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.
MS No.: bg-2014-499
MS Type: Research Article
Special Issue: KEOPS2: Kerguelen Ocean and Plateau Study 2

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 $(\rho Fe: POC)_{bact}^{<25\mu m}$".

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 ($(\rho Fe: POC)^{alone}_{bact}$) 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.