Review BGD – "Microbial Fe uptake in the naturally fertilized waters in the vicinity of Kerguelen

Islands: phytoplankton–bacteria interactions"

Author(s): M.F. Fourquez et al.
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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.