1	Supplementary Information for
2	"Microbial iron and carbon metabolism as revealed by taxonomy-specific functional
3	diversity in the Southern Ocean"
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14	This file includes:
15	Supplementary Methods
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Supplementary Methods

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Environmental conditions and sample collection

- 27 The Marine Ecosystem Biodiversity and Dynamics of Carbon around Kerguelen (MOBYDICK)
- cruise took place in the Indian Sector of the Southern Ocean during the austral late summer
- 29 period from Feb 18th to Mar 29th in 2018. Three stations in contrasting oceanic regions were
- 30 chosen for our study, including one located in the naturally iron-fertilized waters above the
- central Kerguelen Plateau (M2) and two off-plateau stations within the High Nutrient Low
- 32 Chlorophyll (HNLC) waters (M3 and M4; Supplementary Fig. 1A and Supplementary Table
- 1). The timing of the sample collection corresponded to about two months after the peak of the
- summer phytoplankton blooms (**Supplementary Fig. 1B**). In central Kerguelen Plateau waters
- maximum seasonal Chlorophyll a (Chl a) concentrations, based on satellite images, were about
- 36 1.5 μg L⁻¹, and they were substantially lower (0.3 to 0.5 μg L⁻¹) in HNLC waters. During the
- 37 MOBYDICK cruise in late austral summer, differences in the Chl a concentrations among sites
- were less pronounced. As a consequence of the sustained seasonal phytoplankton activity,
- 39 concentrations of dissolved organic carbon (DOC) were higher in Kerguelen Plateau waters as
- 40 compared to those in HNLC waters and during previous investigations in the study region in
- 41 spring [1] (**Supplementary Fig. 1BC**). Concentrations of dissolved iron were shown not to
- differ in the on- and off-plateau surface waters in summer (0.09 nM) [2], due to the rapid
- 43 utilization of iron supplied by natural fertilization [3]. The seasonal phytoplankton activity led
- 44 to a build-up of heterotrophic prokaryotic abundance, associated to higher heterotrophic
- 45 prokaryotic production, in fertilized waters as compared to the HNLC sites. Station M2 was
- visited three times at an 8-day interval, and station M3 and M4 were visited twice at a
- 47 two-week interval. Samples were collected for all visits from surface waters (10 m) with 12 L
- Niskin bottles mounted on a rosette equipped with a conductivity, temperature, depth sensor
- 49 (Seabird SBE-911 plus CTD unit).

50 Microbial community DNA extraction and metagenome library preparation

- Total genomic DNA was extracted from the Sterivex filter units using the AllPrep DNA/RNA
- 52 kit (Qiagen, Hiden, Germany) with the following modifications: Filter units were thawed and
- closed with a sterile pipette tip at the outflow. Lysis buffer was added (40 mM EDTA, 50 mM
- Tris, 0.75 M sucrose) and three freeze-and-thaw cycles were performed using dry ice in ethanol
- and a water bath at 65 °C. Lysozyme solution (0.2 mg mL⁻¹ final concentration) was added, and
- 56 filter units were placed on a rotary mixer at 37 °C for 45 min. Proteinase K (0.2 mg mL⁻¹ final

- 57 concentration) and SDS (1% final concentration) were added and filter units were incubated at
- 55 °C with gentle agitation every 10 min for 1 h. To each filter unit, 1 550 μl RLT plus buffer
- 59 was added and inverted to mix. The lysate was recovered by using a sterile 5 ml syringe and
- loaded in three additions onto the DNA columns by centrifuging at 10 000x g for 30 sec. DNA
- of purification was performed following manufacturer's guidelines. The concentration of
- double-stranded DNA was quantified by PicoGreen fluorescence assay (Life Technologies).
- DNA quality was checked on an Agilent 2100 Bioanalyzer/Agilent Nano DNA chip (Agilent,
- Santa Clara, CA, USA). Triplicate DNA extracts were pooled in equimolar amounts providing
- 1 pooled DNA extract per visit and station. The DNA extracts from the repeated visits (3 at M2
- and 2 at each M3 and M4) were then pooled for each station to achieve 1 µg in 30 µL Tris for
- 67 sequencing purposes.

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Microbial community RNA extraction and metatranscriptome library preparation

- 69 Triplicate samples (each 10 L) for prokaryotic RNA extraction were collected from the Niskin
- bottles and immediately filtered. The filtration procedure did not exceed 10 min and 10 ml of
- 71 RNA-later was added to the filter in a Greiner tube prior to storage at -80 °C. RNA was
- extracted using the NucleoSpin® RNA Midi kit (Macherey-Nagel, Düren, Germany). Filters
- stored in RNA-later were thawed, cut in half, removed from the RNA-later solution, re-frozen
- in liquid nitrogen and shattered using a mortar. The second half of the filter was kept and
- 75 extracted when sufficient RNA could not be obtained in the first round of extraction, resulting
- in different values for normalization per volume (L) used (**Supplementary Table 2**). The
- obtained 'powder-like' filter-pieces were added to the denaturing lysis buffer supplied by the
- NucleoSpin® RNA Midi kit (Macherey-Nagel, Düren, Germany) and vortexed for 2 min. Two
- 79 internal standard RNA molecules of 1 006 nucleotides (nt) were synthesized and added to each
- sample with known copy numbers (Supplementary Table 2 and Supplementary Data), to
- enable absolute quantification of transcripts in the downstream analysis [4, 5]. RNA was treated
- 82 with two rounds of DNA digestion and purified using the RNA Clean & ConcentratorTM-5 kit
- 83 (Zymo Research, OZyme, France). Ribosomal RNA (rRNA) was removed with the RiboZero
- rRNA stranded RNA protocol.

Metagenomic read quality control, assembly, and mapping

- Metagenomic reads was evaluated using FastQC (v0.11.7) [6] and processed with Trimmomatic
- 87 (v0.39) [7]. An amount of 327.8, 336.3, and 285.0 million high-quality reads were retained for
- station M2, M3 and M4, respectively (**Supplementary Table 2**). Sequencing coverage and
- 89 complexity of microbiomes in each metagenomic dataset was assessed using Nonpareil (v3.303)

- 90 [8] with the k-mer based approach (**Supplementary Fig. 2A**). Combined with another 337.3 M
- high-quality reads from station M2 sampled in a previous cruise in early spring, de novo
- oc-assembly was performed using MEGAHIT (v1.0.4) [9] with default settings. CD-HIT-EST
- 93 (v4.7; -c 0.99 -aS 1 -g 1) [10] was used to measure the redundancy of the assembled contigs.
- Metagenomic reads were aligned back to the contigs using Bowtie2 (v2.3.5) [11], with
- duplicates removed by the markdup function of SAMtools (v1.9) [12]. On average, 30.32% of
- reads in each metagenomic dataset could be recruited back to the assembled contigs.

97 Southern Ocean assemblies vs. NCBI nt and nr databases

- We queried all the contigs against the NCBI nt database using BLASTN (v2.7.1) [13] with an
- e-value threshold of 1e-10. The bitscore and percentage of identity of the best alignment for
- each contig were collected. For those contigs which could not find a match in the nt database,
- zeroes were assigned. The statistics of the two features are shown in (Supplementary Fig.
- 102 **2BC**).
- 103 We also searched for homologs of our predicted genes, at both the nucleotide and amino
- acid level, against the NCBI nt database using BLASTN (v2.7.1) [13] and nr database using
- Diamond (v0.9.24; BLASTP mode) with an e-value threshold of 1e-10. The bitscore and
- percentage of identity of the best alignment for each gene were collected. For those genes
- which could not find a match in the nt (or nr) database, zeroes were assigned. The statistics of
- the two features are shown in (**Supplementary Fig. 2D-G**).

109 Metagenome Southern Ocean vs. TARA

- A total of 3 588 draft genomes from the TARA Ocean Global Expedition Project, including 957
- from Delmont, et al. [14] and 2 631 from Tully, et al. [15], were downloaded. Besides, another
- 41 newly released Arctic metagenomic datasets with 11 709 809 contigs were also collected
- 113 [16]. We leveraged FastANI (v1.3) [17] for estimating pairwise ANI values between our 133
- 114 MAGs and the TARA draft genomes (**Supplementary Fig. 4A**). Read recruitment from our
- Southern Ocean samples to the TARA assemblies, including both the draft genomes and the
- un-binned Arctic contigs, were implemented using BBMap (v38.22) [18] to verify the
- differences observed through ANI. The percentage of reads mapped to each assembly dataset,
- with a minimum percentage of identity at 90%, were summarized, and further normalized by
- the size of each dataset to a "per Mbp" unit (**Supplementary Fig. 4BCE**). Sequence similarities
- between our 949 228 non-redundant contigs and the TARA Arctic contigs were assessed using
- BLASTN (v2.7.1) [13]. Matches were identified with a minimum percentage of identity at 90%,
- a minimum coverage of the shorter sequence at 90% and an e-value threshold at 1e-3

123	(Supplementary Fig. 4D). Interestingly, in contrary to the low sequence similarity shared
124	among contigs, the TARA Arctic datasets showed higher abundance through read recruitment
125	than our MOBYDICK assemblies (adjusted P-value < 0.05 by Wilcoxon rank sum test;
126	Supplementary Fig. 4E). To further assess the similarity between our metagenomes and the
127	TARA Arctic ones, we downloaded the 41 TARA Arctic metagenomes and implemented
128	BBMap (v38.22) [18] to map TARA Arctic reads to both the TARA Arctic contigs and our
129	MOBYDICK contigs. The read recruitment statistics are summarized and further normalized by
130	the size of each dataset to a "per Mbp" unit, as shown in Supplementary Fig. 4F. TARA Arctic
131	metagenomes and contigs are classified into two groups based on sampling depth. In addition to
132	the comparisons at the DNA level, we employed OrthoFinder (v2.2.3) [19] to identify
133	orthologous gene clusters among our Southern Ocean MAGs and TARA draft genomes
134	(Supplementary Fig. 4GH). Results and discussion could be found in Supplementary
135	Results.
136	Reconstruction of prokaryotic 16S rRNA markers from metagenomes
137	Reconstruction of prokaryotic16S rRNA markers from metagenomes is a complementary tool
138	for evaluating microbial diversity and abundance. We implemented 16S rRNA-based
139	community characterization using the MATAM (v1.6.0) [20] and phyloFlash (v3.4) [21]
140	assemblers with the SILVA SSU rRNA database (v138.1) [22], resulting 6 groups of SSU
141	assemblies (3 samples \times 2 assemblers). The V4-V5 region of each 16S rRNA assembly was
142	extracted by SSU-ALIGN (v0.1.1) [23]. Non-redundant representative V4-V5 sequences were
143	selected across samples using CD-HIT-EST (v4.7; -c 1 -aS 1 -g 1) [10], among which 1 460
144	were at least 200 bp in length and verified with a prokaryotic origin by the assignTaxonomy
145	function of DADA2 (v1.16.0) with its precompiled SILVA training set (v138.1) [24].
146	The identified archaeal and bacterial 16S rRNA V4-V5 representative sequences were
147	aligned by SSU-ALIGN (v0.1.1) [23] according to their secondary-structure covariance models

The identified archaeal and bacterial 16S rRNA V4-V5 representative sequences were aligned by SSU-ALIGN (v0.1.1) [23] according to their secondary-structure covariance models. We built a phylogenetic tree from the sequence alignment using FastTree (v2.1.10; -slow -nt -gtr -gamma -bionj) [25], with the taxonomic assignment of the sequences as constraints at the order level (-constraints). Metagenomic reads were mapped back to the 16S rRNA V4-V5 representative sequences using Bowtie2 (v2.3.5) [11]. A pseudo-OTU table was generated by jgi_summarize_bam_contig_depths [26], taking the depth of coverage as an approximation to amplicon counts. Visualization and statistics were performed in R (v3.6.1) using the phyloseq package (v1.28.0) [27]: Closely-related taxa were clustered through phylogenetic agglomeration by tip_glom (**Supplementary Fig. 5A**); The pseudo-OTU table was transformed for equal sampling depth by rarefy_even_depth; Relative abundance was calculated with the aid

of transform_sample_counts (**Supplementary Fig. 5B**); Alpha diversity was assessed according to the Shannon index and visualized via plot_richness (**Supplementary Fig. 5C**); To describe the differences across sites, we applied the double principal coordinate analysis (DPCoA) with regard to both phylogenetic structure and relative abundance, and visualized the results using plot_ordination (**Supplementary Fig. 5D**). Environmental vectors (M2, M3 and M4) were added according to the coordinates of the sampling sites reported in the DPCoA result. The first principal component, which accounts for more than 99% of the total variance, is associated positively with M2 and negatively with M3 and M4. Given that the three sampling sites are representatives of the on- (M2) and off-plateau (M3 and M4) environments, the first principal component could primarily measure the major differences that distinguish the contrasting oceanic regions, such as the availability of iron and organic carbon.

Clusters of taxonomic groups are distributed along the first axis. The SAR11 group and Bacteroidota are well separated from each other and other taxonomic groups. A Monte-Carlo permutation test (1 000 replicates) applied to the DPCoA result was used to evaluate whether the observed compositional differences among microbial communities are higher than expected in a random distribution, by implementing the randtest.dpcoa function in the ade4 package (nrep=1000 and alter="greater") [28]. The simulated p-value approximates 0.001, assuring phylogenetic clustering as observed in **Supplementary Fig. 5D** given that phylogeny was used to build the distance matrix for our DPCoA analysis.

We also estimated the root square of Rao's dissimilarity coefficient between samples by the disc function in the ade4 package, using the OTU table and the phylogenetic distance (generated by the cophenetic.phylo function in the ape package) involved in the DPCoA analysis. This resulted a Rao's dissimilarity value of 0.1624 between M2 and M3, 0.1807 between M2 and M4, and 0.0259 between M3 and M4, in consistent with the correlations between the environmental vectors as shown in **Supplementary Fig. 5D**. To evaluate the statistical significance of categorical explanatory variables, such as the on- and off-plateau environments, we performed another permutation test based on constrained DPCoA (cDPCoA) [29]. Our cDPCoA analysis extended the DPCoA result with a partitioning factor of sampling sites (on- and off-plateu), using the bca (between-class) function in the ade4 package. The Monte-Carlo permutation test (1 000 replicates) on the cDPCoA result, using the aforementioned randtest.dpcoa function, resulted in a p-value of 0.3446553 (> 0.05). Considering that we only have three metagenomes, this insignificant outcome is not surprising when the analysis focused on the effect of environmental types. We undertook simulations with pseudo replicates for each sampling sites to examine how the number of samples biased the

- significance test. We considered two cases, including 2 and 3 replicates per site. Assuming that
- biological replicates from the same sampling site should consist of similar microbial
- communities, the pseudo replicate(s) was generated by replacing the original measurement (x)
- with a random number selected using the rnorm function with mean=x and sd=0.25x. The same
- cDPCoA and Monte-Carlo permutation test was performed on each simulated OTU table. A
- total of 100 simulations were carried out in the case of 2 replicates per site, and the mean value
- of the resulted p-values is 0.06286713 (± 0.01416026 standard deviation; mean > 0.05). When 3
- replicates per site were considered, the p-value approximates 0.01042957±0.004679961 (mean
- 199 < 0.05).

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Assembly-free metagenome taxonomic profiling

- One limitation of working with metagenomes is the considerable amount of reads that could not
- be assembled into contigs (**Supplementary Table 2**). Such a limitation might be explained by
- 203 the prevalence of genomes with low genome abundance and the deficiency in de Bruijn
- assembly algorithms [30]. Therefore, we also performed taxonomic profiling solely based on
- 205 unassembled metagenomic reads. We implemented three types of taxonomic classifiers with
- distinct pros and cons [31], including DNA-to-DNA (Centrifuge v1.0.4 [32] with its
- precompiled nt database and Kraken2 v2.0.7-beta [33] with its standard database),
- 208 DNA-to-protein (Kaiju v1.7.0 [34] with its precompiled nr databases), and
- 209 phylogenetic-marker-based (mOTUs2 v2.5.1 [35]) methods. The mOTUs2 intrinsically
- 210 incorporates the correction for copy number and genome length by using universal single-copy
- 211 phylogenetic marker genes [35] (**Supplementary Fig. 5E**). However, to derive accurate relative
- abundance, the other three tools require subsequent normalization by the elusive total sequence
- size of individual taxon in the reference databases. To avoid this, we divided out the effects of
- 214 reference sizes, as well as other multiplicative systematic biases, by considering the fold
- changes between taxon ratios across sampling sites [36]. The fold change is defined as
- 216 $\frac{o_i(s)}{o_j(s)} / \frac{o_i(t)}{o_j(t)}$, where *O* represents the observed number of reads from sample *s* (or *t*) that were
- assigned to individual taxonomic group i (or j) [36]. We used *Candidatus Pelagibacter* as the
- denominator (taxon j) given its prevalence and high abundance across sampling sites. The taxon
- ratios measured the "relative abundance" of a taxonomic group to *Candidatus Pelagibacter*,
- instead of to the whole community. The fold change of taxon ratios, by crossing out potential
- multiplicative systematic biases [36], was used as an indicator of the relative fitness of a taxon
- as compared to the ubiquitous SAR11 population in contrasting environments (M3 vs. M2, or
- 223 M4 vs. M2; **Supplementary Fig. 5F**). Regarding possible detection biases against rare taxa,

- here, we summarized read counts at the genus levels and only considered genera whose read
- counts was no less than 1% of Candidatus Pelagibacter's.

226 Metatranscriptomic read quality control and internal standards quantification

- 227 An initial round of read processing was provided by the company using Trimmomatic (v0.39)
- [7]. The standard Illumina adapters and low-quality bases were removed with the following
- parameters "2:30:10 SLIDINGWINDOW:4:5". Further, we performed another round of quality
- control and refinement with Trim Galore (v0.5.0) using a minimum Phred score of 20 and a
- length threshold of 50 bp. To remove rRNA, tRNA and internal standard sequences, a two-step
- clean-up procedure was performed, starting with SortMeRNA (v2.1b) [37] and followed by a
- BLASTN (v2.7.1) [13] search against a custom database consisting of 545 336 reference rRNA
- and tRNA sequences from diverse taxa along with the 2 internal standard sequences [38].
- Finally, an average of 22.38 M high-quality paired-end protein-coding reads were retained from
- each replicate, and the per-liter calculations were performed based on the recovery rate of
- internal standards as previously described [38-40] (**Supplementary Table 2**).

238 Identification of PUL-like structures and fucose utilization loci

- In this study, we adopted a more general term CAZyme gene clusters (CGCs) [41] to identify
- 240 PUL-like gene clusters in a broader sense, requiring at least one CAZyme accompanied with
- transcription factors (TFs) and/or transporters (TCs) (**Supplementary Fig. 13A-D**). To identify
- fucose utilization loci among Verrucomicrobiae MAGs, the nine *Lentimonas* genomes provided
- in Sichert et al. [42] were downloaded from NCBI GenBank database. Orthologous proteins
- between the MAGs and the *Lentimonas* references were determined using OrthoFinder (v2.2.3)
- 245 [19] (**Supplementary Fig. 13E**).

246 Comparative analysis of Pseudomonadales HTCC2089 pangenomes

- 247 The sequences of 19 Pseudomonadales HTCC2089 draft genomes were downloaded from
- NCBI GenBank database, including 15 from UBA4421 genus and 4 from UBA9926
- 249 (**Supplementary Table 6**). Pangenome is defined on the basis of cluster of orthologous genes
- identified by OrthoFinder (v2.2.3) [19], and comparative analysis between genus UBA4421 and
- 251 UBA9926 was carried out from three aspects, including the core, accessory and singleton genes
- 252 (**Supplementary Table 6**). A gene cluster enhancing the use of light as an energy resource was
- identified exclusively in the UBA4421 genus (**Supplementary Fig. 14A**). A gene encoding
- bacteriorhodopsin was also identified in one of the UBA9926 genome (GCA_002728935), but
- genes around it are not related to light harvesting (**Supplementary Fig. 14A**). Genes encoding

256	bacteriorhodopsin were identified and their protein sequences were retrieved. The top 5
257	homologous bacteriorhodopsin sequences were selected from the MicRhoDE database [43] via
258	BLASTP search. Another two archaeal rhodopsin sequences were obtained from MicRhoDE
259	and used as outgroups for phylogeny analysis. All bacteriorhodopsin proteins were aligned by
260	MAFFT (v7.313) [44] and processed by trimAl (v1.4; -automated1) [45]. Phylogenetic
261	reconstruction was performed using IQ-Tree (v1.6.8; -m MFP -bb 1000 -bnni) [46]. The
262	bacteriorhodopsins from distinct genera are well separated on the phylogeny tree with high
263	support values (Supplementary Fig. 14B).

Supplementary Results

265	Comparison between MOBYDICK metagenome assemblies and the TARA ocean dataset
266	The TARA Ocean Global Expedition Project provides an extensive survey on the global surface
267	ocean microbiomes [47], providing 3 588 draft genomes [14, 15] and another 41 newly released
268	Arctic metagenomic datasets with 11 709 809 contigs [16]. However, to date, only 3 TARA
269	prokaryote-enriched metagenomes from the surface Southern Ocean are publicly available, and
270	their sampling sites were far away from ours. The aforementioned comparisons between our
271	Southern Ocean assemblies and the NCBI databases highlight the uniqueness of the Southern
272	Ocean microbiomes (Supplementary Fig. 2B-G) and further request the comparisons between
273	our Southern Ocean MAGs and the TARA assemblies. Our analyses were performed from three
274	main aspects, including the pairwise average nucleotide identity (ANI) assessment, the
275	Southern Ocean metagenomic read recruitment and the identification of orthologous protein
276	families between the two datasets (see Supplementary Methods). Only 13 of the Southern
277	Ocean MAGs conform to > 95% intra-species ANI values with TARA counterparts, despite the
278	high similarity shared among the TARA MAGs (Supplementary Fig. 4A and Supplementary
279	Table 3) [17]. Metagenomic read recruitment analysis further confirmed that, except for the
280	TARA Southern Ocean samples (SOC), the TARA MAGs recruited a significantly lower
281	amount of our metagenomic reads as compared to our Southern Ocean MAGs (adjusted P-value
282	< 0.05 by Wilcoxon rank sum test; Supplementary Fig. 4BC), assuring that the novelty of our
283	Southern Ocean assemblies is not derived from biases introduced during metagenome assembly
284	and binning. In addition, only 4.18% ($\pm 1.33\%$; S.D.) of our Southern Ocean contigs could find
285	a match with high similarity in the TARA Arctic datasets (Supplementary Fig. 4D).
286	Interestingly, in contrary to the low sequence similarity shared among contigs, the TARA Arctic
287	datasets showed higher abundance through read recruitment than our Southern Ocean
288	assemblies (adjusted P-value < 0.05 by Wilcoxon rank sum test; Supplementary Fig. 4E) and
289	the vice-versa read recruitment analysis confirmed the same pattern (Supplementary Fig. 4F),
290	suggesting that Arctic and Southern Ocean metagenomes may have some taxonomic and
291	functional similarity.
292	Genera with anomalously deviated fold changes between taxon ratios across sampling sites
293	Particularly, <i>Polaribacter</i> presented an intense increase in abundance in the on-plateau M2 site
294	as compared to the HNLC waters (Supplementary Fig. 5F). <i>Polaribacter</i> are heterotrophic
295	bacteria belonging to the family Flavobacteriaceae. They are widely distributed in marine

296 habitats and rely heavily on the phytoplankton-derived dissolved organic matters [48-51]. It can develop rapidly under favorable conditions, and was reported to contribute to a large share of 297 biomass production during the spring phytoplankton blooms surrounding Kerguelen Island [52]. 298 Another interesting Flavobacteriaceae genus is *Formosa* (**Supplementary Fig. 5F**; named as 299 Hell-33-131 under the GTDB taxonomy system [53] in **Supplementary Table 3**), which 300 301 accounted for up to 24% of all bacteria during diatom-dominated spring blooms off the 302 Helgoland Island in North Sea [54]. In general, marine Flavobacteriaceae are active degraders of biopolymers and was observed to be abundant in nutrient-rich habitats [49, 55, 56]. They are 303 key players in Southern Ocean carbon cycling. Therefore, it is important to understand the 304 underlying enzymatic mechanisms and adaptation that drive the specialization of these highly 305 306 competitive bacteria in the on-plateau M2 site. To the contrary, the Gammaproteobacterial 307 genus Psychrobacter were of greater success in HNCL waters, characterized with a log fold change of taxon ratios below 0 (Supplementary Fig. 5F). We have a Southern Ocean MAG 308 (MAG_130) from this genus, which showed an overall high abundance in all three sampling 309 310 sites and were featured with an average coverage of depth per million reads ~12 times higher in M3 and M4 than M2 (Fig. 1 and Supplementary Table 3). Psychrobacter is known to produce 311 siderophores in iron limiting conditions and able to increase growth rate under iron fertilization, 312 313 reassuring its success in both environments [57]. Genomic differences between MAG 103 and MAG 62 314 315 We identified a putative methanogenesis gene cluster in MAG_62, which was conserved in all but one UBA9926 genomes and absent from the UBA4421 genus (Supplementary Table 6). It 316 317 consisted of one trimethylamine methyltransferase (mttB; K14083), a corrinoid 318 methyltransferase, a protein of unknown function, a methyltetrahydrofolate-homocysteine 319 methyltransferase (MTR; K00548), and a ferredoxin. Methylotrophic prokaryotes, which 320 consume methylated compounds such as trimethylamine and dimethyl sulfate, were reported to 321 be common in marine and hypersaline, sulfate-rich sediments [58]. Just adjacent to putative 322 methanogenesis loci, there was another cluster consisting of four sarcosine oxidase-encoding 323 genes (soxADG), which could only be found in one UBA4421 genomes. Another sarcosine 324 oxidase subunit, soxB, was also identified in all UBA9926 genomes and 2 UBA4421 gnomes

suggested a deeper water origin of this species, which is coherent with the higher abundances of

identified as an important process of energy metabolism in sediments and deep-sea vent

but absent from MAG_103. Sulfur oxidation mediated by Sox proteins have also been widely

ecosystems [59, 60]. The co-localization and conservation of the sox and mtt genes in MAG_62

MAG_62 in spring characterized by a deep mixed layer (**Supplementary Table 3**).

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We could not detect fundamental advantages in substrate utilization that would explain the flourishing of MAG 62 in the spring surface waters. Other possible explanations could be related to RecBCD DNA repair enzymes and the biosynthesis of antibiotics. MAG_62 had a gene cluster encoding the three exodeoxyribonuclease V gamma subunits in a row (recB, recC and recD; K03582, K03583 and K03581), which was shared by all UBA9926 genomes but absent from the UBA4421 genus. RecBCD complex is responsible for the repair of DNA double-strand breaks by homologous recombination [61], and was considered to play an effective role in handle antibiotic-induced oxidative DNA damage [62]. Additionally, RecD was also reported to play a critical role for deep-sea bacteria to grow under high pressure [63]. Prokaryotes have evolved various strategies, such as motility, antibiotic synthesis and antibiotic resistance, to increase their probability to survive and increase their competence for larger shared of resources [64]. Both MAG_103 and MAG_62 were capable of producing multiple antibiotics, but their approach to protect themselves from antibiotic-induced damages were not the same (**Supplementary Table 6**). Penicillin-binding proteins (PBPs) are targets of β -lactam antibiotics. Prokaryotes can confer antibiotic resistance by modifying their PBP structures to either reduce the binding capability of antibiotics or directly degrade the antibiotics [65]. Both the UBA9926 and UBA4421 genera could produce penicillin-binding protein 1A (mrcA; K05366) and penicillin-binding protein 2 (mrdA; K05515) which were considered to participate in beta-Lactam resistance. Besides, MAG_62 could produce a putative multi antimicrobial extrusion protein (PF01554.18) which was reported to mediate resistance to multiple antimicrobial agents [66]. As mentioned above, the RecBCD complex also plays are role in antibiotic resistance, therefore, MAG_62 might be better equipped to deal with antibiotic stress.

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Supplementary Figure Legends

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Supplementary Fig. 1 Three sampling sites (M2, M3 and M4) of the MOBYDICK cruise. 353 354 A, Station M2 is located above the central Kerguelen Plateau, where Chlorophyll a (Chl a) concentrations are higher than at the off-plateau stations (M3 and M4). The colour code in (A) 355 represents the monthly (March) climatological mean of Chl a concentration (mg m⁻³) from 356 357 2003-2018. Data was collected from NASA's moderate-resolution imaging spectroradiometer (MODIS-Aqua) dataset (https://oceancolor.gsfc.nasa.gov/data/aqua/). The location of the 358 359 Antarctic Polar Front (APF) is also highlighted in (A), showing that during the MOBYDICK 360 cruise M2 and M4 were located south of the polar front in Antarctic waters whereas M3 was 361 situated in the polar front zone. The monthly APF data was collected from Pauthenet et al., 362 (2018) [67]. The yearly mean latitude of APF at a resolution of 0.1 longitude degree (the black curve in A) was determined by calculating the mean value of all 12-month measurements 363 364 within a 0.2 degree sliding window, which moves 0.1 longitude degree at one time. The standard error of the mean (SEM) was also calculated and is shown as the grey shading around 365 366 the black curve in (A). The seasonal mean and SEM, covering austral summer and autumn, 367 were determined in the same way, except that only the data from December to May was involved (the blue curve with light blue shading in A). The calculation scheme is exemplified in 368 the inset panel of (A), showing how the data at 60 °E were assessed: The blue dots represent 369 370 data for austral summer and autumn, while the orange ones are for austral winter and spring; 371 The mean and SEM values are calculated based on data points falling within the sliding widow which is shaded in grey. **B**, The variation of Chl a concentration (mg m⁻³) of the three sampling 372 sites (M2 in orange, M3 in blue and M4 in green) from October 2017 to March 2018. The 373 background shade in orange displays previous observations from 1998 to 2017, with Mean+S.D. 374 375 as the upper and Mean-S.D. as the lower limits of the shaded region. Data was collected from 376 Copernicus Marine Service (http://marine.copernicus.eu/). C, Depth profiles of temperature 377 (°C), salinity and dissolved organic carbon (DOC, µM) as determined during the MOBYDICK 378 cruise. The measurements from 0 to 200 m are illustrated. The colour scheme for data points 379 from different sampling sites and distinct visits is shown at the bottom. 381

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Supplementary Fig. 2 A, Sequencing coverage and complexity of microbial communities in our samples determined by Nonpareil [8]. Nonpareil examines the redundancy of the reads in metagenomic datasets to assess the average coverage and predict the amount of sequences required to achieve full coverage [8]. Colours indicate different datasets used for the estimation, and "R1/R2" represents the forward/reverse reads. Solid lines show the projection curves of the

estimated coverage per sequencing effort. The empty circles indicate the actual size of each metagenomic dataset (x-axis) and the corresponding fraction of microbiome covered by DNA sequencing (y-axis). The horizontal dashed lines in red indicate the 95% and 100% coverages. The arrows at the bottom indicate the sequence diversity (Nonpareil N_d). Curves on the right display higher sequence diversity than those on the left. **B** and **C**, The 949 228 assembled contigs were queried against the NCBI nt database. The statistics of the bitscore (**B**) and percentage of identity (**C**) of the best alignment for each contig are represented by bar plots. **D** to **G**, The similarity between our 3 003 586 protein-coding genes and the NCBI nt (or nr) database were also assessed. The corresponding statistics of the best hit for each gene are summarized here. **D** and **F**, the x axis represents different ranges of the bitscores, and the y axis shows the percentage of genes falling into each category. Similarly, **E** and **G**, the x axis represents the percentage of identical match, and the y axis illustrates the percentage of genes in each group.

Supplementary Fig. 3 Maximum-likelihood phylogenetic tree of the 133 MAGs (A) and their corresponding sequence-discrete populations revealed by metagenomic read recruitment (B to G). A, Bootstrap values are shown in percentages at internal nodes. Leaves (MAGs) are coloured according to their taxonomic assignment at the class level. B, Fraction of reads recruited to a MAG (y axis) at each percentage of nucleotide identity level (x axis) by using the metagenome reads from Station M2. Bars are coloured in proportion to their heights. MAGs are arranged according to their position on the phylogenetic tree. C to D, Statistics of recruited metagenome reads from Station M3 and M4. E to G, Statistics of recruited metatranscriptome reads from Station M2, M3 and M4.

Supplementary Fig. 4 Comparisons between MOBYDICK Southern Ocean MAGs and the TARA assemblies, including both draft genomes and un-binned contigs, suggesting high genetic diversity at the DNA level but low functional novelty with regards to proteins. A, Pairwise ANI values between our MAGs and the TARA draft genomes were calculated by using fastANI [17]. If one of our MAGs has multiple matches in TARA, only the highest ANI value was used for the statistics. MAGs without a TARA counterpart (ANI < 75%) were not considered. The violin plot describes the distribution and the density trace of the 95 (out of 133) ANI values. The 95 spotted dots are grouped and coloured according to three ranges: "red" represents for > 95% intra-species ANI values; "salmon" are ANI values ranging between 83 and 95%; and "black" shows < 83% (but ≥ 75%) inter-species ANI values [17]. (B, C and E)

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      shows the statistics of the read recruitment analyses against all the metagenome assemblies,
      including 949 228 contigs and 133 MAGs from our study, as well as 11 709 809 contigs and 3
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      588 draft genomes from the TARA Ocean Project [14-16]. Data were summarized according to
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      their sampling locations as specified in the original papers [14-16], and the list of acronyms is
      provided at the end of this paragraph. The boxplots illustrate the distribution of the fraction of
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      reads in each sample (M2, M3 or M4) that could be mapped to the assemblies by BBMap
      (v38.22) [18] with a minimum percentage of identity at 90% and further normalized by the size
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      of each dataset to a "per Mbp" unit. Non-parametric pairwise Wilcoxon rank sum tests with
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      Holm adjustment demonstrated that the non-Southern Ocean TARA draft genomes recruit a
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      significantly lower amount of reads from each sample (M2, M3 or M4), as compared to our 133
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      MAGs (p.adj < 0.05; B and C). In contrary to B and C, the Arctic contigs showed an overall
      higher rate of read recruitment than our assemblies (p.adj < 0.05; E). D, Our 949 228 contigs
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      were queried against the 41 TARA Arctic datasets using BLASTN (v2.7.1) [13]. The violin plot
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      describes the distribution and the density trace of the percentage of our contigs which have an
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      Arctic match with high sequence similarity. In F, Statistics of TARA Arctic metagenome read
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      recruitment to the TARA Arctic contigs and our MOBYDICK contigs. Similar to E, Arctic
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      contigs recruited significantly more reads per Mbp than ours. In G and H, Prodigal (v2.6.3) was
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      used to recover protein sequences from the TARA assemblies under meta mode (-p meta) [68].
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      Protein sequences were pooled according to their sampling locations as mentioned before,
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      resulting one combined site-specific proteome for our study (named as "SO MAGs"), 12 for
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      Delmont, et al. [14] and 10 for Tully, et al. [15]. Orthologous groups shared among these 23
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      site-specific proteomes were identified using OrthoFinder (v2.2.3) [19]. G, A total of 519 468
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      orthologous groups were identified, among which 84 192 were shared by all. Only 71
      orthologous groups were unique to our MAGs (Singletons were not included). H. Barplots
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      illustrate the percentage of proteins in each site-specific proteome that are involved in
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      orthologous groups with at least one member from our MAGs. The TARA SOC MAGs have the
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      highest percentage of shared orthologs, whereas the TARA ASW ones have the lowest
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      percentage. Due to computational limitation, the TARA Arctic datasets were not included in the
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      orthology analysis. Acronyms: Delmont, et al. has 12 locations, including ANE (Atlantic
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      northeast), ANW(Atlantic northwest), ASE (Atlantic southeast), ASW (Atlantic southwest),
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      ION (Indian Ocean north), IOS (Indian Ocean south), MED (Mediterranean), PON (Pacific
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      Ocean north), PSE (Pacific Ocean southeast), PSW (Pacific Ocean southwest), RED (Red Sea)
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      and SOC (Southern Ocean) [14]. And, Tully, et al. consists of 10 sites, including ARS (Arabian
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      Sea), CPC (Chile-Peru coastal), EAC (east Africa coastal), IN (Indian Ocean), MED
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(Mediterranean), NAT (north Atlantic), NP (north Pacific), RS (Red Sea), SAT (south Atlantic) and SP (south Pacific) [15].

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Taxonomic profiling confirmed that natural iron fertilization and Supplementary Fig. 5 DOC availability altered the diversity and abundance of the prokaryotic communities across the sampling sites. A to D, Taxonomic profiling through 16S rRNA assemblies (as defined under the SILVA v138.1 taxonomy). A, The outmost circle shows the phylogenetic tree constructed using FastTree [25] based on the MATAM/phyloFlash-assembled prokaryotic 16S rRNA sequences [20] (Supplementary Methods). Taxonomic assignment of each 16S rRNA is labelled on the tree tip. The taxonomic colour scheme for the labels is shown in the middle. The red numbers on the internal nodes (≥ 0.5) indicate the local support values provided by the Shimodaira-Hasegawa (SH) test [25]. **B**, shows the stacked bar charts of the relative abundance at the class level of the prokaryotic communities from different sampling sites. C, The Shannon index of each sampling site suggests that M3 and M4 share similar and higher species diversity and evenness as compared to M2. **D**, Double principal coordinate analysis (DPCoA), taking both phylogenetic and abundance data into account, reveals a strong clustering of different taxonomic groups by sampling regions (off- vs. on-plateau). **E** and **F**, Taxonomic profiling based on the direct assignment of metagenomic reads to phylogenetic categories (as defined under the NCBI taxonomy). E. The stacked bar charts of the relative abundance at the class level from different sampling sites estimated by mOTUs2 [35]. F, Statistics of the fold changes between taxon ratios across sampling sites. The taxon ratios measured the "relative abundance" of a taxonomic group to that of *Candidatus Pelagibacter*, instead of to the whole community. The fold change of taxon ratios, by crossing out potential multiplicative systematic biases [36], was used as an indicator of the relative fitness of a taxon as compared to the ubiquitous SAR11 population in contrasting environments (M2 vs. M3, or M2 vs. M4). The median value of the log fold change varied between 1.16 and 1.34, indicating approximately one-fold increasing in the relative fitness of the studied genera in comparison with *Candidatus Pelagibacter* in the on-plateau region. Genera with anomalously deviated fold changes could be quintessential representatives of specific environment, such as *Polaribacter*, *Formosa* and *Psychrobacter* (Supplementary Results). The blue and green colors are used to represent different comparison pairs. The shapes are used to distinguish tools. The size of each symbol is in proportion with the total number of reads assigned to the taxonomic group by all tools.

Supplementary Fig. 6 Taxonomic composition and abundance of KEGG Orthology 487 groups (KOs) in the SO metagenomes (A to D) and metatranscriptomes (E to J). In A to D, the 488 relative abundance of a taxonomic category i given a KO_i was measured: A, by the proportion 489 of genes, among all the protein-coding genes belonging to KO_i , assigned to taxonomy i, as 490 defined by the division of $\frac{f_{Counts}(gene \in Tax_i \cap KO_j)}{f_{Counts}(gene \in KO_i)}$; **B to D**, based on gene abundance, in the 491 492 form of FPKM (fragments per kilobase of transcript per million mapped reads) values, as defined by the division of $\frac{\sum_{gene \in Tax_i \cap KO_j} f_{FPKM}(gene)}{\sum_{gene \in KO_j} f_{FPKM}(gene)}$ in each metagenome. In **E** to **G**, the 493 absolute abundance of each KO group in each metagenome was estimated using the normalized 494 transcripts per liter according to the internal standards protocol [40], as defined by the sum of 495 $\sum_{gene \in KO_i} f_{Trascripts L^{-1}}(gene)$. The values of the total transcripts L^{-1} assigned to each KO 496 are shown in the middle of each cell. The colour code, from blue via yellow to red, represents 497 the total number of transcripts L⁻¹ assigned to each KO group from low to hight. In **H** to **J**, the 498 relative abundance of a taxonomic category i given a KO $_i$ was evaluated by the division of 499 $\frac{\sum_{gene \ \in Tax_i \cap KO_j} f_{Trascripts \ L^{-1}}(gene)}{\sum_{gene \ \in KO_j} f_{Trascripts \ L^{-1}}(gene)} \ \text{ in each metatranscriptome. The mean of the three replicates}$ 500 was used to represent their corresponding metatranscriptomic sample. The KO groups in all 501 panels are ordered decreasingly according to the value of transcripts L⁻¹ in M2 (as shown in **E**). 502 Only functional groups consisting of at least 10 genes, out of 3 003 587 protein-coding genes 503 504 predicted from the metagenome assemblies, were shown. 505 Supplementary Fig. 7 Abundance of functional groups in each sample and the relative 506 507 contribution of different taxonomic categories to each functional group were evaluated based on 508 the carbohydrate-active enzymes (CAZyme) [69]. The calculation and the order of panels 509 within each section are the same as shown in **Supplementary Fig. 6**. 510 511 Supplementary Fig. 8 Statistics of functional diversity (A) and shifts in taxonomic 512 composition within functional groups (B) across sampling sites, based on the community-level 513 metagenomic gene abundance represented by FPKM (fragments per kilobase of transcript per 514 million mapped reads) values (**B-D** in **Supplementary Fig. 6-7**). In **B**, the relative contribution (%) of a specific taxonomic category (e.g., Gammaproteobacteria) to a functional group (e.g., 515 516 ferrous iron transporter FeoA) in each sampling station was calculated (Materials and methods).

Shifts in the relative contribution across stations were estimated using the ratio of the relative

contribution in M2 to that in M3 (or M4) and visualized by violin plots. A ratio value less than 1

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519 indicates that the taxonomic category accounts for a larger share of the genes of a functional group in the off-plateau HNLC waters, and vice versa. Multiple databases were considered, 520 including CAZy, FeGenie, KEGG, Pfam, Superfamily and TCDB. Five dominant taxonomic 521 groups in gene pool and transcript inventories across all sampling sites were shown 522 (Supplementary Fig. 6-7). Colour code is the same as Supplementary Fig. 6-7. Only 523 524 functional groups consisting of at least 50 genes, out of the 3 003 587 protein-coding genes predicted from the metagenome assemblies, were used in the calculation. 525 526 Supplementary Fig. 9 A, An exemplary illustration of the influence of genome abundance 527 on the assessment of gene expression levels, showing that fluctuations in transcript abundance 528 529 could be a result of shifting genome copies rather than changes in expression levels. **B**, The pipeline to generate the metagenome-normalized metatranscriptomic pseudo-read count table. 530 All the analyses were performed under R environment (v3.6.1). Initially, two matrices of read 531 counts of genes were generated using featureCounts (v2.0.0) [70]. Each matrix contains 3 003 532 586 rows, representing the total number of protein-coding genes predicted in our 949,228 533 assembled contigs (≥ 1 kbp). The metagenomic matrix consists of 3 columns representing 3 534 sampling sites M2, M3 and M4. The metatranscriptomic matrix has 9 columns corresponding to 535 3 biological replicates × 3 sampling sites. Secondly, genes with low read counts were filtered 536 out. Only genes whose FPM (fragments per million mapped fragments) value is no less than 1 537 in at least 1 sample, which must be satisfied in both metagenomic and metatranscriptomic data, 538 were remained. Variance stabilizing transformation (VST) was carried out on both matrices. 539 Given that $Abundance_{transcript} \sim Abundance_{gene} \times Expression Level (EL_{gene})$, we used 540 the ratio between transcript abundance and gene abundance as approximations of gene 541 expression levels $(EL_{gene} \sim \frac{Abundance_{transcript}}{Abundance_{gene}})$. Here, $Abundance_{transcripts}$ represents the 542 metatranscriptomic read counts of a gene in a sample, and Abundance_{gene} equals to the 543 metagenomic read counts of a gene in a sample. Since the transformed data was on log2 scale, 544 the formula could also be written as $EL_{gene} \sim 2^{VST(Abundance_{transcript}) - VST(Abundance_{gene})}$. Then, 545 in each sample (each column), EL_{gene} values are normalized (divided) by the corresponding 546 sample (column) maximum. Finally, we converted the normalized ratios to pseudo counts by 547 multiplying with 10⁶ and rounding to the nearest integer. C. Heatmap of the sample-to-sample 548 distance, and **D**, Principal component analysis (PCA) based on the normalized read counts after 549 variance stabilizing transformation by DESeq2. 550

Supplementary Fig. 10 Counts of significantly differentially expressed genes (SDEGs) in 552 CAZymes [69] across contrasting oceanic regions (on- and off-plateau). Each row represents 553 one functional group. For CAZymes, enzyme families are further clustered based on their 554 functions, including the breakdown, biosynthesis and modification of carbohydrates and 555 glycoconjugate (e.g., glycoside hydrolases (GHs) hydrolyse the glycosidic bonds between 556 carbohydrates). The three vertical panels show: (1st) The comparison of SDEGs obtained with 557 vs. without the metagenome-based normalization. The black segment of each bar represents the 558 number of SDEGs identified by both methods; the grey segment displays the SDEGs only 559 found based on the metagenome-normalized metatranscriptomic pseudo counts (with 560 normalization); the white segment shows the SDEGs only reported without the normalization 561 procedure. (2nd) The SDEGs based on the metagenome-normalized metatranscriptomic pseudo 562 counts. (3rd) The log2-based fold change values of SDEGs involved in the 2nd panel. In the 563 bi-direction bar plots, the bars pointing to the left indicate the number of genes that are 564 significantly higher expressed in the on-plateau iron-fertilized M2 site, as compared to the 565 off-plateau HNLC M3 and M4 sites. To the contrary, the bars pointing to the right represent 566 genes that are significantly higher expressed in the off-plateau HNLC waters. The colour 567 scheme of taxonomy is shown on top. 568 569 Supplementary Fig. 11 Counts of significantly differentially expressed genes (SDEGs) in 570 571 KEGG Orthology groups (KOs) [71] across contrasting oceanic regions (on- and off-plateau). We built a list of KOs relevant to iron uptake and metabolism, the tricarboxylic acid (TCA) 572 cycle, the Embden Meyerhof-Parnas (EMP) pathway, proposed C1 and methylated compound 573 574 oxidation pathways, and etc (Supplementary Table 4). The vertical panel arrangement and colour schemes are the same as shown in **Supplementary Fig. 10**. Several KOs that are 575 involved in multiple pathways are displayed multiple times. 576 577 Supplementary Fig. 12 The distribution of significantly differentially expressed genes in 578 the 133 MAGs among diverse functional categories related to iron uptake and carbon 579 metabolism. From left to right, the panels represent the phylogenetic tree (the same as shown in 580 Fig. 1), the KOs involved in the EMP glycolysis/gluconeogenesis, C1 and methylated 581 582 compound oxidation pathways, oxidative phosphorylation, nitrogen metabolism, transcription factors, ABC transporters, sulfur metabolism, Sec (secretion) system, type II secretion system, 583

type IV secretion system and the two-component system. The symbol and colour schemes are

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the same as shown in **Fig. 5**.

586 Supplementary Fig. 13 Polysaccharide utilization loci (PULs) identified in MAG 78 (A), 587 134 (B), 3 (C) and 73 (D), as well as fucose utilization loci found in MAG 58, 98, 6 and 17 (E). 588 Arrows represent genes and direction of arrows illustrates their strand locations. The colour 589 scheme is: "orange" represents genes that are significantly higher expressed in the on-plateau 590 591 iron-fertilized water (M2 site); "yellow" shows genes that are higher, but not significantly, expressed in the on-plateau site; "blue" indicates genes that are significantly higher expressed 592 in the off-plateau HNLC waters (M3 and M4 sites); "cyan" are genes that are higher, but not 593 significantly, expressed in the HNLC waters. Genes encoding the fucose metabolic pathway of 594 Lentimonas sp. CC4 [42] is used as the reference to arrange their counterparts identified in 595 596 MAGs from the Verrucomicrobiae class (under the GTDB taxonomy system [53]). Light blue shades between genes are used to indicate orthologous groups and facilitate visualization, 597 because the incompleteness of MAGs leads to the fragmented distribution of genes among 598 contigs. 599 600 Supplementary Fig. S14 (A) A gene cluster potentially engaged in using light as a 601 complementary energy resource was identified exclusively in the UBA4421 genus. A gene 602 encoding bacteriorhodopsin was also detected in one of the UBA9926 genome 603 (GCA 002728935), but genes adjacent to it do not form similar light-harvesting gene clusters 604 as found in the UBA4421 genus. Light blue shades between genes are used to indicate 605 orthologous genes. The colour scheme is: "orange" represents genes that are significantly 606 higher expressed in the on-plateau iron-fertilized water (M2 site); "yellow" shows genes that 607 are higher, but not significantly, expressed in the on-plateau site; "white" are genes from 608 reference genomes. (B) Bacteriorhodopsins from different genera are well separated on the 609 phylogeny tree with high support values, indicating distinct origins of the rhodopsin genes. The 610 611 emergence of the accessory functions of light harvesting might be a result of adaptive evolution. (C) Sequence alignment of transmembrane peptide segment of bacteriorhodopsin sequence of 612 MAG_103 and its top 20 matches in the MicRhoDE database [43], highlighting variation in 613 residues involved in spectral tuning and ion pumping. 614

Supplementary Data

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Sequences of the two internal standards add to RNA libraries:

>MTST51006NT GGGUUCGGUGGUCUAUACUACCUAAGUUGGAUGUACUGGUGGAAGUGCUACCAACACAGUAAUGCUGGUAUAGGUAG GCAACACUACGACUUCAGGAAGAGUCUAACGAAUGUAUGCAUAAUACUAAUGCCUUACAUGUGGAAGCACCCUAUAACGG AUGGAAAAUCAACCUGACGAAAGGAUACUAGCUCAAAUGACGAUAACGGACAGUGACUGGCAACCUGAAGAAUGGUACAAGAAGAGCACCCUGGUGAAAAUGACGUAAUAAGGUGCGUAUACAUAGGUAUAGAAAAUCUAGUAAAUGCUACGUGCC $\tt CUGACAUGGAAGACUACUACGCUAUGACGGGUAAUAAGCCUCUACUAGAACUAAAUAGUAUAGGUCCUUGCACGCAAUGC$ ACGGUACACAAGCUAGAAGGUGUACACUGCAUAUGGUGGAUAGUAAGGAGGGACCACUUCCCUGUACCUAUAAUACAAAUAGUAGACGUAUUCAAUCUAUACAAUUUCGCUAGUGGUACGGUACUAUGCAUACAACACGCUGCUCACCCUUGGGGUGACU GGAUGUUCGACGUACAAUACGAAAGUUGCAGGAUGUACAGGUGGUGGAUGACGAGGAAUGACUGGAGUGGUCCUAAUAAG AGGAGGACGUUCGACUACCCUGACCCUACGGCUUGCGGUCUAGUUU >MTST61006NT ${\tt GGGAAUCUAAUAACGGAACCUAUAUGGUACGUAAGUAUGAGUAGUAGUGGUUUCGGUGUAAAUUUCAGGGACCAACAAGGUGC}$ UAUAGACGUAAGGCUAGCUUGCCUACAAAUAGACCCUAAGGUAUGCCACCAAAGGGGUAGUAAUAGUUACUGGUGGAAUU UGAGGGAAUGCAUGGAAAGUGACCCUCACCAAGACAAUGUAAAGUGGAUAAUAUUCCUAAAGGUAAAUGUAAUAUUCUUC AUGUGGGACAUAUACGUAAGGGCUGAAUGCAAUCUAACGCAACCUUUCAUGUUCUGGAUGAAGCAAGGUUUCAGGUUCCU AAGUUUCACGCACGAAUUCAAUCACGACACGCUACACUGGUACCCAACUAGGUAGUGCUCCUAAUGUAGCUGGUG $\hbox{\tt GUUGCAGGACGAAGAGUUGCCAAACGGACUACAAUUUCAAUCACCCUGACGUAAAUUACCCUAAUCAAUGCGACUUCUGC}$ GGAGUCACCUAUACGAAUACAGUGUAUGCAAUAGGGAAAGGUACACGCUAGAAAUGGUACCUACGGCUCAACCUCACAUA AGGAUAGCUCACAUAAAUUGGGUAUUCGACUUCAGGGUACACGUUU

645 **Supplementary Code Availability**

- All software used in this study are listed below:
- 647 BBTools: https://jgi.doe.gov/data-and-tools/bbtools; Biostrings:
- 648 https://bioconductor.org/packages/release/bioc/html/Biostrings.html; BLAST:
- 649 ftp://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST; Bowtie2: https://sourceforge.net/projects/bowtie-bio/files/bowtie2;
- 650 Centrifuge: https://ccb.jhu.edu/software/centrifuge; CD-HIT: https://github.com/weizhongli/cdhit; CheckM:
- 651 https://ecogenomics.github.io/CheckM; CONCOCT: https://github.com/BinPro/CONCOCT; Circlize:
- 652 https://cran.r-project.org/web/packages/circlize; dbCAN2: http://bcb.unl.edu/dbCAN2/index.php; DADA2:
- 653 https://github.com/benjjneb/dada2; DESeq2: https://github.com/benjjneb/dada2; https://bioconductor.org/packages/release/bioc/html/DESeq2.html; https://bioconductor.org/packages/release/bioc/html/DESeq2.html; https://bioconductor.org/packages/release/bioc/html/DESeq2.html; https://bioconductor.org/packages/release/bioc/html/DESeq2.html; https://bioconductor.org/packages/release/bioc/html; https://bioconductor.org/packages/release/bioc/html; https://discourse.html;
- 654 http://www.diamondsearch.org/index.php; eggNOG-mapper: https://github.com/eggnogdb/eggnog-mapper; FastANI:
- 655 https://github.com/ParBLiSS/FastANI; FastQC: https://github.com/ParBLiSS/FastANI; FastQC: https://www.bioinformatics.babraham.ac.uk/projects/fastqc; FastTree:
- 656 http://www.microbesonline.org/fasttree; FeatureCounts: http://subread.sourceforge.net; FeGenie:
- 657 https://github.com/Arkadiy-Garber/FeGenie; genoPlotR: https://github.com/Arkadiy-Garber/FeGenie; genoPlotR: https://genoplotr.r-forge.r-project.org; GhostKOALA:
- 658 https://github.com/Ecogenomics/GTDBTk; HMMER: https://www.kegg.jp/ghostkoala; GTDB-Tk: https://github.com/Ecogenomics/GTDBTk; HMMER: https://hmmer.org; IQ-Tree:
- http://www.iqtree.org; Kaiju: http://kaiju.binf.ku.dk; KofamKOALA: https://www.genome.jp/tools/kofamkoala; Kraken2:
- 660 https://ccb.jhu.edu/software/kraken2; MAFFT: https://ccb.jhu.edu/software/kraken2; MAFFT: https://mafft.cbrc.jp/alignment/software; MATAM:
- 661 https://github.com/bonsai-team/matam; https://sourceforge.net/projects/maxbin2; MEGAHIT:
- 662 https://github.com/voutcn/megahit; MetaBAT2: https://bitbucket.org/berkeleylab/metabat; https://bitbucket.org/berkeleylab/metabat;
- 663 https://github.com/bxlab/metaWRAP; mOTUs2: https://motu-tool.org; mvoutlier:
- 664 https://cran.r-project.org/web/packages/myoutlier; Nonpareil: https://github.com/lmrodriguezr/nonpareil; OrthoFinder:
- 665 https://github.com/HRGV/phyloFlash; phyloFlash; phyloFlash;
- 666 https://bioconductor.org/packages/release/bioc/html/phyloseq.html; Prodigal; R: https://github.com/hyattpd/Prodigal; https://github.com/hyattpd/Prodigal; https://github.com/hyattpd/Prodigal; https://github.com/hyattpd/Prodigal; https://github.com/hyattpd/Prodigal; https://github.com/hyattpd/Prodigal; http
- 667 https://www.r-project.org; SAMTools: http://www.htslib.org; SortMeRNA: https://bioinfo.lifl.fr/RNA/sortmerna; Superfamily:
- 668 http://supfam.org/SUPERFAMILY; SSU-ALIGN: http://eddylab.org/software/ssu-align; trimAl: http://trimal.cgenomics.org;
- 77 Trimmomatic: http://www.usadellab.org/cms/?page=trimmomatic; Trim Galore:
- 670 https://www.bioinformatics.babraham.ac.uk/projects/trim_galore.

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