

***Interactive comment on “Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*” by L. J. Hoffmann et al.***

**Anonymous Referee #1**

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Review of “Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*”. By L. J. Hoffmann, I. Peeken and K. Lochte

General comments

In this study, Hoffmann and co-authors describe the effect of iron availability on the elemental ratios of particulate organic carbon (POC), nitrogen (PON), phosphorous (POP) and opal (bPSI) using new data from the latest in-situ iron fertilization experiment in the Southern Ocean (EIFEX) and in culture experiments with two Southern Ocean diatoms that dominated phytoplankton assemblage during EIFEX. One of the

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main conclusions from this study is that iron fertilization will affect elemental ratios of particulate matter, in particular the ratios of bPSI to other elements, in ways that cannot be as easily predicted as previously thought. In particular, differences in species composition of diatom assemblages following iron addition will lead to different elemental stoichiometry of bPSI as compared to other elements in particulate organic matter. These results have important consequences for the understanding of changes in the biogeochemistry of the ocean following natural or artificial iron fertilization. I, therefore, believe that these results should be published in Biogeosciences. I have, however, some concerns regarding the interpretation and analysis of data that should be addressed before publication. The major points are listed below. The text (English) also needs some improvement, in particular in the “Materials and methods” and “Results” section (see “Technical corrections”).

#### Specific comments

1) The authors discuss elemental ratios during EIFEX, however, considering that in the field the initial particulate matter contains probably a large fraction of dead or detrital material I would recommend to include a comparison of the ratios of the changes in particulate elements. This might not be possible for out-patch stations as no important changes in biomass were observed, but the authors might find out that elemental ratios do not change that much, inside the fertilized patch. Values obtained with this method should be also more representative of the elemental ratios of the biomass growing during the experiment.

2) p. 255, lines 15-24: Chlorophyll a vs time curves in experiments with *C. dictyota* are similar in all treatments (in particular between day 20 and 35 after some lag phase). Considering that cellular content of chlorophyll a (but also C, N and P) in the no-iron treatment is less than half the values for the iron treatments it seems that growth rates estimates based on cell counts should be higher in the treatment without iron yet the growth rates you present are lower can you explain/discuss that? Your results also suggest that cellular Si “consumption” on a volume basis is also larger in *C. dictyota*

under low iron conditions (see also Leynaert et al., *Limnol. & Oceanogr.*, 49: 1134-1143, 2004).

3) p. 255, lines 15-26: Your growth rates for *C. dictyota* and *F. kerguelensis* are much lower than those given in Timmermans (2004) and Timmermans et al. (2001), why? Are these differences due to experimental conditions? Can you give more information on that (light intensity, temperature etc). Elemental composition of *F. kerguelensis* (Table 3) is also quite different from Timmermans (2004). This is also puzzling to the reader and some explanation (in the discussion) should be given.

4) p. 258, lines 7-11: "We found an increase in cell volume by a factor of 1.3. However, this increase in cell volume would only result in cellular C, N and P concentrations of 2.7, 0.5 and 0.06 pmol cell<sup>-1</sup> and can, therefore, not explain all of the observed increase". Where do these estimates of 2.7, 0.5 and 0.06 pmol cell<sup>-1</sup> come from? Is this conclusion robust?

5) p. 261, lines 4-6: "The bPSI : POC and bPSI : PON ratios of both species were relatively close to those found in the field" I do not see that this is the case for bPSI : PON for *F. kerguelensis*, for example, be more precise. I also doubt that a comparison of field data as calculated in the manuscript (a mixed plankton community including live and detrital material) with cultures of single diatom species makes much sense, especially if you are using bPSI as the reference element for the comparisons further down in the discussion (see also point 1).

6) p. 261, lines 22-24: see point 2) If volume specific values are compared *C. dictyota* also has higher Si under low iron conditions.

7) p. 262, lines 3-4: Yes! What about possible variations in intracellular pools of Si ? The magnitude of the intracellular Si pool can be similar to the cellular content changes in Si measured in your culture experiments and are species-specific (Martin-Jézéquel et. al., 2000). Also intracellular pools might be released upon cellular decay instead of being exported.

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8) p. 262, lines 14-15: see previous point 6).

9) p. 262, lines 15-17: “ As we observed that bPSi : POC , bPSi : PON and  $\check{E}$ , these mechanisms will be of less importance for analysis of nutrient budgets.”. What is the meaning of this sentence? And of the next one?

10) p. 263, lines 1-29: Buoyancy in diatoms is probably strongly regulated and the role of frustule thickness in determining sinking rates of live cells is highly speculative. The same applies to the influence of frustules thickness of *F. kerguelensis* on Si cycling after iron fertilization. It seems that species assemblage shifts might be more important (see Abelmann et al., paleoceanogr., 21, PA1013, doi:10.1029/2005PA001199 and results presented in this manuscript).

11) Data from this study are discussed and interpreted in the light of previous studies. For the sake of clarity it would be helpful to have a table with data from the previous studies mentioned in the manuscript, in particular those concerning the effect of iron on elemental composition both in the field and in culture experiments.

#### Technical corrections

The text needs substantial improvement in the “Materials and methods” and “Results” section. I have made some suggestions for improvement (until p. 254). I recommend more thorough proof reading of the manuscript before resubmission.

p. 251, line 26: Change to “and a decrease of cellular bPSi with increasing iron availability is not always observed (Takeda, 1998).”

p.252, lines 13-17: Change to “A detailed description of the phytoplankton community structure, total and size fractionated POC,PON, POP as well as total bPSi concentrations and corresponding molar ratios during EIFEX is given by Hoffmann et al. (2006)

p. 252, lines 17-22: Change to “Here we present new information on bPSi : POC, bPSi : PON, and the bPSi : POP molar ratios in the size fractions  $>20 \mu\text{m}$  and  $<20 \mu\text{m}$  during EIFEX together with results from culture experiments with the Southern Ocean

diatom species *F. kerguelensis* and *C. dictyota*.”

p.252, line 24: Change to “culturing conditions”

p.252, lines 25-26: Change to “Two experiments with three iron treatments and EDTA were carried out: one treatment with EDTA only, one treatment with EDTA and 100 nM Fe addition and one treatment with EDTA and 1000 nM Fe added. Three replicates were incubated for each treatment.”

p. 253, lines 1-3: start new sentence and change to “In the two iron enrichment treatments, respective free iron concentrations (Fe<sup>+</sup>, including all inorganic Fe species) were 1.55 nM Fe<sup>+</sup> and 15.5 nM Fe<sup>+</sup> as estimated after Timmermans et al. (2001)”.

p. 253, lines 3-5: Change to “An additional experiment was carried out with *F. kerguelensis* without EDTA and iron addition in order to investigate the effect of EDTA on growth and stoichiometry.”

p. 253, lines 10-11: Change to “POC, PON, POP, and bPSi samples from culture experiments were not size fractionated.”

p. 253, lines 11-12: Remove sentence “However, the filters used and sample storage was the same as for the EIFEX samples. “ since no information on samples processing is given in the manuscript.

p. 253, lines 19: Change to “Growth rates of *C. dictyota* and *F. kerguelensis* in culture experiments were calculated as  $\mu$ ”

p. 254, lines 25-27: Change to “During EIFEX, composition of particulate organic matter (POM) inside the iron fertilized patch showed different temporal trends in the >20  $\mu$ m and the < 20  $\mu$ m size fractions (Fig. 1).”

P. 254, lines 1-2: Change to “Almost no changes were found in the > 20  $\mu$ m size fraction during the first 16 days of the experiment. After day 16, bPSi, POC, PON, and POP concentrations increased by a factor of 4.1, 1.9, 4.1 and 1.4, respectively (Fig.

1a).”

p. 254, lines 4-6: Change to “In the size fraction  $< 20 \mu\text{m}$ , no changes in POC, PON, and POP concentrations were observed but bPSi increased continuously until the end of the experiment, on day 37 after the first fertilization. The final bPSi concentration in the size fraction  $< 20 \mu\text{m}$  was 2.5 times the initial value at the end of the experiment (Fig. 1b).”

p. 254, lines 7-9: Change to “Inside the fertilized patch, the bPSi : POC, bPSi : PON and bPSi : POP ratios of the total biomass increased until the end of the experiment. Final values were higher by a factor of 2.1, 1.3 and 2.6, respectively. In non-fertilized water no changes in concentration and composition of particulate organic matter were observed (Table 2).”

p. 254, lines 9-11: Change to “Separation of the total biomass in  $>20 \mu\text{m}$  and  $<20 \mu\text{m}$  size fractions shows that the same trends were found in the molar ratios of both size classes (Fig. 2). “

p. 254, line 13: Change to “at the start of the experiment to 0.4, 2.3, and 40.0 on day 37, inside the fertilized patch.”

p. 254, lines 21-23: Change to “In culture experiments with *F. kerguelensis* and *C. dictyota*, iron addition resulted in a significant increase in photosynthetic efficiency (Fv/Fm), maximum growth rates and chlorophyll concentrations as compared to the control treatments (Fig. 3 and Table 3).”

p. 254, lines 23-25: Change to” Growth rates, Chl concentrations, and Fv/Fm were similar in treatments without iron addition and *F. kerguelensis* (treatments A and B; t-test,  $p=0.3-0.6$ ).”

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