Microbial iron uptake in the naturally fertilized waters in the vicinity of Kerguelen

Islands: phytoplankton-bacteria interactions

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15   **ABSTRACT**

16   Iron (Fe) uptake by the microbial community and the contribution of three different

17   size-fractions was determined during spring phytoplankton blooms in the naturally Fe

18   fertilized area off Kerguelen Islands (KEOPS2). Total Fe uptake in surface waters was on

19   average 34 ± 6 pmol Fe L^{-1} d^{-1}, and microplankton (>25µm size-fraction; 40-69%) and pico-

20   nanoplankton (0.8-25 µm size-fraction; 29-59%) were the main contributors. The contribution

21   of heterotrophic bacteria (0.2-0.8 µm size-fraction) to total Fe uptake was low at all stations

22   (1-2%). Iron uptake rates normalized to carbon biomass were highest for pico-nanoplankton

23   above the Kerguelen plateau and for microplankton in the downstream plume. We also

24   investigated the potential competition between heterotrophic bacteria and phytoplankton for

25   the access to Fe. Bacterial Fe uptake rates normalized to carbon biomass were highest in

26   incubations with bacteria alone, and dropped in incubations containing other components of

27   the microbial community. Interestingly, the decrease in bacterial Fe uptake rate (up to 26-

28   fold) was most pronounced in incubations containing pico-nanoplankton and bacteria, while

29   the bacterial Fe uptake was only reduced by 2- to 8- fold in incubations containing the whole

30   community (bacteria + pico-nanoplankton + microplankton). In Fe-fertilized waters, the

31   bacterial Fe uptake rates normalized to carbon biomass were positively correlated with

32   primary production. Taken together, these results suggest that heterotrophic bacteria are

33   outcompeted by small sized phytoplankton cells for the access to Fe during the spring bloom

34   development, most likely due to the limitation by organic matter. We conclude that the Fe and

35   carbon cycles are tightly coupled and driven by a complex interplay of competition and

36   synergy between different members of the microbial community.

1. INTRODUCTION

Microorganisms in the ocean are characterized by widespread distributions, large abundances and high metabolic rate activities. Consequently they play a pivotal role in biogeochemical cycles of many elements (Arrigo, 2005; Madsen, 2011). Following the pioneering work of Martin (1990), a major achievement in the past decades has been the discovery of the tight, but complex link between the carbon and iron (Fe) biogeochemical cycles in the ocean. Thus, it is not surprising that microorganisms play a crucial role in the functioning and the coupling of both cycles. Autotrophs are a net carbon dioxide (CO_2) sink and heterotrophs are a net CO_2 source, but both require Fe to process carbon. Therefore, the balance between autotrophy and heterotrophy and ultimately the air-sea CO_2 flux should be influenced by Fe availability for microorganisms. This issue is definitively critical in environments receiving low Fe supply, like the high nutrient low chlorophyll regions (HNLC).

The role of heterotrophic bacteria has been far less studied than that of phytoplankton. However, essential data for the understanding of the responses of heterotrophic bacteria to Fe limitation have already been collected. Iron uptake rates, Fe cellular contents and Fe/carbon ratios were determined in various environments (Tortell et al., 1996; Maldonado et al., 2001; Sarthou et al., 2008). Culture experiments (Granger and Price, 1999, Fourquez et al., 2014) have elucidated some of the metabolic pathways affected by Fe limitation which may explain the changes observed in Fe-limited heterotrophic cells or communities. Additionally, the obligate requirement of Fe for heterotrophic bacteria and phytoplankton suggests that both organisms are competing for Fe acquisition. The competition between phytoplankton and bacteria was addressed experimentally (Mills et al., 2008) and conceptually (Litchman et al., 2004) for the access to nitrogen and phosphorus, but this issue has been rarely studied in the case of Fe (Boyd et al., 2012). Beside this possible pure competition, both autotrophic and

62 heterotrophic microorganisms could also benefit from each other. Phytoplankton are a source

63 of carbon for heterotrophic bacteria and the production of ligands by these latter could make

64 Fe available for other microorganisms (Amin et al., 2009; Hassler et al., 2011a, 2011b). The

65 aim of our study was to investigate further the complex interactions between heterotrophic

66 bacteria and phytoplankton, with respect to the carbon and Fe cycling.

67   The Southern Ocean is the largest HNLC region in the world ocean. However, at

68 several places, natural Fe fertilization sustains massive blooms (Blain et al., 2007; Nielsdóttir

69 et al., 2012; Pollard et al., 2009). These natural fertilized regions are exceptional laboratories

70 to study interactions between the Fe and carbon cycling and the role played by

71 microorganisms. The bloom located above the Kerguelen Plateau was investigated in detail

72 during KEOPS1 (Kerguelen Ocean and Plateau compared Study) (Jan-Feb 2005). KEOPS2

73 (Oct-Nov 2011) extended this study to early stages of the bloom and to new investigations in

74 the blooms downstream the island. During KEOPS2 we have determined the Fe uptake of the

75 bulk microbial community and of different size-fractions at stations characterized by a wide

76 range of responses to Fe fertilization. We have also conducted an incubation experiments to

77 specifically study the competition between heterotrophic bacteria and phytoplankton.

78 **2. MATERIALS AND METHODS**

79 **2.1. Site description**

80   This study was carried out as part of the KEOPS2 expedition that took place from 9

81 October to 29 November 2011, in the Indian sector of the Southern Ocean in the vicinity of

82 the Kerguelen archipelago. For the present study 8 stations were sampled (Fig. 1). Station R-2

83 is the reference station located outside the bloom, west of Kerguelen Island (Fig. 1). The

84 stations E were located in a complex meander south of the Polar Front and sampled in a quasi-

85 Lagrangian manner (d'Ovidio et al., 2015). An animation is given in supplement material that

86 shows the development of the bloom over the period of the cruise and position of the stations

87 at the time of sampling (Supplement 1).

88 **2.2. Sampling and manipulation under trace metal clean conditions**

89 Seawater samples were collected with 10-Liters Niskin 1010X-bottles set up on the

90 autonomous Trace Metal Rosette 1018 especially adapted for trace metal work (General

91 Oceanics Inc., USA; Bowie et al., 2014). Each Niskin bottle was acid-washed (2% HCl) and

92 rinsed with milli-Q water before the rosette was deployed. All metal springs are Teflon coated

93 and the crimps are made of aluminium. All samples were carefully manipulated in a clean

94 container under a laminar flow hood (ISO class 5). Within less than 2 hours after sample

95 collection, the seawater was dispersed into 500 mL acid-washed polycarbonate (PC) bottles

96 and the incubations performed as described below. The PC bottles were acid-washed (10%

97 HCl suprapur, Merck) three times, followed by three rinses with milli-Q-water and they were

98 subsequently sterilized by microwaves (5 minutes, 750W). The PC bottles were dried and

99 stored under a laminar flow hood before being used. For the incubation experiments described

100 below, seawater was collected in the surface mixed layer at one depth, and incubated at

101 different levels of surface photosynthetically active radiation (PAR). Characteristics of the

102 stations are given in Table 1.

103 **2.3. Iron uptake experiments**

104 Three types of incubation experiments were performed (Fig. 2). In one set of

105 experiments, 300 mL of unfiltered seawater were amended with Fe as ^{55}FeCl$_3$ (0.2 nM final

106 concentration of ^{55}Fe, specific activity 1.83 x 10^3 Ci mol^{-1}, Perkin Elmer), incubated for 24h

107 at 75%, 25% and 1% surface PAR, and then sequentially filtered through 0.8 µm and 0.2 µm

108 pore size nitrocellulose filters (47mm diameter, Nuclepore)(Fig. 2a). These incubations,

109 performed at station A3-2, E-4E and E-5, provided measurements of the Fe uptake of the bulk

110 community based on the sum of the radioactivity measured on the 0.8μm and 0.2μm filters.

111 The uptake of Fe by heterotrophic bacteria incubated with the whole community (bacteria +

112 pico-nanoplankton + microplankton) was also determined from these incubations. In a second

113 set of experiments, seawater (300 mL) was pre-filtered through a 25 μm mesh before 0.2 nM

114 ^{55}Fe (final concentration) was added. This porosity was chosen to exclude microplankton by

115 retaining them on the 25 μm mesh. Following incubation at 75, 45, 25, 16, 4, and 1% of

116 surface PAR, the seawater was sequentially filtered through 0.8μm and 0.2 μm filters (Fig.

117 2b). The uptake of Fe by pico-nanoplankton (0.8-25 μm), and that of heterotrophic bacteria

118 (0.2-0.8 μm) in the presence of pico-nanoplankton only was derived from these incubations.

119 At the stations where these two types of experiments were performed concurrently (station

120 A3-2, E-4E and E-5), the Fe uptake by microplankton was obtained by the difference between

121 the bulk Fe uptake (Fig. 2a) and the sum of the Fe uptake by pico-nanoplankton and

122 heterotrophic bacteria (Fig. 2b). In a third set of experiments, 300 mL seawater was 0.8 μm

123 pre-filtered prior to the addition of 0.2 nM ^{55}Fe (final concentration), to exclude both

124 microplankton and pico-nanoplankton from the incubation. Following the 24h incubation at

125 1% PAR level, the seawater was filtered on a 0.2 μm filter (Fig. 2c). Based on this type of

126 incubation, we determined the Fe uptake by heterotrophic bacteria in incubations with

127 bacteria alone. This experiment was performed at stations A3-2, E-4E, E-5, E-4W and R-2.

128 For all the incubations, bottles were maintained at *in situ* surface temperature (Table 1)

129 in on-deck incubators supplied continuously with surface seawater. The incubators were

130 equipped with a combination of Nickel screens (LEE Filters, UK) simulating light intensities

131 from 75% to 1%. Incubations were conducted from dawn to dawn.

132 Additionally, to determine if a steady state has been achieved after 24 hours of

133 incubation time we performed a separate set of experiments where Fe uptake by bacteria and

134 bacterial cell abundance was followed over 24, 72, 96h and one week incubation time. Due to

135 the low bacterial Fe uptake rates determined over 24h, we did not perform any time series

136 over shorter incubation times. Our results are therefore based on the assumption of linearity in

137 bacterial Fe uptakes rates over the 24h incubation period.

2.4. Determination of intracellular ^{55}Fe

139   A first step for the assessment of the ^{55}Fe uptake was the removal of ^{55}Fe not

140 incorporated by cells, using a washing solution. Following filtration, the filters were washed

141 with 6 mL of Ti-citrate-EDTA solution (Hudson and Morel, 1989; Tang and Morel, 2006) for

142 2 minutes and subsequently rinsed 3 times with 5 mL of 0.2 μm filtered seawater for 1 minute

143 (Fourquez et al., 2012). The filters were placed into plastic vials and 10 mL of the scintillation

144 cocktail Filtercount (Perkin Elmer) were added. Vials were agitated for 24 hours before the

145 radioactivity was counted with the Tricarb® scintillation counter. Total radioactivity on filter

146 after correction for background represents intracellular ^{55}Fe. For each station, controls were

147 obtained with 300 mL of microwave-sterilized seawater (750 W for 5 minutes repeated 3

148 times) incubated with the same amount of ^{55}Fe and treated in the same way as the live

149 treatments. The radioactivity determined on these filters was considered as background and it

150 is based on the amount of ^{55}Fe absorbed, but not incorporated by cells. Abiotic adsorption of

151 ^{55}Fe onto cells could be influenced by microwave irradiation if cell structures are altered by

152 the treatment. For technical reasons, we could not use formalin to fix the cells at each station,

153 but we performed a series of tests to compare fixation by formalin and by microwave. The

154 background radioactivity of the formalin-killed seawater was similar to that of the

155 microwave-sterilized seawater, validating our control. We performed one control per station

156 maintained for 24h at 75% PAR in the on-deck incubator. The radioactivity measured on the

157 control filters was subtracted from the respective live treatments in all experiments.

7

158   To determine the most appropriate concentration of the radioisotope to be added,

159   different amounts of ^{55}Fe were tested: 0.2, 0.4, 1 and 2 nM of unchelated ^{55}Fe (as ^{55}FeCl$_3$,

160   final concentrations of ^{55}Fe). We determined that the concentration of 0.2 nM ^{55}Fe was the

161   most appropriate as it minimizes changes in dissolved Fe (DFe) and it still allows detection of

162   the incorporated radioactivity by scintillation counting (for 300 mL of seawater). We also

163   observed that adding more than 0.8 nM of ^{55}Fe (final concentration) stimulates the Fe uptake

164   by microorganisms (pico-nanoplankton and bacteria, data not shown). Using our preferred

165   small addition of 0.2 nM, consumption of ^{55}Fe during our incubations was negligible (1-4% of

166   total ^{55}Fe added), and the consumption of the corresponding total dissolved Fe even smaller.

167   The Fe uptake rate (mol Fe L^{-1} d^{-1}) noted ρFe (all symbols are listed in Table 2) was

168   calculated following the equations:

169    $$\rho Fe = \frac{A \times\ ^{55}Fe\ on\ filter}{t\ x\ V} \tag{1}$$

170   with

171    $$A = \frac{mol\ ^{55}Fe\ added + mol\ DFe\ in\ situ}{mol\ ^{55}Fe\ added} \tag{2}$$

172    $$^{55}Fe\ on\ filter\ = \frac{(cpm\ on\ filter\ sample - cpm\ on\ filter\ control)}{^{55}Fe\ specific\ activity} \times \frac{1}{counting\ efficiency} \tag{3}$$

173   V = volume filtered

174   t = incubation time

175   cpm= counts per minute

176   **2.5. Enumeration of heterotrophic bacteria**

177   Subsamples for cell enumeration were taken at the start and at the end of the

178   incubations. To enumerate heterotrophic bacteria, 2 mL samples were fixed with

179   glutaraldehyde (1% final concentration), incubated for 1h at 4°C, and stored at -80°C until

8

180 processed (Obernosterer et al., 2008). Heterotrophic bacterial cell abundance was counted

181 with the FASCCanto II BD flow cytometer (Becton, Dickinson). Heterotrophic bacterial cells

182 were stained with SYBRGreen I (Marie et al., 1997) and enumerated for 1 minute at a rate of

183 30 µL min^{-1}. The machine drift was tested using calibration beads (3 µm). Specific bacterial

184 growth rates were calculated from the slope of log-linear regression between the start and the

185 end of the incubation.

2.6. Carbon content of different microbial size-fractions

187 The cellular carbon content for heterotrophic bacteria was estimated to be 12.4 fgC per

188 cell as reported by Fukuda et al. (1998). The carbon contents for pico-nanoplankton and

189 microplankton were estimated from particulate organic carbon (POC) measured in surface

190 seawater (< 1000 µm) on 300, 210, 50, 20, 5, and 1 µm pore-size filters (see Trull et al. 2014).

191 We assumed the total carbon biomass (representative of the bulk community) to be the sum of

192 all these fractions plus the estimated carbon biomass for heterotrophic bacteria. For pico-

193 nanoplankton we assumed the sum of the POC concentrations on the 1 and 5µm filters,

194 corresponding to the 1-20 µm size-fraction, to be representative of this community. To obtain

195 the carbon biomass for microplankton we subtracted the POC concentration of the 0.2-20 µm

196 size-fraction of the total carbon biomass.

3. RESULTS

3.1. Bulk iron uptake rates and contribution of different size-fractions

199 The Fe uptake rate (ρFe) for the bulk community, determined from incubations of

200 unfiltered seawater (Fig. 2a), was measured at stations A3-2, E-4E and E-5, and the

201 volumetric and integrated values are presented on Tables 3 and 4, respectively. The

202 integration of ρFe over the euphotic layer reveals highest values at station E-5 (1.74 µmol Fe

203   m^{-2} d^{-1}), decreasing to 1.12 µmol Fe m^{-2} d^{-1} at station A3-2 and to 0.86 µmol Fe m^{-2} d^{-1} at

204   station E-4E (Table 4). At these three stations the contribution of heterotrophic bacteria to

205   total ρFe was less than 2% corresponding to a mean daily integrated uptake of 0.018 ± 0.005

206   µmol m^{-2} d^{-1} (Table 4). The contribution of the two other size-fractions was station-dependent

207   (Fig. 3). At station E-4E microplankton and pico-nanoplankton had almost equal contributions

208   to total integrated ρFe (53% and 46%, respectively). At station A3-2 microplankton and pico-

209   nanoplankton accounted for 40% and 59% of total integrated ρFe, respectively. The

210   contribution of microplankton was the highest at station E-5 (69% of total integrated ρFe),

211   whereas the contribution of pico-nanoplankton was the lowest (29% of total integrated ρFe) at

212   this site.

213   To account for differences in the biomass among stations, we normalized ρFe to the

214   concentration of POC of the microplankton and pico-nanoplankton size-classes and to the

215   estimated cellular carbon content for bacteria, and both ratios are referred to ρFe:POC (Table

216   3). For the bulk community, a trend similar to ρFe was observed, with the highest ρFe:POC at

217   station E-5 (5.3±1.1 µmol Fe d^{-1} mol C^{-1}; n=3, mean ± 1 SD of the three PAR levels),

218   decreasing to 3.0±1.0 and 2.5±0.4 µmol Fe d^{-1} mol C^{-1} (n=3, mean ± 1 SD) at stations A3-2

219   and E-4E, respectively. Because this variability in ρFe:POC could in part reflect differences in

220   ρFe and carbon biomass contribution of organisms, we also considered ρFe:POC for the

221   different size classes. At station E-5 microplankton revealed the highest ρFe:POC ratios

222   (5.25-11.56 µmol Fe d^{-1}mol C^{-1}), while at station A3-2 pico-nanoplankton was highest (4.39-

223   7.03 µmol Fe d^{-1}mol C^{-1}). At station E-5, at 75% of PAR, microplankton revealed the highest

224   ρFe:POC of all observed values. This is driven by the Fe uptake rate because carbon biomass

225   was almost equally partitioned between microplankton (47 % of total carbon biomass) and

226   pico-nanoplankton (44 % of total carbon biomass). Heterotrophic bacterial ρFe:POC was

227   quite homogeneous in incubations at different PAR levels at stations E-4E (0.49 ± 0.04 µmol

228   Fe d^{-1} mol C^{-1}) and E-5 (0.73 ± 0.07 µmol Fe d^{-1} mol C^{-1}), but it presented high variability at

229   station A3-2, ranging from 0.21 to 1.69 µmol Fe L^{-1} d^{-1} mol C^{-1} (Table 3). As expected, due to

230   the low contribution of heterotrophic bacteria to total ρFe, their carbon-normalized ρFe was

231   the lowest among the three size-fractions.

3.2. Heterotrophic bacterial iron uptake in the absence of phytoplankton

233   To investigate whether heterotrophic bacteria compete with other members of the

234   microbial community for the access to Fe, the bacterial Fe uptake rates and the bacterial

235   growth rates were also determined during incubations where microplankton and both

236   microplankton and pico-nanoplankton were excluded (experiments (b) and (c) respectively in

237   Fig. 2). The bacterial Fe uptake rates were denoted $(\rho Fe)_{bact}^{whole}$ if incubated with the whole

238   community, $(\rho Fe)_{bact}^{<25\mu m}$ if incubated with pico-nanoplankton only, while $(\rho Fe)_{bact}^{alone}$ refers to

239   bacterial Fe uptake rates measured when bacteria were incubated alone, with neither micro

240   nor pico-nanoplankton. Incubations without microplankton were performed at 6 different light

241   levels. At any given station, the variability of $(\rho Fe)_{bact}^{<25\mu m}$ determined at different light levels

242   did not exceed a factor of 4 (Table 5). The unique noticeable exception was station E-3 where

243    $(\rho Fe)_{bact}^{<25\mu m}$ was about two orders of magnitude higher at 75% light level. To compare

244    $(\rho Fe)_{bact}^{<25\mu m}$ among stations we integrated over the euphotic layer and the mixed layer depths.

245   The outlier value at E-3 (at 75% PAR level) was not considered for the integration. The

246   lowest depth-integrated values were observed at stations R-2 and E-5 (4.7 nmol Fe m^{-2} d^{-1} at

247   both stations; mean of euphotic and mixed layer integrated fluxes) and the highest values

248   were observed at station E-3 (18.4 nmol Fe m^{-2} d^{-1}). Integrated Fe uptake did not show any

249   clear temporal evolution for the stations at the quasi Lagrangien time series E-2, E-3, E-4E

250   and E-5 (Table 5).

251   The bacterial Fe uptake rate normalized to cellular carbon content was also determined

252   in the incubations where microplankton was excluded (noted $(\rho Fe : POC)_{bact}^{<25\mu m}$ in Table 5).

253   The high value of $(\rho Fe)_{bact}^{<25\mu m}$ measured at E-3 (at 75% PAR level) resulted in a high value

254   of $(\rho Fe : POC)_{bact}^{<25\mu m}$ (21.4 µmol Fe d^{-1} mol C^{-1}) that is considered as an outlier. All other

255   values ranged from 0.06 to 2.94 µmol Fe d^{-1} mol C^{-1}, and they were 2 to 8-fold lower than

256   those in the corresponding incubations with the bulk community $(\rho Fe : POC)_{bact}^{whole}$ (Stations

257   A3-2, E-4E, and E-5). The normalization does not modify our general observation that there

258   was no significant difference in the rates between the different light levels and between the

259   different stations (two-tailed, unpaired Student's t-test, p=0.27). In consideration of this, the

260   values at one given station are now treated as biological replicates.

261   At the three stations A3-2, E-4E and E-5 we compared the bacterial Fe uptake when

262   bacteria were incubated with the whole community $((\rho Fe : POC)_{bact}^{whole})$ with that when

263   incubated with pico-nanoplankton only $((\rho Fe : POC)_{bact}^{<25\mu m})$ and that with bacteria alone

264    $((\rho Fe : POC)_{bact}^{alone}$, Fig. 4). For all stations, we found that bacterial Fe uptake was the highest

265   in the absence of any other larger cells and the lowest when incubated with pico-nanoplankton

266   only, with $(\rho Fe : POC)_{bact}^{alone} > (\rho Fe : POC)_{bact}^{whole} > (\rho Fe : POC)_{bact}^{<25\mu m}$. When bacteria where

267   incubated with the entire microbial community, $(\rho Fe : POC)_{bact}^{whole}$ was 2 to 8 times higher than

268   in the incubations with pico-nanoplankton only $((\rho Fe : POC)_{bact}^{<25\mu m})$, but still lower than when

269   bacteria were incubated alone. Similarly to $(\rho Fe : POC)_{bact}^{alone}$, bacterial growth rates were by 2

270   to 5 times higher when bacteria were incubated alone compared to incubations with pico-

271   nanoplankton only (Fig. 4b).

272   **3.3. Growth rates and iron quota of heterotrophic bacteria**

273   In all the incubation experiments the abundance of heterotrophic bacteria was

274   determined at the beginning and at the end of the incubation period. Assuming an exponential

275   growth during the incubation provided an estimate of the growth rates. The lowest growth rate

276   (0.02 d^{-1}) was determined at the station R-2. For the other stations, the growth rate ranged

277   from 0.12 d^{-1} (E-5) to 0.36 d^{-1} (E-3). We also measured $(\rho Fe{:}POC)_{bact}^{alone}$ after 24, 72, 96 h and

278   after 7 days of incubation. The $(\rho Fe{:}POC)_{bact}^{alone}$ was similar after 24h and 96h of incubation

279   and decreased after one week of incubation (data not shown). This suggests that 24h of

280   incubation provides a measurement of steady state Fe uptake rate. Thus, we derived the Fe

281   quota for heterotrophic bacteria (Q_{Fe}) based on the equation $\rho = \mu Q_{Fe}$ (Fig. 5). The Fe quota of

282   heterotrophic bacteria was 4×10^{-20} mol Fe cell^{-1} for stations R-2, E-5, and E-4W, and 8×10^{-20}

283   mol Fe cell^{-1} for station E-2, F-L, A3-2 and E-3.

284   **4. DISCUSSION**

285   **4.1. The microbial Fe demand**

286   In the vicinity of the Kerguelen Islands, natural Fe fertilization produces many blooms

287   with different dynamics resulting from a combination of hydrodynamic and ecological

288   drivers. These sites provide excellent opportunities to investigate the demand of different

289   members of the microbial community for Fe, and how these members interact. During the

290   project KEOPS2 we visited a variety of early spring blooms located above the Kerguelen

291   plateau and in offshore waters north and south of the Polar Front. We start our discussion by

292   putting our results in the context of previous studies related to Fe uptake by the microbial

293   community in the Southern Ocean.

294   In the early spring bloom located above the Kerguelen Plateau (station A3-2), the total Fe

295   demand, defined here as the steady state Fe uptake rate by the bulk community, was 33.2

296   pmol Fe L^{-1} d^{-1} in surface waters. This Fe demand is more than 6 times higher than that

297   determined during KEOPS1 at the same site during the declining phase of the bloom (5.3 ± 1.2

298   pmol L^{-1} d^{-1} for a mean value of A3-4 and A3-5, 50% of PAR, Sarthou et al., 2008). The Fe

299   demand during KEOPS2 is also higher than that measured during the artificial Fe fertilization

300   experiment SOIREE in the Antarctic zone. At about 13 days following the Fe addition, a

301   time-point which corresponded to the growing phase of the bloom, Bowie et al.,(2001)

302   determined an Fe demand of 11.9 pmol L^{-1} d^{-1} (mean mixed layer). The differences in the Fe

303   demand between these three studies likely do not result from differences in biomass, because

304   POC concentrations in the surface mixed layer were similar between studies (10-12 μM;

305   Bowie et al., 2001; Sarthou et al., 2008; Trull et al., 2014).

306   For the KEOPS expeditions, different stages of the bloom provide a temporal framework

307   to interpret these observations. However, this is not the case for the differences observed

308   between KEOPS2 and SOIREE, which were both sampled during the early phase of a bloom,

309   even if the blooms occurred at different seasons. Besides the seasonal differences, the location

310   of the study could explain the variability in the Fe demand. Finally, the results of FeCycle

311   provide a comparison with the Sub Antarctic zone. The Fe demand determined for the steady-

312   state microbial Fe budget was 26-101 pmol L^{-1} d^{-1}, (Strzepek et al., 2005), thus at the upper

313   bound or higher than during KEOPS2, although carbon biomasses were similar (10.2 μM,

314   average for the mixed layer). From all these comparisons it appears that besides the variability

315   driven by temporal or spatial factors, a plankton-based mechanistic explanation is certainly

316   required for a better understanding of the observed differences.

317   Culture studies (Marchetti et al., 2009; Strzepek and Harrison, 2004; Sunda and

318   Huntsman, 1995, 1997) or molecular approaches (Allen et al., 2008) have shown that there

319   are multiple strategies for phytoplankton to deal with Fe limitation. The consequences are that

320   bulk cell properties like the Fe uptake rate, the intracellular Fe concentration or the elemental

321   Fe:C ratio are species dependent. However, the use of this basic knowledge to interpret field

322   results is not straightforward. This is primarily due to the complexity of the natural

323   phytoplankton community, but it is also obscured by possible regional differences as shown

324   by Strzepek et al. (2012). Southern Ocean phytoplankton species responded to Fe-light

325   acclimation differently than temperate species (Sunda and Huntsman, 1997; Strzepek et al.,

326   2012). In the case of heterotrophic bacteria, culture studies (Armstrong et al., 2004; Fourquez

327   et al., 2014; Granger and Price, 1999) and metagenomic analysis (Hopkinson and Barbeau,

328   2012; Toulza et al., 2012) have also provided foundations for our understanding of the

329   responses of bacteria to Fe limitation but extrapolation to field observations face the same

330   constraints as mentioned for phytoplankton.

331   A step forward to obtain some insight into the role of the community composition is to

332   compare parameters in different size-fractions. In Fe-fertilized systems in the Southern Ocean,

333   the largest size-fraction (> 25 μm), named microplankton, is almost entirely composed of

334   diatoms. In the early spring bloom above the Kerguelen plateau, this fraction contributed 40%

335   of the total Fe uptake. This is substantially lower than during the declining phase of the bloom

336   where 62 % of total Fe uptake was accounted for by microplankton (Sarthou et al. 2008). This

337   decrease in the contribution of microplankton is consistent with the idea that the early phase

338   of the bloom is dominated by a succession of rapidly growing diatoms of different sizes, and

339   that larger slow growing, and silicon limited diatoms accumulate at the end of the season

340   (Quéguiner, 2013). At the onset of the bloom above the plateau, pico-nanoplankton were the

341   main contributor to Fe uptake (69%) and this size-fraction also revealed the highest carbon-

342   normalized Fe uptake rates. This fraction contains mainly small diatoms because non-diatom

343   phytoplankton, as determined by flow cytometry, had a minor contribution to POC in this

344   size-fraction at station A3-2 (7.4±0.4 %, n=6). This suggests that the diatoms belonging to

345   this size class are more competitive than larger cells for the conditions prevailing at this

346   period of the season. The same observation holds for the FeCycle experiment in the Sub-

347   Antarctic where the Fe uptake was dominated by photosynthetic pico-nanoplankton during

348   the early bloom (Strzepek et al., 2005, Boyd and Ellwood, 2010).

349   In addition to ρFe:POC, we have also calculated the Fe:C uptake ratios based on *in situ*

350   primary production measurements (Cavagna et al., 2014). In the Southern Ocean, Fe:C uptake

351   ratios (noted here ρFe:ρC) reported in the literature range from ~5 to 50 µmol Fe mol C^{-1}

352   (Sarthou et al., 2005 and references herein) and can reach up to 100 µmol Fe mol C^{-1}, as it

353   was reported in some artificial Fe fertilizations (Boyd et al., 2000). During KEOPS2, the

354   ρFe:ρC ranged from 3.7 (station A3-2) to 22.9 µmol Fe mol C^{-1} (station E-5, Fig. 6). The

355   values determined for the plateau station A3-2 (3.7-11 µmol Fe mol C^{-1}) are similar to those

356   reported for the declining phase of the bloom during KEOPS1 (5.0±2.6 µmol Fe mol C^{-1},

357   average for stations A3-1, A3-4, and A3-5 ,Sarthou et al., 2008). These ρFe:ρC ratios are also

358   consistent with values measured during the two FeCycle studies where ρFe:ρC were

359   comprised between 5.5 and 19 µmol Fe mol C^{-1}, and did not vary much with depth and over

360   time (King et al., 2012; Strzepek et al., 2005). By contrast, at the stations located downstream

361   of the plateau (E-4E and E-5) the ρFe:ρC values were overall higher than above the plateau

362   (range 10 to 22 µmol Fe mol C^{-1}).

4.2. Phytoplankton- bacteria competition for iron acquisition

364   During KEOPS2, heterotrophic bacteria contributed less than 2% to the total Fe uptake

365   (ρFe). This is similar to the low contribution of heterotrophic bacteria of 1 to 5% to the total

366   ρFe during FeCycle (Strzepek et al., 2005), but contrasts with observations from the subarctic

367   Pacific where heterotrophic bacteria dominated the Fe uptake (20-45%, Tortell et al., 1996).

368   Heterotrophic bacterial Fe uptake was negatively affected by the presence of pico- to

369   microplankton, suggesting competition between these members of the microbial community.

370   Competition for the limiting nutrient is not unexpected, however, this issue has rarely been

371   addressed in previous studies (Boyd et al., 2012). Bacterial and pico-nanoplanktonic cells

372   could compete for nutrients as both have comparable metabolic rates (Massana and Logares,

373 2012), and high capacities for resource acquisition. Our observation of the overall low

374 contribution of heterotrophic bacteria to bulk Fe uptake suggests that not only the access to

375 Fe, but also organic carbon could have limited the bacterial response to natural Fe

376 fertilization. This idea is supported by the relation between the extent of stimulation of

377 bacterial Fe uptake in fertilized waters and the increase in primary production (Fig. 7).

378 The bacterial Fe uptake rates were highest when measured in the absence of any larger

379 cells and lowest in incubations where microplankton was excluded and bacteria were

380 incubated with pico-nanoplankton only (Fig. 4a). This was the case for all stations where the

381 experiment was conducted with $(\rho Fe{:}POC)_{bact}^{alone}$ 5 to 26 times higher than $(\rho Fe{:}POC)_{bact}^{<25\mu m}$,

382 except for the reference station R-2. Considering that a higher degree of Fe limitation should

383 result in an increased cellular Fe uptake rate, raises the question of whether different degrees

384 of Fe limitation of bacteria and pico-nanoplankton could explain the observed pattern. To

385 evaluate the degree of Fe limitation, we compared bacterial and pico-nanoplankton Fe uptake

386 rates (Table 6). Two clear features emerge. First, Fe uptake rates for bacteria

387 $((\rho Fe{:}POC)_{bact}^{alone})$ and pico-nanoplankton $((\rho Fe{:}POC)_{pico-nano})$ are very similar for a given

388 station, suggesting that they experienced comparable degree of Fe limitation before the

389 beginning of the incubation experiment. Second, the bacterial Fe uptake rates when incubated

390 alone $((\rho Fe{:}POC)_{bact}^{alone})$ are higher in fertilized waters than at the HNLC site, suggesting that

391 bacteria are not Fe replete at the fertilized stations. The strong correlation between C-

392 normalized bacterial Fe uptake rates when incubated alone and primary production (n=5,

393 r^2=0.97 and p=0.002, Fig. 7), suggests that carbon availability is the main driver of the Fe

394 uptake potential of heterotrophic bacteria. Interestingly, no such correlation was obtained

395 when bacteria were incubated with pico-nanoplankton only (n=5, r^2= 0.31 and p=0.32). These

396 observations strongly suggest that for the stations located in Fe-fertilized regions,

397 phytoplankton, and in particular pico-nanoplankton, competed with bacteria for Fe

398 acquisition.

399 We propose two non-exclusive explanations for the observed positive correlation between

400 these two parameters. First, the increase in primary production could be driven by an increase

401 in Fe availability that may also benefit heterotrophic bacteria when competition with larger

402 cells is alleviated. Second, the increase in primary production could result in an enhanced

403 amount of phytoplankton-derived DOC, which in turn provides energy to synthesize more

404 iron transport molecules to cope with a certain degree of Fe limitation and also stimulates the

405 bacterial Fe demand. In the absence of microplankton, the supply of phytoplankton-DOM is

406 likely to be lower, which could explain the strong decrease in bacterial Fe uptake rates in

407 these incubations $(\rho Fe : POC)_{bact}^{<25\mu m}$. Both mechanisms are likely to occur, as independent

408 experiments during KEOPS2 revealed that bacterial production was stimulated by both, single

409 additions of Fe and organic carbon (Obernosterer et al., 2014).

410   DOC is undoubtedly one of the most important substrates provided by autotrophic

411 phytoplankton cells to heterotrophic bacteria. The amount of DOC produced by

412 phytoplankton during the bloom is likely to play a role in Fe demand by bacteria. Kirchman et

413 al., (2000) suggested that low Fe availability leads to increase the C demand and more

414 recently, Fourquez et al., (2014) have provided some evidence that marine heterotrophic

415 bacteria reallocate their inner resources to sustain this increase of the C demand when Fe

416 limited. Here, we also show that high C availability leads to an increase in Fe demand. Finally

417 we note that the minimum values of $(\rho Fe : POC)_{bact}^{<25\mu m}$ in comparison to whole community

418 $(\rho Fe : POC)_{bact}^{whole}$ and bacteria-only $(\rho Fe : POC)_{bact}^{alone}$ incubations could arise via other

419 microorganism allelopathic interaction mechanisms than competition for Fe. As such, further

420 research is needed to examine interactions between pico-nanoplankton and bacteria across a

421 wider range of conditions, i.e. including non-limiting Fe and carbon substrate levels.

422 Our observation that small diatoms were particularly competitive in removing Fe during

423 the early stage of the spring phytoplankton bloom induced by natural Fe-fertilization in the

424 Southern Ocean suggests an intimate connection between heterotrophic bacteria and pico-

425 nanoplankton. If this is the case, a progressive shift in the community composition from small

426 to larger diatoms in the course of a bloom (Quéguiner, 2013) would affect the bacterial Fe

427 uptake rates over time. This could partly explain why heterotrophic bacteria accounted for 17-

428 27% of the overall Fe-uptake at the late stage of the spring bloom (Sarthou et al., 2008) in

429 contrast to 1-2% at the onset of the bloom. Together, these results demonstrate that the

430 bacterial Fe and carbon metabolism are closely coupled, and that the structure of the microbial

431 community has a marked effect on the extent of bacterially-mediated Fe cycling.

Figure captions

Figure 1

Map of KEOPS2 study area showing the stations sampled for Fe uptake experiments. Dashed line represents the position of the Polar Front. The base map shows the bathymetry in meters.

Figure 2

Schematic representation of experiments to determine Fe uptake by heterotrophic bacteria (0.2-0.8 μm), pico-nanoplankton (0.8-25 μm) and microplankton (>25 μm) during the KEOPS2 cruise (sw for seawater).

Figure 3

Relative contribution of different size-fractions to total Fe uptake (ρFe). The percent contribution was calculated from Fe uptake fluxes integrated over the euphotic layer at plateau (A3-2) and downstream plume (E-4E and E-5) stations.

Figure 4

Bacterial Fe uptake normalized per carbon biomass (a) and bacterial growth rates (b) in incubations conducted with whole community ($(\rho Fe:POC)_{bact}^{whole}$,, unfiltered seawater), with pico-nanoplankton only($(\rho Fe:POC)_{bact}^{<25\mu m}$, 25 μm prefiltered seawater), and when bacteria were incubated alone ($(\rho Fe:POC)_{bact}^{alone}$, 0.8 μm prefiltered seawater). As no significant effect of light on Fe uptake was observed for any station we consider the values measured at the different levels of PAR as replicates. The bars for unfiltered seawater represent the average ± 1 SD of the three light levels (75%, 25% and 1% of surface PAR). The bars for <25 μm seawater represent the average ± 1 SD of all the light levels (n=6 for stations E-4E, E-5, and E-4W; n=5 for stations A3-2 and R-2).

454 **Figure 5**

455 Relationship between the intracellular bacterial Fe quota and growth rate. Black squares:

456 Station E-4W, E-5 and R-2 stations; regression line r^2=0.99, y=4.8x10^{-14} + 8.9x10^{-15}. Grey

457 circles: Station E-2, E-3, A3-2, and F-L stations; regression line r^2=0.99, y=10x10^{-14} + 1.4x10^{-}

458 ^{15}. Calculations are based on bacterial Fe uptake and growth rates measured when incubated

459 with pico-nanoplankton only.

460 **Figure 6**

461 Comparison between total Fe:C uptake ratios noted ρFe:ρC (black bars) and Fe uptake by the

462 bulk community normalized to carbon biomass noted ρFe:POC (grey bars) at 3 different

463 surface PAR levels at stations A3-2 (plateau), E-4E and E-5 (plume).

464 **Figure 7**

465 Relationship between the C-normalized bacterial Fe uptake($(\rho Fe:POC)_{bact}^{alone}$,) and euphotic

466 zone integrated primary production. The plotted line was obtained by least-square regression

467 (r^2=0.97 with p=0.002). Empty symbol represents the reference station R-2 and filled symbols

468 are for Fe-fertilized stations.

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647

648 **Table 1** Location, date, depth of sampling and main biogeochemical properties from studied stations. Experimental approach column refers to
649 Figure 1 with a, b and c related to incubations including the whole community, pico-nanoplankton plus bacteria, and bacteria only, respectively.

Station	Latitude S	Longitude E	Date of sampling (dd/mm/yyyy)	Depth of sampling (m)	SST (ºC)	$NO_3^- + NO_2^-$ [*] ($\mu mol\ L^{-1}$)	PO_4^{3-} [*] ($\mu mol\ L^{-1}$)	$Si(OH)_4$ [§] ($\mu mol\ L^{-1}$)	Chla ∞ ($\mu g\ L^{-1}$)	DFe [†] ($nmol\ L^{-1}$)	Experimental approach [‡]
HNLC reference											
R-2	-50.3590	66.7170	26/10/2011	40	2.3	25.4	1.81	12.1	0.32	0.09	b, c
Kerguelen plateau											
A3-2	-50.6240	72.0560	17/11/2011	20	2.3	25.2	1.75	18.4	1.6	0.18	a, b, c
Polar Front											
F-L	-48.5320	74.6590	07/11/2011	20	4.3	18.5	0.900	6.45	2.8	0.26	b
Downstream plume											
E-2	-48.5230	72.0770	01/11/2011	20	3.0	26.6	1.74	14.5	0.42	0.08	b
E-3	-48.7020	71.9670	02/11/2011	20	3.1	25.4	1.78	15.1	0.079	0.38	b
E-4W	-48.7650	71.4250	12/11/2011	20	2.7	25.3	1.74	17.5	0.56	0.20	b. c
E-4E	-48.7150	72.5630	13/11/2011	20	3.2	24.3	1.62	12.1	1.3	0.19	a, b, c
E-5	-48.4120	71.9000	19/11/2011	20	3.3	25.0	1.73	11.5	1.1	0.06	a, b, c

650

651 * From Blain et al., 2014

652 § From Closset et al., 2014

653 ∞ From Lasbleiz et al., 2014

654 † From Quéroué et al., 2015

655 ‡ see for details Figure 2 and section 2.3

28

656   **Table 2**

657   List of abbreviations used.

Symbols	Explanation
ρFe	Total iron uptake
ρFe_{bact}^{alone}	Bacterial iron uptake determined in incubations with bacterial cells alone (size-fraction < 0.8μm, Fig. 2c)
$\rho Fe_{bact}^{<25\mu m}$	Bacterial iron uptake determined in incubations with pico- and nanoplankton only (size-fraction < 25μm, Fig. 2b)
ρFe_{bact}^{whole}	Bacterial iron uptake determined in incubations with the whole community (unfiltered seawater, Fig. 2a)
$\rho Fe : POC$	Total iron uptake normalized to particulate organic carbon
$(\rho Fe : POC)_{bact}^{alone}$	Bacterial iron uptake determined in incubations with bacterial cells alone (size-fraction < 0.8μm, Fig. 2c) normalized to particulate organic carbon
$(\rho Fe : POC)_{bact}^{<25\mu m}$	Bacterial iron uptake determined in incubations with pico- and nanoplankton only (size-fraction < 25μm, Fig. 2b), normalized to particulate organic carbon
$(\rho Fe : POC)_{bact}^{whole}$	Bacterial iron uptake determined in incubations with the whole community (unfiltered seawater, Fig. 2a) normalized to particulate organic carbon

658

659 **Table 3** Iron uptake rates (ρFe), carbon biomass (POC), and C-normalized Fe uptake rates (ρFe:POC) of the bulk community and the three size-
660 fractions for incubations conducted at 75, 25 and 1% of the photosynthetically active radiation (PAR, % of surface PAR) on unfiltered seawater
661 (see text and Figure 2a for details).

		Fe uptake rate (pmol Fe L^{-1} d^{-1})			C biomass (µmol C L^{-1})			C-normalized Fe uptake rate (µmol Fe d^{-1}mol C^{-1})		
	PAR	A3-2	E-4E	E-5	A3-2	E-4E	E-5	A3-2	E-4E	E-5
Bulk community (>0.2µm)*	75	33.2	28.1	39.5	10.2	10.1	6.2	3.26	2.78	6.33
	25	19.0	26.5	32.7	10.2	10.4	6.2	1.86	2.56	5.27
	1	39.8	22.6	26.3	10.3	11.1	6.2	3.87	2.03	4.23
Microplankton (>25µm)	75	15.5	13.4	33.7	6.9	5.4	2.9	2.25	2.50	11.56
	25	5.1	13.2	22.4	6.8	5.3	2.9	0.75	2.47	7.68
	1	17.9	13.5	15.3	6.9	5.4	2.9	2.60	2.52	5.25
Pico-nanoplankton (0.8-25µm)	75	17.7	14.3	5.3	3.0	12.0	2.7	5.84	1.19	1.93
	25	13.3	12.8	9.9	3.0	12.0	2.7	4.39	1.07	3.61
	1	21.3	8.8	10.1	3.0	12.1	2.8	7.03	0.73	0.39
Heterotrophic bacteria (0.2-08µm)	75	0.07	0.30	0.46	0.3	0.7	0.6	0.21	0.45	0.80
	25	0.60	0.43	0.41	0.4	0.8	0.6	1.69	0.52	0.73
	1	0.57	0.34	0.39	0.4	0.7	0.6	1.37	0.49	0.66

662 * ρFe:POC for bulk community was calculated as the sum of the iron uptake rates of the three size-fractions divided by the sum of particulate organic carbon of each size-
663 fraction.
664

665 **Table 4** Euphotic layer integrated Fe uptake of the bulk community and three size-fractions.

666 The depth of the euphotic layer is 39m for A3-2, 80m for E-4E and 41m for E-5.

	Euphotic layer integrated Fe uptake (μmol Fe $m^{-2} d^{-1}$)			
Station	Bulk community (>0.2 μm)	Microplankton (> 25μm)	Pico-nanoplankton (0.8-25μm)	Heterotrophic bacteria (0.2-0.8μm)
A3-2	1.12	0.44	0.66	0.019
E-4E	0.86	0.45	0.40	0.013
E-5	1.74	1.21	0.51	0.023

Table 5 Bacterial carbon biomass, bacterial Fe uptake rates, C-normalized Fe uptake rates, and integrated Fe uptake (to the euphotic layer depth, Ze; to the mixed layer depth, MLD; average, avg). Values given in the columns $(POC)_{bact}^{<25\mu m}$, $(\rho Fe)_{bact}^{<25\mu m}$, and $(\rho Fe{:}POC)_{bact}^{<25\mu m}$ are relative to incubations with pico-nanoplankton only. Values given in the columns $(POC)_{bact}^{whole}$, $(\rho Fe)_{bact}^{whole}$, and $(\rho Fe{:}POC)_{bact}^{whole}$ are relative to incubations performed with the bulk community. Integrated values are calculated from incubations in absence of microplankton. *n.d*: no data available. Cell numbers refer to the end of the incubation time (24 h).

Station	PAR level	Cell abundance (x10^5 cells mL^{-1})		Biomass (µg C L^{-1})		Fe uptake rate (pmol Fe L^{-1} d^{-1})		C-normalized Fe uptake rate (µmol Fe d^{-1} mol C^{-1})		Integrated Fe (nmol Fe m^{-2} d^{-1})		
		$(POC)_{bact}^{<25\mu m}$	$(POC)_{bact}^{whole}$	$(POC)_{bact}^{<25\mu m}$	$(POC)_{bact}^{whole}$	$(\rho Fe)_{bact}^{<25\mu m}$	$(\rho Fe)_{bact}^{whole}$	$(\rho Fe{:}POC)_{bact}^{<25\mu m}$	$(\rho Fe{:}POC)_{bact}^{whole}$	Ze	MLD	avg
E-4E	75%	10.82	6.49	13.42	8.05	0.10	0.30	0.09	0.45			
	45%	6.74	*n.d*	8.36	*n.d*	0.19	*n.d*	0.28	*n.d*			
	25%	5.89	7.95	7.30	9.86	0.16	0.43	0.26	0.52	**9.7**	**12.8**	**11.3**
	16%	6.89	*n.d*	8.54	*n.d*	0.40	*n.d*	0.56	*n.d*			
	4%	7.80	*n.d*	9.67	*n.d*	0.23	*n.d*	0.28	*n.d*			
	1%	7.07	6.73	8.77	8.35	0.17	0.34	0.23	0.49			
A3-2	75%	*n.d*	3.50	*n.d*	4.34	0.25	0.07	*n.d*	0.21			
	45%	3.52	*n.d*	4.36	*n.d*	0.19	*n.d*	0.51	*n.d*			
	25%	3.75	3.45	4.65	4.28	0.10	0.60	0.26	1.69	**6.6**	**13.1**	**9.9**
	16%	3.60	*n.d*	4.46	*n.d*	0.11	*n.d*	0.30	*n.d*			
	4%	6.39	*n.d*	7.92	*n.d*	0.18	*n.d*	0.27	*n.d*			
	1%	3.78	4.01	4.69	4.97	0.16	0.57	0.40	1.37			
E-5	75%	5.29	5.59	6.56	6.93	0.05	0.46	0.10	0.80			
	45%	5.55	*n.d*	6.88	*n.d*	0.06	*n.d*	0.10	*n.d*			
	25%	5.18	5.39	6.42	6.68	0.07	0.41	0.13	0.73	**5.2**	**4.2**	**4.7**
	16%	5.45	*n.d*	6.76	*n.d*	0.06	*n.d*	0.11	*n.d*			
	4%	6.66	*n.d*	8.26	*n.d*	0.13	*n.d*	0.19	*n.d*			
	1%	5.18	5.74	6.42	7.12	0.14	0.39	0.27	0.66			
R-2	75%	2.84		3.52		0.07		0.23				
	45%	2.55		3.16		0.04		0.14				
	25%	*n.d*	*n.d*	*n.d*	*n.d*	0.00	*n.d*	n.d	*n.d*	**4.4**	**5.0**	**4.7**
	16%	2.90		3.60		0.25		0.82				
	4%	2.85		3.53		0.05		0.16				
	1%	2.65		3.29		0.05		0.19				

Sample	%											
E-2	75%	4.30		5.33		0.07		0.16				
	45%	4.84		6.00		0.05		0.09				
	25%	5.48	*n.d*	6.80	*n.d*	0.06	*n.d*	0.11	*n.d*	**5.8**	**5.8**	**5.8**
	16%	*n.d*		n.d		0.27		n.d				
	4%	5.68		7.04		0.05		0.09				
	1%	5.32		6.60		*n.d*		0.06				
E-3	75%	6.98		8.66		15.50		21.4				
	45%	5.83		7.23		0.25		0.41				
	25%	7.85	*n.d*	9.73	*n.d*	0.41	*n.d*	0.51	*n.d*	**20.0***	**16.8***	**18.4***
	16%	6.96		8.63		0.25		0.35				
	4%	8.49		10.53		0.32		0.36				
	1%	7.27		9.01		0.29		0.39				
F-L	75%	5.23		6.49		0.84		1.56				
	45%	7.80		9.67		0.36		0.45				
	25%	7.80	*n.d*	9.67	*n.d*	0.58	*n.d*	0.72	*n.d*	**14.0**	**18.4**	**16.2**
	16%	0.82		1.02		0.25		2.94				
	4%	3.82		4.74		0.50		1.26				
	1%	22.36		27.73		0.49		0.21				
E-4W	75%	6.63		8.22		0.17		0.25				
	45%	6.70		8.31		0.21		0.30				
	25%	5.07	*n.d*	6.29	*n.d*	0.71	*n.d*	1.36	*n.d*	**13.8**	**16.6**	**15.2**
	16%	19.40		24.06		0.23		0.11				
	4%	13.90		17.24		0.21		0.15				
	1%	7.75		9.61		0.29		0.35				

* Integrated value measured at 75% was excluded of the calculation.

Table 6 Carbon normalized Fe uptake rates for bacteria and pico-nanoplankton. Columns $(\rho\text{Fe:POC})_{\text{bact}}^{<25\mu m}$ and $(\rho\text{Fe:POC})_{\text{bact}}^{\text{alone}}$ are for bacteria incubated with pico-nanoplankton only and bacteria incubated alone, respectively. The column $(\rho Fe:POC)_{pico-nano}$ stands for pico-nanoplankton. We note that this Fe uptake rate was measured during incubations with bacteria. Because pico-nanoplankton largely outcompeted bacteria, this rate is a good approximation of the Fe uptake rate for pico-nanoplankton incubated alone. Values are from incubations performed at 1% of the PAR level.

ρFe:POC (μmol Fe d⁻¹ mol C⁻¹)

Station	$(\rho\text{Fe:POC})_{\text{bact}}^{<25\mu m}$	$(\rho\text{Fe:POC})_{pico-nano}$	$(\rho\text{Fe:POC})_{\text{bact}}^{\text{alone}}$
A3-2	0.40	7.04	5.17
E4-E	0.23	0.73	1.54
E-5	0.27	3.88	1.43
E4-W	0.35	4.13	9.13
R2	0.19	0.14	0.24

Figure 1

Figure 2

Seawater collection

(a)   **(b)**   **(c)**

25 μm sieving   0.8 μm sieving

unfiltered sw   **<25 μm sw**   **<0.8 μm sw**

APPROACH

Addition of ^{55}Fe and incubation for 24h

0.8 μm filter   0.8 μm filter

0.2 μm filter   0.2 μm filter   0.2 μm filter

(a)   (b)   (c)

RESULTS

Fe uptake by heterotrophic bacteria   Fe uptake by heterotrophic bacteria   Fe uptake by heterotrophic bacteria
in presence of larger cells (>0.8 μm)   in absence of microplankton   in absence of any larger cells

Fe uptake by bulk community   Fe uptake by pico-nanoplankton
=heterotrophic bacteria + >0.8 μm
size fraction

(a) - (b)

Fe uptake by microplankton
= bulk community - (pico-nanoplankton + heterotrophic bacteria)

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7