
481 acquisition of siderophore-bound Fe at the end of the season. Among these the gene of the  
482 siderophore receptor *pir* (ferric enterobactin receptor) was assigned to *Flavobacteriaceae*,  
483 and the two *Polaribacter* MAGs harbored both this gene. Many members of  
484 *Flavobacteriaceae*, including *Polaribacter*, are specialized in the degradation of complex  
485 compounds, such as polysaccharides (Xing *et al.*, 2015; Kappelmann *et al.*, 2019). Our  
486 results indicate that the concurrent access to organic substrates and siderophore-bound Fe  
487 could be a strategy that renders these taxa efficient degraders of organic matter in the  
488 Southern Ocean.

489 By considering transporters for different forms of Fe and organic carbon, two elements that  
490 were shown to constrain prokaryotic growth in the Southern Ocean, we provide insights on  
491 temporal dynamics in nutrient acquisition at the community level. The distinct seasonal  
492 patterns in Fe transporters with respect to those of organic substrates suggest that there could  
493 be a switch in nutrient requirements along the season. Our results extend the use of biomarker  
494 genes that have allowed to identify nutrient stress of specific microbial groups on spatial  
495 scales (Saito *et al.*, 2014; Garcia *et al.*, 2020; Ustick *et al.*, 2021). The taxon-specific genomic  
496 capacities to access the different chemical forms of Fe could be the basis for ecological  
497 niches and drive the observed changes in prokaryotic community composition (Liu *et al.*,  
498 2020) as an acclimation to the supply of phytoplankton-derived organic matter in this  
499 naturally fertilized region of the Southern Ocean.

500 **Author contributions**

501 Rui Zhang, Pavla Debeljak, Stephane Blain and Ingrid Obernosterer designed the research.  
502 Bioinformatic analyses were performed by Rui Zhang. Rui Zhang and Ingrid Obernosterer  
503 wrote the first draft of the manuscript. All authors contributed to editing the manuscript.

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516 **Competing of interests**

517 The authors declare no competing interests.

518 **Data availability statement**

519 The data sets generated and analysed during the current study are available in the European  
520 Nucleotide Archive (ENA) repository at <https://www.ebi.ac.uk/ena> under the project ID  
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700

## 701 **Figure Legends**

702 Figure 1. Map of the region around Kerguelen Island and location of the deployment of the  
703 remote autonomous sampler (white dot). Insert shows global position of Kerguelen Island as  
704 indicated by a square. Monthly composite of chlorophyll for November 2016. Modified from  
705 Blain et al. (Blain *et al.*, 2022).

706

707 Figure 2. A. Temporal changes of Chlorophyll a (green shaded area) and abundance of genes  
708 for Fe<sup>3+</sup> and Fe<sup>2+</sup> transporters (A), siderophore-bound Fe and heme transporters, and  
709 siderophore biosynthesis (B) and carbon substrate transporters and carbohydrate-active  
710 enzymes (CAZymes) (C). Normalized gene abundances are given in genes per kilobase  
711 million (GPM) (see Material and Methods for details).

712

713 Figure 3. Abundance of individual genes contributing to Fe<sup>3+</sup> and Fe<sup>2+</sup> transport (A), heme  
714 and siderophore transport (B), organic substrate transport (C) and carbohydrate-active  
715 enzymes (CAZymes, D) at the 12 time points. Transporters of fatty acids, lipoproteins,  
716 ammonia, exopolysaccharides, and urea are pooled as 'others'. CAZyme classes include  
717 auxiliary activities (AA), carbohydrate-binding modules (CBM), carbohydrate esterases  
718 (CE), cohesin, glycoside hydrolases (GH), glycosyltransferases (GT), polysaccharide lyases  
719 (PL), and S-layer homology domain (SLH). Normalized gene abundances are given in genes  
720 per kilobase million (GPM) (see Material and Methods for details).

721

722 Figure 4. Heatmap illustrates the seasonal patterns of genes involved in Fe and organic  
723 substrate transporters and related processes. Similarity between genes is based on Euclidian  
724 distance cluster analysis. Color intensity is determined by Z-score transformed normalized  
725 gene abundances (GPM). The vertical lines separate the four periods.

726

727 Figure 5. Temporal changes of normalized gene abundance (GPM) and relative contribution  
728 of prokaryotic groups to specific genes associated with Fe<sup>3+</sup>, Fe<sup>2+</sup>, heme and siderophore  
729 transport. Prokaryotic groups are based on the family level. Prokaryotic groups discussed in  
730 the text are marked in red.

731

732 Figure 6. Mean coverage of MAGs in the twelve samples and inventories of genes related to  
733 Fe and organic substrate transport and CAZymes. Mean coverage on the upper panel  
734 represents the average depth of coverage across contig (the coverage of each nucleotide in a  
735 contig divided by the length of the contig). Mean coverage on the lower panel represents the  
736  $\Sigma$  coverage of each bp in a gene / gene length.