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Interactive comment on "Fe and C co-limitation of heterotrophic bacteria in the naturally fertilized region off Kerguelen Islands" *by* I. Obernosterer et al.

Anonymous Referee #2

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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 respond 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). 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.

Specific Comments:

1. 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 stimulation (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.

2. 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.

3. 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.

4. 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).

5. 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.

6. 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.

7. 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.

Technical Comments:

1. 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.

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

3. 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.

4. 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.

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

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

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

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

9. Line 20 – consisted "of"

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

11. 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.

12. 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.

13. 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.

14. 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.

Interactive comment on Biogeosciences Discuss., 11, 15733, 2014.