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Comment

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

L. J. Hoffmann et al.

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Authors reply to the review # 1 on the manuscript “Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms *Fragilariopsis kerguelensis* and *Chaetoceros dicaeta*” by L. J. Hoffmann et al.

Reviewers comment # 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

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obtained with this method should be also more representative of the elemental ratios of the biomass growing during the experiment.

Authors reply: We agree that the initial particulate matter probably contains a large fraction of dead or detrital material. The way of data presentation suggested by the reviewer is probably more representative of the total biomass growing during the experiment. However, we feel that in our case, using different size fractions, it is susceptible to errors and not very meaningful. For example in the $> 20 \mu\text{m}$ size fraction almost no changes in the POC, PON, and POP concentrations were found during the first 22 days of the experiment, while BSi ratios already started to increase after day 16. The ratios of the changes compared to the start day are therefore partly negative on day 16 and very high on day 22. Therefore we doubt that this way of data presentation provides new important information since the way to determine the values is highly affected by small changes compared to the initial value. After having carefully evaluated this suggestion we feel that our initial way of data presentation better fits the information of the data and decided not to include a figure as suggested by the reviewer.

Anonymous reviewer # 2: p. 255, lines 15-24: Chlorophyll a vs time curves in experiments with *C. dichæta* 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. dichæta* under low iron conditions (see also Leynaert et al., *Limnol. & Oceanogr.*, 49: 1134-1143, 2004).

Authors reply: The growth rates given in table 3 are the maximum growth rates, calculated based on cell numbers, during the experiments. All treatments showed maximum growth rates during the first 9 days of the experiment and in this period the high iron treatments showed significantly higher growth rates. It is true that growth rates based

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on cell counts are higher in the low iron treatment during day 16 and 30 (0.09 for the low iron treatment and 0.02 for the two high iron treatments). It seems that the iron concentration in our experiments affects the growth behavior of this species. Grown under low iron concentrations, *C. dicaeta* has a longer log phase but grows more constantly over a longer time period. In the high iron treatments the maximum growth rate is significantly higher, but the species is not able so sustain this high growth rate over a longer period and reaches maximum biomass earlier compared to the low iron treatment (compare figure 3). The cellular Si content in *C. dicaeta* is not significantly different between the different treatments, not even on a volume basis. Si per cell volume in the low iron treatment B is 0.44 ± 0.07 and 0.4 ± 0.06 and 0.32 ± 0.11 in the high iron treatments C and D, respectively. We agree that it is more precise to calculate the Si content per cell volume instead of per cell when changes in the cell volume are found. We therefore included the following sentence in the discussion section of the manuscript: “Even though cell volume was lower by a factor of 1.3 under iron limitation in the latter species Si concentrations per cell volume showed no significant difference as well (0.44 ± 0.07 in the low iron treatment B and 0.4 ± 0.06 and 0.32 ± 0.11 in the high iron treatments C and D).”

Anonymous reviewer # 3: p. 255, lines 15-26: Your growth rates for *C. dicaeta* 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 and E 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.

Authors reply: The differences in the growth rates can very well result from differences in the experimental conditions. Timmermans et al. (2001) grew *C. dicaeta* under a constant light intensity of $80 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and changed two different light : dark cycles of 12 : 12 and 20 : 4 hours. *F. kerguelensis* was grown at $60 \mu\text{mol photons}$

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$m^{-2} s^{-1}$ at a light : dark cycle of 16 : 8 hours (Timmermans et al., 2004). In our experiments both species were grown at $30 \mu\text{mol photons } m^{-2} s^{-1}$ and a light : dark cycle of 16 : 8 hours. The experimental conditions of our experiments are now explained in more detail in the “material and methods” section and were chosen in order to represent natural Southern Ocean long day growth conditions under deep mixing. The differences in the elemental composition of *F. kerguelensis* likely results from the different ways this parameter was determined. In our experiment we calculated the cellular elemental composition from direct measurements of POC, PON, POP, bPSi, and cell numbers in the cultures. Timmermans et al. (2004) present the consumption per cell of Si(OH)_4 , NO_3^- , and PO_4^{3-} and determined this parameter by the decrease of the dissolved Si(OH)_4 , NO_3^- , and PO_4^{3-} concentrations and the increase in cell numbers in their experiments. To clarify this to the reader, we included the following sentences in the discussion section: “The data for *F. kerguelensis* reported by Timmermans et al. (2004) are lower than those presented in this study. To avoid confusion it should be noted in this context that the data reported by Timmermans et al. (2004) are cellular NO_3^- and PO_4^{3-} consumption rates estimated by the decrease of these nutrients in the culture medium. These data can therefore not directly be compared to particulate N and P concentrations presented in this manuscript.”

Anonymous reviewer # 4: p. 258, lines 7-11: μDWe found an increase in cell volume by a factor of 1.3 μE However, this increase in cell volume would only result in cellular C, N and P concentrations of 2.7, 0.5 and 0.06 $\mu\text{mol cell}^{-1}$ and can, therefore, not explain all of the observed increase μE . Where do these estimates of 2.7, 0.5 and 0.06 $\mu\text{mol cell}^{-1}$ come from? Is this conclusion robust?

Authors reply: When assuming constant cellular elemental composition, an increase in cell volume by a factor of 1.3 would increase the cellular C, N, P, and Si concentration also by a factor of 1.3. The cellular C, N, and P concentrations of 2.7, 0.5, and 0.06 μmol therefore result from the complete (not rounded) numbers (2.03, 0.39, and 0.047) for the cellular C, N, and P concentrations in the low iron treatment B multiplied by the

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complete (not rounded) factor 1.3183 ($2.03 \times 1.3183 = 2.6761$; $0.39 \times 1.3183 = 0.514$; $0.047 \times 1.3183 = 0.062$). We agree that this way of presentation can be confusing and changed the sentence as follows: “Assuming constant cellular elemental composition, this increase in cell volume would also result in a 1.3 times higher cellular C, N, P, and Si concentration. In fact, the cellular C, N, and P concentration increased 3.0, 3.0, and 2.2 times in the high iron treatment C compared to the low iron treatment B and 1.7, 1.8, and 1.8 times in the high iron treatment D compared to treatment B (compare Table 3). Therefore, the observed increase in cell volume under higher iron availability can not explain all of the observed increase in cellular C, N, and P concentrations.”

Anonymous reviewer # 5: p. 261, lines 4-6: a DThe 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).

Authors reply: We agree that this section is not precise enough. It is definitely right that a direct comparison of elemental ratios from culture experiments with those from natural phytoplankton assemblages is difficult. However, in this case we strongly believe that it is possible since *F. kerguelensis* was the most abundant species during EIFEX. We changed the paragraph as follows: “The BSi : POC and BSi : PON ratios of *F. kerguelensis* were relatively close to those found in the $> 20 \mu\text{m}$ size fraction and in the total community towards the end of the experiment in the field (Fig. 4, 5 and Table 2). This observation is not surprising since *F. kerguelensis* has been the most abundant species during EIFEX (Assmy et al., 2005).”

Anonymous reviewer # 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.

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Authors reply: The volume specific Si concentrations are also not significantly different. See also authors reply to comment # 2. We included the following sentence in the corresponding part of the discussion: “Even though cell volume was lower by a factor of 1.3 under iron limitation in the latter species Si concentrations per cell volume showed no significant difference as well (0.44 +- 0.07 in the low iron treatment B and 0.4 +- 0.06 and 0.32 +- 0.11 in the high iron treatments C and D).”

Anonymous reviewer # 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.

Authors reply: This is an interesting and important point. The intercellular Si pools will definitely affect the BSi:POC, BSi:PON, and BSi:POP ratios and we agree that this should be mentioned. However, since there are huge differences between the species and no information about the species tested here are available in the literature it is difficult to estimate the importance of internal Si pools for this study. Martin-Jézéquel et al. (2000) mention that these pools are sometimes very low since Si is only taken up directly before cell wall formation and not stored over a longer time in the most diatom species. It is questionable if diatoms have a high amount of Si in internal pools when they are not actively growing anymore towards the end of a bloom when massive sedimentation occurs. Nevertheless, we agree that this point should be mentioned and included the following passage in the discussion section: “However, it is important to mention in this context, that not all cellular Si is in the frustule, but that dissolved, internal Si pools can provide a significant amount (Martin-Jézéquel et al., 2000). These pools are usually low since Si is only taken up directly before cell wall formation and not stored over a longer time in the most diatom species (Martin-Jézéquel et al., 2000). We only found significant differences in the BSi : POC, BSi : PON, and BSi : POP ratios between the high and low iron treatments of *F. kerguelensis* at day 34 (see figure

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4) but not earlier during in the growth period at day 20, when higher division rates were observed and thus higher internal Si pools could be expected. We are therefore confident, that the cellular Si concentrations taken from day 34 which is presented in the manuscript mainly represent frustule bound Si and that our estimation concerning export are right. In *C. dictyota* the differences in the BSi : POC, BSi : PON, and BSi : POP ratios between the high and low iron treatments were still significant at day 43, when all treatment had reached highest chlorophyll concentrations. However, in this species the differences were even higher at day 31, when cells were still actively growing, at least in the low iron treatment (compare figure 4). Here, internal Si pools might play an important role.”

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

Authors reply: As described in our reply to the reviewers comment # 2 and 6 there is no significant difference between the volume specific Si content of the high and low iron treatments of *C. dictyota*.

Anonymous reviewer # 9: p. 262, lines 15-17: “ As we observed that bPSi : POC , bPSi : PON and μ 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?

Authors reply: To clarify what we wanted to say we changed the two sentences as follows: “The observed changes in the cellular nutrient concentrations resulted in the same changes in the BSi : POC, BSi : PON, and BSi : POP ratios for both species. Therefore, the changes in the cellular concentrations will be of less importance for analysis of nutrient budgets. However, they can possibly affect the sinking behavior as well as the remineralization of frustules in the sediments.”

Anonymous reviewer # 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

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shifts might be more important (see Abelman et al., paleoceanogr., 21, PA1013, doi:10.1029/2005PA001199 and results presented in this manuscript).

Authors reply: We absolutely agree that changes in species composition are very important concerning export and Si cycling after iron fertilization and already mentioned this topic in the very last sentence of the first version of the manuscript: We also agree that this point should be pointed out and included the following sentence in the discussion: “It seems that the overall budget of carbon export after iron fertilization is mainly influenced by changes in the phytoplankton community structure (see Abelman et al., 2006 and results presented in this manuscript).

Anonymous reviewer # 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.

Authors reply: We agree that it is helpful to present the data from previous studies. However, the data in the literature concerning the effect of iron on elemental composition both in the field and in culture experiments are extensive and difficult to combine since most of them were collected under different culture conditions. We therefore feel that it is beyond the scope of this manuscript to present all of this information since this manuscript is not a review article. For comparison: Timmerman et al. (2004) alone present 232 data points on this subject.

Anonymous reviewer # 12: 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.

Authors reply: We made all the changes listed below as suggested by the reviewer.

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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 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', including all inorganic Fe species) were 1.55nM Fe' and 15.5nM Fe' 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 μ E”

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 $>20 \mu\text{m}$ and the $< 20 \mu\text{m}$ size fractions (Fig. 1).”

P. 254, lines 1-2: Change to “Almost no changes were found in the $> 20 \mu\text{m}$ size fraction during the first 16 days of the experiment. After day 16, bPSi, POC, PON,

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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).”

We did not made the following changes as suggested by the reviewer since the whole text was rewritten due to suggestions made by the other reviewers.

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

Authors reply: We changes this sentence to as suggested by reviewer # 2 to: “While

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this can be caused by decreased cellular Si levels upon release from iron stress (Hutchins and Bruland, 1998; Takeda, 1998) other studies show the effect to be driven mainly by increase in cellular nitrogen and carbon with little or no change in cellular Si (Franck et al., 2003; Takeda, 1998).”

p. 252, lines 17-22: Change to a DHere 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*.”

Authors reply: We changed the sentence to: ”Here we present new information on the POC, PON, POP, and BSi concentrations as well as the BSi : POC, BSi : PON, and the BSi : POP molar ratios in the size fractions $>20 \mu\text{m}$ and the $<20 \mu\text{m}$ 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 a Dculturing conditions“

Authors reply: This sentence was deleted since the paragraph was rewritten.

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, $p=0.3-0.6$).”

Authors reply: We changed the sentence to: “Growth rates, Chl concentrations, and Fv/Fm were not statistically different between treatments with and without iron addition in *F. kerguelensis* (treatments A and B; t-test; $p = 0.3 - 0.6$).”

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