Anonymous Referee #2

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 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.

> According to your comments and comments of D. Hutchins, the comparison with in situ Fe fertilization has been removed. It has been replaced by a comparison with Fe addition experiments along the California coast, where large phytoplankton dominated both control and Fe treated samples at most of the stations (Firme et al., 2003).

Clearly the observation that the elemental composition within one species varies depending on the degree of iron/iron-light co-limitation (percentage of μmax) is novel but falls short in providing a mechanistic explanation for the observed changes in cellular stoichoimetry. 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.

> The Introduction has been extended to better indicate the global biogeochemical context of this study, and the Discussion section now considers our results in a broader oceanographic context.

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.

> As stated above, the comparison with in situ Fe fertilization has been removed.

Specific comments:

Abstract:

Page 7176 line 18: How can the detailed percentage of μmax (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 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 μmax " contradicts the previous sentence.

According to your comments and comments of D. Hutchins, the comparison with in situ Fe fertilization has been removed.

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 (25 g l-1 in10 m mixed layer) and EIFEX (3 g l-1 in100 m mixed layer) are more than compensated when comparing the integrated stocks of 250 and 300 mg Chl a m-2 respectively again illustrating that light was not responsible in setting the upper limit for biomass build-up.

This part of the sentence has been removed (p 4, l 74):
"Fe-light co-limitation occurs in the subarctic Pacific Ocean (Maldonaldo et al., 1999), subantarctic waters (Boyd et al., 1999), central North Atlantic (Moore et al., 2006) and eastern North Pacific (Hopkinson and Barbeau, 2008). Co-limitation by Fe and light may even best describe the HNLC regions than Fe alone (de Baar et al., 2005)."

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.

Precisions have been added (p 5, l 91):

"Batch cultures of the centric diatoms *Thalassiosira oceanica* (CCMP 1005, axenic, small solitary oceanic species from the Sargasso Sea, ca. 80 μm^3) and *Ditylum brightwellii* (CCMP 358, axenic, large solitary coastal species from the Gulf of Mexico, ca. 16,000 μm^3) were grown at 20°C in polycarbonate bottles."

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.

We don't understand if "old" refers to the time since the species was collected from seawater or if it refers to the phase of growth when cells were harvested.

These species are CCMP clones, they were collected a long time ago and obviously are “old” cultures in that sense. However, this is unavoidable when using CCMP clones.
Concerning the filtration, it was done when the cultures were in the mid-exponential phase of growth, and always at the same time of the day to avoid diel cycle variations between the treatments. This precision has been added to the text (p 5, l 101):

"Cultures were sampled in the mid-exponential phase of growth for total cell concentration (CC), biogenic silica (BSi), and particulate (i.e. cellular) carbon (C) and nitrogen (N). Samples were collected at the same time of the day to avoid diel cycle variations between treatments."

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

These two species are model species which have already been used in numerous other culture studies. Besides, they are easy to work with and grow well in AQUIL medium.

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 medium?

Yes, they were acclimated to the culture conditions until their growth rate remained constant over several days. When harvested, at least 10 generations have been grown in the same medium and at an equivalent growth rate. These precisions have been added to the manuscript (p 5, l 98):

“Both species were pre-acclimated to each culture condition (Fe concentration and irradiance level) until their growth rate remained constant over several days. When filtered, at least 10 generations have been grown in the same conditions and at an equivalent growth rate.”

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

The growth rate was not measured above 75 µmol photons m^{-2} s^{-1}. However, μ_{max} of both species are in the high range of what is reported in the literature, as now stated in the text (p 8, l 164):

“Maximum growth rates are within the range of values reported in the literature at the same temperature and higher irradiances for *T. oceanica* (e.g. ~ 0.9 d^{-1} at 180 µmol photons m^{-2} s^{-1}, Peers et al., 2005, 1.1 d^{-1} at 500 µmol photons m^{-2} s^{-1}, Sunda et al., 1991) and *D. brightwellii* (e.g. ~ 1 d^{-1} at 100 µmol photons m^{-2} s^{-1}, Eppley and Rogers, 1970, and 1.2-1.9 d^{-1} at 190 µmol photons m^{-2} s^{-1}, Goldman, 1999).”

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.

We fully agree with the reviewer. However, when considering mean in situ irradiance levels

received by a cell traversing the mixed layer (as estimated by Maldonado et al., 1999, see Table below), our values (7.5 µmol photons m^{-2} s^{-1} and 75 µmol photons m^{-2} s^{-1} ~ 0.65 and 6.5 mol quanta m^{-2} d^{-1}) are in the same range:

Table 3
Mean irradiance levels received by cells traversing the mixed layer (mol quanta m^{-2} d^{-1}), and the mixed layer depth (m) of three locations in the Southern Ocean during austral spring/fall, and Ocean Station PAPA (winter) in the NE subarctic Pacific

Location	Coordinates	Irradiance (mol quanta m^{-2} d^{-1})	Mixed layer depth (m)
Bellingshausen Sea[a] (November 1992)	68.5°S 85°W	0.85 (2 d mean)	60
Subantarctic Pacific[b] (April 1997)	46°S 178.5°E	1.18 (3 d mean)	85
Subantarctic Pacific[b] (October 1997)	46.5°S 178.5°E	2.3 (4 d mean)	90
Ocean Station PAPA[c] (February 1997)	50°N 145°W	0.91 (7 d mean)	80

[a]Boyd et al. (1995b).
[b](Boyd unpubl.).
[c]This study.

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.

"Fe lim" and "Fe-L co-lim" have been replaced by "HL" and "LL" in all Tables and Figures.

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.

As also recommended by Reviewer 1, we changed the tests by calculating the mean and confidence interval over the same range of specific growth rate (p 9, l 188):

"At a given growth rate, the C content was higher under LL than under HL for *D. brightwellii* and almost similar for *T. oceanica*. Indeed, when µ varied between 0.4 and 1.05 d^{-1} for *D. brightwellii* and between 0.4 and 0.75 d^{-1} for *T. oceanica*, the average values of the C content at LL and HL were respectively 53.6 ± 15.7 pmol $cell^{-1}$ (n= 5, CI = 95 %) and 30.0 ± 2.1 pmol $cell^{-1}$ (n= 9, CI = 95 %) for *D. brightwellii*, and 0.70 ± 0.04 pmol $cell^{-1}$ (n= 12, CI = 95 %) and 0.59 ± 0.07 pmol $cell^{-1}$ (n= 5, CI = 95 %) for *T. oceanica*. However, when considering cell volume, C concentration for *T. oceanica* was significantly higher under LL (11.2 ± 0.6 mol $Lcell^{-1}$, n= 12, CI = 95 %) than under HL (8.4 ± 1.1 mol $Lcell^{-1}$, n= 5, CI = 95 %). Under LL, cellular C decreased with Fe limitation for *D. brightwellii* (from ~ 80 pmol $cell^{-1}$ to ~ 30 pmol $cell^{-1}$) but it did not change for *T. oceanica* (0.67 ± 0.07 pmol $cell^{-1}$ and 11.0 ± 1.0 mol $Lcell^{-1}$, mean ± SD, n= 16)."

This has also been done for N and BSi contents.

Page 7178 line 19-20: One data point for D. brightwellii lies close to 80 pmol cell-1.

It has been corrected.

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.

As explained above, new tests have been run using mean values and confidence intervals over a same range of specific growth rates.

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.

This observation has been included in the text (p 13, l 267):

"Once the limitation relieved, the smallest cells should have the highest growth rates according to allometric relationship between μ_{max} and cell volume (Sarthou et al., 2005). However, this relationship is very scattered and in our study the larger diatom would outgrow the smaller one due to its higher maximum specific growth rate."

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 influence on cellular stoichoimetry (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.

This is now discussed in the manuscript. Briefly, using cell volume data for *T. oceanica* does not really change the trends in elemental composition. For *D. brightwellii*, the importance of the potential variation in cell size is now stated, e.g. when discussing C (p 14, l 285) and BSi (p 16, l 354) contents:

"On the contrary, the C content of *D. brightwellii* decreased under LL with increasing Fe limitation. However, although not measured in our study, it is known that the size of this species shows a large plasticity. It can increase by 4-fold under Cu toxicity (from ~25,000 to ~ 100,000 μm^3, Rijstenbil and Gerringa, 2002), and decreases from 4,500 to 3,000 μm^3 when irradiance decreases from 110 to ~10 µmol photons m^{-2} s^{-1} (Waite et al., 1992). The decrease in C content could thus be compensated for by a 2-fold decrease in cell volume."

"Marchetti and Harrison (2007) invoke different mechanisms likely to induce a decrease in biogenic silica under Fe limitation, like the changes in cell volume, cell morphology and the existence of soluble pools.
A change in cell volume with iron and light limitation has indeed been shown for some diatom species (e.g. Hoffman et al., 2008; Timmermans et al., 2001). In our study, the observed decrease in BSi per cell with increasing Fe limitation could be compensated for by a

2.3-fold decrease in cell volume under HL and a 1.4-fold decrease under LL. As stated above, such variations in cell volume can occur for *D. brightwellii* (e.g. Rijstenbil and Gerringa, 2002; Waite et al., 1992)."

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

Actually another study that we performed (Claquin and Bucciarelli, in prep.) showed that only the Fe-L co-limitation increased the G2 phase and silicification in *T. oceanica*. Fe limitation alone did not increase the G2 phase, but we did not observe an increased silicification either. Our purpose here is to point out that Fe limitation does not have the same effect on the cell cycle of *T. oceanica* as other limitations have for *T. pseudonana*. Since "increased limitation = increased G2 phase" does not seem to be a general rule, a decreased G2 phase under Fe limitation might be possible.
The text has been changed for more clarity (p 17, l 380):

"The increase in silicification of *T. oceanica* under the Fe-L co-limitation may indeed be due to an increase in the G2 phase duration (Claquin and Bucciarelli, in prep.). However, limitation does not seem to systematically induce an increase in the G2 phase length, since it was not observed for our Fe-limited cells of *T. oceanica* (Claquin and Bucciarelli, in prep.). If this is not a general rule, then Fe limitation might decrease the length of the G2 phase for species such as *D. brightwellii*, and decrease their silicification. More studies are obviously needed to verify this hypothesis."

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 silicification would not constitute an adaptive trait.

Jensen and Bathman (2007) have recently shown that heavily silicified cells best survived the gut passage of copepods than less silicified species. The text has been accordingly modified, using this recent reference (p 18, l 388):

"Predation avoidance mechanisms include larger size and spines (Irigoien et al., 2005). The frustule is also an effective protection against zooplankton grazing (Hamm et al., 2003). A recent study showed a grazing-induced increase in cell wall silicification in the marine diatom *T. weissflogii* (Pondaven et al., 2007). Under energy limitation (Fe and Fe-L), large cells with spines that are not as sensitive as small ones to grazing may reduce their silicification and save on respiratory energy. On the contrary, smaller cells which are easier to graze may need stronger frustules. Besides, even when small enough to be ingested whole by their predators, more silicified diatoms best survive the gut passage of copepods (Jensen and Bathman, 2007)."

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

It has been removed.

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 μmax.

According to your comments and comments of D. Hutchins, the comparison with in situ Fe fertilization has been removed.

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, Thalasssiotrix antarctica and Thalassiosira lentiginosa. The frustules of these 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 M) to north (close to depletion) in the Southern Ocean whereas nitrate and phosphate concentrations are homogenously high throughout the Southern Ocean.

These parts of the manuscript have been re-written, and a paragraph has been added to discuss more in detail the silica pump (p 20, l 438).

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?

We would rather say that C and N quota per cell at a given degree of Fe limitation are strongly dependent on growth conditions, but that Si content not only depends on the degree of Fe limitation and growth conditions (since environmental parameters influence the silicification, Martine Jézéquel et al., 2000), but is also species specific.
This has been re-written (p 22, l 481):

"We showed that C and N per cell tend to decrease with Fe and Fe-L co-limitation for all species, but an increase in C:N with increasing limitation was only significant for the species we studied. Contrasting results between literature data on C and N contents in Fe-limited diatoms may be more related to growth conditions and cell volume variations than to interspecific differences. (...) On the contrary, there was no significant trend in silica content when comparing different Fe or Fe-L limited diatoms, which suggests that other interspecific differences than Fe-induced variations in cell volume influence the degree of silicification."

Technical corrections:

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

This has been corrected

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

This has been corrected

Page 7188 line 6: …the Fe fertilized…

According to your comments and comments of D. Hutchins, this part has been removed.

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

This has been done.

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

Actually, 600-700 pM Fe are close to the solubility limit where Fe hydroxides are observed to precipitate, which is thus above or close to the value where μ_{max} is reached (Sunda and Huntsman 1997). We decided to focus on the part of the growth curve where limitation and co-limitation occur.

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

All symbols are now bigger and some have been changed.