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***Interactive comment on “Effects of an iron-light co-limitation on the elemental composition (Si, C, N) of the marine diatoms *Thalassiosira oceanica* and *Ditylum brightwellii*” by E. Bucciarelli et al.***

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This is a nicely done study examining and comparing the effects of Fe limitation and Fe/light co-limitation on the elemental composition of two diatoms, including one from the open ocean and one from a coastal environment. The subject is timely, given the recent increased interest in Fe/light co-limitation in the literature, and the examination of Fe and light co-limitation effects on C:N, Si:C, and Si:N ratios is especially novel and makes a good addition to the field. I am much less enthusiastic about the attempt to extrapolate these results to in situ Fe addition experiments, as noted below I don't think this comparison is at all appropriate for several reasons. I also have a

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few methodological questions that should be addressed before publication. In general though, the paper should be suitable for acceptance in Biogeosciences after making these revisions.

#### General comments:

**Introduction:** This section does a good job of briefly introducing some general background about diatoms, their role in ocean biogeochemical cycles, and also of talking about iron limitation in general terms. I thought it should have had a couple of extra paragraphs added (this section is quite short as it is now) also reviewing what is known about iron effects on elemental ratios, after all this is the main subject of the paper. Although some of these references are brought in later in the discussion, it seems logical to introduce readers briefly to what is known about this subject and why it is of interest to study it, up front here in the introduction. I was also a little surprised to see no citation of what is in my opinion probably the best general review out there on diatom biology and biogeochemistry, that is the Sarthou et al. 2005 review in Journal of Sea Research, written by the last author of this paper. This excellent review would be highly appropriate to cite here.

**Materials and Methods:** More information on the culture techniques is needed. For instance, were the stock cultures pre-conditioned by being grown under the appropriate Fe/light conditions for a few generations at least, before being transferred to the experimental incubations? If not, then the results of the single batch cultures used to produce the data run the risk of being compromised by carry-over stored iron in the cells. Doing physiological investigations using batch cultures is very tricky- there is necessarily some subjectivity about choosing when to sample, since the growth rates and cell physiology are continually changing. Although they say that they were sampled only during the exponential phase of growth, because they were batch cultures it would be desirable to actually show the growth curves and indicate when samples were taken. Of course, letting some cultures slip even just a bit farther towards stationary phase than others would greatly affect the results and conclusions.

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It is really too bad they didn't take the small amount of extra effort to measure particulate organic phosphorus (POP) in addition to POC, PON, and BSi. It would not have been a lot of trouble to get the P data and allow them to look at a complete set of major nutrient (C:N:Si:P) elemental ratios. I also have a question about the light levels chosen.  $7.5 \mu\text{Einsteins}$  is without a doubt a low and limiting irradiance, but  $75 \mu\text{Einsteins}$  doesn't strike me as being especially "high". Some phytoplankton can definitely still be in the light-limited portion of the curve at this photon flux density, which calls into question whether the growth rates in these high light treatments (even with plenty of added Fe) truly represent  $\mu$  max values. Do they have Monod-type growth rate versus irradiance curves for both of these species to show that they were truly light-saturated in the "high light" treatments, or at least PE curves? I think this information is needed here, considering the emphasis they put on comparing ratios of  $\mu$  in the experimental treatments to maximum intrinsic growth rates  $\mu$  max later in the manuscript.

Results- Page 7183, lines 7-9. I don't quite understand the text and reasoning here. Are they trying to say that when cells are light-limited they fix more carbon than when photosynthesis is light-saturated? I believe cells acclimate to low light by increasing their light harvesting abilities, not by an increase in C-fixation rates as stated here. By definition, if they are light-limited, growth rates (and thus carbon fixation rates) are lowered, not increased.

Page 7184, lines 2-3. This suggestion that Fe-limited cells use a plasmalemma-bound nitrate reductase to reduce Fe was indeed made in this 2000 paper, but has not been supported or substantiated by any additional evidence since. Please see more recent papers by people like A Kustka and Y Shaked from their work in Francois Morel's lab, or the more recent work by this same author M. Maldonado, in which they demonstrate that in diatoms Fe is instead reduced for uptake by an Fe-specific Cu-containing reductase system, not a nitrate reductase. We know a lot more about the biochemistry of diatom iron uptake now than we did ten years ago, and I think this reference and the explanation built on it is a "red herring" here.

Section 4.3, page 7187. This section is the biggest problem of what is otherwise a very well-done culture study. It simply isn't possible to confidently use physiological results from unialgal lab cultures to explain the biogeochemical responses of entire, complex biological communities in open ocean Fe fertilization experiments. There are many reasons for this. For instance, growth rates measured in cultures are intrinsic or gross growth rates, while those measured in open ocean experiments are net community growth rates, that is they include grazing losses and any other loss terms like sinking, advection and viral lysis. How can the comparison being made here take into account the likely very different grazing rates operating inside and outside of fertilized patches? Second, net growth rates in many of the previous in situ experiments have been shown to be co-limited by not only Fe, but also other factors like light, temperature and Si, the relative importance of which changes both between different experiments and within (over the course of) experiments. Certainly this complicates any simple comparison based on lab experiments in which these other limiting factors are closely monitored and controlled. Finally and most importantly, comparing growth rates of the fertilized community (their " $\mu_{max}$ ") to the  $\mu$  of the outside or pre-fertilization community is not valid because these are usually not the same communities. Typical unfertilized control communities will be dominated by nanoflagellates (and perhaps cyanobacteria in lower latitude environments), while the iron-fertilized community is nearly always dominated by initially rare species of "iron-loving" diatoms. In some in situ experiments, even the fertilized community structure evolves during the course of the experiment, with different groups of diatoms replacing each other. Comparisons of growth rates between these two very different communities are just not the same thing as comparing growth rates in the same species in lab cultures. This whole section needs to be fundamentally re-thought, or removed from the paper completely.

The authors might also be interested in looking at one of our papers, in which we used 44 separate deckboard incubation experiments in the California upwelling to do exactly the same thing they are trying to do here: Quantitatively compare iron-induced changes in growth with changes in diatom elemental ratios. In this paper (Firme et

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al. (2003). Spatial and temporal variability in phytoplankton iron limitation along the California coast and consequences for Si, N, and C biogeochemistry. *Global Biogeochemical Cycles* 17 (1): 10.1029/2001GB001824), we devised a quantitative scale of degrees of iron limitation we called an “iron limitation index”, calculated as the ratio of changes in Chl, POC or PON in the +Fe bottles over those in the control. We then compared these indices to the degree of changes in ratios of community C:N, Si:N and Si:C. Note that we avoided the “apples and oranges” problem of comparing different communities that I discussed above, simply because in this coastal Fe-limited regime both control and +Fe treatments were dominated by the same species of the diatom *Chaetoceros*, so comparing the growth in the two treatments was a valid approach. What we found was that virtually 100% of the changes in Si:N and Si:C after iron addition were explained by changes in community POC and PON, while total BSi concentrations remained fairly constant between the two treatments. In other words, our results support some of the results shown in the present paper, in that BSi per cell was not decreasing after iron addition, rather POC and PON were increasing. Note that we found this strong correlation across all levels of iron limitation though, we didn't see the switches between 40 and 100% of  $\mu_{max}$  below 40% of  $\mu_{max}$ , below 20%, etc. that they claim to see here. Thus I doubt if these trends in their two diatoms can be said to hold true for all diatoms, their culture data may be being a bit over-interpreted here. Although I'm not suggesting the authors need to fill their paper with lots of my own references, this Firme et al. paper is definitely pertinent to their story, since we tried to do the very same thing with natural samples that they are trying to do here with their cultures.

Minor comments:

Line 15, page 7177- “major macronutrients” is redundant.

Treatment nomenclature: The two light treatments are referred to as “Fe limited” and “Fe-light co-limited” throughout the table and figures. Strictly speaking, these are the wrong names for these two treatments. Both actually cover a range of Fe conditions,

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from very limited to completely Fe-replete, certainly the cultures grown at the higher Fe levels are neither Fe-limited, nor Fe/light co-limited. A more accurate way to refer to their two main treatments would be simply “high light” and “low light”.

Lines 9-10, page 7180 and Table 1. The  $K_{\mu\text{Fe}}$  values for *D. brightwellii* certainly look significantly different between irradiances, even though they say they “did not seem to vary significantly”. This should be checked statistically.

Line 17, p. 7183. I assume what is meant here is “nitrate and nitrite reduction”, not just nitrite reduction?

p. 7186, lines 9-11. Since *Phaeodactylum* is a very atypical diatom that can grow just fine with no silicate in the medium and no cellular silicon frustule at all, citing work on this rather bizarre genus to try to understand general principles of silicon physiology in diatoms is probably not a good idea.

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