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Fe and C co-limitation of heterotrophic bacteria in the naturally fertilized region off the Kerguelen Islands

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Abstract. It has been univocally shown that iron (Fe) is the primary limiting nutrient for phytoplankton metabolism in high-nutrient, low-chlorophyll (HNLC) waters, yet the question of how this trace metal affects heterotrophic microbial activity is far less understood. We investigated the role of Fe for bacterial heterotrophic production and growth at three contrasting sites in the naturally Fe-fertilized region east of the Kerguelen Islands and at one site in HNLC waters during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study 2) cruise in spring 2011. We performed dark incubations of natural microbial communities amended either with iron (Fe, as FeCl₃) or carbon (C, as trace-metal clean glucose), or a combination of both, and followed bacterial abundance and heterotrophic production for up to 7 days. Our results show that single and combined additions of Fe and C stimulated bulk and cell-specific bacterial production at the Fe-fertilized sites, while in HNLC waters only combined additions resulted in significant increases in these parameters. Bacterial abundance was enhanced in two out of the three experiments performed in Fe-fertilized waters but did not respond to Fe or C additions in HNLC waters. Our results provide evidence that both Fe and C are present at limiting concentrations for bacterial heterotrophic activity in the naturally fertilized region off the Kerguelen Islands in spring, while bacteria were co-limited by these elements in HNLC waters. These results shed new light on the role of Fe in bacterial heterotrophic metabolism in regions of the Southern Ocean that receive variable Fe inputs.

1 Introduction

Iron (Fe) is an essential element for biological activity but is present at trace amounts in the surface ocean. The role of Fe as a limiting nutrient has been extensively studied in high-nutrient, low-chlorophyll (HNLC) regions, with a focus on phytoplankton productivity and growth. Mesoscale fertilization experiments (see review by Boyd et al., 2007) and investigations in naturally Fe-fertilized regions (Blain et al., 2007; Pollard et al., 2009) have conclusively shown that Fe controls primary productivity and the drawdown of carbon dioxide (CO₂) in large areas of the global ocean. Phytoplankton primary production is intimately linked to heterotrophic bacterial activity in different ways. First, heterotrophic bacteria are potential competitors for the access to limiting nutrients, such as Fe, in the HNLC ocean (Tortell et al., 1996; Maldonado and Price, 1999; Schmidt and Hutchins, 1999; Boyd et al., 2012), and second, bacteria remineralize a substantial fraction of phytoplankton-derived dissolved organic matter (DOM; Ducklow, 2000). Through these processes bacteria contribute to the extent and fate of primary production. However, to date, only few studies have attempted to assess the effects of Fe limitation on heterotrophic bacteria and the potential consequences on the tight coupling between production and remineralization of organic matter.

Heterotrophic bacteria responded to variable extents to Fe addition in mesoscale fertilization experiments and in natural fertilization studies (for an overview see Christaki et al., 2014). Whether the increase in bacterial abundance and production in the Fe-fertilized patches was induced directly by

Table 1. Date, location and environmental characteristics of the study sites. Values are mean \pm SD of the mixed layer (ML).

Station	R2	E-4W	E-3	E-5
Date	27 Oct	11 Nov	3 Nov	19 Nov
Latitude S	50.3834	48.7503	48.7000	48.4002
Longitude E	66.6834	71.4168	71.9666	71.8836
Temperature ML ($^{\circ}$ C)	2.1	2.5	3.1	3.2
Z_{ML} (m)	105 \pm 15	61 \pm 11	38 \pm 9	46 \pm 13
DFe (nM) ^a	0.13 \pm 0.05	0.17 \pm 0.03	0.35 \pm 0.05	0.06 \pm 0.01
DOC (μ M)	48 \pm 1	49 \pm 1	49 \pm 1	48 \pm 1
Chl <i>a</i> (μ g L ⁻¹) ^b	0.3 \pm 0.1	1.3 \pm 0.1	0.6 \pm 0.1	1.2 \pm 0.1
Bacterial abundance ($\times 10^8$ cells L ⁻¹) ^c	2.7 \pm 0.3	6.0 \pm 0.1	5.1	4.6 \pm 0.1
Bacterial heterotrophic production (ng C L ⁻¹ h ⁻¹) ^c	2.6 \pm 1	29 \pm 4	25 \pm 2	27 \pm 1

^a From Qu  rou   et al. (2015); ^b from Lasbleiz et al. (2014); ^c from Christaki et al. (2014).

Fe or indirectly by the enhanced DOM production by phytoplankton, or by combination of both, was difficult to conclude from these observations. Only a few studies have examined the potential role of Fe as a limiting factor for heterotrophic bacteria in dark incubations, with those studies reporting contrasting results. While Fe addition alone did not lead to enhanced bacterial production and growth in HNLC areas such as the coastal Californian and subarctic Pacific (Kirchman et al., 2000; Agawin et al., 2006), in different frontal zones south of Tasmania (Church et al., 2000) and south of the polar front in the Indian sector of the Southern Ocean (Jain et al., 2015), bacterial activity increased upon Fe addition in the Gerlache Strait (Pakulski et al., 1996) and the Ross Sea (Bertrand et al., 2011). Heterotrophic bacterial abundance revealed only a minor response to Fe amendments in incubations performed within the Fe-enriched patch during a mesoscale fertilization experiment in the Pacific Ocean (Agawin et al., 2006). The variable responses of heterotrophic bacteria to additions of Fe compared to phytoplankton in different oceanic environments suggest a more complex interplay between Fe and bacterial metabolism, which could in part be driven by the availability of DOM.

The Kerguelen Ocean and Plateau compared Study 2 (KEOPS2) provided access to naturally Fe-fertilized sites above the Kerguelen Plateau and in offshore waters south and north of the polar front, each with distinct hydrodynamic and geochemical properties (Park et al., 2014). As a consequence, concentrations of dissolved iron (DFe; Qu  rou   et al., 2015), the extent and age of the phytoplankton blooms induced by Fe fertilization (D'Ovidio et al., 2015), and the bacterial responses (Christaki et al., 2014) were variable across sites. The objective of the present study was to examine the role of Fe and C as limiting elements for bacterial heterotrophic activity to better understand the bacterial response to Fe fertilization of the Southern Ocean.

2 Material and methods

2.1 Experimental design

The effect of iron (Fe) and organic carbon (C) additions on bacterial heterotrophic production and growth was determined at three stations located in the naturally Fe-fertilized region east of the Kerguelen Islands (stations E-4W, E-3 and E-5) and at the reference station, R2, in HNLC waters west of the Kerguelen Islands (Table 1; a map of the study region is provided in Blain et al., 2015). At all stations, seawater was collected with 10 L Teflon-lined Niskin-1010X bottles mounted on a 1018 rosette system adapted for trace-metal clean work (Bowie et al., 2014). Sampling depths (surface mixed layer) were 40 m at stations R2 and E-4W, 37 m at station E-3 and 25 m at station E-5. The Niskin bottles were transferred to a trace-metal clean container, where 2 L polycarbonate (PC) bottles were filled with unfiltered seawater. The 2 L PC bottles were transferred to a trace-metal clean lab, and 300 mL of seawater was dispensed into twelve 500 mL PC bottles. All PC bottles were soaked in HCl (10 %) and thoroughly rinsed with Milli-Q water before use. Besides the control, which consisted of unamended seawater, the following three treatments were prepared: seawater + Fe, seawater + C, and seawater + Fe + C. Triplicate incubations were done for all treatments and the control. Iron was added as FeCl₃ (final concentration 1 nM of FeCl₃), and C was added as trace-metal clean glucose (final concentration 10 μ M of glucose). To eliminate trace-metal contamination, the working solution of glucose was passed over a Chelex 100 ion exchange resin (Bio-Rad, 200–400 mesh). The incubations were done in the dark in a temperature-controlled lab at in situ temperature (Table 1). For subsampling, incubation bottles were transferred to the trace-metal clean lab and opened under a laminar flow hood (ISO class 5). Subsamples for bacterial abundance and production were taken at day 0 (T_0), day 2 (T_2), day 4–5 (T_4) at all sites, as well as at day 7 (T_7) at station R2.

2.2 Enumeration of heterotrophic bacteria

For bacterial abundance, 1.8 mL subsamples were fixed with formaldehyde (2 % final concentration), stored in the dark for 20 min and then shock-frozen in liquid nitrogen. The samples were stored at -80°C until analyses by flow cytometry. Counts were made using a FACSCalibur flow cytometer (BD20 Biosciences) equipped with an air-cooled laser, providing 15 mW at 488 nm with the standard filter setup. Heterotrophic bacteria were stained with SYBR Green I, and determined by flow cytometry as described in detail in Obernosterer et al. (2008).

2.3 Bacterial heterotrophic production

Bacterial production was estimated by [^3H] leucine incorporation applying the centrifugation method (Smith and Azam, 1992) as described in Obernosterer et al. (2008). Briefly, 1.5 mL samples were incubated with a mixture of [3,4,5- $^3\text{H}(\text{N})$] leucine (Perkin Elmer, 144 Ci mmol $^{-1}$; 7 nM final concentration) and nonradioactive leucine (13 nM final concentration). Controls were fixed with trichloroacetic acid (TCA; Sigma) at a final concentration of 5 %. Samples were incubated for 2–3 h under the same conditions as the cultures as described above. Incubations were terminated with TCA (5 % final concentration). The radioactivity incorporated into bacterial cells was measured aboard the R/V *Marion Dufresne* in a Tricarb[®] scintillation counter.

2.4 Dissolved organic carbon analyses

In situ samples for dissolved organic carbon (DOC) analyses were taken with Teflon-lined Niskin-1010X bottles adapted for trace-metal clean work (Bowie et al., 2014). Seawater was filtered through two combusted GF/F (0.7 μm nominal pore size, Whatman) filters and 15 mL of the filtrate was stored acidified (H_3PO_4 , final pH = 2) in combusted 20 mL glass ampoules at room temperature in the dark. DOC concentrations were measured on a Shimadzu TOC-VCP analyzer with a Pt catalyst at 680 $^{\circ}\text{C}$ (Benner and Strom, 1993) as detailed in Tremblay et al. (2015).

2.5 Statistical analyses

All statistical comparisons between unamended (control) and amended treatments were performed using one-way analysis of variance (ANOVA) and a post hoc Tukey test. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 Environmental setting of the study sites

The offshore waters east of the Kerguelen Islands represent a region with intense mesoscale activity (Park et al., 2014) that is reflected in variable physical and biological characteristics

of the stations chosen for the present study (Table 1). The three stations within Fe-fertilized waters, all located south of the polar front, revealed considerable variability in concentrations of DFe (up to 5.8-fold in the mixed layer; Quéroué et al., 2015) and of chlorophyll *a* (up to 2.2-fold; Lasbleiz et al., 2014), while bacterial abundance and heterotrophic production were more similar among these sites (Christaki et al., 2014). The reference site, R2, in HNLC waters is located west of the Kerguelen Islands, and concentrations of DFe, Chl *a*, bacterial abundance and production were substantially lower than those in surface waters of the Fe-fertilized stations (Table 1). Concentrations of major inorganic nutrients were present in excess ($> 19 \mu\text{M}$ of nitrate plus nitrite and $> 1 \mu\text{M}$ of phosphate; Blain et al., 2015). Among the parameters of interest to the present study, only concentrations of DOC did not differ between fertilized and non-fertilized sites. This is most likely due to the rapid consumption of phytoplankton-derived DOM in Fe-fertilized waters, as reflected in the marked enhancement of bacterial heterotrophic production (by up to a factor of 11; Table 1).

3.2 Bacterial responses to Fe and C additions

At the HNLC site R2, single additions of Fe and C did not result in a significant enhancement of bulk and cell-specific production, and the combined addition of Fe and C significantly enhanced bulk and cell-specific production (1.9-fold) ($p < 0.05$). By contrast, bacterial abundance was not significantly different in any of the amended treatments compared to the control (Fig. 1a–c). Single and combined additions of Fe and C significantly stimulated bulk and cell-specific production at all fertilized stations, but the temporal patterns and the extent of stimulation varied among experiments. At station E-4W, where Chl *a* and in situ bacterial abundance and production were highest for the stations investigated here, the combined addition of Fe and C rapidly stimulated bulk and cell-specific production at T_2 (1.5-fold), and this enhancement was maintained at T_4 (1.6-fold) (Fig. 1e, f). Additions of Fe and C alone resulted in enhanced bacterial abundance and bacterial production only at T_4 (1.2- to 1.7-fold). Stations E-3 and E-5 have a close geographical position and were sampled in a quasi-Lagrangian manner at a 17-day time interval. The pattern observed at these two stations was strikingly similar, but the extent of the response decreased from E-3 (Fig. 1g–i) to E-5 (Fig. 1j–l). A pronounced response in bulk and cell-specific production to single and combined additions was observed at T_2 (1.7–2.2-fold at station E-3 and 1.3–1.5-fold at station E-5). At the end of the experiment, the enhancement of these parameters was detectable in the C and the combined C and Fe additions but not in the Fe-amended treatments.

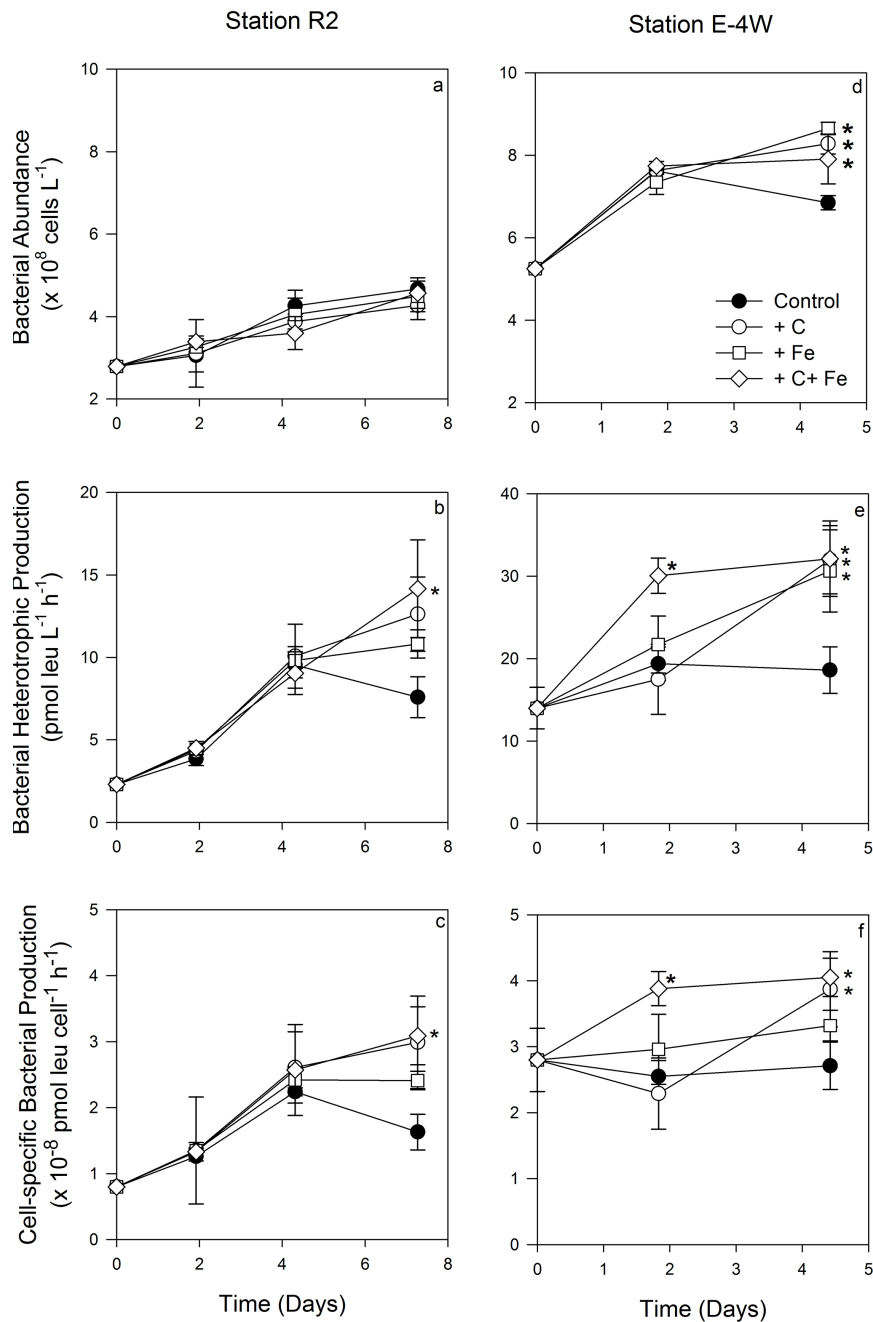


Figure 1.

4 Discussion

4.1 Resource co-limitation

The concept of resource limitation has shifted over the past decades from the theory that a single nutrient limits growth at a given time (Liebig's law of the minimum) to the recognition that co-limitation by multiple resources frequently occurs in the ocean (Arrigo, 2005; Saito et al., 2008; Harpole et al., 2011). Based on theoretical considerations, different

types of nutrient co-limitation of phytoplankton were proposed (Arrigo, 2005; Saito et al., 2008). Here we briefly refer to two types of resource co-limitation: in one case either two or more nutrients are present at concentrations too low to meet the microbial requirements, and in the second case the enhanced concentrations of one limiting resource may facilitate the uptake of another resource (Arrigo, 2005; Saito et al., 2008). In addition, microbial taxa could each be limited by different nutrients due to their specific strategies to access a

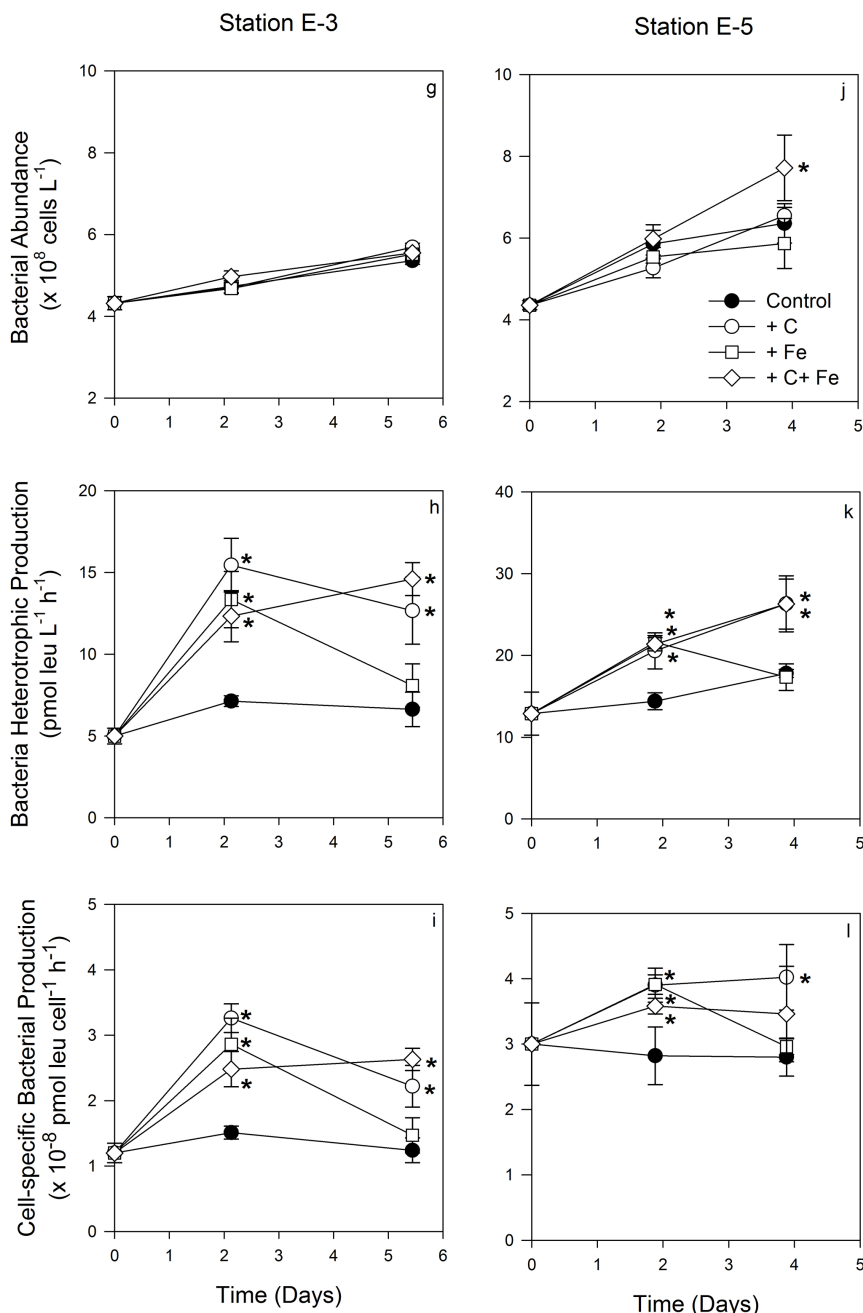


Figure 1. Changes in bacterial abundance (upper panels), bacterial heterotrophic production (middle panels) and cell-specific bacterial production (lower panels) in the control, C-amended, Fe-amended, and both C- and Fe-amended treatments over time. Treatments with an asterisk are significantly different from the control (one-way analysis of variance (ANOVA) and post hoc Tukey; $p < 0.05$).

limiting resource, and this feature could further add to the observation of co-limitation of a diverse microbial community (Sebastián and Gasol, 2013).

How do these concepts apply to the possible co-limitation of heterotrophic bacteria by Fe and C, as suggested previously (Tortell et al., 1996, 1999)? Despite the good performance of chemical analytical methods, the bulk concentrations of DFe and DOC do not provide information on the

biologically available fractions, and therefore it is not possible to determine the limiting concentration of these resources based on in situ concentrations. A simple comparison between the bacterial Fe quota in Fe-limited cultures ($9 \mu\text{mol Fe mol C}^{-1}$; Tortell et al., 1996) and the KEOPS study region ($4\text{--}8 \mu\text{mol Fe mol C}^{-1}$; Fourquez et al., 2015) and the DFe:DOC ratio (range $3\text{--}7 \mu\text{mol DFe mol DOC}^{-1}$; Table 1) indicates similar cellular and in situ molar ratios,

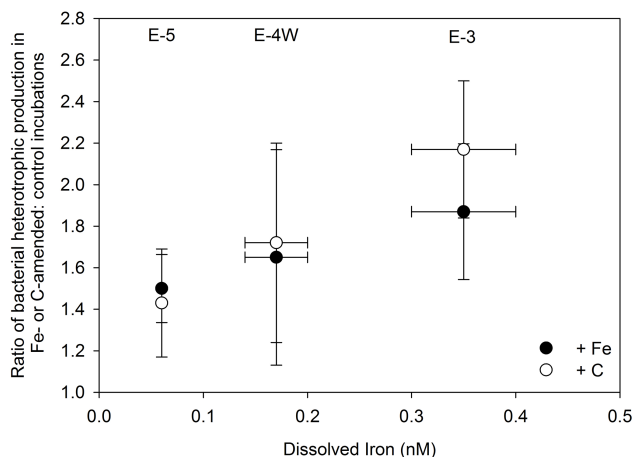


Figure 2. Extent of stimulation of bacterial heterotrophic production by Fe (+Fe) or C (+C) addition and in situ dissolved iron (DFe) concentrations. Ratios of bacterial production in the Fe- or C-amended treatments to the controls correspond to the time points when significant differences were detected for the first time in the cultures (see Fig. 1), and the error bars denote the cumulated error of the bacterial production measurements in the control and the Fe- or C-amended treatments, respectively. For DFe, mean values \pm SD of the surface mixed layer are given (see Table 1).

and thus suggests the potential of both elements to become limiting. The concurrent requirement of DFe by bacteria and phytoplankton could lead to competition for this nutrient between heterotrophic and autotrophic members of the microbial community. Incubation experiments performed during KEOPS2 have indeed shown that the bacterial iron uptake was negatively affected by the presence of phytoplankton (Fourquez et al., 2015). Even though DOC is present in the micro-molar range, the fraction of this bulk DOC that is biologically available is much smaller, in particular under non-bloom conditions in the Southern Ocean. This is due to the permanent upwelling that transports highly refractory DOM from the deep ocean to the surface. As a consequence of the upwelling and the concurrent low phytoplankton primary production, DOC concentrations in the Southern Ocean are the lowest in surface waters of the global ocean (Hansell, 2013). These basic considerations help in understanding the positive response of heterotrophic bacteria to the addition of biologically labile forms of both Fe and C.

4.2 Linking the extent of stimulation to in situ bacterial Fe uptake

To explore the variable extent of stimulation of bacterial production by single Fe and C additions among stations, we determined the respective ratio of bulk and cell-specific bacterial production in the Fe- or C-amended treatments to the controls for the time points when significant differences were detected for the first time in the cultures. This was the case after 2 days of incubation at stations E-3 and E-5 and after

4 days of incubation at station E-4W. The rationale behind doing this is that it takes into consideration the differences in the time lag of the bacterial communities to respond to Fe or C additions at the different sites. We consider these different temporal dynamics of the microbial community part of the response to the question of whether and to what extent they are C- or Fe-limited. Interestingly, the extent of stimulation of bacterial heterotrophic production by single additions of Fe and C revealed an increasing trend with in situ DFe concentrations (Fig. 2). A similar trend was observed for cell-specific production rates (data not shown). No such tendency was observed with in situ chlorophyll *a* concentrations or bacterial abundance or production.

This trend could point to the close coupling between C and Fe for bacterial heterotrophic metabolism, and it could support the idea that the addition of either of these elements facilitates the utilization of the other limiting element (Tortell et al., 1999; Arrigo, 2005; Saito et al., 2008). The response to single C amendment increased at higher in situ DFe concentrations, which could indicate that a larger fraction of the added glucose can be utilized under these conditions. The enhanced response to single Fe addition at higher in situ DFe concentrations could indicate the processing of a larger fraction of the DOM present in situ or the utilization of DOM with a higher efficiency. A synergistic effect, such as the increase in Fe bioavailability by compounds released by phytoplankton, could also be considered (Hassler et al., 2011). Within the bacterial cell, the Fe and C cycles are tightly linked in the TCA cycle and the electron transport system of the respiratory chain, as these key pathways of the cellular carbon metabolism harbor several Fe-containing enzymes. The limitation by Fe can therefore affect the quantity of organic compounds processed by the bacterial cell, and likely also the bacterial growth efficiency (Tortell et al., 1996). Indeed, reduced bacterial growth and respiration under Fe-limited conditions were recently associated with the changes in the expression of Fe-containing enzymes and with the induction of the glyoxylate shunt, a bypass of the TCA cycle, which has important consequences on the fate of organic carbon processed by the bacterial cell (Fourquez et al., 2014; Beier et al., 2015).

The complexity of natural sources of Fe and organic matter and the large differences in concentrations in the ocean require specific metabolic properties such as siderophore production and high-affinity uptake systems for Fe, and enzyme machineries for the cleavage of complex organic carbon compounds. These metabolic capabilities were shown to be associated with specific taxa (Cottrell and Kirchman, 2000; Bauer et al., 2006; Eldridge et al., 2007; Toulza et al., 2012). Thus, it is conceivable to argue that the extent of limitation depends on the metabolic capabilities of the members within the community, and that the bulk response to either addition might be driven by specific taxa.

It was, however, surprising to note that the combined addition of Fe and C did not stimulate bacterial heterotrophic

Table 2. Results from Fe- and C-enrichment experiments in the Southern Ocean and the Pacific Ocean.

Region	Treatment			Experiment description	Study
	+Fe	+C	+Fe +C		
Dark incubations					
Southern Ocean					
Naturally Fe-fertilized region off Kerguelen Islands	+	+	+	Whole seawater, 1 nM Fe, 10 µM glucose, BA, Leu	Present study
Polar front	–	nd	nd	Whole seawater 10 nM Fe, BA	Jain et al. (2015)
Ross Sea	+	nd	nd	<0.65 µm size fraction 1 nM Fe, BA, Leu	Bertrand et al. (2011)
Subtropical front	–	+	+	Whole seawater 2.5 nM Fe	Church et al. (2000)
Subantarctic Zone	–	+	+	1–10 µM glucose BA, Leu, Tdr	
Subantarctic Front	–	+	+		
Antarctic Polar Front	–	–	–		
Gerlache Strait	+	nd	nd	<0.8 µm size fraction 3.8 nM Fe, BA, Leu	Pakulski et al. (1996)
Pacific Ocean					
Subarctic Pacific SERIES				<1 µm size fraction, 4, 20, 40 nM Fe 10 µM glucose	Agawin et al. (2006)
OUT patch	–	–	–	10 µM glutamic acid, BA	
IN patch day 2	–	+	±		
IN patch day 8	–	+	+		
IN patch day 12	–	–	–		
IN patch day 17	+	+	+		
Coastal California				Whole seawater	Kirchman et al. (2000)
1.1 nM Fe in situ	–	+	+	2 nM Fe, 1 µM glucose	
0.2 nM Fe in situ	–	–	+	BA, Leu	
0.06 nM Fe in situ	–	–	+		
Light incubations					
Southern Ocean					
Polar front	+	nd	nd	Whole seawater, artificial light; 16 : 8 h light : dark cycle 10 nM Fe, BA	Jain et al. (2015)
Ross Sea	+	nd	nd	Whole seawater 20 % surface irradiance 1 nM Fe, BA	Bertrand et al. (2011)
Subantarctic	+	nd	nd	Whole seawater 40 % surface irradiance 1.9 nM Fe, Leu	Hutchins et al. (2001)
Naturally Fe-fertilized region off Kerguelen Islands	+	nd	nd	Whole seawater 50 % surface irradiance 1 nM Fe, BA, Leu	Obernosterer et al. (2008)
Pacific Ocean					
South Pacific	+	–	+	Whole seawater 40 % surface irradiance	Kuparinen et al. (2011)
OUT patch				4.9 nM Fe, 6 µM sucrose, Tdr	
SAGE experiment					
Subarctic Pacific	+	nd	nd	Whole seawater 40 % surface irradiance 3.2 nM Fe, BA, Leu	Hutchins et al. (2001)
Californian coastal upwelling	+	nd	nd	Whole seawater 40 % surface irradiance 2.5 nM Fe, BA, Leu	Hutchins et al. (2001)
Equatorial Pacific Ocean transect	+	nd	nd	Whole seawater On-deck incubation 1 nM Fe, BA, Leu	Price et al. (1994)

A positive or negative response of heterotrophic bacteria is indicated by “+” and “–” symbols, respectively; nd – not determined; final concentrations of added Fe and organic carbon source in the incubation experiments are given; Fe was added as FeCl₃; BA refers to bacterial abundance; Leu and Tdr refer to ³[H] leucine and ³[H] thymidine as tracers for bacterial heterotrophic production, respectively.

production to a larger extent than the single additions. Besides Fe and C, temperature, ranging between 3 and 4 °C in the present study, could have limited bacterial heterotrophic activity in surface waters of the study region. We propose that temperature set an upper limit to the potential response to Fe and C additions, as suggested previously (Kirchman and Rich, 1997). Similar experiments performed at higher temperatures (9–14 °C; Church et al., 2000; Kirchman et al., 2000) observed stimulations of bacterial heterotrophic activity severalfold higher than in the present study, which supports the idea of the potential additional control by temperature.

4.3 Spatial and temporal variability in Fe limitation

Our results from the naturally Fe-fertilized region off the Kerguelen Islands add to incubation experiments performed in prominent HNLC regions of the Southern Ocean (Church et al., 2000; Hutchins et al., 2001; Jain et al., 2015) and the Pacific Ocean (Hutchins et al., 2001; Kirchman et al., 2000; Agawin et al., 2006; Kuparinen et al., 2011; Price et al., 1994), as well as in high-nutrient waters off Antarctica such as the Gerlache Strait (Pakulski et al., 1996) and the Ross Sea (Bertrand et al., 2011; see Table 2 for an overview). These previous studies reveal an interesting pattern that appears to be set, in part, by the dark or light incubation regime. While in dark incubations the addition of Fe alone had an effect on bacterial metabolism only in some locations, Fe amendments lead to enhanced bacterial production and growth in all incubations performed in the light and in the presence of autotrophic members of the microbial community. This suggests that the stimulation of phytoplankton by Fe and the associated release of DOM could relieve the organic carbon limitation for heterotrophic bacteria (Kirchman et al., 2000). Taken together, these results point to a strong coupling between organic carbon and Fe in controlling bacterial heterotrophic metabolism in HNLC regions (Tortell et al., 1999).

While Fe was clearly identified as a limiting nutrient for bacterial heterotrophic activity in the present study, the addition of Fe alone did not stimulate bacterial growth along a transect south of Tasmania to the Antarctic Polar Front (Church et al., 2000) and south of the polar front in the Indian sector of the Southern Ocean (Jain et al., 2015). These contrasting findings could suggest that bacterial resource limitation in the Southern Ocean varies among water masses with distinct hydrographic and chemical properties. Besides this possible spatial heterogeneity, the role of Fe as a limiting nutrient could vary with season. In this case, Fe limitation would be more pronounced in early spring, as demonstrated in the present study, than in late summer (Church et al., 2000; Jain et al., 2015). We consider two possible underlying mechanisms to explain this seasonal pattern. First, heterotrophic bacteria and phytoplankton are competing for Fe acquisition (Fourquez et al., 2015). In spring, the phytoplankton com-

munity is dominated by small, fast-growing cells that out-compete heterotrophic bacteria for Fe acquisition, whereas in summer, the lower primary production by less competitive large diatoms could result in a reduction of Fe limitation for heterotrophic bacteria (Quéguiner, 2013; Fourquez et al., 2015). Alternatively, or concomitantly, Fe limitation could be reduced in summer compared to spring due to an overall increased Fe availability resulting from enhanced Fe regeneration mediated by biological activity (Bowie et al., 2014). This could thereby relieve, in part, the limitation by this micronutrient for the summer bacterial community. The idea of seasonal changes in resource limitation is further supported by the higher bacterial Fe quota and cell-specific Fe uptake rates in spring than in summer, which point to enhanced bacterial Fe requirements early in the season (Fourquez et al., 2015). Thus, resource supply and biological interactions determine both the extent of Fe limitation of heterotrophic bacteria, with possible important feedbacks on the Fe and C cycles in the HNLC Southern Ocean.

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