



**HAL**  
open science

## **Microbial Competition in the Subpolar Southern Ocean: An Fe–C Co-limitation Experiment**

Marion Fourquez, Matthieu Bressac, Stacy Deppeler, Michael Ellwood, Ingrid Obernosterer, Thomas W Trull, Philip W Boyd

► **To cite this version:**

Marion Fourquez, Matthieu Bressac, Stacy Deppeler, Michael Ellwood, Ingrid Obernosterer, et al.. Microbial Competition in the Subpolar Southern Ocean: An Fe–C Co-limitation Experiment. *Frontiers in Marine Science*, 2020, 6, 10.3389/fmars.2019.00776 . hal-03831171v1

**HAL Id: hal-03831171**

**<https://hal.science/hal-03831171v1>**

Submitted on 10 Dec 2020 (v1), last revised 26 Oct 2022 (v2)

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# 1 **Microbial Competition in the Subpolar Southern Ocean: an** 2 **Fe-C co-limitation experiment**

3  
4 Marion Fourquez<sup>1,2,3\*</sup> Matthieu Bressac<sup>2,4</sup>, Stacy Deppeler<sup>1,5</sup>, Michael Ellwood<sup>6</sup>,  
5 Ingrid Obernosterer<sup>7</sup>, Thomas W. Trull<sup>8,1,2</sup>, and Philip W. Boyd<sup>1,2</sup>

6 1 Antarctic Climate and Ecosystems CRC, University of Tasmania, Hobart, TAS, Australia

7 2 Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, TAS, Australia

8 3 Department F.-A. Forel for environmental and aquatic sciences, Earth and Environmental Sciences, University  
9 of Geneva, Geneva, Switzerland

10 4 Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, LOV, Villefranche-sur-Mer,  
11 France

12 5 National Institute of Water and Atmospheric Research (NIWA) Wellington, New Zealand

13 6 Research School of Earth Sciences, Australian National University, Canberra 2601, Australia

14 7 CNRS, Sorbonne Université, Laboratoire d'Océanographie Microbienne, LOMIC, F-66650 Banyuls/mer,  
15 France.

16 8 Climate Science Centre, Oceans and Atmosphere, Commonwealth Scientific and Industrial Research  
17 Organisation, Hobart, 7001, Australia

## 18 **Correspondence:**

19 Marion Fourquez

20 \* [marion.fourquez@gmail.com](mailto:marion.fourquez@gmail.com)

21 **Running title:** Carbon availability drives iron competition

22 **Keywords:** Iron, Carbon, Southern Ocean, Competition, Heterotrophic bacteria,  
23 Pico-nanoplankton, Fe uptake, Bacterial production.

## 25 **1. Abstract**

26 Iron (Fe) is a paradox in the modern ocean—it is central to many life-critical  
27 enzymes but is scarce across most surface waters. The high cellular demand and low  
28 bioavailability of Fe likely puts selective pressure on marine microorganisms.  
29 Previous observations suggest that heterotrophic bacteria are outcompeted by small  
30 diatoms for Fe supply in the subantarctic zone of Southern Ocean, thereby  
31 challenging the idea of heterotrophic bacteria being more competitive than  
32 phytoplankton in the access to this trace metal. To test this hypothesis, incubation  
33 experiments were carried out at the Southern Ocean Time Series site (Mar.–Apr.  
34 2016). We investigated (a) whether dissolved organic carbon (DOC), dissolved Fe,

1 or both limit the growth of heterotrophic bacteria and, (b) if the presence of potential  
2 competitors has consequences on the bacterial Fe acquisition. We observed a  
3 pronounced increase in both bulk and cell-specific bacterial production in response to  
4 single (+C) and combined (+Fe+C) additions, but no changes in these rates when  
5 only Fe was added (+Fe). Moreover, we found that +Fe+C additions promoted  
6 increases in cell-specific bacterial Fe uptake rates, and these increases were  
7 particularly pronounced (by 13-fold) when phytoplankton were excluded from the  
8 incubations. These results suggest that auto- and heterotrophs could compete for Fe  
9 when DOC limitation of bacterial growth is alleviated. These interactions between  
10 primary producers and nutrient-recyclers are unexpected drivers for the duration and  
11 magnitude of phytoplankton blooms in the Southern Ocean.

## 12 **2. Introduction**

13 Geological timescales have enabled microbes to develop adaptive solutions to an  
14 evolving environment, and iron (Fe) had a fundamental role in the metabolic  
15 pathways that emerged (Falkowski and de Vargas, 2004; Hunter and Boyd, 2007).  
16 As life appeared in an oxygen-free environment, the primordial ocean provided  
17 sufficient concentrations of readily available Fe (Hunter and Boyd, 2007). However,  
18 the concentration of dissolved Fe (DFe) decreased drastically after two major  
19 irreversible oxygenation events (~2.4 billion years and ~542 million years ago, Ilbert  
20 and Bonnefoy, 2013) and Fe is found at trace levels in most of today's ocean surface  
21 (<0.5nM, Johnson, 1997). Despite these changes, the Fe demand in marine  
22 microorganisms remained high. Nature retained this versatile metal, that can have a  
23 wide range of oxidation states, as an integral part for a wide range of proteins  
24 throughout evolution and many of these proteins are irreplaceable agents for vital  
25 cellular metabolic activities (oxygen transport, electron transport, DNA synthesis,  
26 etc., Morel and Price, 2003).

27 In phytoplankton, the photosynthetic transport chain is one of the most prominent Fe-  
28 dependent processes. One single copy of a photosystem requires 23-24 atoms of Fe,  
29 and overall 80% of Fe is allocated to the photosynthetic transport chain in a cell  
30 (Behrenfeld et al., 2006; Raven et al., 1999; Strzepek and Harrison, 2004). In  
31 heterotrophic bacteria the respiratory chain accumulates more than 90% of the  
32 intracellular Fe (Andrews et al., 2003; Tortell et al., 1999). Phytoplankton and  
33 bacteria play important roles in the ocean and have direct influence on global

1 biogeochemical cycles. Considering that phytoplankton drive ocean CO<sub>2</sub>  
2 sequestration via photosynthesis and downward export, while heterotrophic bacteria  
3 control much of the oceanic release of CO<sub>2</sub> via respiration, the outcome of a  
4 competition for Fe could influence the direction and magnitude of carbon fluxes in  
5 the upper ocean.

6 Despite widespread interest in microbial Fe requirements (Blain and Tagliabue,  
7 2016; Sarthou et al., 2005; Strzepek et al., 2019; Twining and Baines, 2013), there is  
8 no consensus regarding the minimum Fe requirements for phytoplankton or  
9 heterotrophic bacteria. There are two reasons to explain why this question has not  
10 been resolved. First, there have been few studies on the Fe requirements of  
11 heterotrophic bacteria compared with those for phytoplankton. Second, the wide  
12 range of Fe content relative to C biomass (Fe:C ratio) that exist for phytoplankton  
13 does not favor conclusive comparison (Blain and Tagliabue, 2016, and references  
14 herein). What is clear, however, is that DFe in the oceans is overwhelmingly  
15 complexed (99%, Rue and Bruland, 1997) by strong organic ligands with evidence of  
16 them containing Fe-binding functional groups consistent with biologically produced  
17 siderophores (Gledhill and Buck, 2012; Macrellis et al., 2001). Marine microbes  
18 have evolved different mechanisms to cope with the diversity of the Fe-binding  
19 ligand pool, and the capacity to acquire enough Fe for survival in a ‘highly diffusive’  
20 open ocean provides a competitive edge (Desai et al., 2012; Hopkinson and Barbeau,  
21 2012; Toulza et al., 2012). The capacity to produce siderophores is generally  
22 confined to heterotrophic bacteria (Armstrong et al., 2004), but the ability to take up  
23 siderophores may be more widespread than previously thought, and extend to the  
24 phytoplanktonic realm (Hogle et al., 2016; Kazamia et al., 2018; McQuaid et al.,  
25 2018). Recent studies have revealed that distinct siderophores and strategies are  
26 being employed by heterotrophic bacteria (Boiteau et al., 2016, 2019; Bundy et al.,  
27 2018; Debeljak et al., 2019). But to date, no eukaryotic phytoplankton have been  
28 found to produce or release siderophores. For this reason heterotrophic bacteria are  
29 commonly reported as highly efficient competitors, especially in severely Fe-limited  
30 environments (Braun and Killmann, 1999).

31 The Southern Ocean is the largest High Nutrient, Low Chlorophyll (HNLC) region in  
32 the world ocean, mainly because of Fe limitation. In the Southern Ocean, chlorophyll  
33 levels remain low year-round, but phytoplankton blooms occur in areas in the

1 vicinity of land masses (Blain et al., 2007). During austral spring 2011, the KEOPS2  
2 project aimed at exploring different phytoplankton blooms east of Kerguelen island.  
3 Over the course of the bloom, the release of dissolved organic carbon (DOC) derived  
4 from primary production increased the Fe demand of heterotrophic bacteria  
5 (Fourquez et al., 2015) which were Fe-limited (Obernosterer et al., 2015). The  
6 availability of a labile C source may have led to a higher bacterial Fe demand. These  
7 findings have led to the hypothesis that labile organic carbon exacerbated the  
8 potential competition between small-sized phytoplankton cells (pico- and  
9 nanophytoplankton) and heterotrophic bacteria for Fe (Fourquez et al., 2015). The  
10 present work aimed to test the above hypothesis. For this, our experimental design  
11 was based on the joint assumption that (1) heterotrophic bacteria are outcompeted for  
12 Fe by pico- and nanophytoplankton and (2) that DOC availability to heterotrophic  
13 bacteria influences the strength of this relationship.

### 14 **3. Materials and Methods**

#### 15 **3.1. Site description**

16 This study was carried out as part of the V02-IN2016 voyage of the R.V. Investigator  
17 (March 11 to April 17, 2016). During the expedition, we visited on two occasions  
18 (March 19 and 29) the Southern Ocean Time Series (SOTS, 47°S, 142°E) site that is  
19 located within a low current region in the subantarctic Zone (SAZ) north of the  
20 subantarctic Front (SAF) that marks the northern edge of the Antarctic Circumpolar  
21 Current (Figure 1). This area represents a large portion of the total area of the  
22 Southern Ocean that serves as a strong sink for atmospheric CO<sub>2</sub>. Conditions at  
23 SOTS are typical and representative of the Indian sector SAZ, from ~90 to 145°E.  
24 The absence of Fe is regarded as the primary cause that restricts primary production  
25 and constrains the biological pump in the area (Cassar et al., 2011; Sedwick et al.,  
26 1997; Trull et al., 2019). The relief of the Fe limitation can occur by aerosol Fe  
27 supply in summer in the region that differs in this way from mechanisms of deep  
28 mixing and/or sediment input/resuspension that enhances Fe concentrations in  
29 surface waters at the vicinity of subantarctic islands such as Kerguelen (Blain et al.,  
30 2008; Rembauville et al., 2015).

### 3.2. Experimental strategy

The study involved two separate sets of incubation experiments: Experiment 1 and 2 (Figure 2 and Suppl. Material Figure 1). Because there is a potentially confounding influence of Fe and C limitation on bacterial processes, the objective of Experiment 1 was to first determine whether Fe, C, or both are limiting or co-limiting factors at SOTS while the objective of Experiment 2 was to determine whether the presence of larger cells (especially pico- and nanoplankton) influences bacterial activities. In addition to the one control that consisted in unamended nutrient seawater (no addition), the following 3 treatments were prepared as triplicates: +Fe, +C, and +Fe+C. Iron (+Fe) was added as  $\text{FeCl}_3$  (final concentration of  $\text{FeCl}_3$  1 nM), and carbon (+C) was added as trace-metal clean glucose (final concentration of glucose  $10 \mu\text{mol L}^{-1}$ ). Hence, the addition was  $16.6 \mu\text{molFe molC}^{-1}$  to attain the bacterial Fe quota observed in Fe-replete bacterial cultures ( $16.1 \pm 2.3 \mu\text{molFe molC}^{-1}$ , Fourquez et al. 2014). For experiment 1, incubations were performed directly on unfiltered seawater (bacteria were incubated with micro and pico-and nanoplankton communities). Additional incubations were performed on  $20\mu\text{m}$ -prefiltered seawater ( $<20\mu\text{m}$  condition), and on  $1\mu\text{m}$ -prefiltered seawater ( $<1\mu\text{m}$  condition) for Experiment 2. We use the term “condition” throughout the manuscript to refer to the different size fractionation treatments (summary in Table 1). The biological response of heterotrophic bacteria was monitored from sub-samples drawn from these incubation bottles, and analysed for several parameters as described in sections below.

### 3.3. Sampling procedures

Seawater was pumped from the surface ocean ( $\sim 5\text{m}$  depth) using a trace metal clean towed fish sampler. Samples were collected directly into a trace metal clean laboratory (clean room, ISO5) where 300 mL of seawater was dispensed into 500 mL acid-washed polycarbonate (PC) bottles under a laminar flow hood (ISO class 5). Overall, 12 (for experiment 1) and 54 (for experiment 2) independent replicates were prepared in bottles capped and sealed with Parafilm. All plastic materials used were acid-washed following GEOTRACES procedures in our home laboratory (GEOTRACES cookbook, (Cutter et al., 2017)). Briefly, PC bottles were soaked for one week in the alkaline detergent Decon 90, then rinsed four times with deionized water and three times with milliQ water. They were subsequently filled with 10%

1 hydrochloric acid (Suprapur, Merck) for one week. After that time, bottles were  
2 rinsed 5 times with highly purified water. The PC bottles were dried, and UV  
3 sterilized for 15-30 minutes under a laminar flow hood and then stored in triple  
4 plastic bag before being used.

5 To minimize risks of potential contamination of samples with metals or dissolved  
6 organic matter as an artefact of filtration in preparation for Experiment 2, seawater  
7 was filtered at very low pressure (<5 Hg). All incubations were performed at *in situ*  
8 temperature (13.5°C). Bottles were placed in a water bath within a controlled  
9 temperature room (13.5°C) to avoid temperature fluctuations. For experiment 1,  
10 incubations were performed in total darkness. For experiment 2, incubations were  
11 conducted under 12:12 light-dark condition, and we employed neutral density  
12 screens to attenuate the light intensity. Low levels of light can reduce rates of  
13 photosynthesis and the release of DOC associated and may alter Fe uptake rates in  
14 photosynthetic cells. Therefore, we opted to use low light intensity (average 4.5  
15  $\mu\text{mol photon m}^{-2} \text{ s}^{-1}$ , ~1W) in experiment 2 to (a) increase potential competition for  
16 Fe stocks between autotrophic pico- and nanoplankton cells and heterotrophic  
17 bacteria, and to (b) avoid stimulation of large phytoplankton growth and subsequent  
18 organic enrichment artefacts. For subsampling, incubation bottles were transferred to  
19 the clean container and opened under a laminar flow hood (ISO class 5). Subsamples  
20 from each triplicate for bacterial abundance and production were taken at T0, T1  
21 (+24h), T2 (+48h), T3 (+72h) and at T4 (+110h) for Experiment 1 and at T0, T1  
22 (+36h), and T2 (+120h) for Experiment 2. The time points for experiment 2 were  
23 chosen following results collected during Experiment 1. For both experiments  
24 bacterial heterotrophic production, cell abundance, and Fe uptake were measured at  
25 several time points. We also measured heterotrophic bacterial respiration at the end  
26 of the incubation in Experiment 1 to estimate the bacterial growth efficiency in the  
27 different treatments (Figure 2). Methods for each parameter measured are detailed in  
28 sections below.

### 29 **3.4. Cell abundance**

30 For each biological replicate, 4.5 mL subsamples were fixed with glutaraldehyde  
31 (0.5% final concentration), kept in the dark at 4°C for 20 min and then shock-frozen  
32 in liquid nitrogen. The samples were stored at -80°C until analyses by flow  
33 cytometry. Flow cytometry was performed following the protocols in Marie et al.,

1 (1997 and 2005). Frozen samples were rapidly thawed in a water bath at 70 °C for 3  
2 min and aliquots taken for autotrophic and/or prokaryote cell counts. Sample aliquots  
3 were kept on ice in the dark and promptly analysed on a Becton Dickinson FACScan  
4 flow cytometer fitted with a 488 nm laser. MilliQ water was used as sheath fluid for  
5 all analyses. Before and after each run, samples were weighed to  $\pm 0.0001$  g to  
6 determine the volume of sample analysed.

7 Samples for autotrophic cell abundance were prepared by aliquoting 998  $\mu\text{L}$  of  
8 sample into a clean 5 mL polycarbonate tube, with 2  $\mu\text{L}$  of PeakFlow Green 2.5  $\mu\text{L}$   
9 beads (Invitrogen) added as an internal fluorescence and size standard. Each sample  
10 was run for 5 min at a high flow rate of  $\sim 40 \mu\text{L min}^{-1}$ . Autotrophic cell populations  
11 were separated into regions based on their chlorophyll autofluorescence in red (FL3)  
12 versus orange (FL2) bivariate scatter plots. *Synechococcus* cells were determined  
13 from their high FL2 and low FL3 fluorescence. Pico- and nano-phytoplankton  
14 communities were determined from their relative cell size in side scatter (SSC)  
15 versus FL3 fluorescence bivariate scatter plots. Final cell counts in cells  $\text{L}^{-1}$  were  
16 calculated from event counts in the identified regions and analyzed volume.

17 Samples for prokaryote cell abundance were prepared by aliquoting 995  $\mu\text{L}$  of  
18 sample to a clean 5 mL polycarbonate tube. Samples with high prokaryote cell  
19 counts were diluted to 1:10 with 0.2  $\mu\text{m}$  filtered seawater (FSW) to remove  
20 underestimation of cell concentration from coincidence (100  $\mu\text{L}$  sample in 900  $\mu\text{L}$   
21 FSW). Cells were stained for 20 min with 5  $\mu\text{L}$  of SYBR Green I (Invitrogen) at a  
22 final dilution of 1:10,000. An additional 2  $\mu\text{L}$  of PeakFlow Green 2.5  $\mu\text{L}$  beads  
23 (Invitrogen) was added to the sample as an internal fluorescence and size standard.  
24 Each sample was run at a low flow rate of  $\sim 12 \mu\text{L min}^{-1}$  for 3 min and prokaryote cell  
25 abundance was determined from bivariate scatter plots of SSC versus green (FL1)  
26 fluorescence. Final cell counts in cells  $\text{L}^{-1}$  were calculated from event counts in the  
27 identified regions and analyzed volume.

### 28 **3.5. Heterotrophic bacterial production**

29 Bacterial production was estimated by [ $^3\text{H}$ ] leucine incorporation applying the  
30 centrifugation method (Martinez et al., 1996) as described in Obernosterer et al.,  
31 (2008). Briefly, 1.5-mL samples were incubated with a mixture of radioactive  
32 leucine, L-[3,4,5- $^3\text{H}$ (N)] (PerkinElmer, specific activity 123.8  $\text{mCi}\cdot\text{mol}^{-1}$ ) and



1 nonradioactive leucine at final concentrations of 20 nM. Duplicates plus one “killed  
2 sample” were incubated in the dark at the respective *in situ* temperatures for 2–3 h.  
3 Linearity of leucine incorporation over this time period was tested in parallel and at  
4 two occasions (at the beginning of Experiment 1 and 2). Incubations were terminated  
5 by the addition of trichloroacetic acid (TCA; Sigma) to a final concentration of 5%.  
6 To facilitate the precipitation of proteins, bovine serum albumin (BSA; Sigma, 100  
7 mg L<sup>-1</sup>, final concentration) was added prior to centrifugation at 16,000 g for 10 min  
8 (Van Wambeke et al., 2002). After discarding the supernatant, 1.5 mL of 5% TCA  
9 solution was added and the samples were subsequently vigorously shaken on a  
10 vortex and centrifuged again. The supernatant was discarded again and 1.5 mL of  
11 UltimaGold™ uLLt (PerkinElmer) was finally added. The radioactivity incorporated  
12 into bacterial cells was counted in Hidex 300SL Liquid Scintillation Counter. A  
13 factor of 1.55 kg C mol leucine<sup>-1</sup> was used to convert the incorporation of leucine to  
14 carbon equivalents, assuming no isotope dilution (Kirchman et al., 1993). Isotopic  
15 dilution ranged from 1.0 to 1.3 as determined on three occasions using a kinetic  
16 approach.

### 17 **3.6. Fe uptake rates**

18 Following the subsampling for bacterial production, Fe uptake experiments were  
19 initiated by adding 0.2 nmol L<sup>-1</sup> at final concentration of <sup>55</sup>Fe (as <sup>55</sup>FeCl<sub>3</sub>,  
20 PerkinElmer specific activity 2.46 x 10<sup>3</sup> Ci mol<sup>-1</sup>) after 36h and 120h of incubations  
21 (independent replicates). After a 24-hour incubation with <sup>55</sup>Fe, microorganisms were  
22 filtered through a stack of nitrocellulose filters (Whatman) of 0.2 and 0.8µm  
23 porosity, separated with 20µm mesh filters. These filter porosities were chosen to  
24 separate phytoplankton (including *Synechococcus*, >0.8µm) from heterotrophic  
25 bacteria (0.2–0.8µm). Before running dry, the filters were rinsed with 0.2-µm filtered  
26 Ti(III) citrate EDTA solution (Tovar-Sanchez et al., 2003) for 2 minutes to dissolve  
27 any extracellular Fe, followed by three consecutive rinses with 5 mL of 0.2µm  
28 filtered seawater for 1 min (Fourquez et al., 2012, 2015). The filters were placed into  
29 plastic vials and 10 mL of the scintillation cocktail Filtercount (Perkin Elmer) was  
30 finally added. Vials were agitated for 24h before the radioactivity was counted with  
31 the Hidex 300SL scintillation counter. Radioactivity on filters was corrected for  
32 background (<sup>55</sup>Fe adsorbed into the filter and/or onto particles and not being  
33 efficiently washed out by the washing solution) using <sup>55</sup>Fe-radiotracer medium with

1 dead cells, also called “killed control”. Killed controls were treated the same as above  
2 but microorganisms were fixed with 1% of glutaraldehyde (left for 1 hours at 4°C)  
3 prior addition of  $^{55}\text{Fe}$ . DFe concentration in each incubation bottle was also assessed  
4 prior incubation with  $^{55}\text{Fe}$ . Subsamples (~40 mL) were measured by flow injection  
5 with online preconcentration and chemiluminescence detection (adapted from Obata  
6 et al., 1993). An internal acidified seawater standard was measured every day in  
7 order to control the stability of the analysis. The detection limit was  $40 \text{ pmol kg}^{-1}$  and  
8 the accuracy of the method was controlled by analyzing the SAFe S ( $0.110 \pm 0.036$   
9  $\text{nmol kg}^{-1}$  ( $n = 3$ ); consensus value  $0.093 \pm 0.008 \text{ nmol kg}^{-1}$ ), and SAFe D1 ( $0.66 \pm$   
10  $0.06 \text{ nmol kg}^{-1}$  ( $n = 4$ ); consensus value  $0.67 \pm 0.04 \text{ nmol kg}^{-1}$ ) seawater standards.  
11 DFe concentration were employed to correct  $^{55}\text{Fe}$  uptake estimates from cold DFe  
12 present in incubation bottle at the time of the measurement. In incubation bottles, the  
13 DFe concentration changed over time also suggesting that there was remineralization  
14 taking place. To correct for this, we have multiplied the results from these  
15 experiments by the calculated proportion of  $^{55}\text{Fe}$  from the total DFe. Calculation  
16 details can be found in Fourquez et al. (2015).

### 17 **3.7. Bacterial respiration**

18 Rates of respiration were determined from dissolved oxygen consumption in 24  
19 hours dark incubations at the end (T4, +110h) of Experiment 1 using Winkler  
20 titration method. In order to keep the bacteria only for the measurement, all samples  
21 were carefully pre-filtered onto  $0.8\mu\text{m}$  acid-washed PC filter. Two out of the three  
22 biological replicates belonging to each treatment (control, +Fe, +C, and +Fe+C) were  
23 used for measurement. Last replicate was employed as a T0 by adding manganese  
24 chloride followed by alkaline iodide prior incubation. To estimate the consumption  
25 in dioxygen ( $\text{O}_2$ ), the amount of  $\text{O}_2$  measured in bottles after 24 hours of incubation  
26 was subtracted from T0 measurement. All incubation bottles were opened and gently  
27 shaken under flow laminar hood to optimize  $\text{O}_2$  level inside and homogeneity  
28 between treatments. After these steps, the samples were subsequently transferred into  
29 cleaned and acid-washed glass biological oxygen demand stoppered bottle.  
30 Incubations lasted for 24 hours in the dark in a temperature-controlled incubator set  
31 at  $13.5^\circ\text{C}$  (*in situ* temperature). At the end of the incubation time, subsampling (1.8  
32 mL) for flow cytometry analysis was taken quickly just prior to add the reactive.  
33 Dissolved oxygen concentration was measured based on the whole-bottle modified

1 Winkler titration of Carpenter (1965) plus modifications by Culberson (1991).  
2 Bacterial respiration rates were normalized to C biomass by considering the bacterial  
3 cell abundance in each incubation bottle.

### 4 **3.8. Carbon biomass and conversion factor**

5 Direct carbon contents for pico-nanoplankton and microplankton were estimated  
6 from particulate organic carbon (POC) measurements. In total, 3 L of seawater  
7 sample were first filtered through 20 $\mu$ m and subsequently passed through 1.2 $\mu$ m  
8 (diameter 25mm) Sterlitech silver membrane filters, and dried at 60 °C. The samples  
9 were acidified, dried and analyzed by high-temperature combustion (1000°C) to  
10 determine POC. The analysis for total nitrogen, carbon and hydrogen was determined  
11 using a Thermo Finnigan EA 1112 Series Flash Elemental Analyzer.

12 In parallel, the carbon content was also indirectly estimated using conversion factors.  
13 Photosynthetic pico-nanoplankton cell abundance was converted to carbon biomass  
14 using constant cell-to-carbon conversion factors based on the literature. Conversion  
15 factors used were respectively 255 and 2590 fgC cell<sup>-1</sup> for the cyanobacterium  
16 *Synechococcus* and for picoeukaryotes (Buitenhuis et al., 2012), and 183 fgC cell<sup>-1</sup>  
17 for nanoeukaryotes (Caron et al., 1994, 2017). We assume the cyanobacterium  
18 *Synechococcus* represented the majority of the resident cyanobacteria. For  
19 heterotrophic bacteria, the carbon content was estimated using 12.4 fgC cell<sup>-1</sup> as  
20 reported by Fukuda et al. (1998).

### 21 **3.9. Statistical analyses**

22 All statistical comparisons were performed using one-way analysis of variance  
23 (ANOVA) and a post hoc Tukey test. Differences were considered statistically  
24 significant at  $p < 0.05$ . To evaluate the differences between treatments, statistics were  
25 individually performed between nutrient unamended (control) and amended  
26 treatments (+Fe, +C, and +Fe+C). We also evaluated statistical differences between  
27 conditions by comparing unfiltered (control) to other conditions (<20 $\mu$ m or <1 $\mu$ m).

## 28 **4. Results**

### 29 **4.1. Environmental settings of the study site**

30 As is typical for HNLC regions, at SOTS site the C biomass was dominated by small  
31 cells (<20 $\mu$ m) which represented 1.51  $\mu$ mol L<sup>-1</sup> of the 1.90  $\mu$ mol L<sup>-1</sup> total POC in

1 surface waters. The concentration of DFe was  $0.081 \pm 0.02$  nM at 5m depth.  
2 Concentration of dissolved organic carbon (DOC) were not measured during the  
3 cruise. Nevertheless, the Southern Ocean surface waters exhibit a DOC concentration  
4 range of  $\sim 40\text{-}50$   $\mu\text{molC L}^{-1}$  (Hansell et al., 2009). If we consider the upper range of  
5  $50$   $\mu\text{molC L}^{-1}$  to be representative of what can be found at SOTS, the resulting  
6 DFe:DOC molar ratio was  $\sim 1.62$   $\mu\text{mol mol}^{-1}$ .

## 7 **4.2. Experiment 1. Responses of bacteria to Fe and C additions**

### 8 **4.2.1. Cell-specific bacterial production**

9 In Experiment 1, we investigated the responses of bacteria to Fe, C and concomitant  
10 Fe and C additions. Sole additions of Fe did not result in any significant  
11 enhancement of bulk nor cell-specific BP (Figure 3). In accordance with these  
12 results, bacterial abundance in the Fe-amended treatment did not differ from the  
13 control (Figure 3a). However, single (+C) and combined (+Fe+C) additions of C  
14 significantly stimulated bulk and cell-specific BP. We note a pronounced response in  
15 cell-specific BP to single (+C) and combined (+Fe+C) additions of carbon over the  
16 time of the experiment (1.3–56-fold and 1.6–26-fold higher than control in +C and  
17 +Fe+C, respectively). The results from treatments +Fe+C and +C were not  
18 statistically different from each other apart from T3 (+72h,  $p=0.012$ ). The bacterial  
19 cell abundance also increased, however, the magnitude of the stimulation was less  
20 than that measured for the BP. Cell abundance is a complex function between  
21 growth and mortality rates; this decoupling is therefore not surprising. At the end of  
22 the experiment, the enhancement of these parameters was still detectable in the +C  
23 and +Fe+C treatments but a decrease in cell-specific BP was also observed after 72  
24 hours of incubation.

### 25 **4.2.2. Bacterial respiration and growth efficiency**

26 Bacterial respiration (BR) rates were measured at the end of the Experiment 1. BR  
27 varied from  $0.39 \pm 0.15$  and  $1.63 \pm 0.34$   $\text{fmolO}_2 \text{ cell}^{-1} \text{ d}^{-1}$  (standard error, SE;  $n=2$ ). It  
28 was intriguing to note that the highest BR rate was measured for +Fe addition alone,  
29 which shows also the lowest (3.2%) bacterial growth efficiency (BGE) due to low  
30 BP rate (table 2). The highest BGE estimation was measured for the +Fe+C  
31 treatment with 57% (Table 2).

### 1        **4.3. Experiment 2. Responses of bacteria when phytoplankton is absent or** 2        **present**

3        To make the Results section concise and easy to follow, all results presented in  
4        figures and text description correspond to the first time point for Experiment 2 (+36h  
5        of incubation). Similar conclusions can be formulated from the second time point  
6        (+120h of incubation) and data are accessible in the supplementary materials.

#### 7        **4.3.1. Bacterial production in presence and absence of phytoplankton**

8        Cell-specific BP rates across all treatments and conditions are shown in Figure 4a. In  
9        the control treatment (no nutrient addition), the cell-specific BP ranged from 4 to 9  
10        ( $\times 100 \text{ fmolC cell}^{-1} \text{ d}^{-1}$ ) and no significant difference was found between unfiltered  
11        and  $<20\mu\text{m}$  nor  $<1\mu\text{m}$  conditions, which is evidence that the presence of  
12        phytoplankton did not affect cell-specific BP. However, in the nutrient amended  
13        treatments two differences were significant: a decrease in BP in the +Fe treatment  
14        and an increase in the +Fe+C treatment, respectively. In the +Fe treatment, the cell-  
15        specific BP was highest when the whole community was present (unfiltered) and the  
16        lowest when bacteria were incubated solely with pico- and nanoplankton ( $<20\mu\text{m}$ ).  
17        The difference between these two conditions was significant ( $p=0.04$ , Figure 4a).  
18        Cell-specific BP in +Fe+C treatment were higher than all other treatments, ranged  
19        from 19 to 48 ( $\times 100 \text{ fmolC cell}^{-1} \text{ d}^{-1}$ ) and was the highest when bacteria were  
20        incubated solo. Cell-specific BP was about 2 times higher in  $<1\mu\text{m}$  compared to  
21        unfiltered condition, and this difference was highly significant ( $p=0.005$ , Figure 4a).

22        Significant differences were also found when comparing  $<20\mu\text{m}$  and  $<1\mu\text{m}$   
23        conditions in +Fe and +Fe+C treatments. Indeed, the  $<1\mu\text{m}$  condition showed  
24        significantly higher rates compared to the  $<20\mu\text{m}$  condition with respectively  $9\pm 1$   
25        versus  $4\pm 0.4$  and  $48\pm 6$  versus  $19\pm 1$  ( $\times 100 \text{ fmolC cell}^{-1} \text{ d}^{-1}$ ) for +Fe and +Fe+C  
26        treatment. Overall, cell-specific BP was negatively affected by the presence of pico-  
27        and nanoplankton cells in the +Fe and +Fe+C treatments while no effect was found  
28        in the control (no addition) treatment.

#### 29        **4.3.2. Microbial Fe uptake**

30        To investigate whether heterotrophic bacteria compete for Fe with other members of  
31        the microbial community, the bacterial Fe uptake rates were determined for

1 incubations where microplankton (<20 $\mu$ m condition) or both micro- and pico- and  
2 nanoplankton (<1 $\mu$ m condition) were excluded from incubation. Results were  
3 compared to the treatment where all the members of the microbial community were  
4 present (unfiltered condition). During Experiment 2, we also compared the  
5 contribution of two size-fractions to Fe uptake. Figure 4b shows results for bacteria  
6 on a cell-specific basis, and Figure 5 combines data for phytoplankton (<0.8 $\mu$ m) and  
7 bacteria (0.2-0.8 $\mu$ m) on the volumetric basis for better comparison. Data used to  
8 create figure 5 can also be found in detail in Table 3.

### 9 **4.3.3. Cell-specific Fe uptake by bacteria**

10 The response of bacteria to size-fractionation (condition) and nutrients amendments  
11 (treatment) were overall similar for cell-specific Fe uptake (Figure 4b) to those  
12 presented for bacterial production (Figure 4a). While no significant differences were  
13 found for the control treatment, the uptake of Fe by bacteria was respectively  
14 lowered and enhanced in +Fe and +Fe+C treatments. In +Fe treatment, Fe uptake by  
15 bacteria is 3 times lower in presence of pico-nanoplankton (<20 $\mu$ m condition) and  
16 decreased from  $2.1 \pm 0.5$  to  $0.6 \pm 0.1$  ( $\times 100$  amolFe cell<sup>-1</sup> d<sup>-1</sup>) compared to the  
17 unfiltered condition. This difference was significant ( $p=0.006$ ). Across all datasets,  
18 the Fe uptake by bacteria was the highest for the +Fe+C treatment and the <1 $\mu$ m  
19 condition. This result makes precise the sense in which the availability of C together  
20 with the removal of potential competitors had the greatest effect on the uptake of Fe  
21 by bacteria. Interestingly, the concomitant addition of Fe and C did not enhance the  
22 bacterial Fe uptake for the other conditions (unfiltered and <20 $\mu$ m). Values were  
23 respectively  $4.7 \pm 0.3$  and  $8.5 \pm 3.5$  ( $\times 100$  amolFe cell<sup>-1</sup> d<sup>-1</sup>) for unfiltered and <20 $\mu$ m  
24 conditions while it reached up to  $61 \pm 21$  ( $\times 100$  amolFe cell<sup>-1</sup> d<sup>-1</sup>) for the <1 $\mu$ m  
25 condition. Considering all data together, the size fractionation used in the incubation  
26 (i.e., presence or absence of phytoplankton) had a greater effect than addition of  
27 growth-limiting nutrients.

### 28 **4.3.4. Fe uptake by phytoplankton**

29 Iron uptake by cells larger than 0.8 $\mu$ m is presented as the Fe uptake by  
30 phytoplankton in Figure 5 and specific Fe uptake by pico-nanoplankton (0.8-20 $\mu$ m)  
31 is given in Table 3. If we consider the unfiltered condition with no nutrient addition  
32 to be the closest representation of the natural system, phytoplankton contributed to

1 66±6% of the total Fe uptake at the SOTS site. In terms of percentage contribution,  
2 phytoplankton Fe uptake increased to more than 80% when Fe or Fe plus C was  
3 added (82±1% and 83±1% for +Fe and +Fe+C treatment, respectively).

4 In the +Fe treatment, the Fe uptake by phytoplankton was slightly lower in <20µm  
5 condition compared to unfiltered condition which is explained by the removal of  
6 about 20% of the phytoplankton initial biomass. However, the concomitant addition  
7 of Fe and C clearly led to the increase of Fe uptake by phytoplankton. As the Fe  
8 uptake is higher by 22% in unfiltered conditions compared to <20µm, which is again  
9 explained by the removal of larger cells, we consider that the microphytoplankton  
10 also benefited from the +Fe+C treatment in some ways (e.g. Fe regenerated by  
11 bacteria).

## 12 **4.4. Carbon biomass and Fe:C ratios**

### 13 **4.4.1. Contribution to carbon biomass at initial conditions**

14 We examined the carbon biomass partitioning of the pico- and nanoplankton  
15 communities across treatments and size fractions, and in relation to the heterotrophic  
16 bacteria. First, we compare estimates of C biomass of small photosynthetic cells  
17 based on conversion factors and flow cytometry numbers with direct measurements  
18 of POC as described before. We found 1.49±0.05 µmolC L<sup>-1</sup> (estimate) versus 1.51  
19 µmolC L<sup>-1</sup> (measure). Given the comparable results we are confident in using  
20 conversion factors to investigate variations in carbon biomass in our incubation  
21 bottles.

22 Among the pico-and nanoplankton community, picoeukaryotic cells were the most  
23 abundant in surface waters at SOTS and represented 71±0.2% (n=9) of carbon  
24 biomass while cyanobacteria made up 26±0.3% (n=9) and photosynthetic  
25 nanoeukaryotes 2±0.1% (n=9). After 36 hours of incubation, no notable differences  
26 in these contributions were found when comparing size fractions and treatments. In  
27 the case of total carbon biomass, heterotrophic bacteria were dominant (averaging  
28 58%) at the start of the experiment, with values ranging from 1.85 to 2.33 µmol L<sup>-1</sup>.

### 29 **4.4.2. Contribution to Fe uptake for bacteria and pico-nanoplankton**

30 In this section, we only consider values measured in <20µm condition as we did not  
31 directly measure the carbon content of larger cells (microplankton) so that we cannot

1 evaluate accurately their contribution. Heterotrophic bacteria represented about 52%  
2 (control), 56% (+Fe), and 59% (+Fe+C) of the total C biomass (Table 3). However,  
3 their contribution to the total uptake of Fe did not reflect this dominance (Figure 5).  
4 For the unfiltered condition, heterotrophic bacteria were only responsible for 25, 20  
5 and 32% of the total Fe uptake in control, +Fe and +Fe+C treatments, respectively.

#### 6 **4.4.3. Fe:C ratio**

7 We normalized Fe uptake rate to carbon biomass and the resulting Fe:C ratio is  
8 presented in Table 3. Comparison of these Fe:C ratios for bacteria among the  
9 different treatment and condition indicate that heterotrophic bacteria had a higher Fe  
10 content in the +Fe+C treatment. However, this Fe:C ratio also varied from 46 to 591  
11  $\mu\text{molFe molC}^{-1}$  (+Fe+C treatment, Table 3) which shows that heterotrophic bacteria  
12 can assimilate a substantial amount of Fe when phytoplankton is removed from the  
13 experiment. For instance, the Fe:C ratio of heterotrophic bacteria in the  $<20\mu\text{m}$   
14 condition was nearly twofold higher than in the unfiltered condition, and it was close  
15 to 13-fold higher in the  $<1\mu\text{m}$  condition. These high Fe:C ratios also indicate that  
16 heterotrophic bacteria have rapidly upregulated their Fe acquisition machinery  
17 relative to C to acquire more dissolved Fe or that their uptake systems were already  
18 activated.

19 The Fe:C ratios were also estimated for photosynthetic pico- and nanoplankton cells  
20 (including cyanobacteria). As for heterotrophic bacteria, we observed a pronounced  
21 increase of Fe:C ratio in the +Fe+C treatment, but no notable difference between  
22 unfiltered and  $<20\mu\text{m}$  condition (Table 3). These estimates ranged from 32 to 59  
23  $\mu\text{molFe molC}^{-1}$  for both control and +Fe treatments considered versus a range of  
24 231-250  $\mu\text{molFe molC}^{-1}$  in +Fe+C treatment.

25 Overall, our calculation of Fe:C ratios show that pico- and nanoplankton constitute a  
26 larger fraction of biogenic Fe compared to heterotrophic bacteria in all incubations.  
27 However, the highest Fe:C ratio measured in this study was when bacteria were  
28 incubated alone ( $591\pm 208$ ,  $n=3$ ; +Fe+C treatment) and it was more than 2-fold  
29 higher than the maximum we calculated for pico-and nanoplankton ( $250\pm 50$ ,  $n=3$ ;  
30 +Fe+C treatment for  $<20\mu\text{m}$  condition).

## 31 **5. Discussion**



1 In environmental science, the concept of bioavailability for one or several resources  
2 is generally associated with chemical features, in particular, in the case of Fe. This  
3 micronutrient is present in multiple chemical forms and redox states (Morel and  
4 Price, 2003). However, we show here that biological interactions matter as well. To  
5 discuss the results of this study, we first comment on the concept of co-limitation.  
6 Next, we discuss the nature of the interspecific interactions that most likely explain  
7 our results. Finally, we close this section on implications for future perspectives of  
8 research.

### 9 **5.1. Does carbon availability offset Fe limitation in heterotrophic bacteria?**

10 One particular feature of the Southern Ocean is that both bioavailable Fe and organic  
11 carbon can be at growth-limiting concentrations for heterotrophic bacteria in surface  
12 waters (Church et al., 2000; Obernosterer et al., 2015). At first sight, our results  
13 suggest that heterotrophic bacteria were primarily limited by organic carbon at  
14 SOTS. Iron could have a role, however, in affecting BP and bacterial metabolism  
15 when the supply of organic carbon is adequate and Fe concentrations are low.  
16 Indeed, while these results lead us to the conclusion that heterotrophic bacteria were  
17 firstly C-limited, the argument for Fe limitation of heterotrophic bacteria is not so  
18 clear. A simple comparison between *in situ* molar DFe:DOC ratio ( $2.61 \mu\text{mol mol}^{-1}$ )  
19 and Fe:C ratios of Fe-limited cultures (e.g.  $0.43 \pm 0.1$  and  $7.52 \pm 1.65 \mu\text{molFe molC}^{-1}$   
20 for oceanic strains in Fourquez et al., 2014 and Tortell et al., 1996, respectively)  
21 would suggest that both nutrients may become limiting. In the present study, Fe  
22 alone had no effect on rates of BP rates or cell abundance. But Fe did affect these  
23 variables when added together with glucose.

24 Consistent with the hypothesis that bacteria may have been co-limited by Fe and C,  
25 we observed that cell-specific BP was positively correlated ( $r=0.98$ ,  $n=6$ ,  $p=$   
26  $0.000373$ , Pearson correlation) with bacterial Fe uptake in the +Fe+C treatment. In  
27 contrast, there was no significant correlation in the control ( $r=-0.33$ ,  $n=6$ ,  $p=0.58$ ),  
28 and in the +Fe treatment ( $r=0.29$ ,  $n=6$ ,  $p=0.56$ ). Such a high correlation in studies  
29 using natural communities is uncommon. An explanation for our results is that  
30 bacterial growth became Fe-limited, but only after C-limitation was alleviated by the  
31 addition of glucose. This can be explained by the increase in the C demand induced  
32 by cellular remodeling to support growth and maintenance under Fe stress conditions  
33 (Fourquez et al., 2014; Kirchman et al., 2000).

1 There is growing evidence that the expression of alternative pathways is a  
2 widespread strategy for heterotrophic bacteria in low Fe environments (Beier et al.,  
3 2015; Debeljak et al., 2019; Fourquez et al., 2014; Koedooder et al., 2018). A  
4 comparative proteomics approach revealed that Fe limitation leads cells to utilize C  
5 through the glyoxylate cycle (Fourquez et al., 2014). This alternative pathway not  
6 only bypasses two important Fe-containing enzymes in the Krebs's cycle, but also  
7 the two steps where carbon is lost as CO<sub>2</sub>. Redirection of glucose into the Entner-  
8 Doudoroff pathway also allows Fe-limited cells to supply the Krebs's cycle with  
9 substrates while bypassing the first step of glycolysis which is ATP-consuming  
10 (Fourquez et al. 2014). Other biomass recycling processes such as amino and organic  
11 acid catabolism contribute as well to the regulation of energy production (Fourquez  
12 et al. 2014). These underlying mechanisms may explain why BP was stimulated by C  
13 addition, strongly stimulated by concomitant Fe and C additions, but not stimulated  
14 by the addition of Fe alone in the present study. It also indicates that Fe-C co-  
15 limitation for heterotrophic bacterial growth is a predominant feature in the Southern  
16 Ocean, but that it can be masked by conventional experimental approaches.

## 17 **5.2. A minimum Fe:C quota to support bacterial growth?**

18 In our study we explicitly examined the bacterial Fe uptake together with the BP. As  
19 we observed there is a good agreement in the trends for each response variable,  
20 bacterial growth and Fe uptake rates are likely to be related. However, the link  
21 between growth and nutrient uptake is not straightforward in natural communities,  
22 and variation in maximum growth rate and minimum cell quota can greatly  
23 complicate this relationship. Here we propose a threshold value to reconcile these  
24 two variables. Based on the correlation between cell-specific Fe uptake and BP, we  
25 derived a minimum Fe:C quota for heterotrophic bacteria of 37  $\mu\text{molFe molC}^{-1}$  that  
26 we propose as a threshold limit value to define Fe or C limitation (Suppl. Material  
27 Figure 2). Based on this assumption, Fe is the primary limiting element for a cellular  
28 quota that is below 37  $\mu\text{molFe molC}^{-1}$ , and C is the primary limiting element for a  
29 quota above to this value. To understand the boundaries of implication for this  
30 threshold limit, it is important to consider how heterotrophic bacteria utilize Fe and  
31 organic substrates to gain energy. However, the vastness of biogeochemical  
32 gradients—both spatially and temporally—that govern the composition of microbial  
33 communities, and the plethora of metabolic strategies among taxa (Hopkinson and

1 Barbeau 2011, Hogle et al.; Debeljak et al. 2019) require similar studies in other  
2 ocean regions in order to investigate the spectrum of heterotrophic bacterial Fe:C  
3 quotas.

### 4 **5.3. Carbon availability increases Fe demand: the starting point of** 5 **competition?**

6 When DOC is no longer a limiting resource, the competition for Fe between  
7 autotrophic pico- and nanoplankton and heterotrophic bacteria negatively affect the  
8 latter (Fourquez et al. 2015, this study). There were also intriguing results in the  
9 outcomes of the experiment regarding phytoplankton. In the present study, the uptake  
10 of Fe by phytoplankton was similar for the control and the +Fe treatment but  
11 increased by nearly five times in the +Fe+C treatment. We have two explanations for  
12 this intriguing finding:

13 *1) Phytoplankton had a higher Fe uptake rate in +Fe+C treatment because Fe*  
14 *regenerated by bacteria became available*

15 Fe availability influences the growth and abundance of auto- and heterotrophic  
16 microorganisms, and heterotrophic bacteria can modify its speciation by the  
17 synthesis of organic ligands (Gerringa et al., 2008; Rue and Bruland, 1997). In this  
18 context, heterotrophic bacteria could act either as competitors with phytoplankton  
19 (Kirchman, 1994; Thingstad, 2000), or on the contrary, facilitate their assimilation of  
20 Fe in maintaining Fe solubility within the ecological niche they share (Amin et al.,  
21 2009; Hopkinson and Morel, 2009).

22 The high rates of BP in +Fe+C treatment could in part be due to remineralization of  
23 Fe during the incubation. The regenerated Fe may become available for  
24 phytoplankton. Since the DFe concentration was measured in the incubation bottles  
25 at the beginning, after +36h and at the end of the incubation (120 hours, Suppl.  
26 Material Figure 3), we were able to directly compare these values with total Fe  
27 uptake by microorganisms. The DFe concentrations decreased over time during the  
28 incubations (Suppl Material Figure 3). We used a simple approach to (1) verify that  
29 remineralization occurred during the incubation and (2) to provide an estimate of the  
30 Fe regeneration rate. The regeneration rate of Fe was estimated by subtracting the  
31 amount of Fe consumed by the entire microbial community (phytoplankton and  
32 bacteria) from the initial Fe concentration as follows:

1 
$$DFe_{regenerated} = DFe_{measured} - DFe_{expected}$$

2 
$$\text{With } DFe_{expected} = DFe_{initial} - [Total Fe_{uptake} \times t]$$

3 Where DFe initial is the concentration of DFe at the start of the experiment, Total Fe uptake is the  
4 amount of Fe consumed by phytoplankton and bacteria during the incubation, and DFe measured is  
5 the DFe concentration at the end of the incubation.

6 If we consider the amount of “missing” DFe as the regenerated Fe, we obtain rates of  
7 Fe regeneration of 0.48 to 0.92 nmolFe L<sup>-1</sup> d<sup>-1</sup> (respectively unfiltered and <1µm  
8 condition, Suppl. Material Table 1).

9 Iron regeneration within the microbial loop (also termed the “ferrous wheel”;  
10 Kirchman, 1996) represents a key term in the Fe budget (Boyd and Ellwood, 2010;  
11 Strzepek et al., 2005). For instance, in the SAZ (FeCycle voyage, see Strzepek et al.,  
12 2005), it was found that between 30 to 100% of the microbial Fe demand could be  
13 met by Fe regeneration mediated by grazers (Boyd et al., 2005). In the naturally Fe  
14 fertilized waters off Kerguelen Island, Fe regeneration accounted for roughly 50% of  
15 the Fe demand (Sarhou et al., 2008). Heterotrophic bacteria and viruses contribute as  
16 much as grazers to Fe recycling (Obernosterer et al., 2008; Poorvin et al., 2004).  
17 Many of the metabolites originating from microorganisms can possess Fe-binding  
18 properties that can exert strong control on Fe speciation (Boyd et al., 2010; Dalbec  
19 and Twining, 2009; Poorvin et al., 2004). Unlike larger cells of phytoplankton (e.g.  
20 diatoms), pico- and nanoplankton are equally adept to heterotrophic bacteria at  
21 accessing either new or regenerated Fe (Boyd et al. 2012). Ultimately, niche  
22 differentiation of bacteria and phytoplankton related to Fe-speciation might act as a  
23 selection process (Debeljak et al., 2019; Hogle et al., 2016; Hopkinson and Barbeau,  
24 2012).

25 Over the course of a phytoplankton bloom there is a transition from the utilization of  
26 new Fe (i.e., winter reserve Fe stocks) to regenerated Fe (Boyd et al., 2012) which  
27 maintains primary productivity. During this transition, rapidly growing heterotrophic  
28 bacteria may quickly shift to Fe limitation if phytoplankton-derived organic carbon is  
29 available, resulting in their enhanced ability to compete for Fe. As the bloom status  
30 moves towards senescence and cells exude DOC, competition between pico- and  
31 nanoplankton and heterotrophic bacteria may result in different amounts of Fe  
32 regenerated. Significantly, In the present study we calculated a Fe regeneration rate

1 nearly two-fold larger for the  $<1\mu\text{m}$  ( $0.92 \text{ nmolFe L}^{-1} \text{ d}^{-1}$ ) versus  $<20\mu\text{m}$  ( $0.53$   
2  $\text{nmolFe L}^{-1} \text{ d}^{-1}$ ) or in unfiltered seawater ( $0.48 \text{ nmolFe L}^{-1} \text{ d}^{-1}$ ). It is reported that  
3 organic Fe complexes are available to few phytoplankton species (Kranzler et al.,  
4 2011; Lis et al., 2015; Shaked and Lis, 2012). This could suggest that the bloom  
5 duration is primarily set by DOC availability and competition for DFe between  
6 heterotrophic bacteria and phytoplankton.

## 7 2) *Synechococcus like it organic*

8 Many studies make operational distinctions based on size-fractionated samples. In  
9 the study of Strzeppek et al. 2005 (FeCycle), flow cytometric analyses revealed that  
10 both picophytoplankton and eukaryotic phytoplankton were  $>1\mu\text{m}$ , and heterotrophic  
11 bacteria were submicron in size. We used the same size cutoffs of  $1\mu\text{m}$  to separate  
12 phytoplankton and heterotrophic bacteria in this study. It remains, however, difficult  
13 to draw clear distinctions between the size-fractionation and assigning  
14 microorganisms to eukaryotes or prokaryotes. At SOTS, most pico- and  
15 nanoplankton (70%) were cyanobacteria (most likely *Synechococcus*).  
16 *Synechococcus* is one of the most prominent genera of picoplanktonic marine  
17 cyanobacteria (Buitenhuis et al., 2012) that have particularly high Fe demands  
18 relative to heterotrophic bacteria and eukaryotic phytoplankton (Lis et al., 2015;  
19 Morrissey and Bowler, 2012; Raven, 1990). There is growing evidence of the ability  
20 of the genera of *Synechococcus* to assimilate organic nutrients (Yelton et al., 2016),  
21 and more broadly there are reports that suggest some of the photosynthetic  
22 picoeukaryotes are mixotrophs (Farnelid et al., 2016). Cyanobacteria may be  
23 responsible for an important part of the total Fe taken up by the phytoplankton  
24 fraction ( $>0.8\mu\text{m}$ ), and that due to their ability to take up organic compounds for  
25 their metabolism they may also have benefited from the +Fe+C treatment, as did  
26 their heterotrophic counterparts. In our experiment, cyanobacteria, but none of the  
27 pico- and nanoeukaryotes had significantly increased in cell abundance in the +Fe+C  
28 treatment (Suppl. Material Figure 4). This incubation was performed under very low  
29 light intensities which suggests a complementary mechanism such as mixotrophy.  
30 This raises the question of whether a mixotrophic capacity can become an  
31 advantageous for these microorganisms, and if they could become also competitors  
32 for C availability if this is proven.

#### 1        **5.4. The fate and duration of phytoplankton blooms in the Southern Ocean** 2        **driven by interspecific relationships.**

3 Interactions between autotrophic and heterotrophic microbes could affect the  
4 dynamics of nutrient-limited phytoplankton blooms. This hypothesis originates from  
5 an investigation of what was initially perceived as an isolated event in the vicinity of  
6 the Kerguelen plateau (Fourquez et al. 2015). As we reached similar conclusions in  
7 the present study, these joint findings raise the issue of whether such interactions are  
8 widespread across the Southern Ocean?

9 One stand-out result of our study is the amount of Fe taken up by heterotrophic  
10 bacteria in the absence of competition. Our reported bacterial Fe uptake rates are  
11 well beyond the range of those previously reported for Southern Ocean microbes. For  
12 example, in comparison to the Fe uptake by the entire microbial community, the rate  
13 of heterotrophic bacteria alone is by 12-fold higher than that measured during  
14 FeCycle (HNLC waters southeast of New Zealand) and more than 100fold higher  
15 than that measured above the Kerguelen plateau during summer (KEOPS2; Table 4).  
16 The observation of the present study underlines the potential of heterotrophic  
17 bacteria to control the decline of the bloom in the absence of competition with pico-  
18 and nanoplankton.

19 It is only recently that the potential influence of the composition of phytoplankton  
20 community and its interactions with heterotrophic microbes has been taken into  
21 consideration (Bunse et al., 2016; Farnelid et al., 2016; Lima-Mendez et al., 2015;  
22 Liu et al., 2019; Zhou et al., 2018). For instance, Liu et al. (2019) showed a  
23 pronounced association between assemblages of diatoms and heterotrophic microbes  
24 at the onset of spring phytoplankton blooms occurring in the region off Kerguelen  
25 Island. The quality and quantity of DOC derived from phytoplankton exudates  
26 (Landa et al., 2015) and resource competition for Fe (Fourquez et al. 2015) are the  
27 two explanations put forward to explain how diatom assemblages shape the habitat  
28 type for their heterotrophic counterparts (Liu et al. 2019).

29 Uncertainties remain on the effects of climate change on the composition of  
30 phytoplankton assemblage; but there is compelling evidence that all regions of the  
31 Southern Ocean will encounter changes in phytoplankton community composition  
32 (Deppeler and Davidson, 2017; Hays et al., 2005). Models project that waters of the

1 Southern Ocean will become warmer, and that rising temperatures will cause rates of  
2 grazing to increase more rapidly than rates of phytoplankton growth (Behrenfeld,  
3 2014; Cael and Follows, 2016; Caron and Hutchins, 2012; Evans et al., 2011;  
4 Sarmiento et al., 2010). Increasing temperature is also expected to increase bacterial  
5 respiration rates (Vázquez-Domínguez et al., 2007). Thus, phytoplankton standing  
6 stocks are likely to decline and the proportion of primary production respired in near-  
7 surface waters by heterotrophic bacteria will increase (Cavan et al., 2019; Cavan and  
8 Boyd, 2018; Deppeler and Davidson, 2017). The study of Cavan and Boyd (2018),  
9 which have predicted an increase in POC-normalised respiration, estimates that the  
10 biological pump efficiency (POC export scaled to primary production) would  
11 decrease by  $17 \pm 7\%$  (SE) by 2100 for the subantarctic site SOTS. Such reports of  
12 increased rates in bacterial respiration are enzymatic reactions as the temperature  
13 increases, but are also supported by the enhanced release DOC-derived from  
14 phytoplankton at higher temperatures to support heterotrophic bacteria (Hutchins et  
15 al., 2019).

16 For microbial ecologists, the existence of interactions between primary producers  
17 and bacteria that shape the activity and the diversity of both partners is well  
18 recognised (Amin et al., 2015), but the mechanisms of such interactions remain  
19 mostly unknown. The term “interactive co-limitation” was first proposed by Bertrand  
20 et al. (2015) to describe scenarios in which at least two limiting “nutrients cycle are  
21 affected by one another through interactions among different microbial functional  
22 groups” (Bertrand et al., 2015). The findings of our study appear to be an example of  
23 interactive colimitation. Moreover, they expand on the emerging recognition that  
24 interaction between microorganisms is an ecological trait to be considered in the  
25 study of Fe and C biogeochemistry. As Fe sources — including the inputs, amounts  
26 and the nature of Fe — are often considered to be the primary drivers of Southern  
27 Ocean productivity, our study teaches us that Fe bioavailability for microorganisms  
28 is not a simple matter of chemistry.

## 29 **Acknowledgement**

30 The authors sincerely thank the reviewers for their help and thorough  
31 reviews. We especially thank Reviewer #2 and Reviewer #4 for taking the time to  
32 thoroughly read the manuscript and offer tremendously valuable suggestions. The  
33 authors also wish to thank Alice Della Penna (CSIRO) who prepared filters for POC

1 measurements and Diana Davies (CSIRO) who analysed them, and Audrey  
2 Guéneuguès (LOMIC) for the preparation of the trace-metal clean stock solution of  
3 Glucose. We are also grateful to Robert Strzepek, Sam Eggins, and Cassie  
4 Schwanger (IMAS/CSIRO) for their valuable help at sea and Christel Hassler for her  
5 suggestions regarding the manuscript. Finally, we also would like to thank Laurent  
6 Besnard (IMOS) and Sébastien Moreau for Figure 1, Pierre Poisson (PLUM) for his  
7 encouragement in this work, and Andrew Davidson for his kindness in facilitating  
8 the use of the Australian Antarctic Division (AAD) facilities. M.F and P.B were  
9 primarily supported by the Australian Research Council through a Laureate  
10 (FL160100131), and this research was also supported under the Australian Research  
11 Council's Special Research Initiative for Antarctic Gateway Partnership (project ID  
12 SR140300001). M.B was funded from the European Union Seventh Framework  
13 Program ([FP7/2007-2013]) under grant agreement no. [PIOF-GA-2012-626734]  
14 (IRON-IC project)).



## References

- Amin, S. A., Green, D. H., Hart, M. C., Küpper, F. C., Sunda, W. G., Carrano, C. J., et al. (2009). Photolysis of iron – siderophore chelates promotes bacterial – algal mutualism. *Proc. Natl. Acad. Sci. U. S. A.* 106, 17071–17076. doi:10.1073/pnas.0905512106.
- Amin, S. A., Hmelo, L. R., van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., et al. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature* 522, 98–101. doi:10.1038/nature14488.
- Andrews, S. C., Robinson, A. K., and Rodríguez-Quiñones, F. (2003). Bacterial iron homeostasis. *FEMS Microbiol. Rev.* 27, 215–237. doi:10.1016/S0168-6445(03)00055-X.
- Armstrong, E., Granger, J., Mann, E. L., and Price, N. M. (2004). Outer-membrane siderophore receptors of heterotrophic oceanic bacteria. *Limnol. Oceanogr.* 49, 579–587. doi:10.4319/lo.2004.49.2.0579.
- Behrenfeld, M. J. (2014). Climate-mediated dance of the plankton. *Nat. Clim. Chang.* 4, 880–887. doi:10.1038/nclimate2349.
- Behrenfeld, M. J., Worthington, K., Sherrell, R. M., Chavez, F. P., Strutton, P. G., McPhaden, M., et al. (2006). Controls on tropical Pacific Ocean productivity revealed through nutrient stress diagnostics. *Nature* 442, 1025–1028. doi:10.1038/nature05083.
- Beier, S., Gálvez, M., Molina, V., Sarthou, G., Quéroúé, F., Blain, S., et al. (2015). The transcriptional regulation of the glyoxylate cycle in SAR11 in response to iron fertilization in the Southern Ocean. *Environ. Microbiol. Rep.* 7, 427–434. doi:10.1111/1758-2229.12267.
- Bertrand, E. M., McCrow, J. P., Moustafa, A., Zheng, H., McQuaid, J. B., Delmont, T. O., et al. (2015). Phytoplankton-bacterial interactions mediate micronutrient colimitation at the coastal Antarctic sea ice edge. *Proc. Natl. Acad. Sci. U. S. A.* 112, 9938–43. doi:10.1073/pnas.1501615112.
- Bertrand, E. M., Saito, M. A., Lee, P. A., Dunbar, R. B., Sedwick, P. N., and

- Ditullio, G. R. (2011). Iron limitation of a springtime bacterial and phytoplankton community in the Ross Sea: Implications for vitamin B12 nutrition. *Front. Microbiol.* 2, 160. doi:10.3389/fmicb.2011.00160.
- Blain, S., Quéguiner, B., Armand, L., Belviso, S., Bombled, B., Bopp, L., et al. (2007). Effect of natural iron fertilization on carbon sequestration in the Southern Ocean. *Nature* 446, 1070–1074. doi:10.1038/nature05700.
- Blain, S., Queguiner, B., and Trull, T. (2008). The natural iron fertilization experiment KEOPS (KErguelen Ocean and Plateau compared Study): An overview. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 559–565. doi:10.1016/j.dsr2.2008.01.002.
- Blain, S., and Tagliabue, A. (2016). *Iron Cycle in Oceans*. John Wiley & Sons, Incorporated doi:10.1002/9781119136859.
- Boiteau, R. M., Fansler, S. J., Farris, Y., Shaw, J. B., Koppenaar, D. W., Pasatolic, L., et al. (2019). Siderophore profiling of co-habiting soil bacteria by ultra-high resolution mass spectrometry. *Metallomics* 11, 166–175. doi:10.1039/C8MT00252E.
- Boiteau, R. M., Mende, D. R., Hawco, N. J., McIlvin, M. R., Fitzsimmons, J. N., Saito, M. A., et al. (2016). Siderophore-based microbial adaptations to iron scarcity across the eastern Pacific Ocean. *Proc. Natl. Acad. Sci.* 113, 14237–14242. doi:10.1073/pnas.1608594113.
- Bowie, A. R., Maldonado, M. T., Frew, R. D., Croot, P. L., Achterberg, E. P., Mantoura, R. F. C., et al. (2001). The fate of added iron during a mesoscale fertilisation experiment in the Southern Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 48, 2703–2743. doi:10.1016/S0967-0645(01)00015-7.
- Boyd, P. W., and Ellwood, M. J. (2010). The biogeochemical cycle of iron in the ocean. *Nat. Geosci.* 3, 675–682. doi:10.1038/ngeo964.
- Boyd, P. W., Law, C. S., Hutchins, D. A., Abraham, E. R., Croot, P. L., Ellwood, M., et al. (2005). FeCycle: Attempting an iron biogeochemical budget from a mesoscale SF 6 tracer experiment in unperturbed low iron waters. *Global Biogeochem. Cycles* 19, 1–13. doi:10.1029/2005GB002494.

- Boyd, P. W., Strzepek, R., Chiswell, S., Chang, H., DeBruyn, J. M., Ellwood, M., et al. (2012). Microbial control of diatom bloom dynamics in the open ocean. *Geophys. Res. Lett.* 39, 1–6. doi:10.1029/2012GL053448.
- Boyd, P. W., Strzepek, R., Fu, F., and Hutchins, D. a. (2010). Environmental control of open-ocean phytoplankton groups: Now and in the future. *Limnol. Oceanogr.* 55, 1353–1376. doi:10.4319/lo.2010.55.3.1353.
- Braun, V., and Killmann, H. (1999). Bacterial solutions to the iron-supply problem. *Trends Biochem. Sci.* 24, 104–109. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10203757>.
- Buitenhuis, E. T., Li, W. K. W., Vaultot, D., Lomas, M. W., Landry, M. R., Partensky, F., et al. (2012). Picophytoplankton biomass distribution in the global ocean. *Earth Syst. Sci. Data* 4, 37–46. doi:10.5194/essd-4-37-2012.
- Bundy, R. M., Boiteau, R. M., McLean, C., Turk-Kubo, K. A., McIvin, M. R., Saito, M. A., et al. (2018). Distinct Siderophores Contribute to Iron Cycling in the Mesopelagic at Station ALOHA. *Front. Mar. Sci.* 5, 1–15. doi:10.3389/fmars.2018.00061.
- Bunse, C., Bertos-Fortis, M., Sassenhagen, I., Sildever, S., Sjöqvist, C., Godhe, A., et al. (2016). Spatio-Temporal Interdependence of Bacteria and Phytoplankton during a Baltic Sea Spring Bloom. *Front. Microbiol.* 7, 517. doi:10.3389/fmicb.2016.00517.
- Cael, B. B., and Follows, M. J. (2016). On the temperature dependence of oceanic export efficiency. *Geophys. Res. Lett.* 43, 5170–5175. doi:10.1002/2016GL068877.
- Caron, D. A., Connell, P. E., Schaffner, R. A., Schnetzer, A., Fuhrman, J. A., Countway, P. D., et al. (2017). Planktonic food web structure at a coastal time-series site: I. Partitioning of microbial abundances and carbon biomass. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 121, 14–29. doi:10.1016/J.DSR.2016.12.013.
- Caron, D. A., Dam, H. G., Kremer, P., Lessard, E. J., Madin, L. P., Malone, T. C., et al. (1994). The contribution of microorganisms to particulate carbon and nitrogen in surface waters of the Sargasso Sea near Bermuda. 42.

- Caron, D. A., and Hutchins, D. A. (2012). The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *J. Plankton Res.* 35, 235–252. doi:10.1093/plankt/fbs091.
- Carpenter, J. I. (1965). The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* 10, 135–140. doi:10.4319/lo.1965.10.1.0135.
- Cassar, N., DiFiore, P. J., Barnett, B. A., Bender, M. L., Bowie, A. R., Tilbrook, B., et al. (2011). The influence of iron and light on net community production in the Subantarctic and Polar Frontal Zones. *Biogeosciences* 8, 227–237. Available at: <http://www.biogeosciences.net/8/227/2011/>.
- Cavan, E. L., and Boyd, P. W. (2018). Effect of anthropogenic warming on microbial respiration and particulate organic carbon export rates in the sub-Antarctic Southern Ocean. *Aquat. Microb. Ecol.* 82, 111–127. doi:10.3354/ame01889.
- Cavan, E. L., Henson, S. A., and Boyd, P. W. (2019). The Sensitivity of Subsurface Microbes to Ocean Warming Accentuates Future Declines in Particulate Carbon Export. *Front. Ecol. Evol.* 6. doi:10.3389/fevo.2018.00230.
- Church, M. J., Hutchins, D. A., and Ducklow, H. W. (2000). Limitation of bacterial growth by dissolved organic matter and iron in the Southern Ocean. *Appl. Environ. Microbiol.* 66, 455–466. doi:10.1128/AEM.66.2.455-466.2000.
- Culbertson, C. (1991). Dissolved Oxygen. *WHPO Publ.* 91.
- Cutter, G., Sciences, A., Dominion, O., Casciotti, K., Croot, P., Sciences, O., et al. (2017). Sampling and Sample-handling Protocols for GEOTRACES Cruises.
- Dalbec, A., and Twining, B. (2009). Remineralization of bioavailable iron by a heterotrophic dinoflagellate. *Aquat. Microb. Ecol.* 54, 279–290. doi:10.3354/ame01270.
- Debeljak, P., Toulza, E., Beier, S., Blain, S., and Obernosterer, I. (2019). Microbial iron metabolism as revealed by gene expression profiles in contrasted Southern Ocean regimes. *Environ. Microbiol.*, 1462-2920.14621. doi:10.1111/1462-2920.14621.
- Deppeler, S. L., and Davidson, A. T. (2017). Southern Ocean Phytoplankton in a Changing Climate. *Front. Mar. Sci.* 4, 40. doi:10.3389/fmars.2017.00040.

- Desai, D. K., Desai, F. D., and LaRoche, J. (2012). Factors Influencing the Diversity of Iron Uptake Systems in Aquatic Microorganisms. *Front. Microbiol.* 3. doi:10.3389/FMICB.2012.00362.
- Evans, C., Thomson, P. G., Davidson, A. T., Bowie, A. R., van den Enden, R., Witte, H., et al. (2011). Potential climate change impacts on microbial distribution and carbon cycling in the Australian Southern Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 58, 2150–2161. doi:10.1016/j.dsr2.2011.05.019.
- Falkowski, P. G., and de Vargas, C. (2004). Genomics and evolution. Shotgun sequencing in the sea: a blast from the past? *Science* (80-. ). 304, 58–60. doi:10.1126/science.1097146.
- Farnelid, H. M., Turk-Kubo, K. A., and Zehr, J. P. (2016). Identification of Associations between Bacterioplankton and Photosynthetic Picoeukaryotes in Coastal Waters. *Front. Microbiol.* 7, 339. doi:10.3389/fmicb.2016.00339.
- Fourquez, M., Obernosterer, I., and Blain, S. (2012). A method for the use of the radiotracer  $^{55}\text{Fe}$  for microautoradiography and CARD-FISH of natural bacterial communities. *FEMS Microbiol. Lett.* 337, 132–139. doi:10.1111/1574-6968.12022.
- Fourquez, M., Obernosterer, I., Davies, D. M., Trull, T. W., and Blain, S. (2015). Microbial iron uptake in the naturally fertilized waters in the vicinity of the Kerguelen Islands: phytoplankton–bacteria interactions. *Biogeosciences*. doi:10.5194/bg-12-1893-2015.
- Fourquez, M., Schaumann, A., Gueneugues, A., Jouenne, T., and Obernosterer, I. (2014). Effects of iron limitation on growth and carbon metabolism in oceanic and coastal heterotrophic bacteria. 59, 1–14. doi:10.4319/lo.2014.59.1.0000.
- Fukuda, R., Ogawa, H., Nagata, T., and Koike, I. (1998). Direct determination of carbon and nitrogen contents of natural bacterial assemblages in marine environments. *Appl. Environ. Microbiol.* 64, 3352–8.
- Gerringa, L. J. A., Blain, S., Laan, P., Sarthou, G., Veldhuis, M. J. W., Brussaard, C. P. D., et al. (2008). Fe-binding dissolved organic ligands near the Kerguelen Archipelago in the Southern Ocean (Indian sector). *Deep Sea Res. Part II* 55, 606–621. doi:10.1016/j.dsr2.2007.12.007.

- Gledhill, M., and Buck, K. N. (2012). The organic complexation of iron in the marine environment: a review. *Front. Microbiol.* 3, 69. doi:10.3389/fmicb.2012.00069.
- Hansell, D. A., Carlson, C. A., Repeta, D. J., and Schlitzer, R. (2009). Dissolved Organic Matter in the Ocean Carbon Cycle. *Oceanography* 22, 202–211. doi:10.1029/2015eo033011.
- Hays, G. C., Richardson, A. J., and Robinson, C. (2005). Climate change and marine plankton. *Trends Ecol. Evol.* 20, 337–344. doi:10.1016/j.tree.2005.03.004.
- Hogle, S. L., Thrash, J. C., Dupont, C. L., and Barbeau, K. A. (2016). Trace Metal Acquisition by Marine Heterotrophic Bacterioplankton with Contrasting Trophic Strategies. *Appl. Environ. Microbiol.* 82, 1613–1624. doi:10.1128/AEM.03128-15.
- Hopkinson, B. M., and Barbeau, K. A. (2012). Iron transporters in marine prokaryotic genomes and metagenomes. *Environ. Microbiol.* 14, 114–128. doi:10.1111/j.1462-2920.2011.02539.x.
- Hopkinson, B. M., and Morel, F. M. M. (2009). The role of siderophores in iron acquisition by photosynthetic marine microorganisms. *Biometals* 22, 659–669. doi:10.1007/s10534-009-9235-2.
- Hunter, K. A., and Boyd, P. W. (2007). Iron-binding ligands and their role in the ocean biogeochemistry of iron. *Environ. Chem.* 4, 221. doi:10.3389/fmars.2016.00027.
- Hutchins, D. A., Jansson, J. K., Remais, J. V., Rich, V. I., Singh, B. K., and Trivedi, P. (2019). Climate change microbiology — problems and perspectives. *Nat. Rev. Microbiol.* 17, 391–396. doi:10.1038/s41579-019-0178-5.
- Ilbert, M., and Bonnefoy, V. (2013). Insight into the evolution of the iron oxidation pathways. *Biochim. Biophys. Acta - Bioenerg.* 1827, 161–175. doi:10.1016/j.bbabi.2012.10.001.
- Johnson, K. (1997). What controls dissolved iron concentrations in the world ocean? Authors' closing comments. *Mar. Chem.* 57, 181–186. doi:10.1016/S0304-4203(97)00047-9.

- Kazamia, E., Sutak, R., Paz-Yepes, J., Dorrell, R. G., Vieira, F. R. J., Mach, J., et al. (2018). Endocytosis-mediated siderophore uptake as a strategy for Fe acquisition in diatoms. *Sci. Adv.* 4, eaar4536. doi:10.1126/sciadv.aar4536.
- Kirchman, D. L. (1994). The uptake of inorganic nutrients by heterotrophic bacteria. *Microb. Ecol.* 28, 255–271. doi:10.1007/BF00166816.
- Kirchman, D. L. (1996). Microbial ferrous wheel. *Nature* 383, 303–304.
- Kirchman, D. L., Keil, R. G., and Simon, M. (1993). Biomass and production of heterotrophic bacterioplankton in the oceanic subarctic Pacific Samples for bacterial abundance and incorporation rates were taken from CTD Niskin bottles . These bottles all had silicone tubing during the cruises reported here . *Deep. Res.* 40, 967–988.
- Kirchman, D. L., Meon, B., Cottrell, M. T., Hutchins, D. A., Weeks, D., and Bruland, K. W. (2000). Carbon versus iron limitation of bacterial growth in the California upwelling regime. *Limnol. Oceanogr.* 45, 1681–1688. doi:10.4319/lo.2000.45.8.1681.
- Koedooder, C., Guéneuguès, A., Van Geersdaële, R., Vergé, V., Bouget, F.-Y., Labreuche, Y., et al. (2018). The Role of the Glyoxylate Shunt in the Acclimation to Iron Limitation in Marine Heterotrophic Bacteria. *Front. Mar. Sci.* 5, 1–12. doi:10.3389/fmars.2018.00435.
- Kranzler, C., Lis, H., Shaked, Y., and Keren, N. (2011). The role of reduction in iron uptake processes in a unicellular, planktonic cyanobacterium. *Environ. Microbiol.* 13, 2990–9. doi:10.1111/j.1462-2920.2011.02572.x.
- Landa, M., Blain, S., Christaki, U., Monchy, S., and Obernosterer, I. (2015). Shifts in bacterial community composition associated with increased carbon cycling in a mosaic of phytoplankton blooms. *ISME J.* 10, 1–12. doi:10.1038/ismej.2015.105.
- Lima-Mendez, G., Faust, K., Henry, N., Decelle, J., Colin, S., Carcillo, F., et al. (2015). Determinants of community structure in the global plankton interactome. *Science* (80-. ). 348, 1262073–1262073. doi:10.1126/science.1262073.

- Lis, H., Shaked, Y., Kranzler, C., Keren, N., and Morel, F. M. M. (2015). Iron bioavailability to phytoplankton: an empirical approach. *ISME J.* 9, 1003–1013. doi:10.1038/ismej.2014.199.
- Liu, Y., Debeljak, P., Rembauville, M., Blain, S., and Obernosterer, I. (2019). Diatoms shape the biogeography of heterotrophic prokaryotes in early spring in the Southern Ocean. *Environ. Microbiol.* 21, 1452–1465. doi:10.1111/1462-2920.14579.
- Macrellis, H. M., Trick, C. G., Rue, E. L., Smith, G. J., and Bruland, K. W. (2001). Collection and detection of natural iron-binding ligands from seawater. *Mar. Chem.* 76, 175–187.
- Marie, D., Partensky, F., Jacquet, S., and Vaulot, D. (1997). Enumeration and cell cycle analysis of natural populations of marine picoplankton by flow cytometry using the nucleic acid stain SYBR Green I. *Appl. Environ. Microbiol.* 63, 186–193.
- Marie, D., Simon, N., and Vaulot, D. (2005). “Phytoplankton Cell Counting by Flow Cytometry,” in *Algal Culturing Techniques* (Elsevier), 253–267. doi:10.1016/B978-012088426-1/50018-4.
- Martinez, J., Smith, D. C., Steward, G. F., and Azam, F. (1996). Variability in ectohydrolytic enzyme activities of pelagic marine bacteria and its significance for substrate processing in the sea. *Aquat. Microb. Ecol.* 10, 223–230.
- McQuaid, J. B., Kustka, A. B., Oborník, M., Horák, A., McCrow, J. P., Karas, B. J., et al. (2018). Carbonate-sensitive phytotransferrin controls high-affinity iron uptake in diatoms. *Nature* 555, 534–537. doi:10.1038/nature25982.
- Morel, F. M. M., and Price, N. M. (2003). The Biogeochemical Cycles of Trace Metals in the Oceans. *Science* (80- ). 300, 944–947. doi:10.1126/science.1083545.
- Morrissey, J., and Bowler, C. (2012). Iron utilization in marine cyanobacteria and eukaryotic algae. *Front. Microbiol.* 3, 1–13. doi:10.3389/fmicb.2012.00043.
- Obata, H., Karatani, H., and Nakayama, E. (1993). Automated Determination of Iron in seawater by chelating resin concentration and chemiluminescence detection.



*Anal. Chem.* 65, 1524–1528. doi:10.1021/ac00059a007.

Obernosterer, I., Christaki, U., Lefevre, D., Catala, P., Vanwambeke, F., and Lebaron, P. (2008). Rapid bacterial mineralization of organic carbon produced during a phytoplankton bloom induced by natural iron fertilization in the Southern Ocean. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 55, 777–789. doi:10.1016/j.dsr2.2007.12.005.

Obernosterer, I., Fourquez, M., and Blain, S. (2015). Fe and C co-limitation of heterotrophic bacteria in the naturally fertilized region off the Kerguelen Islands. *Biogeosciences* 12, 1983–1992. doi:10.5194/bg-12-1983-2015.

Poorvin, L., Rinta-Kanto, J. M., Hutchins, D. A., and Wilhelm, S. W. (2004). Viral release of iron and its bioavailability to marine plankton. *Limnol. Oceanogr.* 49, 1734–1741. doi:10.4319/lo.2004.49.5.1734.

Raven, J. A. (1990). Predictions of Mn and Fe use efficiencies of phototrophic growth as a function of light availability for growth and of C assimilation pathway. *New Phytol.* 116, 1–18. doi:10.1111/j.1469-8137.1990.tb00505.x.

Raven, J. A., Evans, M. C. W., and Korb, R. E. (1999). The role of trace metals in photosynthetic electron transport in O<sub>2</sub>-evolving organisms. *Photosynth. Res.* 60, 111–150. doi:10.1023/A:1006282714942.

Rembauville, M., Salter, I., Leblond, N., Gueneugues, A., and Blain, S. (2015). Export fluxes in a naturally iron-fertilized area of the Southern Ocean – Part 1 : Seasonal dynamics of particulate organic carbon. *Biogeosciences* 12, 3153–3170. doi:10.5194/bg-12-3153-2015.

Rue, E. L., and Bruland, K. W. (1997). The role of organic complexation on ambient Fe chemistry in the equatorial Pacific Ocean and the response of a mesoscale Fe addition experiment. *Limnol. Oceanogr.* 42, 901–910.

Sarmiento, H., Montoya, J. M., Vázquez-Domínguez, E., Vaqué, D., and Gasol, J. M. (2010). Warming effects on marine microbial food web processes: how far can we go when it comes to predictions? *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 365, 2137–49. doi:10.1098/rstb.2010.0045.

Sarthou, G., Timmermans, K. R., Blain, S., and Tréguer, P. (2005). Growth

- physiology and fate of diatoms in the ocean: a review. *J. Sea Res.* 53, 25–42. doi:10.1016/j.seares.2004.01.007.
- Sarthou, G., Vincent, D., Christaki, U., Obernosterer, I., Timmermans, K. R., and Brussaard, C. P. D. (2008). The fate of biogenic iron during a phytoplankton bloom induced by natural fertilisation: Impact of copepod grazing. *Deep Sea Res. Part II* 55, 734–751. doi:10.1016/j.dsr2.2007.12.033.
- Sedwick, P. N., Edwards, P. R., Mackey, D. J., Griffithst, F. B., and Parslow, J. S. (1997). Iron and manganese in surface waters of the Australian subantarctic region. *Deep Sea Res. Part I* 44, 1239–1253.
- Shaked, Y., and Lis, H. (2012). Disassembling iron availability to phytoplankton. *Front. Microbiol.* 3, 1–26. doi:10.3389/fmicb.2012.00123.
- Strzepek, R. F., Boyd, P. W., and Sunda, W. G. (2019). Photosynthetic adaptation to low iron, light, and temperature in Southern Ocean phytoplankton. *Proc. Natl. Acad. Sci. U. S. A.* 116, 4388–4393. doi:10.1073/pnas.1810886116.
- Strzepek, R. F., and Harrison, P. J. (2004). Photosynthetic architecture differs in coastal and oceanic diatoms. *Nature* 403, 689–692. doi:10.1038/nature02954.
- Strzepek, R. F., Maldonado, M. T., Higgins, J. L., Hall, J., Safi, K., Wilhelm, S. W., et al. (2005). Spinning the “Ferrous Wheel”: The importance of the microbial community in an iron budget during the FeCycle experiment. *Global Biogeochem. Cycles* 19, 1–14. doi:10.1029/2005GB002490.
- Thingstad, T. (2000). Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems. *Limnol. Oceanogr.* 45, 1320–1328. doi:10.4319/lo.2000.45.6.1320.
- Tortell, P. D., Maldonado, M. T., Granger, J., and Price, N. M. (1999). Marine bacteria and biogeochemical cycling of iron in the oceans. *FEMS Microbiol. Ecol.* 29, 1–11. doi:10.1111/j.1574-6941.1999.tb00593.x.
- Tortell, P. D., Maldonado, M. T., and Price, N. M. (1996). The role of heterotrophic bacteria in iron-limited ocean ecosystems. *Nature* 383, 330–332. doi:10.1038/383330a0.
- Toulza, E., Tagliabue, A., Blain, S., and Piganeau, G. (2012). Analysis of the global

- ocean sampling (GOS) project for trends in iron uptake by surface ocean microbes. *PLoS One* 7, e30931. doi:10.1371/journal.pone.0030931.
- Tovar-Sanchez, A., Sanudo-Wilhelmy, S. A., Garcia-Vargas, M., Weaver, R. S., Popels, L. C., and Hutchins, D. A. (2003). A trace metal clean reagent to remove surface-bound iron from marine phytoplankton. *Mar. Chem.* 82, 91–99. doi:10.1016/S0304-4203(03)00054-9.
- Trull, T. W., Jansen, P., Schulz, E., Weeding, B., Davies, D. M., Bray, S. G., et al. (2019). Autonomous Multi-Trophic Observations of Productivity and Export at the Australian Southern Ocean Time Series ( SOTS ) Reveal Sequential Mechanisms of Physical-Biological Coupling. *Front. Mar. Sci.* 6, 1–17. doi:10.3389/fmars.2019.00525.
- Twining, B. S., and Baines, S. B. (2013). The trace metal composition of marine phytoplankton. *Ann. Rev. Mar. Sci.* 5, 191–215. doi:10.1146/annurev-marine-121211-172322.
- Van Wambeke, F., Heussner, S., Diaz, F., Raimbault, P., and Conan, P. (2002). Small-scale variability in the coupling/uncoupling of bacteria, phytoplankton and organic carbon fluxes along the continental margin of the Gulf of Lions, Northwestern Mediterranean Sea. *J. Mar. Syst.* 33–34, 411–429. doi:10.1016/S0924-7963(02)00069-6.
- Vázquez-Domínguez, E., Vaqué, D., and Gasol, J. M. (2007). Ocean warming enhances respiration and carbon demand of coastal microbial plankton. *Glob. Chang. Biol.* 13, 1327–1334. doi:10.1111/j.1365-2486.2007.01377.x.
- Yelton, A. P., Acinas, S. G., Sunagawa, S., Bork, P., Pedrós-Alió, C., and Chisholm, S. W. (2016). Global genetic capacity for mixotrophy in marine picocyanobacteria. *ISME J.* 10, 2946–2957. doi:10.1038/ismej.2016.64.
- Zhou, J., Richlen, M. L., Sehein, T. R., Kulis, D. M., Anderson, D. M., and Cai, Z. (2018). Microbial Community Structure and Associations During a Marine Dinoflagellate Bloom. *Front. Microbiol.* 9, 1201. doi:10.3389/fmicb.2018.01201.

## Legends

**Figure 1** Study site of R/V Investigator voyage 02. Dot represents location of the SOTS site where experiments were conducted (42°S, 47°S, 51°S, and 54°S at 141°E). SOTS was visited on two occasions on 19 (experiment 1) and 29 March 2016 (experiment 2). SAF-N, SAF-S stands for the northern and southern branches of the subantarctic front, and PF for polar front, respectively. Sea surface height was used to characterize fronts position (AVISO Satellite Data Altimetry). Sea surface Chl a composite image for March 2016 was derived from the Moderate Resolution Imaging Spectroradiometer aboard NASA's Aqua satellite and obtained from the ocean color data distribution site [https://oceandata.sci.gsfc.nasa.gov/MODIS-Aqua/Mapped/Monthly/4km/chlor\\_a/](https://oceandata.sci.gsfc.nasa.gov/MODIS-Aqua/Mapped/Monthly/4km/chlor_a/).

**Figure 2** Overview of the setup experiment. (1) and (2) respectively refer to Experiment 1 and Experiment 2 with water collected on the same site (SOTS).

**Figure 3** Changes in bacterial cell abundance (left panel) and cell-specific bacterial heterotrophic production (right panel) for unamended (control) and amended treatments (+Fe, +C, and both +Fe+C) over time for the experiment 1. Treatments with an asterisk are significantly different from the control (one-way analysis of variance (ANOVA) and post hoc Tukey test; \*  $p < 0.05$ , \*\*  $p < 0.01$ ,  $p < 0.001$ ).

**Figure 4** Cell-specific heterotrophic production (a) and Fe uptake (b) by bacteria (0.2-0.8  $\mu\text{m}$  fraction-size) for the three different treatments (control, +Fe, +Fe+C) and conditions (unfiltered,  $< 20\mu\text{m}$ ,  $< 1\mu\text{m}$ ). Values represent average number of the three biological replicates. Error bars represent the standard deviation of the three biological replicates. Treatments with an asterisk are significantly different from the unfiltered condition (one-way analysis of variance (ANOVA) and post hoc Tukey test; \*  $p < 0.05$ , \*\*  $p < 0.01$ ).

**Figure 5** Contribution of the two size-fraction ( $> 0.8\mu\text{m}$  and 0.2-0.8 $\mu\text{m}$ ) to Fe uptake in incubation for the three conditions (unfiltered, and prefiltered on  $< 20\mu\text{m}$  or  $< 1\mu\text{m}$  seawater) and for the three treatments (control (no addition), +Fe, +Fe+C). Bars and errors bars represent respectively the average and the standard deviation of biological triplicates for the Fe uptake measured following 36h (T1) of incubation in conditions described in the materials and methods section. sw, seawater.

Table 1 List of abbreviations used.

<i>Abbreviation</i>	<i>Explanation</i>
Unfiltered	Raw seawater. Microplankton, pico-nanoplankton and heterotrophic bacteria were incubated together.
<20µm	Seawater prefiltered on 20µm mesh-size. Microplankton was excluded from incubation. Pico-nanoplankton and heterotrophic bacteria were incubated together.
<1µm	Seawater prefiltered on 1µm mesh-size. Heterotrophic bacteria were incubated alone.

Table 2 Specific bacterial production, cell specific bacterial respiration and bacterial growth efficiency at the end on the incubation experiment. \* Bacterial respiration rates have been converted from O<sub>2</sub> into C units using a RQ value of 1. Samples were prefiltered on 1µm and kept in the dark to measure specifically the consumption of O<sub>2</sub> by heterotrophic bacteria. Means values +/- SE of two biological replicates are given for specific BR and BGE. Means values +/- SD of three biological replicates are given for specific BP.

<i>Treatment</i>	<i>Specific BP</i> ( <i>fmol C cell<sup>-1</sup> d<sup>-1</sup></i> )	<i>Specific BR</i> ( <i>fmol C cell<sup>-1</sup> d<sup>-1</sup></i> )*	<i>BGE</i> (%)
Control	0.04 ± 0.02	0.39 ± 0.15	10 ± 0.1
+Fe	0.05 ± 0.01	1.63 ± 0.34	3.2 ± 1.5
+C	0.77 ± 0.21	0.66 ± 0.21	52 ± 15
+Fe+C	0.54 ± 0.10	0.40 ± 0.09	57 ± 10

Table 3 Iron uptake rates, carbon biomass, and C-normalized Fe uptake rates (Fe:C ratio) of photosynthetic cells (cyanobacteria plus pico- and nanoeukaryotes) and heterotrophic bacteria. Values represent the average  $\pm$  standard deviation of the three independent biological replicates for each treatment and condition after 36hours of incubation.

Condition	Treatment	Fe uptake rate ( $\mu\text{molFe L}^{-1} \text{D}^{-1}$ )		C biomass ( $\mu\text{molC}^{-1} \text{L}^{-1}$ )		Fe:C ratio ( $\mu\text{molFe molC}^{-1}$ )	
		Phot. cells	H. bacteria	Phot. cells	H. bacteria	Photo. cells	H. bacteria
Unfiltered	control	103 $\pm$ 5.4 <sup>‡</sup>	52 $\pm$ 16	1.84 $\pm$ 0.34	1.29 $\pm$ 0.19	51 $\pm$ 0.2 <sup>‡</sup>	41 $\pm$ 15
	+Fe	79 $\pm$ 9.3 <sup>‡</sup>	21 $\pm$ 3.6	1.65 $\pm$ 0.23	1.06 $\pm$ 0.12	49 $\pm$ 10 <sup>‡</sup>	20 $\pm$ 4
	+Fe+C	393 $\pm$ 23 <sup>‡</sup>	101 $\pm$ 5.1	1.79 $\pm$ 0.16	2.20 $\pm$ 0.19	231 $\pm$ 24 <sup>‡</sup>	46 $\pm$ 3
<20 $\mu\text{M}$	control	114*	38*	1.44 $\pm$ 0.90	1.54 $\pm$ 0.90	59*	15*
	+Fe	60 $\pm$ 10	15 $\pm$ 2.1	1.86 $\pm$ 0.04	2.34 $\pm$ 0.09	32 $\pm$ 6	6 $\pm$ 1
	+Fe+C	383 $\pm$ 47	181 $\pm$ 21	1.56 $\pm$ 0.28	2.37 $\pm$ 0.67	250 $\pm$ 50	83 $\pm$ 34
<1 $\mu\text{M}$	control		89 $\pm$ 65		2.10 $\pm$ 0.86		39 $\pm$ 20
	+Fe	n/a	40 $\pm$ 3	n/a	2.62 $\pm$ 0.16	n/a	15 $\pm$ 2
	+Fe+C		1258 $\pm$ 136		2.25 $\pm$ 0.55		591 $\pm$ 208

\*only one replicate available

<sup>‡</sup> Calculation were done on the basis that photosynthetic cells for size fraction comprised between 0.8-20 $\mu\text{m}$  represented 80% of Fe uptake (as it was measured they represented 80% of POC in unfiltered condition)

*Table 4 Total Intracellular Fe uptake rates uptake in this study compared to published studies of natural and Fe-fertilized surface waters in the Southern Ocean.*

<i>Experiment location (acronym)</i>	<i>Fe uptake rate (pmolFe L<sup>-1</sup> d<sup>-1</sup>)</i>	<i>References</i>
SAZ waters - SOTS	96-156 847-1258*	This study – unfiltered control This study - <1µm +Fe+C
Kerguelen Plateau (KEOPS)	4.4–6.2	Sarthou et al. (2005)
Kerguelen Plateau (KEOPS2)	19–39.8	Fourquez et al. (2015)
South of Australia (SOIREE)	3.07–11.9	(Bowie et al., 2001)
Southeast of New Zealand (FeCycle)	26.2–101	Strzepek et al. (2005)

\* Heterotrophic bacteria only



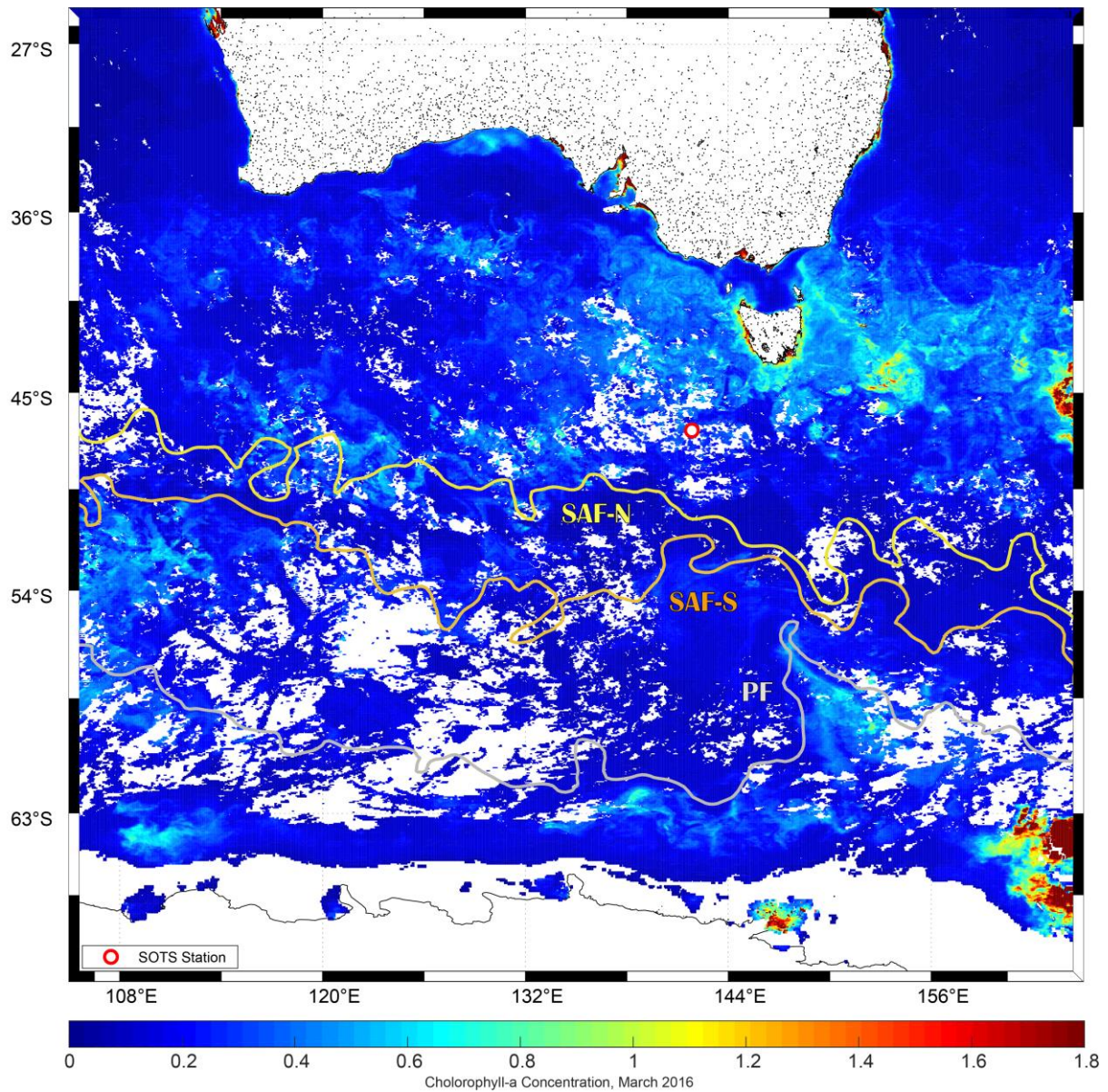


Figure 1

	Conditions	Treatments	Parameters
Experiment (1)	<div style="border: 1px solid black; padding: 2px; display: inline-block;"> <span style="background-color: #cccccc; width: 20px; height: 10px; display: inline-block;"></span> Unfiltered         </div>	X <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-left: 10px;">             ● No addition              ○ +Fe              ○ +C              □ +Fe+C           </div>	<ul style="list-style-type: none"> <li>• Heterotrophic bacterial production (1) + (2)</li> <li>• Bacterial cell abundance (1) + (2)</li> <li>• Bacterial respiration rate (1)</li> <li>• Bacterial Fe uptake rate (1+ 2)</li> <li>• Cell abundance of small photosynthetic cells (cyano, pico and nanoeukaryotes) (2)</li> </ul>
Experiment (2)	<div style="border: 1px solid black; padding: 2px; display: inline-block; margin-right: 10px;"> <span style="background-color: #cccccc; width: 20px; height: 10px; display: inline-block;"></span> Unfiltered  <span style="background-color: #cccccc; width: 20px; height: 10px; display: inline-block; border: 1px dashed black; border-style: dotted;"></span> &lt;20µm  <span style="background-color: #cccccc; width: 20px; height: 10px; display: inline-block; border: 1px dashed black; border-style: dotted;"></span> &lt;1µm         </div>	X <div style="border: 1px solid black; padding: 2px; display: inline-block; margin-left: 10px;">             ● No addition              ○ +Fe              □ +Fe+C           </div>	<ul style="list-style-type: none"> <li>• Fe uptake rate for microplankton (&gt;20µm) and pico-nanoplankton (0.8-20µm) (2)</li> <li>• DFe concentration in incubation bottles (2)</li> </ul>

Figure 2

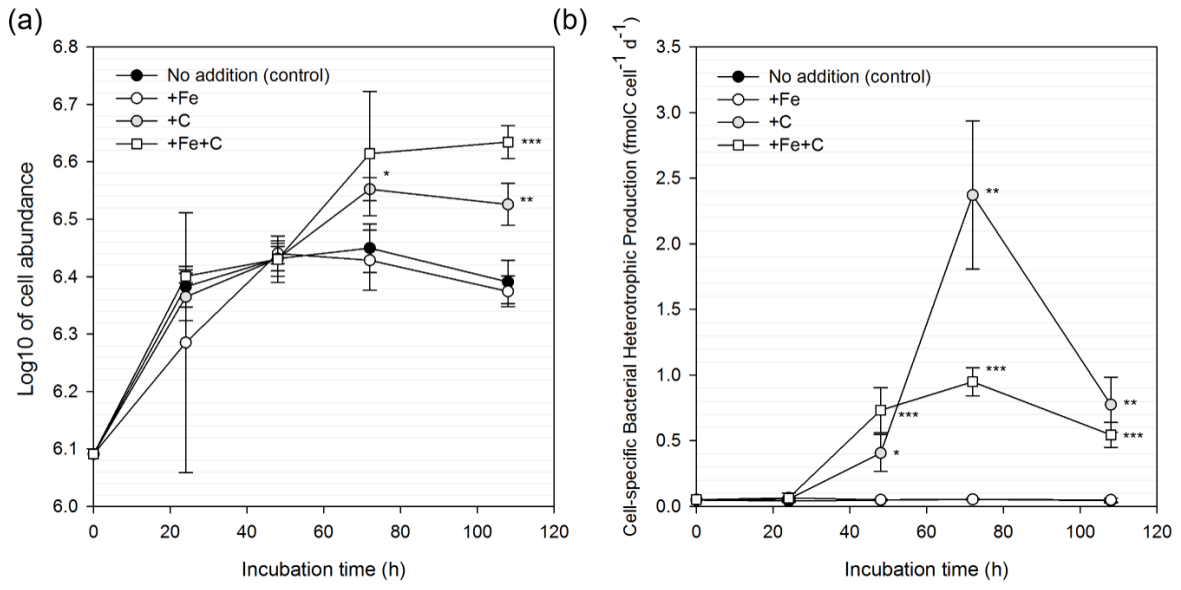


Figure 3

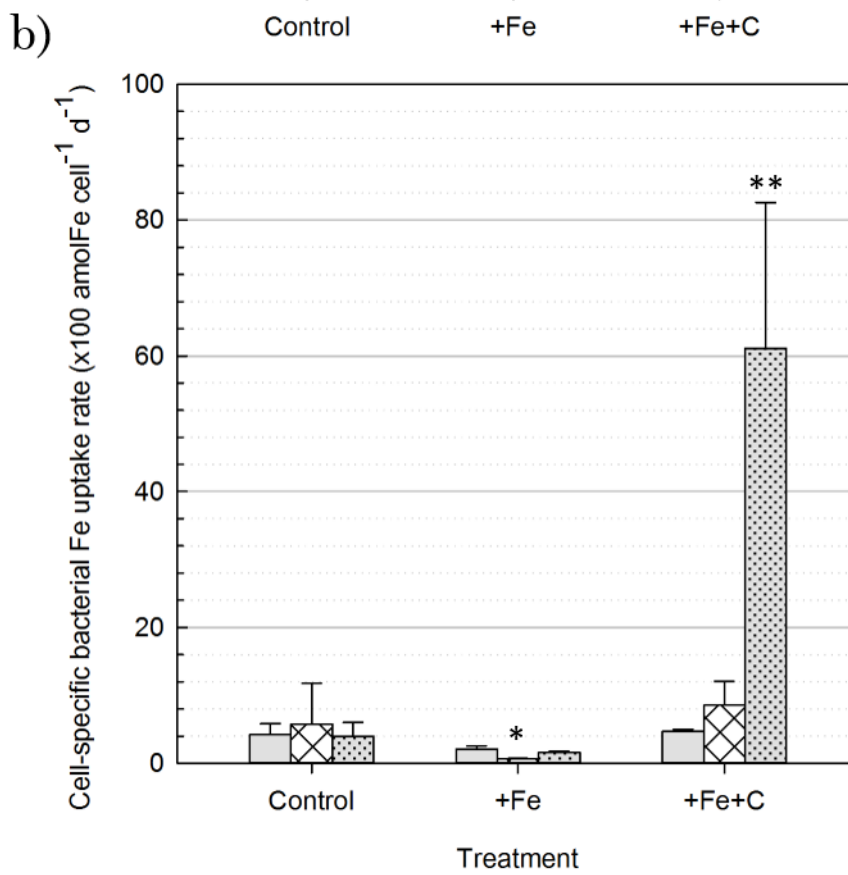
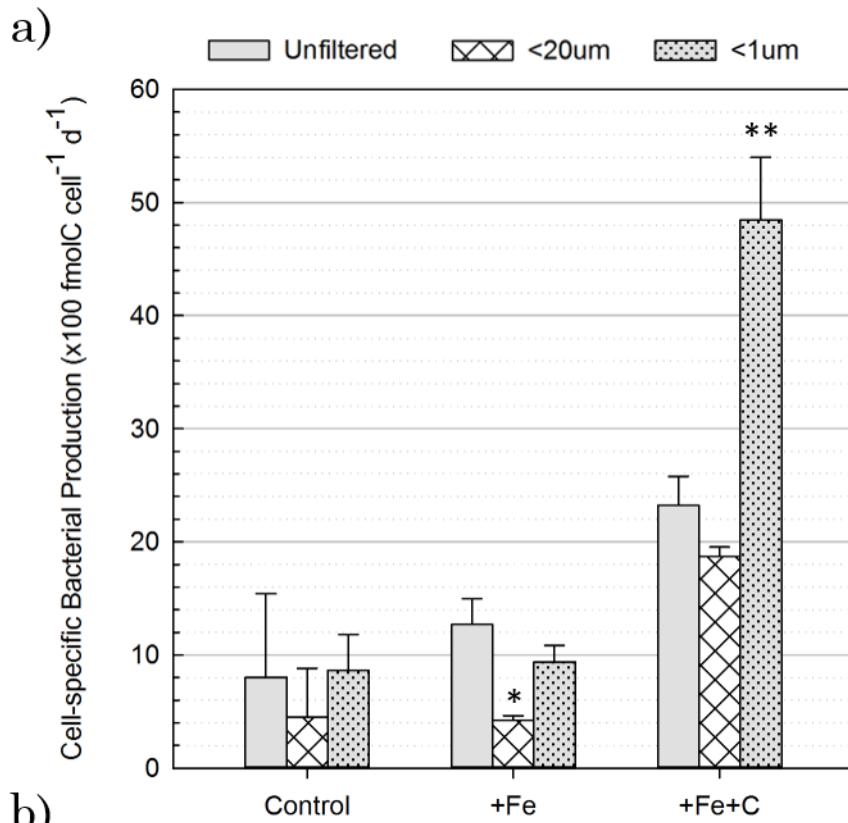


Figure 4

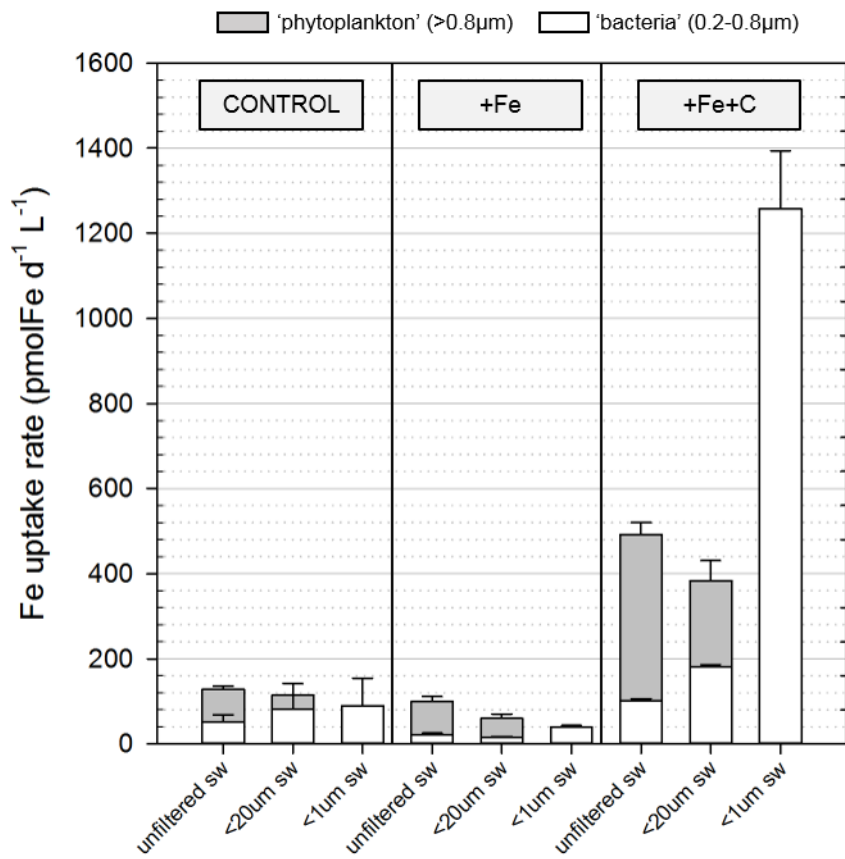
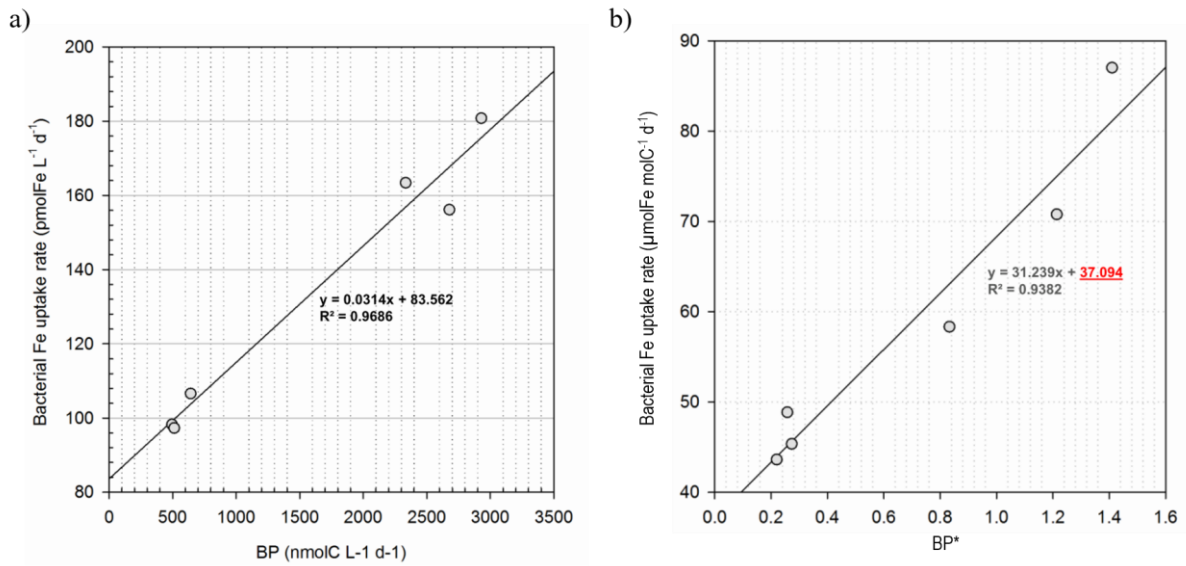
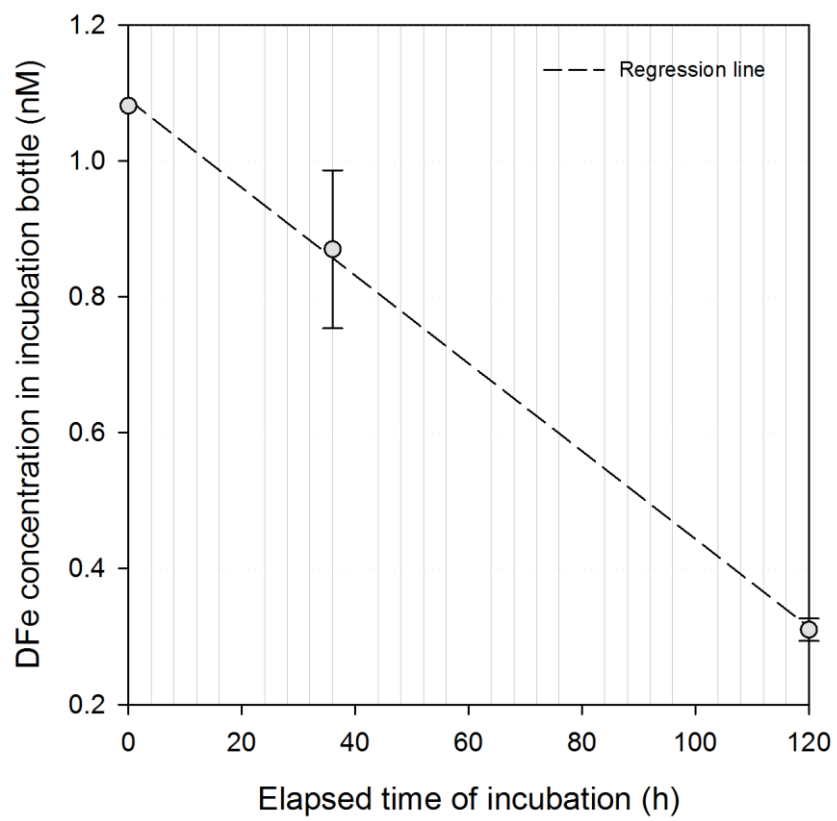


Figure 5



Supplementary Fig 1



Supplementary Fig 2