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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.

I. Obernosterer et al.

ingrid.obernosterer@obs-banyuls.fr

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We thank the Reviewer for the time invested and the comments made on a previous version of the manuscript.

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), ex-

cept 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 : 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 preconditioning 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

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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

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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

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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

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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.

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

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