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Biogeosciences
Discussions

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.

L. J. Hoffmann et al.

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Authors reply to the review # 3 on the manuscript "Effects of iron on the elemental stoichiometry during EIFEX and in the diatoms Fragilariopsis kerguelensis and Chaetoceros dichaeta" by L. J. Hoffmann et al.

Initial authors comment: The manuscript presents novel data on the effect of iron avalability upon the mechanisms responsible for changes in stoichiometry of major elements in diatoms and therefore deserves publication. However my opinion is that the exploitation of data has been too fast in several poitns and then need to be sddressed more cardully. Authors often use shortcuts which focuse on the conclusions they want to reach. Also on such a subject like phytoplankton elemental stoichiometry, the litterature review has to be exhaustive while, as is often obervsed, the litterature analyis seems to me to be resttricted to the recently started e-litterature era (i.e. considering only works $>$ 1995). It is very surprising that the classical paper by Brzezinski (1985) has been omitted. Also a large part of the manuscript addresses the question of vertical export, a point which is not supported by any new data in this paper. My opinion is that the manuscript has to be rewritten and is not acceptable as it is. It would probably benefit from a fusion with the other companion paper submitted at the same time to Biogeoscience. One has to avoid the dispersion of expeimental data between several papers and to focus on more synthetic approaches aimed at facilitating the analysis by colleagues and foster faster progress in knowledge and comprehension increase.

Authors reply: From the following detailed comments by the reviewer we can not understand the upbraiding that we often use shortcuts which focus on the conclusions we want to reach. Additionally we want to emphasize that it was not our intention to write a review article about phytoplankton elemental stoichiometry. This manuscript solely deals with the effect of iron limitation on the elemental stoichiometry of diatoms, a topic that has not been studied before the data of the first iron fertilization experiment were published (Martin and Fitzwater, 1988). That is the only reason why mainly recent literature has been cited. We feel that it is beyond the scope of this manuscript to present an extensive review of the complete literature concerning phytoplankton elemental composition, especially since this topic has been reviewed quite often recently. However, we agree that the introduction should be more detailed and rewrote it, taken the suggestions made by the reviewer into account. We also included the paper by Brzezinski (1985). It is true that we do not provide any direct data on export in this manuscript. However, the elemental composition and silicification of dominant Southern Ocean diatoms is definitely of interest concerning the export of organic matter after an iron induced bloom. We therefore feel that this topic should be discussed in this context. We definitely do not see how and why we should combine this manuscript with the other one submitted at the same time to Biogeosciences. Both manuscripts are based on completely different datasets, which resulted from independent experiments. The experiments were performed to investigate different questions and, except for C. dichaeta, different species were used. It is therefore not true that we dispersed data between different manuscripts.

Reviewers comment # 1: Important : Refering to biogenic silica, please use the acronym BSi which is widely accepted instead of introducing the confusing "bPSi" terminology.

Authors reply: We changed the terminology as suggested by the reviewer.

Reviewers comment # 2: English phrasing should be carefully checked.

Authors reply: Two native speakers read the manuscript.

Reviewers comment # 3: Introduction This part has to be reconsidered in more details and strengthened. My impression is that authors have been tempted to extract only yhe recent litterature and in that amount of publications have been tempted to choose what sticked closer to theire impressions. For example, it has never been argued in the past (and certainly Redfield did not) that Redfield ratios are supposed to be representative of living organisms. Rather, studies conducted in the 70's-80's tended to demonstrate that Redfield ratios ar emore specific of aged organic matter and are individually found in senescent populations or detritus accumululation levels. Perhaps this should be mentioned with appropriate references.

Authors reply: As mentioned in our reply to the initial statement by the reviewer this manuscript solely deals with the effect of iron availability on diatom elemental stoichiometry. The reason why mainly new literature was cited in the introduction is that this topic has only been studied since the first classic experiments by Martin and co-workers (Martin and Fitzwater, 1988). We feel that it is beyond the scope of this manuscript to present an extensive summary of the complete literature concerning phytoplankton elemental composition. However, we extended the introduction and especially described the physiological effects of iron on nitrate, carbon, and silicate uptake in more detail

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(see authors reply to the next reviewers comment). We can not agree with the statement by the reviewer, that is has never been argued in the past, that Redfield ratios are supposed to be representative for living organisms. Based on the dataset of R. H. Fleming from 1940, Redfield concluded that the elemental composition of plankton was uniform and that the inorganic C : N : P ratio of the seawater “were almost entirely a result of the synthesis or decomposition of organic matter” (Redfield, 1958). Despite the extensive literature describing exceptions from Redfield’s assumption under certain unfavorable growth conditions, it is commonly expected that living marine phytoplankton organisms on average incorporate macronutrients according to the Redfield ratio and this assumption is widely used in biogeochemical models (e.g. Armstrong, 1999).

Reviewers comment # 4: Examples of enzymatic processes presented are only examples and not exclusive of other important processes at the cellular level (chelatase, eventual nitrogenase functions, etc. . .). For example, the increase in Chl a per cell is related to resuming by Fe addition of both chlorophyll synthesis and nitrate utilization.

Authors reply: We agree that we should be more detailed in this point and included the following passages to the introduction: ”Iron is needed in the nitrogen metabolism of phytoplankton cells. For the synthesis of amino acids nitrate has to be reduced to ammonium. This occurs in a two step reduction, where the energy is derived from Fe-dependent photosynthetic redox reactions. Both enzymes involved, nitrate and nitrite reductase, have a high iron content. Additionally nitrite reductase uses reduced ferredoxin, an iron-sulfur redox protein, or the non-iron-containing flavodoxin to reduce nitrite to ammonium. Therefore, iron limitation leads to reduced nitrate uptake rates (Price et al., 1994) and lowers nitrate reductase activity (Timmermans et al., 1994). However, it is not known if the latter is due to a direct reduction in the enzyme activity or indirectly via a reduced supply of the reductant from photosynthesis. Besides nitrate, phytoplankton cells can directly take up ammonium and incorporate it into amino acids without the use of iron containing enzymes. Therefore the iron demand of phytoplankton cells is higher when growing on nitrate compared to ammonium as N source

(Maldonado and Price, 1996; Raven, 1988). This implies that in low Fe waters like the SO ammonium uptake is preferred and new production is suppressed (Maldonado and Price, 1996) despite the surplus of nitrate. To fulfill the higher iron requirements for nitrate uptake, phytoplankton cells have higher iron uptake rates when growing on nitrate compared to ammonium (Wang and Dei, 2001)." "Besides nitrate uptake, iron affects the efficiency of the photosynthetic apparatus and thus probably carbon uptake. Iron is an essential part of the iron-sulfur proteins and ferredoxin of the photosystems and the heme and iron-sulfur proteins of the cytchrom b6f complex. It therefore plays an important role in the photosynthetic electron transfer and is essential for photosynthetic energy supply (Greene et al., 1991; Greene et al., 1992; Greene et al., 1994). Under iron limitation a visible decrease in chlorophyll concentration (chlorosis) as well as a decrease in the photosynthetic efficiency (Fv/Fm) is generally observed." "It is generally assumed that higher silicification is caused by a reduction in growth rate and an increased duration of the cell in the G2 + M phase of the cell cycle during which Si uptake occurs (Martin-Jézéquel et al., 2000). Therefore, the effect of iron on the BSi : POC, BSi : PON, and BSi : POP ratios is an indirect one and the same effect is observed for other factors influencing growth such as temperature, light intensity (Brzezinski, 1985), photoperiod, and macronutrient limitation (reviewed by Ragueneau et al., 2000)."

Reviewers comment # 5: "Therefore in High Nutrient Low Chlorophyll (HNLC) regions like the SO, where iron limits phytoplankton growth, higher POC : PON and lower PON : POP ratios compared to the Redfield ratio may be expected." : this is far too simplistic, regrading the different cellular sites of Fe action and the differences in environmental factors (e.g. light/verticla mixing) among the different HNLC areas.

Authors reply: This sentence is only an assumption that could be made based on the effects of iron on N metabolism explained in the paragraph before. To emphasize that this assumption is not correct and that other factors influence the elemental composition we included the following sentence: "However, as many other abiotic factors

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such as macronutrient concentration, daylength, irradiance, salinity and temperature also have a wide influence on the elemental stoichiometry the overall situation is more complicated (Geider and La Roche, 2002)."

Reviewers comment # 6: Material and Methods "We additionally grew F. kerguelensis without iron and EDTA addition," The sentence ecan be confusing and I propose you be more precise, like "We additionally grew F. kerguelensis without iron addiation and with EDTA addition,". At a first look I got the impression that no Fe nor EDTa had been added.

Authors reply: Your first look was right. As shown in table 1 F. kerguelensis was grown without iron and without EDTA addition in treatment A. In treatment no iron but 10 μM EDTA were added.

Reviewers comment # 7: Please give hte refs of POC, PON, POP and BSi measurements (Hoffman et al., 2006, is not a classical methodological reference).

Authors reply: We included the original references in the "material and methods" section.

Reviewers comment # 8: Results Table 2 should be replaced by a figure. If one do so, one as to consider that the in and out patch data are not that different at the end of the experiment (i.e. around day 35) although the previous evolutions are clearly differentiated. IT is my opinion that out patch waters are developing a biological activity at the end of the experiment which adds more complexity about the interpretation of in patch evolution.

Authors reply: We replaced table 2 by a figure (now figure 2). It is true that there is some patchiness in the out-patch data. The BSi:POC, BSi:PON, and BSi:POP values of the out-patch waters are higher at day 12 and 36. However, there is no increasing trend in the BSi:POC, BSi:PON, and BSi:POP ratios outside the fertilized patch and there is absolutely no evidence for any increase in the biological activity in the out-patch waters towards the end of the experiment. Neither chlorophyll nor any other biomass indicator such as POC, PON, POP, and BSi nor Fv/Fm increased outside the fertilized patch during EIFEX (Hoffmann et al., 2006).

Reviewers comment # 9: “In F. kerguelensis and C. dichaeta cultures, iron fertilization resulted in a significant increase in maximum growth rate, chlorophyll concentrations, and photosynthetic efficiency (Fv/Fm) compared to the non fertilized treatments (Fig. 3 and Table 3) ".I do not totally agree because the elemental composition at the cell level of Ch dichaeta are ambigous as treatment B and D give more or less the same results with opposite treatments. Also why is the experiment A lacking for Ch dichaeta?

Authors reply: In the sentence “In F. kerguelensis and C. dichaeta cultures, iron fertilization resulted in a significant increase in maximum growth rate, chlorophyll concentrations, and photosynthetic efficiency (Fv/Fm) compared to the non fertilized treatments (Fig. 3 and Table 3)" nothing is said about the elemental composition of the species tested. The reviewer is right that the cellular elemental composition of C. dichaeta is not significantly different between treatments B and D but in this sentence we only concentrated on maximum growth rates, chlorophyll concentrations and Fv/Fm. As shown in table 1, neither iron nor EDTA was added to treatment A while treatment B was without iron but with EDTA addition. The initial aim of these two treatments was to test if there is an effect of EDTA on growth and elemental composition. EDTA has obviously no such effect since we found no significant difference between treatment A and B for F. kerguelensis. We only had a very limited amount of natural Southern Ocean seawater which we used as growth medium and therefore decided not repeat treatment A for C. dichaeta.

Reviewers comment # 10: Discussion 4.1 Deviation from the Redfield ratio “These uncertainties lead us to the suggestion to use PON : POP and POC : POP ratios with great caution in terms of nutrient drawdown ratios and for biogeochemical modeling. 3 This is not really your resutls but is self-contained in the Fu et al paper.

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Authors reply: We never meant to present this as solely our result. In the sentences before the one cited by the reviewer we emphasized that other researchers, such as Fu et al., showed the high variability of PON:POP and POC:POP ratios. Together with our results this lead to the suggestion mentioned above.

Reviewers comment # 11: “The general observation that the POC:PON ratio is less affected by environmental conditions and generally closer to the Redfield ratio makes it a far better vironmental proxy for these purposes." I disagree with this information which arises from an uncomplete review of the extensive litterature on C/N/P ratios in the particulate matter (see my remark in introduction). I think that you should think about reviewing the litterature between 1960 and 1995.

Authors reply: We followed the advice by the reviewer and carefully reviewed the literature between 1960 and 1995. However, we still feel that our statement is correct. Of course there are also large variations in the C : N ratio of different phytoplankton species, however the variation from the Redfield ratio are never that extreme as reported for the N : P and C : P ratios. A recent review concerning the elemental composition of phytoplankton in the field and in laboratory experiments came to the same result (Geider and La Roche, 2002). In this review the authors clearly stated: “In fact, the evidence for biochemical or physiological constraining imposing a N : P ratio of 16 : 1 or C : P of 106 : 1 on phytoplankton production is weak as best. Ě In contrast, the C : N ratio is much more tightly constrained by the data [ ] and theoretical analysis [ ] to a value near the Redfield ratio of 6.6.” We therefore feel confident that our statement is correct.

Reviewers comment # 12: 4.2 Impact of iron on silicification Be carefull about the “opal paradox" which has been more or less completely ruled out by several papers icnluding the Pondaven et al paper in Nature in relation to sediment focusing processes affecting the sedimentary budgets of Si. Although I am fully convinced that phytoplankton export to depth is a major point of interest regarding Fearticiallly- induced fertilization, I diagree this subject has to be addressed in this paper as authors do not provide any data on

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export.

Authors reply: We agree that the discussion about the "opal paradox" should be mentioned and included the following sentences: "It is discussed that the "opal paradox" may result from an underestimation of BSi production and an overestimation of BSi burial rates in the sediments (Pondaven et al., 2000). However, more recent research supports the exceptionally high BSi accumulation rates in SO sediments (Rickert et al., 2002)." As we explained in our reply to the initial statement of the reviewer we feel that export should be discussed in this manuscript as the elemental composition and frustule silicification of dominant Southern Ocean diatom species will have effects on carbon export after artificial iron fertilization events.

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