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Response to Dr. Maldonado's comments

We thank Dr. Maldonado for her comments and provide our responses below (in italics).

General comments: This manuscript presents the first exhaustive comparison of field-based plankton iron quotas determined with three different, but well established methods. This study took place during an in situ phytoplankton bloom in the subtropical Pacific Ocean off the coast of New Zealand. The data presented are of very high quality, and are nicely discussed in the manuscript. The corrections applied to the data are well thought out. The manuscript is well written. This manuscript would be a very significant contribution to the field of Fe biogeochemical cycles. I thank the authors for taken the initiative to complete this nice study. This research was long overdue and would be greatly appreciated by the scientific community. My comments below are meant to improve this, already excellent, manuscript; as well as to clarify some parts of the manuscript.

Materials and Methods
page 9387, line 6. Somewhere in the manuscript, maybe right here, the authors should define the light levels of their sampling depths (30 and 60 m). Since the bottles for the determination of Fe:C using radioisotopes were incubated at 30% Io, it would be nice to see how this light intensity compares to the depth were the phytoplankton were originally collected from.

Response: We will add the range of light intensities that were measured on the year days on which sampling occurred (~14-30% of surface intensity for the mixed layer).

page 9387, line 10. Something is not right in this sentence; it reads "Each filter was then individually washed with oxalate reagent to remove both extracellular Fe (Tovar-Sanchez et al. 2003). " Either add the other element that is removed with the oxalate wash, or remove "both".

Response: The word 'both' will be removed.

page 9387, line 12. Where is this trace metal clean seawater from? Is this chelexed, artificial seawater Aquil? Or is this seawater from a HNLC region? Do you have the background Fe concentration? This is not important for the washes of the 55Fe:14C ratios, but it will be important for the washes of the particulates collected on filters for the ICPMS-based Fe:C/Fe:P determinations.

Response: We will add text to clarify the origin of the <0.2 µm rinse seawater. The rinse water was collected periodically from within the study region by a trace metal clean fish pumping system and filtered through a 0.2 µm in-line filter in a trace metal clean work area. We do not have background Fe concentration values for the rinse water. However, according to our calculations, even if the rinse water had 0.6 nM DFe (max DFe observed during study), and assuming that all of the DFe in the rinse water scavenged onto particles/filters during the rinse (which is not likely), it would result in a maximum addition of 15 pM to our particulate Fe measurements that ranged from ~0.2-1.3 nM.

*0.6 nM DFe * 0.025 L [5 ml * 5 rinses] / 1 L [smallest volume filtered for ICPMS work] = 0.015 nM Fe*

page 9388, line 9-12 & page 9389, line 10-15. According to the methods described here, the ICPMS filters were treated with the oxalate wash to remove extracellular Fe. Does this include

some lithogenic Fe? If it does, then the ICPMS Fe data is overcorrected for lithogenic Fe, as the oxalate wash would remove some lithogenic Fe, and the ICPMS Fe data is then corrected using a crustal Fe:Al ratio (that assumes no oxalate wash). Is like the data are corrected twice for lithogenic Fe, one using the oxalate wash, and another one using the crustal Fe:Al ratio. The authors should include some discussion about whether the oxalate wash also removes extracellular Al. If the oxalate wash removes to the same extent extracellular Al and Fe, then using the crustal Fe:Al correction after the oxalate wash would be fine, as the two elements are affected equally by the wash.

Response: We are uncertain of what degree the oxalate wash removes lithogenic Fe from the particles we filtered. This issue is discussed in further detail in the Discussion (section 4.3).

Results

page 9393, line 19. It would be helpful to know what mediated the decline of the phytoplankton bloom? Was it Fe limitation, Si limitation and/or light limitation (due to deepening of the mixed layer)? A sentence here with some insights from the authors would be helpful.

Response: We will include text referencing the decline of the bloom due to Fe limitation (Boyd et al., in prep.).

page 9394, line 16. Please add "Oxalate-washed" at the beginning of the sentence "Total P values by ICPMS..."

Response: 'Oxalate-washed' will be added to the beginning of sentence to clarify that total P values by ICPMS were determined from oxalate-washed samples.

page 9395, line 5-7. It is also worth noticing that the total C uptake rate on day 266 was relatively low compared to this study range (3.9 to 8.8 E-7 μmol C μg chla-1 h-1), so probably fast Fe uptake rates combined with low C uptake rates account for the elevated Fe:C ratios on day 266.

Response: We will add text in reference to the contribution of lower C uptake rates on day 266 to higher Fe:C uptake ratios.

page 9395, line 8-10. I will rephrase the sentence as "The results from day 266....were driven largely by higher DFe at this depth, *which probably resulted in faster Fe uptake rates, since the ambient DFe were well below the half saturation constant for Fe uptake (4 nM Fe', Maldonado et al. 2001)*". The present statement is misleading, as it is true that higher DFe will lower the specific activity of the radiotracer but this should not affect your calculated uptake rates, as they were corrected with the daily ambient DFe concentrations. To clarify this, please also add "daily" after "and" in *page 9387, line 16*, where you described how Fe uptake rates were calculated using ambient DFe.

Response: We will move the text about higher Fe uptake rates for the day 266, 30m sample to the Discussion (section 4.2). In section 4.2, we plan to add text regarding higher Fe uptake rates due to higher DFe and cite the half saturation constant for Fe uptake (Maldonado et al., 2001). However, we still contend that if the measured DFe concentration did not match in vitro *DFe, then <u>calculated</u> Fe uptake rates would be higher than <u>actual</u> Fe uptake rates. For example, if 0.2 nM ^{55}Fe was added to seawater with 0.2 nM DFe (0.4 nM total DFe), and 0.1 nM ^{55}Fe uptake was measured, then calculated uptake would be 0.2 nM Fe. If 0.2 nM ^{55}Fe was added to seawater with 0.6 nM DFe (0.8 nM total DFe) with a measurement of 0.1 nM ^{55}Fe uptake, then calculated uptake would be 0.4 nM Fe. The mismatch in*

measured and in vitro DFe could have been an issue due to the temporal discrepancy between the collection of samples for uptake experiments and samples for DFe measurements.

page 9395, line 2-4 & page 9396, line 8-10 & Figure 2 and 3. In general, the radioisotope and ICPMS derived Fe:C ratios showed a trend of higher ratios for the 30 m samples than the 60m samples. Can the authors provide an explanation for this? In terms of Fe quotas and light availability, I would have expected the opposite, higher Fe:C at lower light (60m).

Response: While there were a few cases of significant differences between Fe quotas of samples from 30 m and 60 m, we do not believe that the trend is significant. There are several reasons why a trend might not be likely. 1) For most sampling days, both the 30 m and 60 m samples were collected within the mixed layer. 2) Fe:C radioisotope experiments from both 30 m and 60 m were conducted at the same light level (~30% surface irradiation). 3) SXRF-determined Fe quotas did not detect a significant difference between depths.

page 9396, line 14-16, & 26. Why does lithogenic Fe become more important as size increases? I would have expected the opposite, as smaller particles would have a higher surface area to volume ratio for Fe to adsorb to them. Is this telling us that the lithogenic Fe and Al in this region is mainly comprised of large particles? Is this typical of lithogenic Fe and Al? A reference here supporting the findings of the present study would be useful. Or is this due to the oxalate wash used in this study? Did the oxalate wash get rid of most of the lithogenic/extracellular Fe in the small particles but not on the larger size particles?

Response: Dust particles collected from central Australia have been found to be in the 2-10 µm size fraction (Heese and McTainsh, 1999). During the same study as ours (Ellwood et al., in prep.), non-oxalate washed particulate Al was also higher in the >20 µm fraction in comparison to the 0.2-20 µm fraction. Also, from a nearby region during a 2003 study, Frew et al. (2006) reported that ~50% of LFe was in the >20 µm fraction.

This suggests that there are mixed-layer processes that redistribute Fe and/or Al from lithogenic particles to the rest of the suspended particle field. We plan to add text to this effect. As mentioned above and in the Discussion, we are uncertain what effect the oxalate wash has on lithogenic Fe/Al on our particular samples.

Frew, R.D., Hutchins, D.A., Nodder, S., Sañnudo-Wilhelmy, S., Tovar-Sanchez, A., Leblanc, K., Hare, C.E., and Boyd, P.W.: Particulate iron dynamics during FeCycle in subantarctic waters southeast of New Zealand, Global Biogeochem. Cycles, 20, GB1S93, doi:10.1029/2005GB002558, 2006.

Heese, P.P., McTainsh, G.H.: Last Glacial Maximum to early Holocene wind strength in the mid-latitudes of the Southern Hemisphere from Aeolian dust in the Tasman Sea, Quat. Res. 52, 343-349, 1999.

page 9397 and 9398, section SXRF Fe quotas. In this section there is no discussion of the Fe:C ratios derived using this method. This is worth a sentence here. For example, I found interesting that the Fe:C converted values (using the in situ 133 mol C: mol P ratio), ranging from 17-56, were similar to the ratios I calculated (using the Fe:P and the typical Redfield ratio of 106 mol C:1 mol P), ranging from 21-70. It seems that in general the Fe:C derived using biovolume C may underestimate the ratios. This is briefly mentioned in the discussed.

Response: We will add a sentence to this section referring the reader to the Discussion for text about converting SXRF-determined P to C.

Discussion

page 9398, line 25. Include "oxalate washed" before "total ICPMS-determined BFe:P...

Response: We will add this to the revision.

page 9399, line 1. After "...ICPMS-determined BFe:POC" a parenthesis should be added to specify that the POC was not derived from the ICPMS but from the CHNS elemental analyzer.

Response: This change will be made.

page 9399, line 12. Do you mean 273 instead of 274? There are no data for day 274.

Response: Yes, we erroneously wrote day 274 instead of day 273. This will be corrected in the revision.

page 9401, line 15. Why do you limit the discussion of 14C uptake here to the photosynthetic picoplankton? So far in this paragraph you are discussing Fe:C ratios in general, so why narrow this discussion to the picoplankton. It seems as if a sentence describing Fe:C ratios in the small size fraction is missing here. However, in general the data presented in Table 3 do not support that the Fe:C ratios were the highest in the 0.2-2 μm size fraction, so I am not sure this discussion is necessary here. I would simply mentioned that the 0.2-2 μm size fraction Fe:C ratios include Fe uptake from the heterotrophic and autotrophic bacteria, but only C uptake from the autotrophic bacteria, so the Fe:C ratios in the smallest fraction might be an overestimate of the true ratios of the 0.2-2 μm phytoplankton.

Response: We were in fact referencing the 0.2-2 μm size fraction, as the reviewer deduced. We will edit this paragraph to correctly refer to the 0.2-2 μm size fraction.

page 9402, line 13-15. The most useful information for the reader here is by how much did you increase the ambient DFe as a result of the 55Fe addition. According to my calculation, the 55Fe addition increased the ambient DFe by 1.33 and 7.6 fold. I would change the sentence in line 13-15 to incorporate this information.

Response: We will change the ^{55}Fe addition in terms of 1.3-7.6-fold, as suggested.

page 9402, line 17-18. I do not agree with this statement. See my previous comment for *page 9395, line 8-10.* The result here is due to higher DFe which results in faster Fe uptake rate.

Response: We will edit this to include the suggested edit (also see response to comment for page 9395, lines 8-10).

page 9402, line 20. Replace "affect" with "enhance"

Response: We will make this replacement.

page 9402, line 23. "...in which phytoplankton were observed to be Fe-limited..". Can you provide Fv/Fm for the phytoplankton in these days? This could support your statement on the Fe limited condition of the phytoplankton on day 272 and 275.

Response: Unfortunately, we do not have Fv/Fm measurements from year days 272 and 275. We will either quantitative comment to support the observation of Fe limitation from the deckboard bottle experiments (Wilhelm et al., in prep.,) or we will remove this sentence in the revision.

page 9403, line 1-5. The key here is that the Fe addition should be as minimal as possible to avoid changes in the ambient (and speciation) Fe concentration. If this is achieved, the cells will be in their steady-state, and the Fe and C uptake rates should reflect the in situ uptake rates. As a result the ratio of this rates should be the steady-state Fe:C phytoplankton ratios. Adding Fe bound to an organic ligand is one way to achieve this, but the Fe and the organic ligand concentrations added, as well as the organic ligand of choice need to be carefully thought out.

Response: We will include these ideas regarding Fe uptake experiments in the revised text.

page 9403, line 25. It is ok to use the Fe:Al ratio of the crustal material to correct the data even if dissolution occurs, the key is that the dissolution of both Fe and Al needs to occur and to the same extent. This complements the comment above, see *page 9388, line 9-12 & page 9389, line 10-15.*

Response: We agree with this statement and will add more discussion to this point. Some text regarding the extent of Fe and Al dissolution is already in the proceeding sentence (p9403, lines 26-28).

page 9406, line 14. Here would be a good place to include information about the dissolution of Fe relative to Al in lithogenic particles? In the cited manuscript, do the author report % of solubilisation of Fe relative to Al?

Response: We will include % solubilization of Fe and Al from lithogenic particles (soils) as reported in McKeague (1967). They reported that a relatively small, but equal fraction of Fe and Al was recovered from a variety of amorphous inorganic substances (1.5% of Fe and 1.3% of Al).

McKeague, J.A.: An evaluation of 0.1 M pyrophosphate and pyrophosphate-dithionite in comparison with oxalate as extractants of the accumulation products in podzols and some other soils, Canadian Journal of Soil Science, 47, 95-99, 1967.

page 9406, line 26-27. It is not clear what this last sentence is describing. Is it describing data from the present study or data for the *Trichodesmium* study/es? Please modify the sentence to clarify.

Response: The last sentence is describing data from the present study. We will restructure the end of the paragraph to be clearer.

page 9408, line 28. days 265 and 276 are not included in Table 1. Is this a typo here? According to Table 1, the highest DFe were found on days 263, and 266, and maybe also 273 and 275.

Response: DFe concentrations from days 265 and 276, while not reported in this article, are reported by Ellwood et al. (in prep.). Because Fe quotas were not measured on these particular year days, DFe values from these days are not reported in this manuscript. The appropriate explanation and citation will be added.

page 9410, line 10-11. Adding 55Fe bound to an organic ligand could be suggested here, as a mean to not disturb the system.

Response: We will add this idea to the Recommendations text.

page 9410-9411. Given this beautiful data set, which include Fe:P and Fe:C, I think the authors are in a unique position to comment on what is best to normalize biogenic Fe data. I believe a paragraph should be included on the pros and cons of using P or C…. This is especially important for the ICPMS data, as the remineralization of organic C and P is different in the water column. This should be discussed somewhere here.

Response: We agree that commentary regarding the use of P or C for normalizing Fe quotas is relevant to the manuscript. In the revision, we will add a paragraph pertaining to the advantages/disadvantages of using particulate P or C for Fe quota normalization. In support for the use of C for normalization, we believe that C directly links atmospheric CO_2 and the oceanic biological pump. The longer remineralization time scale of C (relative to P) is potentially important for assessing Fe quotas of sinking particles. Measuring C uptake (if using the Fe:C uptake ratio technique) is a much less daunting task in comparison with ^{32}P-uptake. P, on the other hand, is generally less plastic than cellular C content. P can also be directly measured by techniques that also measure Fe (ICPMS, SXRF).

Tables

Table 2. The numbers reported in this table under the heading "chla % of total" need to be multiplied by 100 in order to reflect true %. This comment also applies to Supp. Table 2, 3, and 4.

Response: We will change the "% of total" heading to "fraction of total" to accurately reflect values in the table.

Table 3. More information is needed in this legend. Please include "oxalate-washed, ICPMS determined" before "BFe:P.."

Response: We will include "oxalate-washed" in the legend.

Table 5. Spell out LS at the beginning of the legend.

Response: The acronym, LS, will be spelled out in the revision.

Table 7. I would include some advantages and disadvantages here about what it is used to normalize the Fe data for each one of the three methods. Normalization is clearly more problematic for the ICPMS derived data. In the radioisotope method I would emphasize the need to careful designed the additions of Fe (with or w/out an organic ligand) to not disturb the steady-state condition of the phytoplankton.

Response: We will add phrases to the table in reference to normalization-related issues, as well as the suggestions for Fe:C uptake ratios.

Figures

Figure 1. In this legend nothing is said about significant differences between 30 and 60m. Is this because there are no significant differences? If so, please mention it at the end of the legend.

Response: Significant differences between 30 m and 60 m of total POC and oxalate-washed particulate P values will be indicated with an asterisk and mentioned in the legend. For POC, this occurred on year days 266, 267, 269, 270, and 278. For oxalate-washed particulate P, this occurred on year days 267, 269, 270, 272, and 273.

Figure 2. I am assuming that the data presented in Panel A were corrected for ambient DFe concentrations so these are the Fe:C ratios derived from radioisotope experiments. I will change the Y-axis label in Panel A to "µmol Fe:mol C" instead of "µmol 55Fe:mol 14C". Also the Y-label of Panel B should be changed from "µM Fe uptake h-1 µgchla-1 L-1" to "µmol Fe µgchla-1 h-1 ". Similarly, the Y-label of Panel C should be changed from "M C uptake h-1 µgchla-1 L-1 " to "µmol C µgchla-1 h-1". This comment also applies to the legend of Supp. Table 3

Response: We will change the y-axis labels in Fig. 2 and the legend of Supp. Table 3 to reflect the simplified uptake units, as suggested.

Figure 3. Emphasize "oxalate washed" ICPMS based BFe:P…

Response: "Oxalate-washed will be added to the Fig. 3 legend.

Figure 5. In order to see better the SXRF and radioisotope derived Fe:C ratios, please change the Y-axis range in Panel A from 0-30 to 0-25 and in Panel B from 0-200 to 0-150. In the legend insert "total" after "Comparisons of.."

Response: We viewed Figure 5 with the scale on the Y-axes reduced as suggested, but this resulted in the error bars having greater values than the axes range. We will therefore leave the axes unchanged.

Supplementary Table 3. I would change the units of the legend in this table in order to remove all these E-6, E-7 or E-8 next to the numbers. For example instead of reporting 2.3E-6 pmol Fe ug chla-1 h-1, report 2.3 amol Fe ug chla-1 h-1. Similarly, for the C instead of reporting 1.6 E-7 µmol C µg chla-1 h-1 , report 0.16 pmol C µgchla-1 h-1.

Response: This change in units will be made to Supp. Table 3.