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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.***

**Anonymous Referee #2**

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General comments:

The manuscript by Bucciarelli et al. presents interesting findings on changes in cellular stoichiometry of two centric diatoms, one oceanic and one coastal species, in response to iron and iron-light co-limitation. Since only small deviations of phytoplankton elemental composition from the classical Redfield ratio will have large consequences for biogeochemical cycles and in particular the efficiency and strength of the biological carbon pump studies investigating the factors that determine elemental composition in marine phytoplankton are highly warranted. Previous studies have focused on iron limitation alone whereas this study integrates both iron and light limitation as both are

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tightly coupled on the physiological level. The present study is generally well written and structured, although part of the results and figures are tedious to read and interpret respectively. The authors put their findings into context by comparing their results with previous culture studies as well as in situ iron fertilization experiments. Nevertheless I would be cautious to generalize the findings from species maintained in culture to processes occurring in natural assemblages. Clearly the observation that the elemental composition within one species varies depending on the degree of iron/iron-light co-limitation (percentage of  $\mu_{max}$ ) is novel but falls short in providing a mechanistic explanation for the observed changes in cellular stoichiometry. Interspecific differences are large and culturing conditions, as acknowledged by the authors, can have strong influence on the experimental outcome. Thus this study adds another interesting but puzzling aspect to the role of iron (and now light) in regulating the elemental composition of phytoplankton. The consequences of iron limitation/alleviation of iron limitation for cellular processes proposed to date have been manifold and range from increased silification under iron stress, increase in cellular C and N content, iron-induces changes in morphometrics, changes in species composition etc. but none of them has provided a conceptual framework that integrates the various findings into an ecological meaningful context. Unfortunately this manuscript falls short of making such an attempt and could clearly be improved by better integrating the present study into the large context of previous findings. Furthermore I am sceptical in comparing elemental ratios of unialgal cultures with those of natural assemblages containing tens to hundred of phytoplankton species during iron fertilization studies.

Specific comments:

Abstract:

Page 7176 line 18: How can the detailed percentage of  $\mu_{max}$  (from 100% to below 20%) be inferred from iron fertilization experiments?

Page 7176 line 18 and Table 2: The growth rates from iron fertilization experiments

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were mainly derived from increases in chlorophyll. These however do not represent in situ growth rates but are in fact accumulation rates or net growth rates because losses due to mortality, sinking and dilution are already included. A direct comparison with maximum growth rates of cultures is therefore misleading.

Page 7176 Line 20: The sentence “Between 15 and 30% of  $\mu_{\max}$  . . . . .” contradicts the previous sentence.

Introduction:

Page 7177 line 22: Iron fertilization experiments EisenEx and EIFEX in the Southern Ocean have induced large diatom blooms despite cloudy skies and deeply mixed layers of over 80 m depth thus illustrating the pivotal role of iron as compared to light and illustrating that Southern Ocean diatoms are shade adapted. Roughly ten-fold differences in chlorophyll concentrations between SEEDS ( $\sim 25 \mu\text{g l}^{-1}$  in 10 m mixed layer) and EIFEX ( $\sim 3 \mu\text{g l}^{-1}$  in 100 m mixed layer) are more than compensated when comparing the integrated stocks of 250 and 300 mg Chl a  $\text{m}^{-2}$  respectively again illustrating that light was not responsible in setting the upper limit for biomass build-up.

Materials and methods:

The M&M section is missing a general description of both species used in the experiments. E.g.: habitat preferences, cell size, biovolume, chain-forming vs. solitary etc.

2.1 culture conditions:

How old were the cultures when the experiments were conducted? Old cultures generally attain a deformed status not representative of the species in nature. The same will hold true for their elemental composition.

What were the criteria to choose the two species used in this study (availability)?

Page 7178 line 6: Were the cultures acclimated to the culture conditions prior to the start of the experiment? How many times were the cultures transferred into fresh

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medium?

Page 7178 line 9: Is the high light irradiance of  $75 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  growth saturating for both species?

Page 7178 line 9: Only two light intensities are not representative of natural conditions. In the ocean cells experience a range of light intensities on a daily basis due to differences in cloud cover and/or mixing depth. Thus over a daily cycle cells might experience light intensities ranging from saturating to limiting levels but on average under iron-replete conditions net population growth rates sustain biomass build-up even under cloudy skies and in deeply mixed water columns.

Results:

Confusing terminology: In the figure legends the terms Fe lim and Fe-L lim are used whereas in the text LL and HL are used.

Page 7180 line 16-17: For *T. oceanica* the differences between LL and HL are not obvious despite statistical backing. The data points cluster very close together.

Page 7178 line 19-20: One data point for *D. brightwellii* lies close to  $80 \text{ pmol cell}^{-1}$ .

Page 7181 line 9-10: Differences in BSi content per cell are difficult to tell from the data. The same as above applies to lines 15-16, page 7181.

Furthermore there is quite a scatter in the data.

Discussion:

Page 7182 line 19-20: Just from simple surface to volume considerations *T. oceanica* should realize higher growth rates even under non-limiting conditions than the much larger *D. brightwellii*.

Page 7185 line 23: One major flaw of the study is actually that they have not considered cell morphology. Changes in cell size (morphometrics) can have considerable

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influence on cellular stoichiometry (see paper by Marchetti and Cassar 2009 in Geobiology). Furthermore instead of Si, C and N content per cell the content per cell volume and/or surface area would have been more comparable between two species that differ substantially in size. Changes in cell size could well explain the observed differences in elemental composition.

Page 7186 line 17: Why should Fe limitation increase the G2 phase in *D. brightwellii* when it has the opposite effect in *T. oceanica*?

Page 7186 line 23-24: Smaller cells do not necessarily need stronger frustules as compared to larger cells because they can be ingested whole by their predators. In this case stronger silification would not constitute an adaptive trait.

Page 7187 line 16-17: The increase in R(Si:C) below 20% of  $\mu_{max}$  is not very convincing considering the intrinsic scatter of the data and that it is only represented by two data points.

Page 7187 line 20-21: There are only few data points from iron fertilization experiments as compared to culture studies and they do not cover the whole data range.

Page 7188 line 16-18: Changes in Si:N ratios during EIFEX were clearly due to shifts in diatom species composition and not due to the hypothesised specific growth rates between 15 and 30% of  $\mu_{max}$ .

Page 7189 whole paragraph: This paragraph is not very enlightening and brings in new aspects (grazing, aggregate and TEP formation) that are not clearly linked to presented study.

Page 7189 line 25-28: I suspect that changes in growth rate have a strong influence on the decoupling of Si and N in the Southern Ocean. The modern HNLC Southern Ocean is a major silicon sink whereas most of the nitrogen is recycled in the surface layer due to the properties of the dominant diatom species present, e.g. *Fragilariopsis kerguelensis*, *Thalassiotrix antarctica* and *Thalassiosira lentiginosa*. The frustules of these

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heavily silicified species sequester most of the silicon in the deep ocean and sediments whereas their cell contents (C and N) are retained in the surface. This is reflected in the steep gradient in silicic acid concentrations from south (80  $\mu\text{M}$ ) to north (close to depletion) in the Southern Ocean whereas nitrate and phosphate concentrations are homogeneously high throughout the Southern Ocean.

Conclusions:

Page 7190 lines 5-10: What do your conclusions indicate? C and N content is strongly dependent on growth conditions whereas Si content is species-specific?

Technical corrections:

Page 7177 line 6: .....contribute up to 40%.....

Page 7185 line 19: .....in terms of silification.....

Page 7188 line 6: .....the Fe fertilized.....

The authors should include the study by Marchetti and Cassar 2009 (Geobiology)

Figure 1: There is a large data gap between roughly 150 pM Fe and 600-700 pM Fe!

Figure 2: The symbols are difficult to differentiate (the same applies for figures 3, 4 and 5)

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