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Dear Professor Herndl,

We submit hereby a revised version of our manuscript entitled "Microbial iron uptake in the naturally fertilized waters in the vicinity of Kerguelen Islands: phytoplankton-bacteria interaction" by Marion Fourquez, Ingrid Obernosterer, Diana Davies, Tom Trull, and Stéphane Blain to be considered for publication in Biogeosciences in the special issue KEOPS2.

In this revised version, we have taken into consideration the comments of the reviewers and adopted all of their editorial corrections. In particular, we have changed the terminology and extended our discussion on the potential competition for iron between heterotrophic and autotrophic microbes as required by Reviewer#2. We provide the details of the specific changes to the manuscript in the point-by-point responses to the Reviewers here below.

Our revised manuscript now contains 6 tables, 7 figures and two supplementary materials (one table and one animation).

We want to thank the reviewers for their thoughtful comments on our first version of the manuscript.

Best Regards,

Marion Fourquez

Response to Reviewer#1

Review for Fourquez et al.
General comments
The paper is generally well written and expands and explores the very scare data of intracellular iron uptake in phytoplankton and bacteria. The comments are only minor corrections and I can thoroughly recommend its publication.

We thank the reviewer #1 for his/her positive feedback and constructive comments. Here below we answer point by point the different comments or remarks he/she raised.

In between numbers and unit there is no -, ie it is 500 mL acid washed bottles and not 500-mL ...
Add n= XX each time an average and standard deviation are given.

This has been corrected through the manuscript.

1. Introduction
Line 37: Reference needed

References follow in the next sentence (Tortell et al., 1996; Maldonado et al., 2001; Sarthou et al., 2008).

Line 53: Since there are only 3 Southern Ocean island system investigated, it would be apropriate to reference all three, hence add Nielsdottir et al 2012.

Nielsdottir et al. (2012) was added in the revised version.

Line 54: change to 'natural ferilized regions'

Change was made in the revised version.

Line 58: As a reader I would prefer figure 1 to show surface chl with the incubation locations superimposed, so the reader can see 'the blooms downstream the island'.

We agree with the purpose of this comment. However, because all stations were not sampled at the same date, we think that it would not be rigorous to superimpose the stations where incubations were performed on a snapshot of blooms coming from a composite of satellite images.
We therefore indicate the concentration of Chl *a* in Table 1, and we now propose an animation as supplementary material that shows the development of the bloom over the period of the cruise. In this video, stations that were sampled in this study are highlighted in red when they were visited (date is provided at the left top). We think this supportive material will help the reader to see the blooms downstream the island as required by reviewer#1.

Line 61: Delete 'a few'
Done.

2. Material and Methods
Line 77: 500 mL acid washed polycarbonate
Done.

Line 80: change to: three times, followed by three rinses
Done.
Line 111: Bran or company for the Nickel screens needed

Company was added.

Line 119: remove – between filtered seawter, change ‘during’ to ‘for’
Change was done in the revised paper.

Line 141: Ryan-Keogh et al 2013 have shown that 0.2 nM Fe added is sufficient to stimulate growth.

In Ryan-Keogh et al. 2013 the authors show a stimulation of the Fv:Fm ratio of the photosynthetic community after addition of 0.2 nM of Fe. The relationship between this ratio and the growth rate is, however, not straightforward. The value Fv:Fm is more frequently used to appreciate the physiological state of the cells. The authors acknowledge this point as follows: “an empirical relationship between Δ(Fv:Fm)+2.0 Fe and ΔμChl should not be taken to infer any universal relationship between the absolute value of Fv:Fm and phytoplankton growth rates”.

Line 157: Reference for method needed

“To enumerate heterotrophic bacteria, 2 mL samples were fixed with glutaraldehyde (1% final concentration), incubated for 1h at 4°C, and stored at -80°C until processed “.

Flow cytometric analyses were done following the protocol described in Obernosterer et al. (2008). We added this reference in the revised version.

3. Results

Line 175: Remove’as’
Done.

Line 179: Space needed between umol and Fe
Done.

Line 193: how many replicates, please write n = ...
We added n=3 before the mention mean ± SD of the three PAR levels in the revised version.

Line 198: Change to: while at station A3-2 pico-nanoplankton was highest ( ) and remove ‘this was the case for’
Change was made in the revised manuscript.

Line 200: Exchange ‘measured’ with ‘observed’
Done.

Line 217: Replace ‘a’ with any, so At any given station...
Done.

Line 241: New line wrongly placed
This is a Word issue, it doesn’t appear in the Biogeoscience version

Line255-258: Work by Zubkov et al 2007 of bacterial 55Fe uptake shows that iron uptake is linear for 10-12 h, but after that it takes the shape of an exponential curve. Also, work by Maldonado et al 2005 showed variance in iron uptake over 24 h. I would suggest you go back to your data and look at iron uptake and not the Fe:POC uptake and compare it with other published data. Also, other published data would suggest that the way you derived the Fe quota for heterotrophic bacteria, if you calculate it for 24 h. However, if you use data for shorter time periods, e.g. 10 h it is a different matter.

The reviewer highlights an important issue, which is the linearity of iron uptake over time. In the present study, a 24h incubation time was required to obtain measurable bacterial iron uptake rates (i.e. significant differences in the "live" as compared to the "killed" treatments. For this reason, we did not attempt to perform incubations of shorter duration. We therefore do not have the possibility to calculate the iron quota, based on shorter incubation times. We have taken this issue into consideration in the revised version of the manuscript by adding the following paragraph: "Due to the low bacterial iron uptake rates determined over 24h, we did not perform any time series over shorter incubation times. Our results are therefore based on the assumption of linearity in bacterial iron uptakes rates over the 24h incubation period."
One major difference with the work of Zubkov et al. (2007) is that the authors have performed incubations for determining bacterial iron uptake using ^{55}Fe complexed with citrate. Therefore it is very difficult to extrapolate their conclusions to other studies like ours. Also the final concentration of ^{55}Fe that was added in the work of Zubkov et al. (2007) is unknown (the authors only mention that 3.9 Mbq of ^{55}Fe was spiked but they do not mention the specific activity or the actual final concentration in incubation). For all these reasons we did not mention the work of Zubkov et al. (2007) as it is hardly comparable to ours. In the work of Maldonado et al. (2005), incubations were performed at temperatures between 13 and 14°C which may explain why the authors showed variance in bacterial Fe uptake after 24h. In our experiments, incubations were performed between approximatively 2 and 4.5°C and we observed linear C-normalized Fe uptake rates for bacteria after 140 hours of incubation, and then followed by a plateau (data not shown but mentioned in the text).
The rationale behind the normalization of the iron uptake to POC (which equals C-normalized cell numbers) is to make our results comparable among sites and between treatments. We consider this normalized value also of interest for comparison with other studies. Iron uptake by bacteria on a volumetric basis is compared to other studies and discussed in the manuscript (see point 4.2).

4. Discussion

Line 294: List Sunda and Huntsman 1995 too
Done.

Line 308: Replace ' first step forward, even if not perfect', with 'A step forward'
Done.

Line 308-326: Reads more like a review and instead of discussing the data of this paper in context with other studies.
The aim of this paragraph is to discuss the results of the varying seasonal contributions to total iron uptake in the context of changes in the phytoplankton community composition. We point out some previous observations from the same study region (KEOPS1) to make the reader familiar with the seasonal context. For a good comprehension of the discussion, we consider it appropriate to maintain this paragraph.

Line 331: Are there no more Southern Ocean studies with a Fe:C ratio?
On line 338, we provide a range of ρFe:ρC reported for the Southern Ocean (5-50 µmolFe molC^{-1}) and we now refer clearly to the work by Sarthou et al. 2005 and references herein.

Line 338: Incert 'of' located downstream of the plateau
Change was made in the revised paper.

Line 340-344: Please explain this further with data included
The results of the phytoplankton community composition are presently prepared for a different publication and they can therefore not be presented and discussed in more detail here. For this reason we removed this part.

Line 353: Boyd et al 2012?
Yes thanks for pointing this mistake. We changed the reference for Boyd et al. (2012).

Line 365-367: I am not sure I buy the argument that primary productivity and bacterial Fe uptake are negatively correlated, as the bacteria also need the phytoplankton for all its organic nutrient needs. And with only 3 points it is almost possible to make any correlation positive.
This argument is based on the assumption that organisms compete for the access to Fe and that an increase in primary production would negatively affect the Fe bacterial uptake (thus leading to increase Fe-limitation for bacteria). In the revised version of the manuscript, we have modified this part of the Discussion: We have eliminated this sentence and we present the correlation coefficient of the relation between C-normalized bacterial Fe uptake rates and primary production, that is based on 5 data points.

Line 373: change to 'may also benefit heterotrophic bacteria...'
Change was made in the revised manuscript.

Line 382: Incert ' play a role...'
Change was made in the revised manuscript.

Line 386: I can only find a reference to the Obernosterer paper in this issue that 'high C availability leads to an increas in Fe demand', so if you want to keep this sentence I suggest you add some of that data, or keep it out
The references Kirchman et al. (2000) and Fourquez et al. (2014) follow in the next sentence in the original manuscript to support this idea.

Line 394: Remove in so it reads ' Southern Ocean suggests an intimate connection...'
Figure Captions
Done.

Line 420: Incert ± 1 SD
Done.

Line 425: Station E-4W
Done.

Line 426: Grey circels: Station E-2
Done.

Line 436: r2=0.97
Done.

Figure 3: Percent (%) contribution to the totaltal Fe uptake...
Done.

Comment to Table 1: Table text should decribe the table content. Therefore Experimental approach a,b,c should be described succintly.
We have now added a brief description of the signification of a, b, and c.

Standard deviations or standard error should be given listed including number of samples(n) used to derive the average. OR, my personal opiniton is that it would give more sense to give the nutrient concentration at that particular station where the incubation was initiated, instead of an average (but also with ST and n).
Thanks to Reviewer#1 for pointing out a mistake in the table caption with his/her comment. The table 1 gives the value at the corresponding depth of the sampling, not an average. It was a

mistake in the table caption. We changed "mean biogeochemical properties" for "main biogeochemical properties" in the revised manuscript.

Line 579: Should read Table and not Tableau
Change was made in the revised paper.

Response to Reviewer#2

This paper describes an extensive field effort of Fe uptake by size fractionated microorganisms in the vicinity of Kerguelen Islands. The scientific level is high and the methods are suitable and well conducted. The writing, however, should be improved. Terminology can be simplified and shortened. Some more data should be shown and discussion can be expanded. I recommend publication once these corrections are made.

Thanks to the Reviewer for a supportive and helpful review. Here below we respond to his/her comments.

Abstract: Terminology is awkward (had to read the sentence 4 times): "Bacterial Fe uptake rates normalized to carbon biomass were highest when bacteria were incubated in the absence of both micro- and pico-nanoplankton. The absence of microplankton resulted in a decrease in bacterial Fe uptake rates by up to 20-fold, while in incubations with the whole microbial community bacterial uptake rates were reduced by 2- to 8-fold". What about "Bacterial Fe uptake rates normalized to carbon biomass were highest in incubations with bacteria only, and dropped in incubations containing other components of the microbial community. Substantial decrease in bacterial Fe uptake rate (up to 20 fold) was found in incubations containing pico-nanoplankton…"

We have rewritten the Abstract according to the Reviewers' suggestion.

Trying to re-write these sentences, I see that I do not get it. How come that pico-nanoplankton only, resulted in lower Fe uptake rates (i.e stronger competition) than those with whole water (which contains pico-nanoplankton +microplankton – in which case similar or stronger competition is expected?

We agree with the Reviewer, this is a rather unexpected finding. To highlight this finding in the Abstract, we slightly modified the respective sentence.

We make two interesting observations:

1) Bacterial Fe uptake rates are lower in the presence of phytoplankton than with bacteria alone. This observation suggests that phytoplankton outcompete heterotrophic bacteria for the access to Fe.

2) Bacterial Fe uptake rates are higher in the presence of the entire microbial community than in the presence of pico-nanoplankton only.

For the second observation, we propose the following possible explanations:

a) Interactions between pico-nanoplankton and microplankton

Allelopathic interactions could directly or indirectly affect competition for Fe among microorganisms. Phytoplankton and bacteria produce and excrete chemical substances that affect the metabolism of other microorganisms in negative or positive ways (Legrand et al., 2003). Allelopathic interactions are commonly observed within phytoplankton communities and between phytoplankton and bacteria, however, these processes remain still poorly understood.

We have mentioned this possibility briefly in the discussion "incubations could arise via other microorganism allelopathic interaction mechanisms than competition for Fe. As such, further research is needed to examine interactions between pico-nanoplankton and bacteria across a wider range of conditions, i.e. including non-limiting Fe and carbon substrate levels", but because we do not have any supportive data on how this could affect the Fe-uptake rates, we have not explored in more detail this hypothesis.

b) Differences in DOM supply

The absence of microplankton during the 24h incubation could have resulted in a lower supply in phytoplankton-derived DOM, explaining the lower bacterial Fe uptake rates in the incubations bacteria + pico-nanoplankton. The importance of DOM in regulating the bacterial Fe uptake is illustrated by the strong relationship between the bacterial Fe uptake and primary production. As suggested by the Reviewer, an increased bacterial C-limitation in this type of incubation could lead to slower bacterial growth and a lower bacterial Fe demand.

We have added this possible explanation in the discussion part of the revised manuscript, "In the absence of microplankton, the supply of phytoplankton-DOM is likely to be lower, which could explain the strong decrease in bacterial Fe uptake rates in these incubations $(\boldsymbol{\rho Fe}: \boldsymbol{POC})_{\boldsymbol{bact}}^{\boldsymbol{<25\mu m}}$".

In general (throughout the text) I think the choice of words – in the absence of, rather than excluding or bacterial cells only, is awkward. Similarly the choice of symbols for that purpose (Fe bact <25um etc) is not good. Why not - Fe bact for bacteria only, Fe bact <25um for bacteria in the presence of nano-plankton only and Fe bact whole for bacterial uptake with the whole community.

We changed our annotations according to Reviewer#2's comments.

I think that adding the carbon biomasses of phytoplankton although calculated from another study, to a table such as Table 2, is very useful. The calculated bacterial biomass is really a must, while cell numbers will also be great. We want to judge for ourselves if indeed we have very little bacteria that take Fe at "normal" rates, or for example there are more bacteria in one place, but since they are not Fe limited they acquire Fe at slow rates etc…

The carbon biomasses were added to table 2 and the bacterial cell abundances were added in table 4.

Table 2 uptake by 0.8-25 is calculated – it is noted in the methods but not in the table.

The Fe uptake by the 0.8-25µm size-fraction is now given in Supplementary data.

I liked the discussion part on the DOC limitation and I'd like to emphasize a point that may be missing from the discussion. The rates of Fe uptake we measure when we add Fe represent the pre-conditioning of the organism, and not necessarily its competence. It means that if the bacteria are Fe replete they'll show slow uptake rate per cell compared to Fe deplete cells. Bacteria can be Fe replete due to high Fe supply (e.g above the plateau), or due to slow growth as a result of limiting DOC. Slow growth will slow their Fe bio-dilution and hence inhibit the expression of transport molecules. This explanation indeed goes well with the observed link between primary production and bacterial Fe uptake rate. It has however some implications on the interpretation of the competitive ability of

bacteria against phytoplankton. It probably only implies that pico-nanoplankton were more Fe limited than bacteria and hence upregulated more transport systems. So that when supplied with 55Fe they were able to outcompete bacteria for this source.

We thank the Reviewer for this interesting point of consideration. There are two issues to be examined:

1) Are bacteria Fe replete before the start of the incubations?
In a separate set of experiments we have investigated the bacterial response to additions of Fe (dark incubations of the whole microbial community; Obernosterer et al. BGD of the Special Issue). These experiments revealed a clear positive response to Fe-alone additions, indicating that bacteria are to some extent Fe limited.
As suggested by the Reviewer, Fe-replete cells should be present at the fertilized stations (above the plateau and in the plume). At all these stations, the cell-specific bacterial Fe-uptake rate, determined in bacteria-alone incubations, is higher than at the HNLC station. This does not support the idea that bacterial cells above the plateau are Fe replete, because they appear to have more Fe transport molecules available than cells at the HNLC station. As discussed previously, the difference may again be explained by the availability of carbon that is higher above the plateau. This higher supply of carbon provides energy to synthesize more transport molecules to cope with a certain degree of limitation.

2) Are the Pico-Nanoplankton more Fe-limited than bacteria (before incubation)?
To respond to this question the Fe-uptake rates of Pico-Nanoplankton and bacteria can be compared. Bacterial Fe uptake rates are available for bacteria-alone incubations, however no incubations where Pico-Nanoplankton were incubated alone could be performed. If we consider that in the incubations of bacteria + Pico-Nanoplankton the Pico-Nanoplankton largely outcompete bacteria, the Fe uptake rate measured for Pico-Nanoplankton in these conditions is a good approximation for the Fe uptake rate for Pico-Nanoplankton alone. As illustrated in the Table below, bacteria alone and Pico-Nanoplankton have similar uptake rates. This does not support the idea that Pico-Nanoplankton was more severely Fe limited than bacteria.

Consequently we do not think that the observation of the reduced Fe uptake rate of bacteria in presence of picoplankton resulted from preconditioning conditions where bacteria were Fe replete or where Pico-Nanoplankton were more severely limited than bacteria.

These arguments are now added in the revised version of the manuscript "To evaluate the degree of Fe limitation, we compared bacterial and pico-nanoplankton Fe uptake rates (Table 6). Two clear features emerge. First, Fe uptake rates for bacteria ($(\rho Fe: POC)_{bact}^{alone}$) and pico-nanoplankton ($(\rho Fe: POC)_{pico-nano}$) are very similar for a given station, suggesting that they experienced comparable degree of Fe limitation before the beginning of the incubation experiment. Second, the bacterial Fe uptake rates when incubated alone ($(\rho Fe: POC)_{bact}^{alone}$) are higher in fertilized waters than at the HNLC site, suggesting that bacteria are not Fe replete at the fertilized stations". with the following table (Table 6 in the revised manuscript).

Table 6 Carbon normalized Fe uptake rates for bacteria and pico-nanoplankton. Columns $(\rho Fe: POC)_{bact}^{<25\mu m}$ and $(\rho Fe: POC)_{bact}^{alone}$ 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	$(\rho Fe: POC)_{bact}^{<25\mu m}$	$(\rho Fe: POC)_{pico-nano}$	$(\rho Fe: POC)_{bact}^{alone}$
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

The discussion mostly compares the new data to other studies, but hardly deals with the uptake itself and the differences between stations (not only for bacteria).

Our data on the bulk Fe uptake rates are limited to 3 Stations, which renders it difficult to discuss this part of the manuscript in more detail. These results are integrated in a companion paper on the Fe budget (Bowie et al.; BGD of the Special Issue) where they are compared to other Fe-related fluxes and discussed in a more general context.