Response to Reviewers

Authors' Reply to Reviewer #1

General comments:
Reviewer Query 1) This MS presents results of the impact of Fe and C (glucose) on heterotrophic bacterial uptake of leucine and on bacterial cell numbers in experiments performed in 0.5 L bottles, at in situ temperature and in the dark. Samples had been drawn from different stations (4) from surface mixed layer at 25-40 m depths. Incubations lasted for 4-5 days, except in the reference station R2 for 7 days. Measurements from subsamples were done 3 times (day 0, day 2, day 4-5), except from the station R2 four times (day 0, day 2, day 4-5 and day 7). The observations (Figure 1, Table 1) do not support well the given discussion. The main problem arise from the incubation conditions and sampling frequency, growth has been detected from three samplings at other stations, but R2 and thus the third sampling with intensive bacterial growth, e.g. station E-3, has been taken when severe resource limitations appears (growth between day 0 and day 2 suggest of higher cell numbers on the third sampling).

Authors' Response :
We thank the Reviewer for the time invested and the comments made on a previous version of the manuscript.
The main objective of the present manuscript was to investigate whether C and/or Fe are limiting factors for bacterial heterotrophic growth and metabolism in a naturally iron fertilized region of the Southern Ocean. We addressed this question using incubation experiments that have proven useful in many previous studies. Further, all incubations were done in triplicates, a prerequisite to test statistical differences between treatments. Our results and conclusions are based on statistical differences, using a one-way ANOVA and post hoc Tukey at a confidence interval of 95%, between treatments at a given time point of sampling.

Depending on the treatment and the station, significant differences are detected after 2 days and/or after 4-7 days (as illustrated in Fig. 1). 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. These variable responses are most likely driven by the initial environmental conditions, and the consequent pre-conditioning of the microbial community. We have listed the parameters that appear of importance in this context, such as concentrations of Chla, DOC and DFe, and bacterial heterotrophic production. The combination of these and other factors are likely to set in part the temporal evolution in the incubation experiments.

Reviewer Query 2) Environmental variables have been given in the Table 1, but not the basic nutrient (N&P) levels, nor the dominant algal species in the studied waters. This information is needed for the discussion about Fe, C or other limiting factors and thus carbon co-limitation becomes very speculative and is not based on the observations in this study. Discussion on Fe&C limitation, co-limitation during different seasons, pages 10-11 without N&P and species data is loose and speculative and does not reflect the observations from this study.

Authors' Response :
The idea that the micronutrient iron is a limiting factor for biological activity in the surface waters of the Southern Ocean is well known and supported by a large body of literature. The

origin of this idea is the observation that primary productivity is low despite the high concentrations of major inorganic nutrients, such as N and P, in surface waters of the HNLC Southern Ocean. The same environmental context holds for the present study. Concentrations of nitrate+nitrite and phosphate were present in excess (> 19 μM of nitrate and nitrite and > 1 μM of phosphate, presented in the companion paper by Blain et al. 2015), and these nutrients are unlikely limiting for bacterial heterotrophic activity.
In response to the Reviewers' comment, we have added a sentence in the first paragraph of the Results Section that briefly describes the concentration of major inorganic nutrients in the study region.
We are not convinced that the presentation of phytoplankton species composition is relevant in the context of our study, as all the incubations were performed in the dark.

Reviewer Query 3) The statements on C limitation and Fe&C co-limitations cannot be based on the third (final) sampling as other limitations are evident at that time, based on growth rates between the first and second sampling.

Authors' Response :
We base our conclusion and discussion on the entire time series, and not only on the final time point. This is illustrated by Fig. 1, which indicates the time points for which statistical differences between a given treatment and the control were observed.

Reviewer Query 4) Species succession in Southern Ocean normally proceed from diatom blooms in spring to smaller cells in summer, thus the authors should present data to support their contacting speculation on p. 11.

Authors' Response :
We only partly agree on this point with the Reviewer. Several studies, reviewed in Quéguiner (2013) have shown that the early phase of phytoplankton blooms is dominated by a succession of rapidly growing diatoms of different sizes, and that larger slow growing, and silicon limited diatoms accumulate at the end of the season (Quéguiner, 2013). This reference is now cited in the text.

Reviewer Query 5) Specific comments: Table 1 gives values of Chl. a and bacteria, two of the sites have low Chl. a (0.3 and 0.6), but tenfold higher heterotrophic production (2.6 and 24.9) and twice the cell counts (2.7 and 5.1). They are both highly stimulated by the carbon and Fe additions (E3 day 2, R2 day 7 due to slower growth). Why so? Is this related to the age/fate of the blooms and availability of carbon? I would be very careful to conclude C-impacts based on the final sampling (except at R2),(p.7 before the 3.3) as the community has been in the darkness for 4-5 days in 0.5 L bottle and the day 2 growth suggests of higher numbers and activities for the final sampling. (See general comment above).

Authors' Response :
The Reviewer wonders why bacteria are stimulated by C and Fe additions despite the differences in initial conditions. This is an interesting question that we tried to address by relating the variable extents of stimulation to biotic and abiotic environmental parameters. The extent of stimulation appears to be linked to bacterial Fe uptake rates and dissolved iron concentrations, but not to Chla concentrations or in situ bacterial metabolism. These results

are shown on Fig. 2 and mentioned in the Discussion Section. We think the stimulation by C or Fe is strongly coupled and therefore driven by the in situ availability of these two nutrients. We agree, this could in part be related to the age of the bloom, but we do not have any firm support for this.
As stated above, all our results and conclusion are based on the entire time series, and not only on the final time point. This is illustrated by Fig. 1, which indicates the time points for which statistical differences between a given treatment and the control were observed.

Reviewer Query 6)
Discussion on temperature control of the co-limitation by Fe and C on p. 10 is not supported by the study, the combined effect gives highest values for leucine incorporation, but at station E-3 on day 2. Samples come from the mixed layer and active bacteria are adapted to their environment, moreover other carbon sources than glucose are available (algal exudates), which makes the speculation even more loose.

Authors' Response :
This rather short paragraph aimed to briefly discuss the observation that combined additions did not yield significantly higher bacterial production rates than single additions, as observed in several previous studies. We agree with the Reviewer, the bacterial community is most likely adapted to the low temperatures, but we do not think that this prevents the community from taking advantage from an additional supply of readily available organic carbon or iron. We refer now to a study that demonstrates an increase in the bacterial response to nutrient amendment at higher temperatures.
If phytoplankton-derived DOM was sufficient to meet the bacterial carbon requirements, we would not have observed a significant response to glucose additions. Another possible explanation of our results is to consider glucose as a primer that stimulates the degradation of refractory organic matter. However, we do not have any support for a potential priming effect. Our result therefore supports the idea that, even during the early phytoplankton bloom, organic carbon was a limiting factor for bacterial heterotrophic metabolism.

Reviewer Query 7)
Figure 1 statistics: the Student0s t-test is not a valid for testing the treatment effects as data comes from time series incubations in which each observation is dependent on the previous value. There are more relevant statistics to test the significance in time series incubations.

Authors' Response :
As suggested by the Reviewer, we re-analyzed our results using a different statistical test. The main aim of the statistical analyses was to identify the treatment effect at a given time point during the incubation. So, for each time point there is only one factor in question, and this is the treatment. We therefore performed a one-way ANOVA and a post hoc Tukey test. The results from these analyses overall confirm our results, with the exception of Station R2 (See Fig. 1). In the revised version of the manuscript, we have slightly changed the presentation of these statistical analyses. We highlight only the treatments that are significantly different to the control (at 95% confidence interval).

Reviewer Query 8)
Figure 1. The growth rates and values would be better comparable if variable scales were not used in each subfigure. E.g. two different cell growth y-axis scales could be up to 6 and 12 and two scales for heterotrophic production, 20 and 40. Also x-axis scales could be more

realistic, ending at 6 (E stations) and 8 (station R3).

Authors' Response :
As suggested by the Reviewer, we have homogenized the y-axes among stations, whenever possible.

Authors' Reply to Anonymous Reviewer #2

We thank the Reviewer for the time invested and the numerous comments that helped improve our manuscript. We appreciate the Reviewers' overall positive feedback on the importance of the scientific objective addressed in the present study.

General Comments:

The paper by Obernosterer et al. reports new data on Fe and C limitation of heterotrophic bacteria in the Southern Ocean. It addresses an important scientific question and provides the first strong support for the co-limitation hypothesis of Tortell et al.(1996; 1999). Other tests of this hypothesis in different regions of the sea by Church et al 2000 and Kirchman et al 2000 showed bacteria were C-limited and did not re-spond to Fe addition alone. A few additional studies have also tested the co-limitation hypothesis and found support for it (or not) and these need to be acknowledged. The submitted manuscript does a poor job of crediting the research and ideas of other scientists who have contributed to this field of study (see Technical Comments).

Reviewer Query 1)

One of the most serious shortcoming of the paper is the lack of information about the Fe uptake measurements, which make it impossible to understand what was actually done and how to interpret the results. I would rate the scientific significance, good; the scientific quality, poor; and the presentation quality, fair.

The Reviewer raises 2 major critical comments.

Authors' Response :

1) **Lack of information on the Fe uptake rates.** We would like to clarify a misunderstanding. The Fe uptake data presented in Fig. 2 of the initially submitted manuscript were determined by Fourquez et al. (2014), and they are not part of the core results of the present manuscript. The companion paper by Fourquez et al. (now accepted for publication in the Special Issue KEOPS2 in BG) is entirely dedicated to the Fe uptake by the microbial community in the study region. It describes in detail the experimental setup and methods applied, and it discusses the results in the context of previous studies, in particular the papers highlighted by the Reviewer. Even though we have mentioned this in our manuscript, the full reference of Fourquez et al. (2014) could not be provided, because the manuscript was still in the editorial processing. We recognize that the use of these data without a complete reference was to some extent misleading. Upon the Reviewers' comment, we have re-considered the use of the Fe uptake data for the discussion of our findings in the present manuscript, as it might not

be straightforward to understand these data without having read the paper by Fourquez et al. (2014). We have therefore modified Fig. 2 in the revised version of the manuscript (see also more specific comment below).

2) **Discussion of relevant literature.** We produced Table 2 to review previous results on similar types of incubation experiments, and thereby set the context for our own study. We consider this a suitable way, because the information is easily accessible for any reader. We are, however, aware, that such an exercise of a « Review Table » contains the risk of missing the one or other published study. We thank the Reviewer for pointing out the publication by Agawin et al (2006), which, together with a publication in press (Jain et al. 2015) has now been included in Table 2 in the revised version. As suggested by the Reviewer, we now mention more often the conclusions from these previous studies in the main text.

Specific Comments:

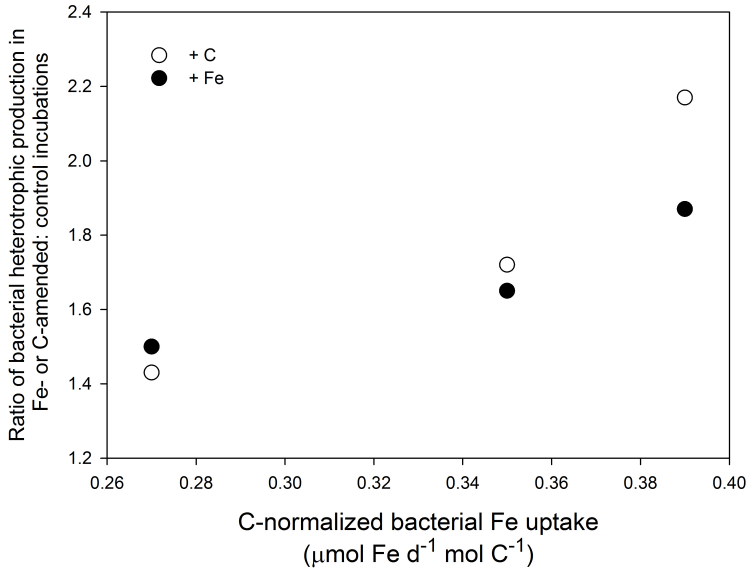
Reviewer Query 2)

There is no way to evaluate what the Fe uptake measurements mean. I think that the wrong paper has been cited here (page 15740, line 2: Fourquez?) (and elsewhere), but even if it is correct, readers need to know some details about the method and how it was applied. Figure 2 reports water-column integrated Fe uptake rate – but over what depth (ML?) and how many sample depths? Why would the maximum extent of stim-ulation (MEOS) of bacterial production (BP) by Fe and C be related to water-column integrated Fe uptake? I would have thought that the MEOS should be compared to Fe uptake of samples taken from the same depth (a volumetric rate)?? Some justification is required. The Fe uptake rates are also referred to as in situ rates – but what does this mean? Was the 55Fe complexed to some ligand or added in the inorganic form? I suspect that Fe uptake was measured by adding 55Fe at a total Fe concentration equal to or higher than the in situ concentration, but this is not reported. The rates are unlikely to be true in situ rates and are probably closer to saturated rates, but not enough information is provided for readers to judge. Knowing which of these rates was actually measured will completely alter how the MEOS results are interpreted.

Authors' Response :

We acknowledge that the Fe uptake rates are not straightforward to understand without having read the companion paper by Fourquez et al. (2014). We consider a full description of the 55Fe uptake measurements redundant with the paper by Fourquez et al. (2014), and we have therefore eliminated these data and the observed trend from the manuscript. A revised version of the Figure 2 now shows the extent of stimulation vs DFe concentration (see answer to Reviewer Query 4).

The seawater for the Fe uptake measurements and our incubation experiments were taken from the same cast in the surface mixed layer. Fe-uptake rates were then determined at different irradiance levels. In response to the Reviewers' comment, we present below the extent of stimulation vs the bacterial Fe uptake rates, normalized to cell biomass, on a volumetric basis (determined at 1% light level). We basically obtain the same trend as for the integrated values. The aim of this figure was to illustrate the potential relationship between these two independent measures of bacterial activity related to Fe and C, and thereby to point to the idea of the strong coupling between these cycles through microbial activity.



Reviewer Query 3)

As it stands now the bacteria Fe uptake rate is not normalized to bacteria density, which varies by a factor of 2 among sites. Since the water column integrated rate will depend on the uptake rate per cell and the bacterial abundance, then shouldn't this be factored in? In a co-limited community, Fe uptake rate per cell should somehow be related to the degree to which bacteria are limited by Fe and C which influences the MEOS.

Authors' Response :

In the above figure, the Fe uptake rates are normalized to cell biomass, based on cell abundances and a carbon conversion factor of 12.4 fg C cell $^{-1}$ (Fukuda et al. 1998).

Reviewer Query 4)

Reporting the MEOS seems completely arbitrary and potentially biased. We have no way of knowing whether the values are really the maxima, since samples were only taken at days 2 and 5 and if I understand correctly, some of the values plotted in Figure 2 are from samples that were taken at d2 and others at d5. What if the maximum stimulation occurred on d3 or d4 at Station E-4W for example? Then the true maximum would be missed (look at the data from E-3 which shows a peak at d2 and then decline by d5, so that the MEOS can vary quite substantially). We could be completely misled if the maximum was not measured at all stations. I think the only way to circumvent this problem is to construct this graph using BP measured either at d2 or d5 for all stations. Since water temperature is the same at all sites the kinetics of the bacteria metabolic response should be similar and so shouldn't confound the results.

Authors' Response :

The term « maximum extent of stimulation » was intended to provide a relative indication for the responses to a given treatment in the incubation experiments. We agree, in absolute terms,

this is not an appropriate term. In response to the Reviewers' concern, this term is not used any more in the revised version of the manuscript.

We propose a different way of looking at our data, which does not change the overall conclusion presented in the initially submitted manuscript. We now use the ratio of 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 4d of incubation at Station E-4W. The rationale behind 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. These variable responses are most likely driven by the initial environmental conditions. We have listed the parameters that appear of importance in this context, such as concentrations of Chla, DOC and DFe and in situ bacterial heterotrophic production. The combination of these and other factors are likely to set in part the temporal evolution in the incubation experiments. For this reason, and also because the time that separates the sampling is not exactly the same among experiments, we consider it not appropriate to choose only one time point for all incubation experiments for the calculation of the extent of stimulation.

The corresponding paragraph and Figure 2 have been moved to the Discussion Section, and we refer to the « extent of stimulation ». As an aside, for a given experiment, the extent of stimulation, whenever significant differences are observed, does not vary substantially between time points (<10%).

Reviewer Query 5)

Although the paper claims that the MEOS "was also positively related to in situ DFe concentrations", I can't believe that this is correct. The author's will need to report statistical analyses to back this up, although as I suggested in comment 3 the approach is currently flawed. Using the DFe concentration reported in Table 1 the values are: 0.13 nM Fe (1.4, 1.65-fold increase); 0.06 nM Fe (1.4, 1.5); 0.17 nM Fe (1.6, 1.6); 0.35 nM Fe (1.85, 2.05).

Authors' Response :

Fig. 2 of the revised version of the manuscript shows the extent of stimulation vs DFe concentration. The aim of this figure is to illustrate a tendency between these parameters, not to show a correlation. Due to the low number of observations, we purposefully did not calculate any correlation coefficient in the initially submitted and revised version of the manuscript. We agree with the Reviewer, the term « related » is not appropriate here. In the revised version, this term is replaced by "observation of a trend or tendency". The results for Station R2 are not shown on this graph, because single additions did not reveal a significant difference to the control (see answer below).

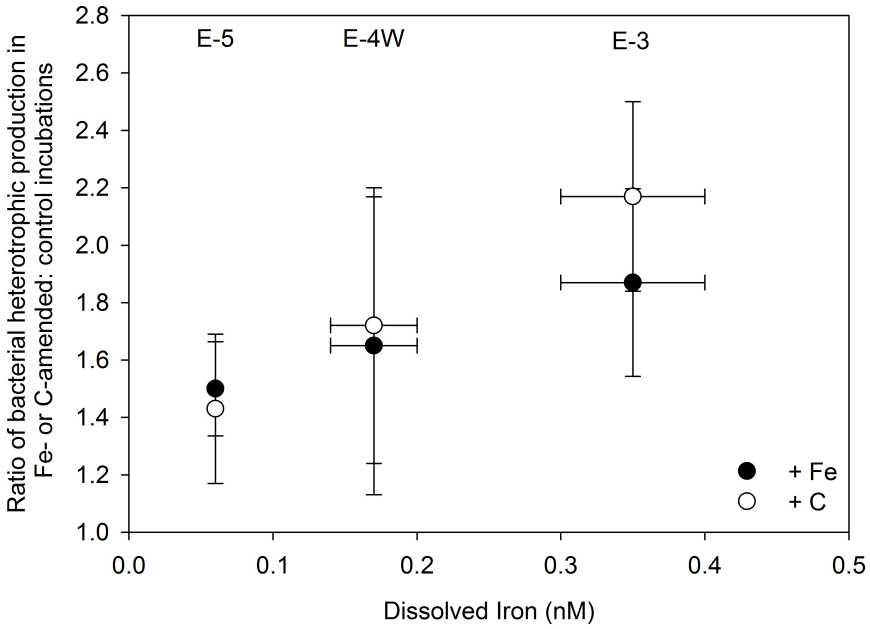


Fig. 2_revised. Extent of stimulation of bacterial heterotrophic production by Fe (+Fe) - or C (+ C)-addition and in situ dissolved iron (DFe) concentrations. Ratios of bacterial production in the Fe- or C-amended treatments to the controls correspond to the time points when significant differences were 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 ±SD of the surface mixed layer are given (see Table 1).

Reviewer Query 6)

I fail to see how the ratio of DFe:DOC "clearly identifies Fe as a potentially limiting resource for heterotrophic bacteria". Since we don't know the fraction of DOC or Fe that is utilizable, this ratio is not very informative. The authors acknowledge the problems with bulk DOC analysis in the next sentence. Delete.

Authors' Response :

The objective of this paragraph is to provide some ideas on why the addition of both Fe and C could stimulate bacterial heterotrophic metabolism. We do so by comparing the molar ratios of Fe:C of bacterial cells to those of their resource that are DFe and DOC, similar to what is commonly done for N:P-ratios for phytoplankton. We entirely agree with the Reviewer, the bioavailable fractions of both DFe and DOC are unknown, and thereby this ratio is not as straightforward to apply as for inorganic nutrients. However, we still consider this an interesting exercise, and the similarity in the molar ratios provides some clues on the potential limitation of Fe and C in this environmental context.

In response to the Reviewers' comment, we have rewritten this paragraph in the revised version of the manuscript, pointing out the concern raised by the Reviewer.

Reviewer Query 7)

It doesn't look like the t-tests were corrected for multiple comparisons – a 2-way ANOVA with time and treatment as fixed factors would be more appropriate or perhaps a repeated-measures ANOVA.

Authors' Response :

As suggested by the Reviewer, we re-analyzed our results using a different statistical test. The main aim of the statistical analyses was to identify the treatment effect at a given time point during the incubation. So, for each time point there is only one factor in question, which is the treatment. We therefore performed a one-way ANOVA and a post hoc Tukey test. The results from these analyses overall confirm our results, with the exception of Station R2 (See Fig. 1). In the revised version of the manuscript, we have slightly changed the presentation of these statistical analyses. We highlight only the treatments that are significantly different to the control (at 95% confidence interval).

The Reviewer suggests to perform a two-way ANOVA, using time and treatment as factors. However, days cannot be used as a factor level, because measurements taken on a given day are not independent of those taken already on the previous sampling date. Time is therefore not an independent factor. For the same reason, it is not appropriate to perform a repeated measures ANOVA.

Reviewer Query 8)

Page 15741, lines 17-22. There is no way to evaluate whether these hypotheses have any merit because of the lack of information about Fe uptake.

Authors' Response :

We do not present bacterial Fe uptake rates in the revised version of the manuscript, and these ideas have been reformulated accordingly.

Technical Comments:

Reviewer Query 9)

Page 15735, line 5 – Schmidt and Hutchins 1999 and Tortell et al. 1996 should be given credit here as they were the first (along with Maldonado et al.) to quantify the relative rates of Fe uptake by heterotrophs.

Authors' Response :

These references were added in the revised version of the manuscript.

Reviewer Query 10)

Line 7 – some citation is needed here to support this statement (Ducklow, Kirchman?).

Authors' Response :

A citation has been added in the text.

Reviewer Query 11)

Line 20 – Kuparinen et al. 2011 presented results that showed a positive effect of Fe addition and argued for C and Fe co-limitation – I'm surprized it has not been referenced here. It must be included. Agawin et al. 2006 also looked at Fe and C co-limitation in dark incubations in the subarctic Pacific Ocean, it too should be cited –if not here in the text, then in the Table.

Authors' Response :

This brief overview of bacterial responses to Fe addition refers only to incubation experiments performed in the dark. The reason for this is that in incubations in the light, the direct effect of Fe cannot be distinguished from the indirect effect due to Fe-stimulated production of phytoplankton-DOM. Kuparinen et al. (2011) have performed light incubations only. The publication by Kuparinen et al. (2011) is cited in Table 2. The publication by Agawin et al. (2006) and a report in press (Jain et al. DSR) have been added to the Introduction and also to Table 2.

Reviewer Query 12)

Line 21 – As far as I can tell, a single seawater sample was collected using a Niskin bottle and then dispensed into replicate sample bottles – are these then pseudoreplicates or true replicates? A more powerful analysis of the effect of Fe and C enrichment would be to consider the results from each station as truly independent samples and then combine the stations to test for a significant treatment effect. Some normalization of the data may be required for this sort of analysis.

Authors' Response :

We consider these samples as true replicates, as they represent independent biological incubations. Our experimental protocol respects the most fundamental rules of replication for any addition experiment one can conceive and this consists in 3 replicates.
We do not quite understand the approach suggested by the Reviewer, because we test the treatment effect (+C, + Fe or +C+Fe) compared to a control, and each of the unamended controls is representative only for a given site.

Reviewer Query 13)

Page 15736, line 1 – Queroue et al., 2014 is missing from the references.

Authors' Response :

The manuscript by Quéroué et al. is part of the KEOPS2 Special Issue. At the time when we submitted our manuscript, the paper of Quéroué et al. was not submitted, and it could therefore not be cited. It has been added to the References in the revised version of the manuscript.

Reviewer Query 14)

Line 14 – Bowie et al., 2014 is missing from the references.

Authors' Response :

The manuscript by Bowie et al. is part of the KEOPS2 Special Issue. At the time when we submitted our manuscript, the one of Bowie et al was not submitted, and it could therefore not be cited. It has been added to the References in the revised version of the manuscript.

Reviewer Query 15)

Line 15 - "The Niskin bottles were transferred to a trace-metal clean container" – I'm not sure if you mean lab instead of container?

Authors' Response :

The sentence is correct as it is: The Niskin bottles were transferred to a trace-metal clean container. The container had two sections, separated from each other: one where the Niskin bottles were sampled, and one where the analyses and incubations were performed. This latter part of the container could be considered as a trace-metal clean lab. Details of the trace-metal clean work are provided in the manuscript by Bowie et al. (2014).

Reviewer Query 16)

Line 18 – "dispensed" would be better than "dispatched"

AR : DONE

Reviewer Query 17)

Line 20 – consisted "of"

AR : DONE

Reviewer Query 18)

Page 15739, line 6 – awkward wording please change "the most contrasted station"

AR : DONE

Reviewer Query 19)

Page 15740, second paragraph. The idea of Fe and C co-limitation was originally advanced by Tortell et al. (1996, 1999) and needs to be referenced here. Kuparinen et al. 2011 should also be included, since they obtained some support for this hypothesis in field experiments in the sub-Antarctic. Church et al. 2000 also observed a Fe/C interaction in enrichment experiments. This part of the discussion minimizes the contributions of other researchers and makes it sound like the idea of Fe/C co-limitation has its origins here. It would make sense to introduce the co-limitation hypothesis in the Introduction.

Authors' Response :

As stated earlier, the overview Table 2 was made with the intention to appreciate previous reports on C and Fe enrichment experiments in various ocean regimes, and the publications highlighted by the Reviewer (Kuparinen et al. 2011 ; Church et al. 2000) are cited in this

Table. To address the Reviewers request, we have now added Tortell et al. (1996, 1999) and more citations in the text that was modified accordingly.

Reviewer Query 19)

Page 15742, line 18 – A temperature and organic substrate interaction was originally advanced by Pomeroy and colleagues in the late 80's and I fail to see how the proposal made here is any different than the original idea. Cite them.

Authors' Response :

This rather short paragraph aimed to briefly discuss the observation that combined additions did not yield significantly higher bacterial production rates than single additions, as observed in several previous studies. We refer now to a study that demonstrates an increase in the bacterial response to nutrient amendment at higher temperatures.
The studies by Pomeroy and Deibel (1986) and Wiebe et al. (1992) suggest that bacteria require higher concentrations of labile organic matter at low temperatures, which does not exactly reflect the idea presented in this paragraph.

Reviewer Query 20)

Page 15743, line 8. Here again the authors need some appropriate citations. The idea that the relief of Fe limitation of phytoplankton could increase the flow of C to bacteria has been around for some time.

Authors' Response :

This sentence was accompanied by a citation.

Reviewer Query 21)

Table 1 reports that bacterial production (ng C/L/h) is roughly equal at the E stations and 10 times lower at the R station. Yet, in Figure 1 the relative production values (here reported as leucine uptake) are quite different. If the same conversion factor was applied to compute the C rates, then something is odd. The leucine rates at the E stations differ by a factor of 3 and the R station is not too different from E-4W. Comment please.

Authors' Response :

We present BHP rates in ng C 1^{-1} h^{-1} in Table 1, because these data are from Christaki et al. (2014) and we wanted to maintain the same units as in the initial paper. By contrast, in our incubation experiments, leucine incorporation was used as a measure to determine the bacterial response to C, Fe and C+Fe additions, and we used this measure only in a comparative manner among treatments. We therefore consider it more appropriate to present the leucine uptake rates prior to the use of a carbon conversion factor.

When calculating our leucine uptake rates in carbon units, we obtain similar results (at time zero) as those given in Christaki et al. (2014) for 3 sites: Station R-2: 3.6±0.2 vs 2.6±0.5 ng C 1^{-1} h^{-1} ; Station E-4W: 26.5±3.9 vs 29.1±3.9 ng C 1^{-1} h^{-1} ; Station E-5: 19.9±4.1 vs 27.4±1.3

ng C 1^{-1} h^{-1}. At Station E-3, our values 7.7±0.7 ng C 1^{-1} h^{-1} are indeed lower than those given in Christaki et al. (2014) 24.9±1.7 ng C 1^{-1} h^{-1}.

Given that the two independent bacterial production measurements were done on water samples collected from different CTD casts, the overall coherent results point out an excellent reproducibility of the production measurement. The different values obtained at one site could be attributed to the differences in the collection of seawater (trace-metal clean vs common Niskin bottle), which could have affected instantaneous rates of BHP at this site. We consider, however, that this difference in rates of BHP does not affect the interpretation of our results in the incubation experiments.

Revised version submitted February 27 2015

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

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Keywords : Iron limitation, Carbon limitation, bacterial heterotrophic activity, natural iron fertilization, Southern Ocean

Running title: Bacterial limitation in the Southern Ocean

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2

Abstract

It has univocally been 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 Kerguelen Islands and at one site in HNLC waters during the KEOPS2 (Kerguelen Ocean and Plateau Compared Study2) cruise in spring 2011. We performed dark incubations of natural microbial communities amended either with iron (Fe, as $FeCl_3$), 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 2 out of the 3 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 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 for bacterial heterotrophic metabolism in regions of the Southern Ocean that receive variable Fe inputs.

Ingrid 10/2/15 15:38
Supprimé: three out of four

Ingrid 10/2/15 16:24
Supprimé: bacterial growth was enhanced only in two out of four occasions. The extent of stimulation of bulk bacterial heterotrophic production by single Fe or C additions increased with increasing in situ bacterial Fe uptake rates in the surface mixed layer.

Ingrid 10/2/15 16:26
Supprimé: HNLC and

Ingrid 10/2/15 16:30
Supprimé: observation that the extent of stimulation by both elements was related to Fe-uptake rates highlights the tight interaction between the C- and Fe-cycles through bacterial heterotrophic metabolism in the Southern Ocean.

1. Introduction

Iron (Fe) is an essential element for biological activity, but present at trace amounts in the surface ocean. The role of Fe as a limiting nutrient was extensively studied in High Nutrient Low Chlorophyll (HNLC) regions with 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 (CO_2) 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 & 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, up to date, only few studies have tempted 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.

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Heterotrophic bacteria responded to variable extents to Fe addition in mesoscale fertilization experiments and in natural fertilization studies (see for overview Christaki et al., 2014). Whether the increase in bacterial abundance and production in the Fe-fertilized patches was induced directly by Fe, or indirectly by the enhanced DOM production by phytoplankton, or a by combination of both was difficult to conclude from these observations. Only a few studies have examined the potential role of Fe as limiting factor for heterotrophic bacteria in

dark incubations, 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 the 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 than phytoplankton to additions of Fe in different oceanic environments suggests 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, Quéroué 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 were determined at three stations located in the naturally Fe-fertilized region east of Kerguelen Island (Stations E-4W, E-3 and E-5) and at the reference station R2 in HNLC waters west of Kerguelen Island (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 40m at Stations R2 and E-4W, 37m at Station E-3 and 25m 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 to 12 x 500 ml PC bottles. All PC bottles were soaked in HCl (10%) and thoroughly rinsed with Milli Q water before use. Besides the control that consisted of unamended seawater, the following 3 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 $FeCl_3$ (final concentration 1 nM of $FeCl_3$), and C was added as trace-metal clean glucose (final concentration 10 µM 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 and at Day 7 (T_7) also 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

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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 15mW at 488 nm with the standard filter set-up. Heterotrophic bacteria were stained with SYBRGreen 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 [^{3}H]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}H(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 2-3h 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 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 2 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°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 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 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 Kerguelen Island, 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 µM of nitrate plus nitrite and > 1 µM 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

by 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 (by 1.5-fold), and this enhancement was maintained at T_4 (by 1.6-fold) (Fig. 1e, f). Additions of Fe and C alone resulted in enhanced bacterial abundance and bacterial production only at T_4 (by 1.2- to 1.7-fold). Stations E-3 and E-5 have close geographical position and were sampled in a quasi-Lagrangian manner with a 17 days 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 was observed to single and combined additions at T_2 (by 1.7-2.2-fold at Station E-3 and by 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.

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). We refer here briefly to two types of resource co-limitation

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Supprimé: By contrast, Fe and C additions lead to increased bacterial abundance only at 2 out of the 4 stations (E-4W and E-5). At the HNLC-site R2, single additions of Fe and C stimulated bulk and cell-specific production at T_7 (by 1.4 to 1.8-fold), and the combined addition of Fe and C resulted in an enhancement of 1.9 as compared to the unamended control (Fig. 1a-c). By contrast, bacterial abundance was not significantly different in any of the treatments.

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Supprimé: 3.3. Linking the extent of stimulation to in situ bacterial Fe uptake. To further explore the variable extent of stimulation of bacterial production by single Fe and C additions among stations, we determined the respective maximum ratio of bacterial production in the Fe- and C-amended to the control treatments for each experiment. Single additions of both Fe and C resulted in the most pronounced responses at Station E-3 (1.9- and 2.0-fold, respectively), followed by Stations E-4W (1.7-fold for Fe and C) and E-5 (1.50-fold for Fe and C). The maximum extent of stimulation at Station R2 was overall in the range of those observed in Fe-fertilized waters, but the difference in the response to C (1.7-fold) and to Fe (1.4-fold) was most pronounced at this site. Interestingly, the maximum extent of stimulation of bacterial heterotrophic production by single additions of Fe and C was positively related to in situ bacterial Fe uptake rates as determined by 24h incubations of the microbial community with ^{55}Fe (Fourquez et al., this issue), and this relationship is particularly well established across the Fe-fertilized sites (Fig. 2). A similar relation was observed for cell-specific production rates (data not shown). The maximum extent of stimulation was also positively related to in situ DFe concentrations, but not to in situ Chl *a* or bacterial abundance and production.

where 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 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; Tortell et al. 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 µmolFe $molC^{-1}$ (Tortell et al., 1996) and in the KEOPS study region (4-8 µmolFe $molC^{-1}$; Fourquez et al., 2014) and the ratio DFe:DOC (range 3 – 7 µmol DFe $molDOC^{-1}$; Table 1) indicates similar cellular and in situ molar ratios, and thus suggests the potential of both elements becoming 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. 2014). 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

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281 considerations help to understand the positive response of heterotrophic bacteria to the
282 addition of biologically labile forms of both Fe and C.

283 **4.2. Linking the extent of stimulation to in situ bacterial Fe uptake.** To explore the
284 variable extent of stimulation of bacterial production by single Fe and C additions among
285 stations, we determined the respective ratio of bulk and cell-specific bacterial production in
286 the Fe- or C-amended treatments to the controls for the time points when significant
287 differences were detected for the first time in the cultures. This was the case after 2 d of
288 incubation at Stations E-3 and E-5, and after 4 d of incubation at Station E-4W. The rationale
289 behind this is that it takes into consideration the differences in the time lag of the bacterial
290 communities to respond to Fe- or C- additions at the different sites. We consider these
291 different temporal dynamics of the microbial community part of the response to the question
292 of whether and to what extent they are C- or Fe-limited. Interestingly, the extent of
293 stimulation of bacterial heterotrophic production by single additions of Fe and C revealed an
294 increasing trend with in situ DFe concentrations (Fig. 2). A similar trend was observed for
295 cell-specific production rates (data not shown). No such tendency was observed with in situ
296 chlorophyll a concentrations or bacterial abundance or production.

297 This trend could point to the close coupling between C and Fe for bacterial
298 heterotrophic metabolism, and it could support the idea that the addition of either of these
299 elements facilitates the utilization of the other limiting element (Tortell et al. 1999; Arrigo
300 2005; Saito et al., 2008). The response to single C amendment increased at higher in situ DFe
301 concentrations, which could indicate that a larger fraction of the added glucose can be utilized
302 under these conditions. The enhanced response to single Fe addition at higher in situ DFe
303 concentrations could indicate the processing of a larger fraction of the DOM present in situ or
304 the utilization of DOM with a higher efficiency. A synergistic effect, such as the increase in
305 Fe bioavailability by compounds released by phytoplankton could also be considered (Hassler

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Supprimé: Single additions of both Fe and C resulted in the most pronounced responses at Station E-3 (1.9- and 2.0-fold, respectively), followed by Stations E-4W (1.7-fold for Fe and C) and E-5 (1.50-fold for Fe and C). The maximum extent of stimulation at Station R2 was overall in the range of those observed in Fe-fertilized waters, but the difference in the response to C (1.7-fold) and to Fe (1.4-fold) was most pronounced at this site.

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Supprimé: The maximum extent of stimulation was also positively related to in situ DFe concentrations, but not to in situ Chl *a* or bacterial abundance and production.

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et al., 2011). Within the bacterial cell, the Fe and C cycles are tightly linked in the tricarboxylic acid (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 to the changes in the expression of Fe-containing enzymes, and to the induction of the glyoxylate shunt, a bypass of the TCA cycle, that has important consequences on the fate of organic carbon processed by the bacterial cell (Fourquez et al., 2014; Beier et al., in press).

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 & 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 production to a larger extent than the single additions. Besides Fe and C, temperature, ranging between 3-4°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 several fold 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 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), and also 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).

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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 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., 2014). In spring, the phytoplankton community is dominated by small, fast growing cells that outcompete 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. 2014). 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, that point to enhanced bacterial Fe requirements early in the season (Fourquez et al., 2014). 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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Figure Legends

Fig. 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; $p<0.05$).

Fig. 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 ±SD of the surface mixed layer are given (see Table 1).

Ingrid 17/2/15 15:24
Supprimé: the same letters are not statistically different, while different letters indicate treatments that are statistically different (Student's t-test; $p<0.05$)

Ingrid 24/2/15 08:57
Supprimé: Relationship between the maximum

Ingrid 17/2/15 15:32
Supprimé: uptake rates as determined by 24h incubations of the microbial community with ^{55}Fe (Fourquez et al., this issue)