Anonymous Referee #1

Bucciarelli et al. present an interesting study, investigation the effect of iron and iron light co-limitation on marine diatom growth rates and elemental composition. This topic is of high importance and the results are interesting and well presented. The authors provide a very helpful comparison of their data with field data from in situ iron fertilization experiments, which is a major strength of this manuscript. I only have a few questions and comments, mainly about a possible change in cell size during the experiment which should be discussed. Apart from that I recommend publication of this manuscript.

General comments:

It would be good to include a table with the [Fe'] used or the mention the concentrations in the material and methods section. From figure 1 it is not possible to tell the difference in [Fe'] for the Fe-limited treatments (below about 100 pmol L-1) and since this is where the main change in growth rate was observed it would be very helpful for the reader to have the exact concentrations.

Table 1 and legend have been added :

Fe' (pmol L^{-1})			
Thalassiosira oceanica		*Ditylum brightwellii*	
HL	LL	HL	LL
0.9	0.7	8.7	11*
2.2	2.0	11*	19*
7.8	6.8	12	37
43	13	13*	98*
112	37	15*	610
154	98	44	
698	135	113*	
	610	699	

Table 1: Inorganic Fe concentrations in the medium ([Fe'], in pmol L^{-1}) at high light (HL) and low light (LL) for *T. oceanica* and *D. brightwellii*. Starred values indicate that specific growth rates were measured at these concentrations but not elemental composition.

Material and methods:

The authors should mention the size of the diatom species used. Later in the manuscript the authors discuss the difference between large and small diatoms (page 7182 line 18) but a reader who is not familiar with marine diatom species might not know that D. brightwellii is much bigger and how important this can be.

This has 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. "

Results:

page 7180 line 16: the authors state: At a given growth rate, the C content was higher under LL than under HL… this is not obvious to me from figure 2. As far as I can see there are only three HL data points within the range of the LL data points (between about 0.25 and 0.75 µ). These three data points are indeed in the lower range of the LL data but from the figure it seems that they never have exactly the same growth rate as any LL data point. I therefore question if such a statistic comparison can be made.

Given that the growth rates were indeed never exactly the same, we changed the tests by calculating the mean and confidence interval over the same range of specific growth rate, which seems more suitable (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.

Discussion:

The authors only briefly mention the importance of cell size in their discussion. If possible the authors should show the concentration of C, N and BSi per cell volume instead of per cell since a change in cell volume with iron and light limitation was found in some diatom species (e.g. (Hoffmann et al. 2007; 2008; Timmermans et al. 2001). If this is not possible the authors should carefully discuss the matter and the possible effects of changing cell size on their results. Some differences in cellular C, N and BSi between the different light and iron treatments are relatively minor and changes in cell volume could easily affect the results and the outcome of the statistical analysis. This is especially important in the BSi section, where a change in silicification of diatom cells under Fe and light limitation is discussed. If cell size changes with limitation, changes in Si/N and Si/C can not be directly taken as changes in cell wall silicification. The work of (Strzepek and Price 2000) on Fe and light and of (Hoffmann et al. 2008) on Fe, light and Si co-limitation should be included in the discussion.

Cell size was only measured for *T. oceanica*, as now stated in the Results section (p. 8, l. 169) and shown in Figure 2:

"The volume per cell of *T. oceanica* did not vary significantly under Fe limitation (V_{cell} = 79.0 ± 1.2 μm^3, n = 15, CI = 95 %) except at the lowest specific growth rate (V_{cell} = 62.9 ± 2.3 μm^3, n = 2, CI = 95 %) (Fig. 2). The volume per cell of this species decreased significantly when the irradiance decreased (t-test, $p < 0.01$) and it remained stable at low light whatever the Fe

concentration (V_{cell} = 61.2 ± 2.1 µm^3, n = 16, CI = 95 %). In other Fe-limited experiments at HL, µ decreased down to 0.1 d^{-1}, and values of V_{cell} were similar to those observed here under LL (Bucciarelli, unpubl.). The difference between HL and LL in the present study is thus most likely due to a decrease in the specific growth rate, and not to a direct effect of light limitation.
While we did not measure any change in cell volume due to glutaraldehyde preservation for *T. oceanica*, the use of glutaraldehyde induced an increase (up to 3-fold) in cell volume of *D. brightwellii* that could not be corrected. As a result, the elemental compositions of both diatoms are presented on a per cell basis to allow interspecific comparisons. Data of *T. oceanica* are also discussed on a per cell volume basis."

The elemental composition per cell volume of *T. oceanica* is now presented in the text (Results section) and discussed, but it does not really change the trends and conclusions.
A paragraph has also been added to discuss the effect of cell volume of *D. brightwellii* on the decrease in 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)."

The works of Strzepek and Price (2000) and of Hoffmann et al. (2008) have been included in the discussion.

Page 7183, line 6-9: The study by Sunda and Huntsman cited here showed that a decrease in photoperiod from 14 to 7 h resulted in an increase of the specific C-fixation rate. A decrease in the duration of the photoperiod is often directly compared to a decrease in light intensity in the literature. I am very sceptic that these two processes can be directly compared and and I doubt that they will result in the same type of light limitation. I think it should at least be mentioned here that the cells in the Sunda and Huntsman study were not acclimated to low light but to a shorter photoperiod. As mentioned above, the possible effect of changes in cell size on cellular C should be mentioned/discussed here.

This paragraph has been re-written in order to take into account this comment and D. Hutchins' comment (p 13 l 275) :

"It has also recently been shown that *T. oceanica* uses the copper-containing plastocyanin instead of the functionally equivalent Fe-containing cytochrome c6 (Peers and Price, 2006), and has a different photosynthetic apparatus from a coastal species, i.e. lower cellular

concentrations of Fe-rich cytochrome b6/f and PSI (Strzepek and Harrison, 2004). This could also explain how cellular C remained constant for *T. oceanica* with increasing Fe limitation under LL (Fig. 2a). Cells acclimatize to low light by increasing their Fe content and Fe:C ratio, i.e. their photosynthetic capacity (Strzepek and Price, 2000; Sunda and Huntsman, 1997). Its photosynthetic apparatus allows *T. oceanica* to decrease its cellular iron requirements but not its photosynthetic rates (Strzepek and Harrison, 2004), which may help this species to maintain its C content under LL and increasing Fe limitation."

Specific comments:

page 7178 line 7: change "xenic" to "axenic"

This has been done.

page 7181 line 15-16: I think the names of the two species were mixed up here

Actually they were not, but the new tests (mean and confidence interval) changed the outcome for *T. oceanica* (p 11, l 229):
"When the specific growth rate 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 value of the molar ratio C:N was lower at LL than at HL for *D. brightwellii* (respectively 5.84 ± 0.32 mol mol^{-1}, n= 5, CI = 95 %, and 6.58 ± 0.28 mol mol^{-1}, n= 9, CI = 95 %) and similar at LL than at HL for *T. oceanica* (respectively 12.33 ± 0.56 mol mol^{-1}, n= 12, CI = 95 %, and 10.06 ± 1.77 mol mol^{-1}, n= 5, CI = 95 %) (Fig. 4a, b)."

page 7183 line 24: : : : exclude that singular point: : : it is two and not one data point

It has been changed.

page 7186 line 23: what do the authors mean by "limited cells" Fe limited?

Not only, it might be any limitation that reduces growth rate and decreases cell volume (Fe but also other trace metals, light, nitrate, ...). Because part of this paragraph has been rewritten, this term has been removed from this sentence (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 7188 line 6: change fertlized to fertilized

This part has been removed to take into account comments of reviewer 2 and D. Hutchins.

Table 1 and Figures 1-6: I think the use of Fe lim and Fe-L co-lim throughout the manuscript is very confusing for the reader, especially in this table 1. The maximum growth rate is certainly not reached under Fe or Fe-L co-limitation but under Fe-replete growth conditions. I suggest that the authors use HL and LL instead and only use the term Fe lim Fe-L co-lim in those treatments where a clear limitation was observed.

This has been changed in all Tables and Figures.