**Interactive comment on “Synergistic effects of iron and temperature on Antarctic plankton assemblages” by J. M. Rose et al.**

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Received and published: 14 October 2009

This manuscript presents results on a set of experiments from the Ross Sea examining the response of the natural phytoplankton community to changes in iron and temperature. The results are interpreted in terms of previous experiments in the Antarctic and the possible effects on Antarctic phytoplankton under future climate change are discussed. The overall findings were that synergies between Fe and temperature exist and the response is not a simple linear combination of the two effects. I find the experimental work well designed and executed and the descriptions of this generally well described in the text. The paper is also well written and concise. This work has clear and obvious implications for future field work and modelling studies of biogeochemistry in the Antarctic and will be an important contribution to this field. I would
recommend acceptance of this manuscript after a few minor revisions were made as specified below.

General Comments

Temperature/Iron influences on growth rate/nutrient uptake: I think the authors do a good job of explaining and summarising their data but I would like to see a little more on the possible influence of temperature and/or iron on nutrient stoichiometry (see also below). I think given the data we have from lab studies and from field work in the Southern Ocean it should be possible to have one paragraph on how temperature and iron might specifically alter nutrient stoichiometries particularly with regard to Si to nutrient/metal ratios. This could help explain some of the synergies between iron and temperature limitation of growth rates.

The effects of temperature and iron on nutrient stoichiometry are discussed on page 5868 (page 17-18 in the manuscript). We revised this section considerably based on the suggestions and references provided by Dr. Croot in this comment and below, and added in nearly a page of new text.

Season and Community response: I would also like to some discussion of how the outcome may be influenced by the timing of the experiment with respect to the seasonal cycle of the phytoplankton in the Ross Sea. By this I mean if the experiment was performed in November or December how might the response have been different due to a pre spring bloom phytoplankton community, rather than a possibly post spring bloom community.

In response to this comment and the questions about Figure 6 below, we added the following text to the Discussion section: “This experiment was conducted during austral summer, with a diatom-dominated phytoplankton community that was likely post-spring bloom. The community response to increased iron and temperature would likely have been affected by the season in which the experiment was performed. For example, if the experiment had been conducted in early spring, ambient iron concentrations may
have been higher (Sedwick et al., 2000), which could have resulted in a more iron-replete initial community and the effects of iron additions may have been lessened. At the same time, if the work had been conducted in early spring, ambient sea surface temperature would have been lower and the effect of increased temperature may have been greater. Additionally, the early spring phytoplankton community in the Ross Sea is generally dominated by *Phaeocystis antarctica* rather than diatoms. We observed different effects of iron in particular on the *P. antarctica* and diatom fractions of the community (Figures 3A and 3B), suggesting that results observed for a community dominated by *P. antarctica* may have been very different.”

Main Effects: Throughout the manuscript the term ‘main effects’ is used but without explanation, as many readers may not be aware that this is a statistical term and not simply an adverbial description (e.g. the main event) it would be useful to clarify this in the text. Something simple such as, at the start of section 2.5 stating that: Main effects (the effect of an independent variable on a dependent variable averaging across the levels of any other independent variables) for iron and temperature. . .

We added the suggested text.

Specific Comments

P5853, line 14. Freshwater inputs to the Ross Sea do appear to be changing (Jacobs et al., 2002) and this information should be provided here.

We have added the following text to this section of the introduction (page 5 of the manuscript): “It has been suggested that the Ross Sea is freshening due to increased precipitation, decreased sea ice production and increased melting of the West Antarctic Ice Sheet ..........(Jacobs et al., 2002). All of these can be significant sources of iron, so iron inputs to the Ross Sea may already be changing. Additionally, there is evidence that existing aeolian inputs from more distant continental sources such as Australia may change with a changing climate ...............(Revel-Rolland et al., 2006). “
P5853, line 24. The work of Raiswell and others is of more relevance here with regard to bioavailable iron in icebergs and should be cited (Raiswell et al., 2008; Raiswell et al., 2006). Also can the authors comment in the manuscript specifically about the potential sources of iceberg melt (Gladstone et al., 2001), for instance the mega iceberg B-15 originated in the Ross Sea, or Aeolian dust (Revel-Rolland et al., 2006) to the Ross Sea.

We agree that melting icebergs may be an important source of bioavailable iron to the upper water column in the Ross Sea and have incorporated the Raiswell references into this section of the introduction. We also agree with Dr. Croot that the potential sources of bioavailable iron to the Ross Sea due to iceberg melt (such as B-15) or aeolian dust pose interesting research questions, but our intention in this section of the introduction is a short speculation that existing sources of bioavailable iron may change, and we unfortunately can’t go into an in-depth discussion quantifying changes in these sources in the text of the introduction. We have added some text discussing the potential for changes in the continental source of aeolian dust inputs with future climate change based on the Revel-Rolland referen(see answer to last comment).

P5857, line 19. What was the precision on the nutrient analysis?

The precision for the nutrient analysis is as follows:

- Phosphate: 0.7%
- Nitrite: 2.5%
- Nitrate+nitrite: 1.0%
- Silicic acid: 0.5%

This information was added to the methods section.

P5858, line 2. What borate buffer? This is the first time it is mentioned, so the exact
molar strength should be reported. Also with so much ammonia in the sample it would be a mixed borate-ammonia buffer.

We added the following text to the methods section: “A 5 mM salicylaldoxime (SA: Aldrich, ≥ 98%) solution was first prepared in quartz-distilled methanol (Q-MeOH) and stored in the refrigerator. A final concentration of 25 μM SA was used for total dissolved Fe measurements. A 1.5 M borate buffer was made in 0.4 mol L quartz-distilled ammonium hydroxide (Q-NH4OH) as previously described (Ellwood and van den Berg 2000)”

P5858, line 9. What is the precision of this technique for replicate samples? Can this method replicate the consensus values for the SAFe intercalibration samples?

The precision of the technique is less than 4%. This method has been used on the SAFe samples, for reference please see the Fe section here: http://es.ucsc.edu/~kbruland/GeotracesSaFe/kwbGeotracesSaFe.html. We added the precision of this technique to the methods section.

P5859, line 1. Biogenic sulfur seems an all encompassing term when here it only refers to DMS and DMSP and not to other species such as glutathione or cysteine which could be more significant biogenic S sources (Dupont et al., 2006). I would suggest the Biogenic sulfur term be exchanged to simply “DMS/DMSP”.

We changed this heading to DMS/DMSP.

P5861, line 12. Replace of with for.

Changed text.

P5867, line 6. Higher temperatures also lead to lower inorganic iron solubility (Liu and Millero, 1999, 2002) and there may also be other temperature dependent processes affecting iron bioavailability and this should be discussed within the present manuscript.
Higher temperature can lead to lower inorganic iron solubility (Liu and Millero, 1999, 2002), which could affect iron bioavailability. However, the overwhelming majority of dissolved iron in the Ross Sea is bound in high-affinity organic complexes (>99%, M. Lohan personal comm), and little is known about temperature effects on iron organic chelation. It is possible that there are other, unknown temperature-dependent chemical or biological processes affecting iron solubility as well.

P5868, line 14. Change in community stoichiometry are often critically related to growth rate which in turn is related to Fe and temperature. Given the results here is it interesting to see if the changes in Si uptake observed in the high temperature treatment relative to the high iron treatment represents the fast accumulation of Si at elevated temperature but a still iron-limited growth rate leading to increased BSi:C ratios. Obviously this would require a temperature dependent Si uptake transporter in diatoms and the authors are referred to 3 review papers that have summarised the knowledge on this (Martin-Jezequel et al., 2000; Thamatrakoln and Hildebrand, 2008; Thamatrakoln and Kustka, 2009). This topic could be further explored here in line with other relevant field work (Twining et al., 2004a; Twining et al., 2004b) and recent laboratory experiments (Ho et al., 2003; Quigg et al., 2003). Comparison with the Twining et al. work would seem appropriate as the current treatments are roughly related to the conditions in the SOFeX north and south patches.

We agree that this subject needed further discussion, and thank the reviewer for pointing out these relevant references, most of which we have now added to the paper. We’ve included an extensive new section of text on page 17-18 in the manuscript to examine how our findings compare to the information in the literature on iron and temperature effects on diatom Si utilization. We don’t think our results can be explained by a straightforward temperature effect on a Si uptake transporter; one of the new references Dr. Croot pointed out to us shows that at very high silicic acid concentrations (>30 μM) such as those in our experiments, diatoms instead shift to a non-saturable, diffusion-mediated uptake system (Thamatrakoln and Hildebrand 2008). In our exper-
iment initial silicic acid levels were near 80 $\mu$M, and final concentrations were not less than 45 $\mu$M in any treatment. The diffusion-driven uptake system that seems likely to be operating at these high Si concentrations should be much less affected by temperature increases than active biological transport.

In this new text we also added some comparison of our results to the single cell Si quota measurements of Twining et al (2004). We agree that our temperature treatments could be considered comparable to the SoFeX north and south patches, but unfortunately the Twining et al. 2004 paper presents SXRF results for only the south patch, and we have been unable to locate any similar published results for the north patch. The other Twining et al. 2004 reference focuses only on Fe quota measurements, which we did not make in our experiments; similarly the Ho et al. 2003 reference examined various trace metal quotas as a function of major algal taxonomic grouping, and the Quigg et al. 2003 reference presents similar findings for C:N:P ratios. Although both of these are certainly classic papers, their applicability to this question about Si stoichiometry in our study is not obvious since neither measured Si quotas, or used Fe limitation or temperature changes as experimental treatments.

P5868, line 20. Thus it could be suggested here that warming of the Ross Sea in the absence of iron supply could lead to increased silica drawdown.

We added this speculation to our revised section on nutrient ratios in the Discussion.

P5870, line 9. Some results with rates of microzooplankton grazing are available for both EisenEx (Henjes et al., 2007a; Henjes et al., 2007b; Schultes et al., 2006) and EIFeX (Jansen et al., 2006; Kragefsky et al., 2009).

We thank the reviewer for pointing out the Henjes reference for microzooplankton grazing in EisenEx, and we have included the microzooplankton herbivory results from EisenEx in our discussion of the microzooplankton community on page 19. Unfortunately, the papers by Schultes, Jansen and Kragefsky focus on copepods, which were
largely excluded from our study by the 200 µm pre-screening described in the methods section and so we are unable to make a comparison with the work described here.

Figure 4. The Fv/Fm was relatively high at the start. Does this indicate that you were looking at a post spring bloom community as the initial conditions? Does this have any bearing on the phytoplankton community that was there and would the final communities have been different if the experiment had started in November? Some discussion on this in the text would be useful about the timing of the experiment.

We agree that the results of this experiment are likely seasonally-dependent. In response to these questions and the comment above about Season and Community response, we added the following text to the Discussion: This experiment was conducted during austral summer, with a diatom-dominated phytoplankton community that was likely post-spring bloom. The community response to increased iron and temperature would likely have been affected by the season in which the experiment was performed. For example, if the experiment had been conducted in early spring, ambient iron concentrations may have been higher (Sedwick et al., 2000), which could have resulted in a more iron-replete initial community and the effects of iron additions may have been lessened. At the same time, if the work had been conducted in early spring, ambient sea surface temperature would have been lower and the effect of increased temperature may have been greater. Additionally, the early spring phytoplankton community in the Ross Sea is generally dominated by Phaeocystis antarctica rather than diatoms. We observed different effects of iron in particular on the P. antarctica and diatom fractions of the community (Figures 3A and 3B), suggesting that results observed for a community dominated by P. antarctica may have been very different.”

Figure 4. The Fv/Fm of the high iron, high temperature treatment rises slightly faster than the high iron treatment but starts to drop by day 5. Is this an expected response at 4C? During SOIREE for instance Fv/Fm did not rise as high but nor did it drop rapidly after attaining a maximal value (Boyd and Abraham, 2001). Do the authors have any explanation for this drop that could be explained in the manuscript?
We believe that this response was observed because the increased temperature in the high iron, high temperature treatment increased the growth rates of the phytoplankton assemblage. This resulted in more rapid depletion of available nitrate and iron in these bottles than the treatment at lower temperatures, and thus an earlier decline in Fv/Fm. It is likely that if SOIREE had been run at temperatures 4°C above ambient, the Fv/Fm may have dropped more quickly as well. We added some text to the discussion section making this point more clear.

Figure 6. Are there error bars for the iron results? If not thus should be reported in the figure legend. If yes then the above comment on the precision for the Fe work is also relevant here.

We changed the text in the figure legend to make it clear that there are only error bars for C, N, P and Si.

Interactive comment on Biogeosciences Discuss., 6, 5849, 2009.