Effects of an iron-light co-limitation on the elemental composition (Si, C, N) of the marine diatoms Thalassiosira oceanica and Ditylum brightwellii

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Effects of an iron-light co-limitation on the elemental composition (Si, C, N) of the marine diatoms *Thalassiosira oceanica* and *Ditylum brightwellii*

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Abstract. We examined the effect of iron (Fe) and Fe-light (Fe-L) co-limitation on cellular silica (BSi), carbon (C) and nitrogen (N) in two marine diatoms, the small oceanic diatom *Thalassiosira oceanica* and the large coastal species *Ditylum brightwellii*. We showed that C and N per cell tend to decrease with increasing Fe limitation (i.e. decreasing growth rate), both under high light (HL) and low light (LL). We observed an increase (*T. oceanica*, LL), no change (*T. oceanica*, HL) and a decrease (*D. brightwellii*, HL and LL) in BSi per cell with increasing degree of limitation. The comparison with literature data showed that the trend in C and N per cell for other Fe limited diatoms was similar to ours. Interspecific differences in C and N quotas of Fe limited diatoms observed in the literature seem thus to be mostly due to variations in cell volume. On the contrary, there was no global trend in BSi per cell or per cell volume, which suggests that other interspecific differences than Fe-induced variations in cell volume influence the degree of silicification. The relative variations in C:N, Si:C and Si:N versus the relative variation in specific growth rate (i.e. μ:μ_{max}) followed the same patterns for *T. oceanica* and *D. brightwellii*, whatever the irradiance level. However, the variations of C:N under Fe limitation reported in the literature for other diatoms are contrasted, which may thus be more related to growth conditions than to interspecific differences. As observed in other studies, Si:C and Si:N ratios increased by more than 2-fold between 100% and 40% of μ_{max}. Under more severe limitation (HL and LL), we observed for the first time a decrease in these ratios. These results may have important biogeochemical implications on the understanding and the modelling of the oceanic biogeochemical cycles, e.g. carbon and silica export.

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1 Introduction

Warming of the climate system is now unequivocal and very likely due to the atmospheric increase of greenhouse gases such as carbon dioxide (CO_2) (IPCC, 2007). The rate of change in atmospheric CO_2, depends, however, not only on human activities but also on oceanic biogeochemical processes (Falkowski et al., 2000). Oceanic ecosystems indeed strongly affect the composition of the atmosphere, through CO_2 uptake by phytoplankton, and the export of that organic carbon from the surface to the ocean interior. In this regard, the phytoplanktonic group of the diatoms is thought to play a major role (Sarthou et al., 2005). These siliceous species contribute up to 40% of the global oceanic primary production of carbon (Nelson et al., 1995) and the termination of their massive blooms export large quantities of organic carbon and biogenic silica from upper layers to the deep ocean (Smetacek, 1999). These export events may partly control the partitioning of carbon in the atmosphere-ocean-sediment system over geological timescales (Barber and Hiscock, 2006; Falkowski et al., 1998). Since the 1990s, it has been convincingly shown that the subnanomolar oceanic concentrations of iron (Fe) are low enough to limit primary production and in particular diatom growth in at least 40% of the ocean (de Baar et al., 2005). Iron limitation also induces a decoupling in the use of macronutrients by phytoplankton, likely to influence the cycling of the major biogeochemical

cycles (C, N, P, Si, S) over geological time scales (de Baar and La Roche, 2003). For example, it is now well admitted that Fe-limited diatoms generally increase their cellular Si:N and Si:C ratios, which may have large consequences for biogeochemical cycles, e.g. the efficiency and strength of the biological carbon pump, and the depletion of Si before N, driving the system towards Si limitation (Marchetti and Cassar, 2009; Sarthou et al., 2005). This increase has been attributed to increased Si content and decreased C and N content (e.g. Takeda, 1998; Timmermans et al., 2004). It is then usually assumed in biogeochemical models that diatom Si content, Si:N and Si:C ratios increase under limiting conditions (e.g. Aumont et al., 2003; Moore et al., 2004). However, two recent studies indicated no change or even a weak decrease in cellular biogenic silica of Fe-limited cells of *Chaetoceros dichaeta* (Hoffmann et al., 2007) and some clones of *Pseudonitzschia* (Marchetti and Harrison, 2007). In those cases, an increase in Si:N and Si:C ratios under Fe limiting conditions was due to a greater decrease in C and N contents than in biogenic silica. Marchetti and Cassar (2009) proposed that such discrepancies between studies may be due to the level of Fe deficiency, different culture conditions and implemented methodology, in situ shifts in diatom species composition, interspecific differences, and/or change in cell size and diatom morphology.

Other abiotic parameters, like irradiance, also control primary production and influence the elemental stoichiometry of phytoplankton (Geider and La Roche, 2002). Besides, irradiance may be a determining factor in diatom species succession and distribution (Timmermans et al., 2007). 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 better describe the HNLC regions than Fe alone (de Baar et al., 2005). Iron and light indeed interplay at the biochemical level, because phytoplanktonic cells need higher Fe:C for growth under low light (Sunda and Huntsman, 1997). However, despite the importance of this environmentally relevant co-limitation, very few studies explored its impact on the coupling of the major biogeochemical cycles.

In the present study, we examined the effect of Fe limitation and Fe-light co-limitation on cellular silica, carbon and nitrogen in the small oceanic diatom *Thalassiosira oceanica* and the large coastal species *Ditylum brightwellii* over a large range of Fe limitation and at two different irradiances. We then compared our results to literature data, taking into account the degree of Fe limitation.

2 Materials and methods

2.1 Culture conditions

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. Cultures were grown under cool white fluorescent light at an irradiance of 75 (high light: HL) and 7.5 µmol photons $m^{-2}\,s^{-1}$ (low light: LL) and a 14 h:10 h light:dark cycle. The culture media (see below) were sterilized by micro wave treatment (Keller et al., 1988). Cultures were grown as duplicates or triplicates at each Fe concentration. Cultures were gently mixed twice a day by hand to prevent cell sedimentation. 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 (see Supplementary Material: http://www.biogeosciences.net/7/657/2010/bg-7-657-2010-supplement.pdf). 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.

2.2 Culture media

The complete medium consisted of artificial AQUIL seawater enriched with 300 µmol L^{-1} nitrate, 10 µmol L^{-1} phosphate, 100 µmol L^{-1} silicate, 0.55 µg L^{-1} vitamin B_{12}, 0.5 µg L^{-1} biotin, 100 µg L^{-1} thiamin, 10 nmol L^{-1} selenite and 100 nmol L^{-1} molybdate (Price et al., 1988/1989). The medium also contained a trace metal ion buffer system consisting of 100 µmol L^{-1} ethylene diamine tetra acetic acid (EDTA), 19.6 nmol L^{-1} Cu, 50.3 nmol L^{-1} Co, 79.7 nmol L^{-1} Zn and 121 nmol L^{-1} Mn. The buffer system generated free ion concentrations of Cu, Co, Zn and Mn of $10^{-13.79}$, $10^{-10.88}$, $10^{-10.88}$ and $10^{-8.27}$ mol L^{-1}, respectively, at pH 8.1 (Price et al., 1988/1989). Added iron concentrations to the medium ranged from 0 (no addition) to 500 nmol L^{-1}. Background iron in the medium without EDTA (0.61 nmol L^{-1}) was measured by ICP-MS after preconcentration onto an 8-HQ resin. Total iron concentration was computed from the sum of added iron and the background iron concentration. In this medium, inorganic iron concentrations ([Fe′]) can be estimated from total iron concentrations, and depend on the irradiance (Sunda and Huntsman, 1997). They varied between 0.9 and 699 pmol L^{-1} at HL and between 0.7 and 610 pmol L^{-1} at LL (Table 1).

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.

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	

2.3 Specific growth rate and volume per cell

No Coulter counter was available on site, and cellular concentrations (CC, cells mL^{-1}_{medium}) were determined by microscopic counts. Specific growth rate (μ, d^{-1}) was determined by linear regression of the natural log CC versus time. The cells exhibited a constant daily specific growth rate over several days before the experiment. During the samplings, 10 ml were fixed with 600 µl of 25% glutaraldehyde. The volume per cell (V_{cell}, μm^3) was measured a week later with a Z2 Coulter electronic particle counter for *Thalassiosira oceanica* (LPI, Station Expérimentale d'Argenton d'Ifremer, France). The cylinder shape of *Ditylum brightwellii* does not allow to use a Coulter counter: fifty randomly selected cells were digitized on an inverted microscope using an analogic Leica camera and analyzed with software image analysis (Visilog 5) to determine the maximal width (a, µm) and length (A, µm). The average cell volume was determined using the geometric formula for a cylinder: Volume=π a^2 A.

2.4 Cellular nitrogen and carbon

All the glassware used for cell carbon and nitrogen determination (filter holders, filtration funnels, and vials) were washed with 10% HCl, rinsed with Milli-Q water and dried. They were then pre-combusted at 450 °C for 4.5 h. Cells from culture samples were filtered as duplicates onto GF/F filters (pre-combusted as the glassware) and rinsed with artificial seawater containing no nutrient. Samples were stored frozen at −20 °C and were dried before analysis. Samples were analyzed using a Carlo-Erba NA-1500 elemental analyzer.

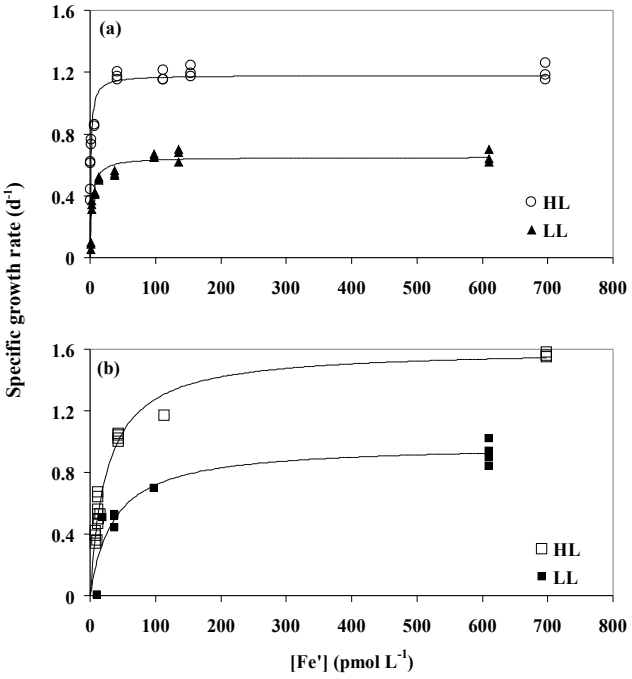

Fig. 1. Specific growth rate (μ, d^{-1}) of **(a)** *Thalassiosira oceanica* and **(b)** *Ditylum brightwellii* versus inorganic iron concentration in the medium ([Fe′], pmol L^{-1}) under high light (HL, open symbols) and low light (LL, closed symbols).

2.5 Biogenic silica (BSi)

Culture samples were filtered as duplicates onto 0.6 µm polycarbonate membrane and rinsed with artificial seawater containing no nutrient. The filters were oven dried at 60 °C for 24 h, digested for 7 days in 2.9 mol L^{-1} HF, and the resulting orthosilicic acid was measured by spectrophotometry (Ragueneau and Tréguer, 1994).

3 Results

3.1 Specific growth rate and volume per cell

The specific growth rate decreased with the irradiance and the inorganic iron concentrations in the medium for both species (Fig. 1a, b). The maximum specific growth rate (μ_{max}) and the half-saturation constant for growth with respect to iron ($K_{\mu Fe'}$) were determined using a Monod saturation function (Table 2). When the irradiance decreased by 10-fold, μ_{max} decreased by 1.8-fold and 1.6-fold for *T. oceanica* and *D. brightwellii*, respectively. In the same time, $K_{\mu Fe'}$ increased by 2.3-fold for *T. oceanica*. Despite the large standard error at low light, a 1.4-fold increase in $K_{\mu Fe'}$ was significant for *D. brightwellii* between HL and LL (ANOVA, $p < 0.001$, F=51.1). Maximum growth rates are within the range of values reported in the

Table 2. Maximum specific growth rate μ (d^{-1}) and half-saturation constant for growth with respect to iron ($K_{\mu Fe'}$) for *T. oceanica* and *D. brightwellii* under high light (HL) and low light (LL). The squared correlation coefficient of the Monod saturation function versus inorganic Fe concentration (R^2) and the number of data used for the regression (n) are also given.

	Thalassiosira oceanica		*Ditylum brightwellii*	
	HL	LL	HL	LL
μ_{max} (d^{-1})	1.18±0.02	0.65±0.02	1.60±0.04	0.98±0.07
$K_{\mu Fe'}$ (pmol L^{-1})	1.29±0.17	3.01±0.43	25.4±1.99	36.5±9.79
n	20	24	19	10
R^2	0.92	0.93	0.97	0.88

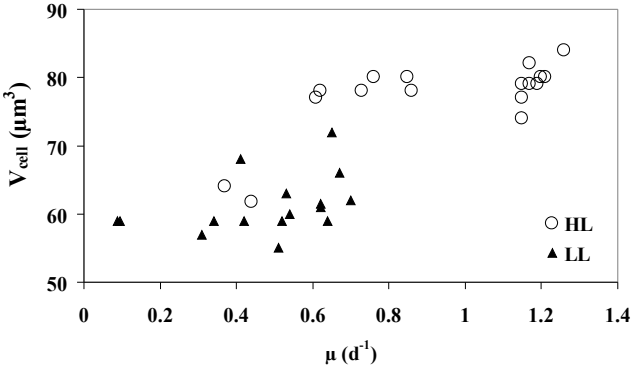

Fig. 2. Cell volume (V_{cell}, μm^3) of *Thalassiosira oceanica* versus specific growth rate (μ, d^{-1}) under high light (HL, open symbols) and low light (LL, closed symbols).

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).

The volume per cell of *T. oceanica* did not vary under Fe limitation at HL (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 between HL and LL (t-test, $p < 0.01$) and remained stable at LL 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, unpublished data). 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.

3.2 Cellular nitrogen and carbon

The trends in variations in cellular carbon were similar for both diatoms under HL. Cellular C decreased with Fe limitation under HL from ∼1 pmol $cell^{-1}$ to ∼0.5 pmol $cell^{-1}$ for *T. oceanica* (Fig. 3a) and from ∼50 pmol $cell^{-1}$ to ∼30 pmol $cell^{-1}$ for *D. brightwellii* (Fig. 3b). 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 L_{cell}^{-1}, n=12, CI=95%) than under HL (8.4±1.1 mol L_{cell}^{-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 did not change for *T. oceanica* (0.67±0.07 pmol $cell^{-1}$ and 11.0±1.0 mol L_{cell}^{-1}, mean±SD, n=16).

For both species and light conditions, at μ higher than 0.1 d^{-1}, the nitrogen content decreased with Fe limitation (Fig. 3c, d). At a given specific growth rate, the average value was similar for *T. oceanica* under LL and HL, on a per cell and on a cell volume basis (for μ between 0.4 and 0.75 d^{-1}, 0.057±0.004 pmol $cell^{-1}$ and 0.92±0.06 mol L_{cell}^{-1}, n=12, CI=95%, under LL, and 0.061±0.013 pmol $cell^{-1}$ and 0.84±0.11 mol L_{cell}^{-1}, n=5, CI=95%, under HL). It decreased from ∼0.1 pmol $cell^{-1}$ at 1.2 d^{-1} to ∼0.04 pmol $cell^{-1}$ at 0.3 d^{-1}. At the most severe Fe-L co-limitation (μ=0.09 d^{-1}), the nitrogen content of *T. oceanica* increased up to 0.07 pmol $cell^{-1}$ (i.e. 1.2 mol L_{cell}^{-1}). For *D. brightwellii*, the average value was higher at LL than at HL (for μ between 0.4 and 1.05 d^{-1}, 9.32±3.04 pmol $cell^{-1}$, n=5, CI=95%, and 4.56±0.36 pmol $cell^{-1}$, n=9, CI=95%, respectively).

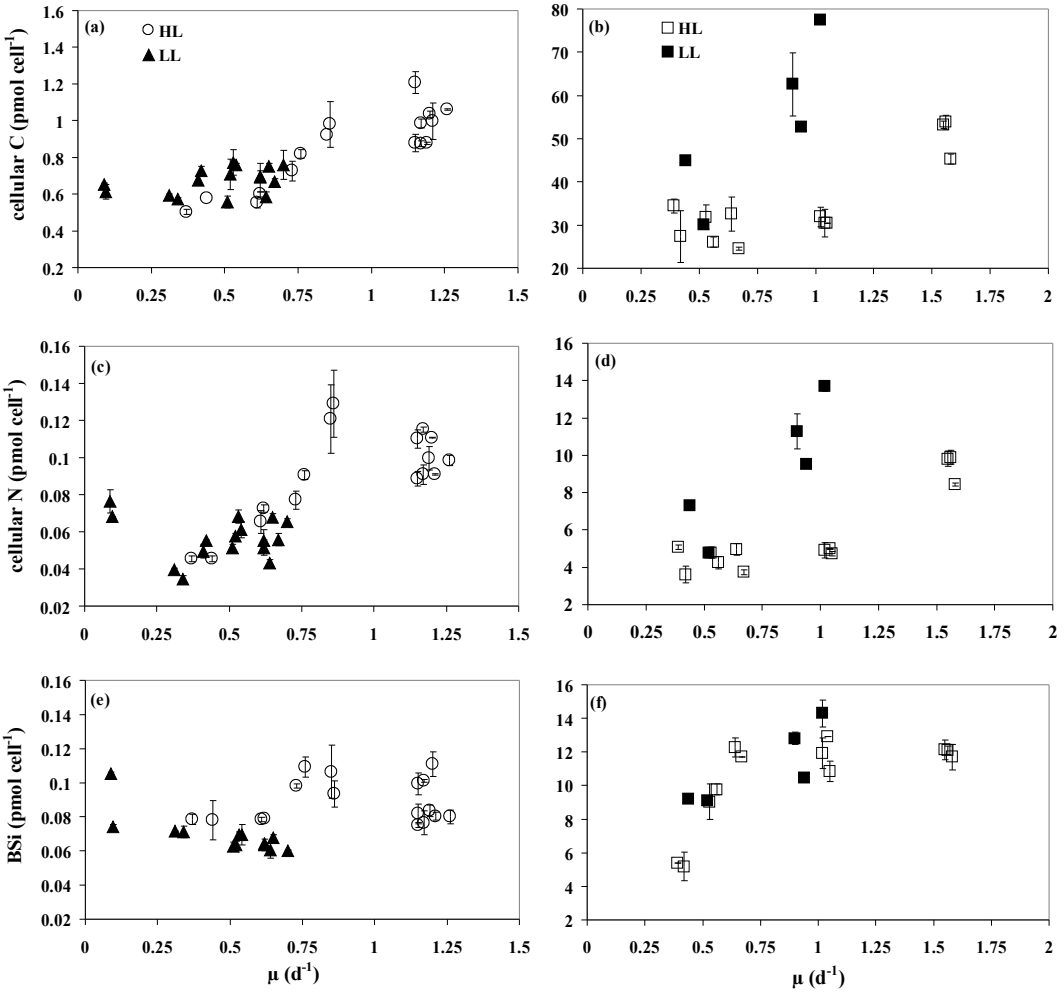

Fig. 3. Carbon **(a, b)**, nitrogen **(c, d)** and biogenic silica **(e, f)** per cell (in pmol cell^{-1}) versus specific growth rate (μ, d^{-1}) of *Thalassiosira oceanica* (left panels) and *Ditylum brightwellii* (right panels) under high light (HL, open symbols) and low light (LL, closed symbols).

It decreased approximately from 11.5 to 5 pmol cell^{-1} at 1 d^{-1} (LL and HL, respectively) and from 6 pmol cell^{-1} to 4.5 pmol cell^{-1} at 0.5 d^{-1} (LL and HL, respectively).

3.3 Biogenic silica (BSi)

The BSi content of *T. oceanica* was scattered under Fe limitation at HL and did not change significantly with the specific growth rate, either on a per cell (Fig. 3e) or per cell volume basis ($r^2 < 0.1$, $p > 0.25$, n=17). For μ between 0.4 and 0.75 d^{-1}, the average BSi per cell was lower under LL than under HL on a per cell basis (0.065±0.002 pmol cell^{-1}, n=9, CI=95%, and 0.083±0.008 pmol cell^{-1}, n=5, CI=95%, respectively) but similar on a per cell volume basis (1.06±0.05 mol L_{cell}^{-1}, n=9, CI=95% and 1.16±0.11 mol L_{cell}^{-1}, n=5, CI=95%). Under LL, it increased with Fe limitation for μ decreasing from 0.7 to 0.1 d^{-1}, per cell (r^2=0.60, $p < 0.01$, n=13) and per cell volume (r^2=0.72, $p < 0.01$, n=13). The reverse trend was observed for *D. brightwellii*, with a decrease with Fe limitation ($\mu < 0.6$ d^{-1}) for both light conditions (Kruskall Wallis test, χ^2=12.55, p=0.0057, n=18) and similar values at a given specific growth rate under low and high light (for μ between 0.4 and 1.05 d^{-1}, 11.2±2.0 pmol cell^{-1}, n=5, CI=95%, and 9.89±1.89 pmol cell^{-1}, n=9, CI=95%, respectively) (Fig. 3f).

3.4 Elemental ratios C:N, Si:C and Si:N

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 and 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).

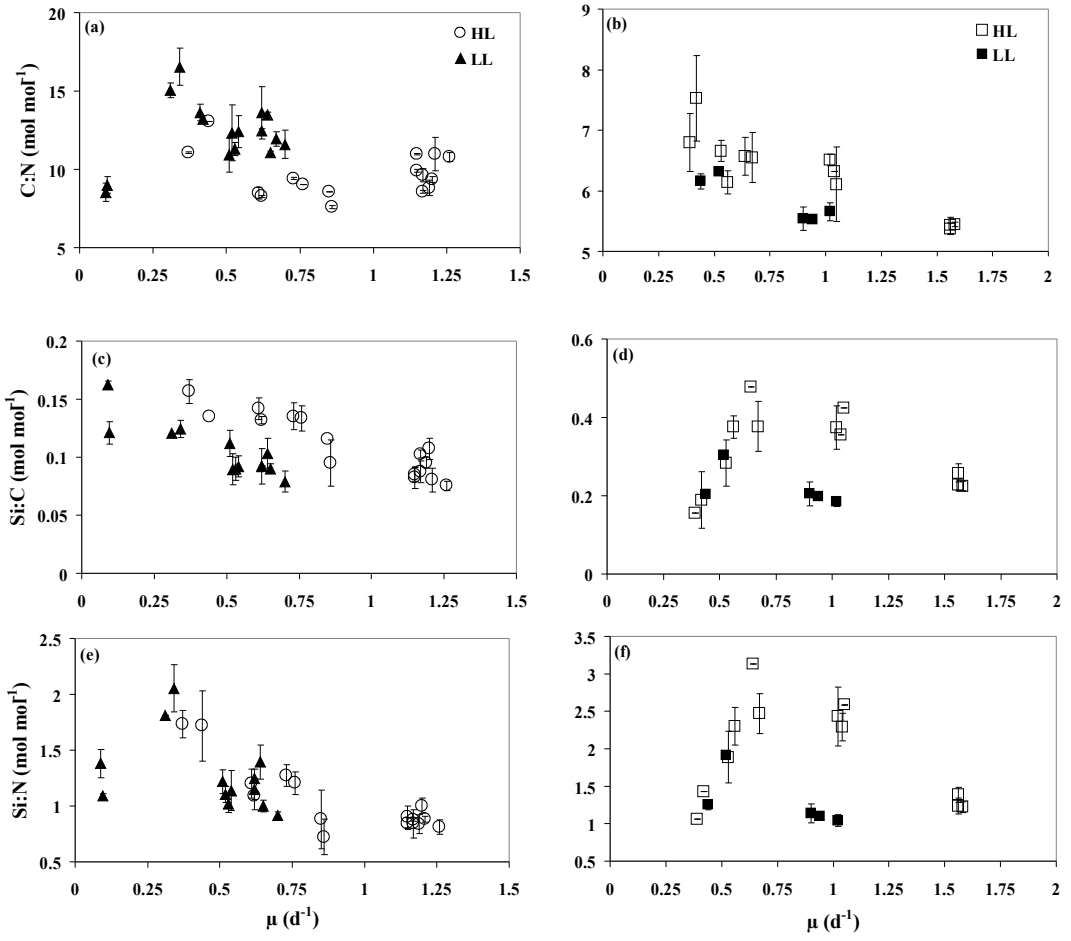

Fig. 4. Molar ratios of C:N **(a, b)**, Si:C **(c, d)** and Si:N **(e, f)** versus specific growth rate (μ, d^{-1}) of *Thalassiosira oceanica* (left panels) and *Ditylum brightwellii* (right panels) under high light (HL, open symbols) and low light (LL, closed symbols).

It increased for both diatoms when the specific growth rate decreased (∼1.6-fold for *T. oceanica* and 1.4-fold for *D. brightwellii*), except at the most severe Fe-L co-limitation for *T. oceanica*, where it equalled the non limited value. Molar ratios Si:C (Fig. 4c, d) and Si:N (Fig. 4e, f) did not follow the same patterns for the two species. For *T. oceanica*, Si:C increased by ∼1.8-fold and 1.4-fold under Fe limitation and Fe-L co-limitation respectively, and Si:N increased by ∼2-fold and 1.5-fold under Fe limitation and Fe-L co-limitation respectively, with the exception of the most severe Fe-L co-limitation where a decrease was observed. When the specific growth rate varied between 0.4 and 0.75 d^{-1} for *T. oceanica*, the average value of Si:C was lower under LL than under HL (respectively 0.09±0.01 mol mol^{-1}, n=9, and 0.14±0.01 mol mol^{-1}, n=5, CI=95%), while the average value of Si:N was equivalent under LL and HL (respectively 1.13±0.09 mol mol^{-1}, n=9, and 1.40±0.26 mol mol^{-1}, n=5, CI=95%). For *D. brightwellii*, Si:C and Si:N increased respectively by 2-fold and 2.4-fold under Fe-limitation down to a specific growth rate of 0.6 d^{-1}. Below 0.6 d^{-1}, the ratios decreased to similar values as for non limited conditions. Under Fe-L co-limitation, the same pattern was observed, but it was harder to characterize due to the lower number of values. Besides, Si:C and Si:N were lower at LL than at HL for $\mu > 0.6\,d^{-1}$, and similar for $\mu < 0.6\,d^{-1}$.

To compare the effects of the limitations on the two diatoms, we will use in the discussion section the R ratio, defined as the relative variation of a given parameter between a limiting condition and the Fe-replete condition (e.g., at a specific growth rate μ, $R(Si:N)_\mu$=$(Si:N)_\mu$: $(Si:N)_{\mu max}$). The degree of Fe limitation will be defined by its impact on the growth rate using the ratio μ:μ_{max} (i.e. $R(\mu)$), with the value of maximum growth rate measured at the highest Fe concentration, either under HL (e.g.: $\mu_{max} \sim 1.2\,d^{-1}$ for *T. oceanica*), or under LL (e.g.: $\mu_{max} \sim 0.7\,d^{-1}$ for *T. oceanica*).

4 Discussion

4.1 Growth parameters and the decoupling of cellular C and N

The half-saturation constants for growth with respect to iron ($K_{\mu Fe'}$, Table 2) agree well with previous studies, showing a much lower value, i.e. a better adaptation to limitation (Fe and Fe-L) of a small diatom than of a large one (Sunda and Huntsman, 1995; Timmermans et al., 2001b, 2004). Once the limitation is 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 allometric relationship is very scattered and in our study the largest diatom would outgrow the smallest one due to its higher maximum specific growth rate. The better adaptation of *T. oceanica* can be explained by a more favorable surface to volume ratio for a small species than for a bigger one (Hudson and Morel, 1990) and a general lower Fe requirement for growth in the oceanic species than in the coastal species (Sunda and Huntsman, 1995; Sunda et al., 1991). For example, oceanic diatoms can synthesize flavodoxin instead of ferredoxin (La Roche et al., 1995). 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. 3a). 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. 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 increases by 4-fold under Cu toxicity (from ∼25 000 to ∼100 000 µm^3, Rijstenbil and Gerringa, 2002), and decreases from 4500 to 3000 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.

The inefficiency of photosynthesis also reduces the efficiency of nitrate and nitrite reduction by lowering the amount of reductants. This directly disrupts the metabolism of nitrogen, whose energetic needs are important (Muggli et al., 1996; Timmermans et al., 1994). Besides, Fe is the metal at the center of the nitrate and nitrite reductases. These combined effects of Fe limitation on N metabolism may explain why we observed a stronger effect of Fe on N than on C of Fe and Fe-L (co-)limited cells, except at the most severe degree of limitation for *T. oceanica* (i.e. at the highest degree of Fe-L co-limitation). If we exclude these two singular points, N content indeed decreased by 60% and 50% for *T. oceanica* and *D. brightwellii* respectively (same relative decrease at LL and HL, Fig. 3c, d) while C content decreased by ∼40% for *T. oceanica* and *D. brightwellii* at HL and did not vary (*T. oceanica*) or decreased by 40% (*D. brightwellii*) at LL. As indicated above, however, at the highest degree of Fe-L (co)-limitation for *T. oceanica*, the N content doubled while C remained stable. This sharp increase might be explained by the high level of Fe limitation, even more important under LL. It has indeed been suggested that under severe Fe stress, *T. oceanica* may produce a Fe reductase that is also a plasmalemma bound form of nitrate reductase (Maldonado and Price, 2000). In that case, severely Fe-limited cells might increase their N quota while increasing Fe uptake. Our results give support to this hypothesis.

Many other studies focused on the intracellular C and/or N quota of Fe-limited diatoms. Their conclusions are rarely similar, even for the same species. To better compare all of these studies, we considered the relative variation in C and N per cell (i.e. R(C) and R(N)), versus the relative variation in the specific growth rate, i.e. $R(\mu)$ for 14 other Fe-limited species in six other studies (5 species of *Pseudonitzschia*: Marchetti and Harrison, 2007; 6 species of *Thalassiosira* including *T. oceanica*: Gallinari et al., 2010; Maldonado and Price, 1996; Timmermans et al., 2004; *Actinocyclus* sp.: Muggli et al., 1996; Timmermans et al., 2004; *Fragilariopsis kerguelensis*: Hoffmann et al., 2007; Timmermans et al., 2004; *Corethron pennatum* : Timmermans et al., 2004; and *Chaetoceros dichaeta*: Hoffmann et al., 2007). Results are reported on Fig. 5a and b. If we exclude the two values of *T. oceanica* at the most severe limitation, R(C) and R(N) tend to decrease when Fe or Fe-L co-limitation increases (for R(C): r^2=0.58, $p < 0.00001$, n=63, and R(N): r^2=0.54, $p < 0.00001$, n=87). However, when considering the N or C quota per cell volume (when available: Gallinari et al., 2010; Maldonado and Price, 1996; Marchetti and Harrison, 2007; Muggli et al., 1996), there is no significant trend in R(C) or R(N), as also observed by Price (2005) for *T. weissflogii*. Interspecific differences in C and N quotas of Fe-limited diatoms observed in the literature seem thus to be mostly due to variations in cell volume.

Given the importance of cell volume in comparing the different species and studies, we considered the relative variation in C:N, i.e. R(C:N), versus the relative variation in the specific growth rate, i.e. $R(\mu)$. In our study, and excluding the two values of *T. oceanica* at the most severe limitation, we observed a similar increase with limitation for both species and both limitations (r^2=0.31, p=0.0001, n=47) (Fig. 6a). The relative variation in C:N of the other species cited above, however, does not show any dependency on $R(\mu)$ (r^2=0.003, p=0.8, n=24, data not shown). Growth conditions and species difference have been invoked to explain

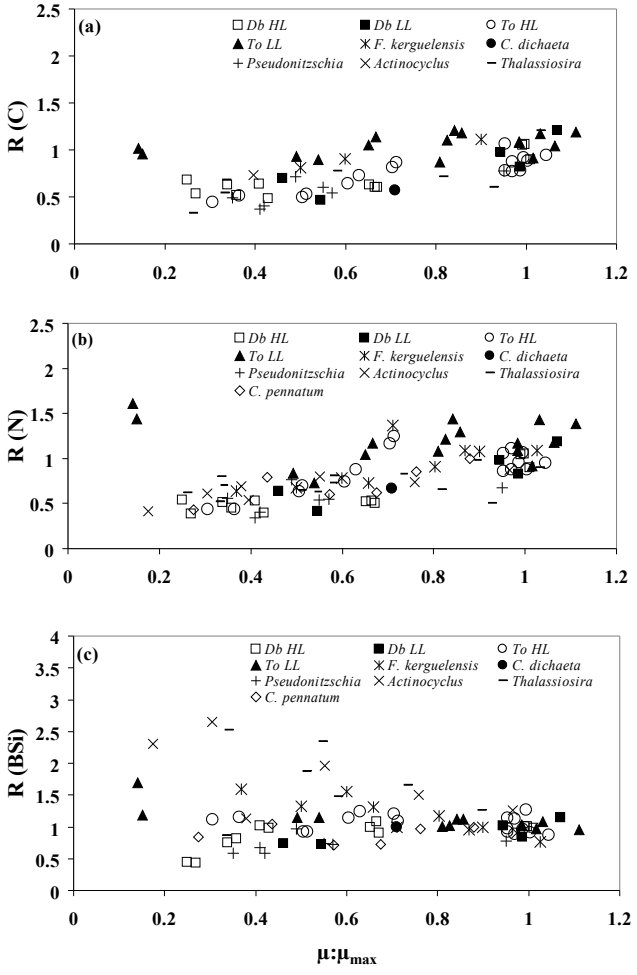

Fig. 5. Relative variation in **(a)** cellular C (R(C)), **(b)** cellular N (R(N)) and **(c)** cellular BSi (R(BSi)) versus relative variation in specific growth rate (μ:μ_{max}) for *Thalassiosira oceanica* (*To*, HL: open circles, LL: closed triangles), *Ditylum brightwellii* (*Db*, HL: open squares, LL: closed squares), *Fragiloriopsis kerguelensis* (*F. kerguelensis*, *, Hoffmann et al., 2007; Timmermans et al., 2004), *Chaeotoceros dichaeta* (*C. dichaeta*, •, Hoffmann et al., 2007), *Pseudonitzshia heimii* type 1, *Pseudonitzshia* cf. *heimii* type 2, *Pseudonitzshia* cf. *turgidula*, *Pseudonitzshia multiseries*, *Pseudonitzshia* cf. *calliantha* (*Pseudonitzshia*, +, Marchetti and Harrison, 2007), *Actinocyclus* sp. (*Actinocyclus*, x, Muggli et al., 1996; Timmermans et al., 2004), *Thalassiosira partheneia*, *Thalassiosira pseudonana*, *Thalassiosira weissflogii*, *Thalassiosira subtilis*, *Thalassiosira oceanica* 13-1, *Thalassiosira oceanica* 1003, *Thalassiosira* sp. (*Thalassiosira*, –, Gallinari et al., 2010; Maldonado and Price, 1996; Timmermans et al., 2004), *Corethron pennatum* (*C. pennatum*, ◇, Timmermans et al., 2004).

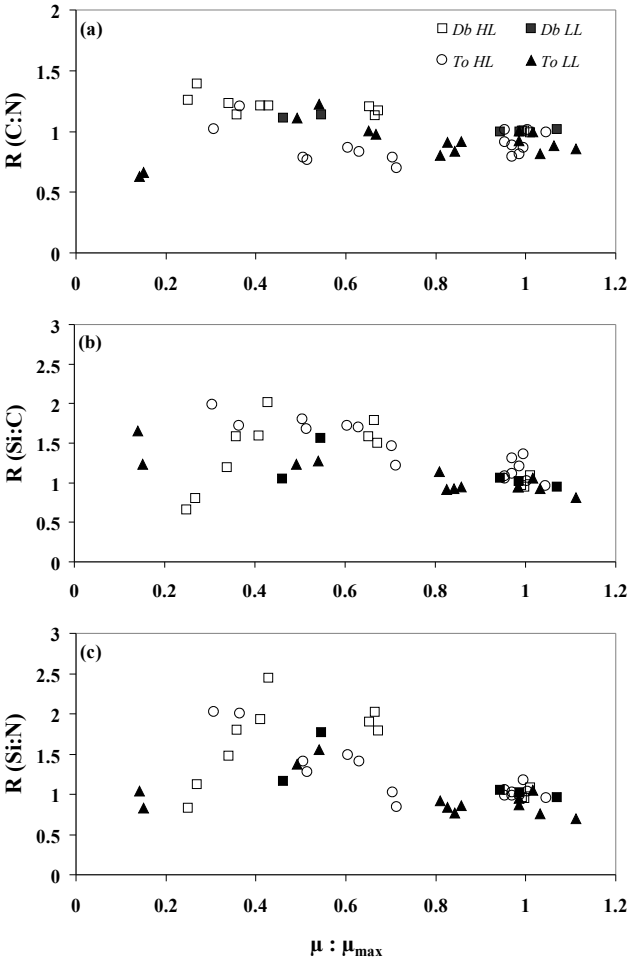

Fig. 6. Relative variation in molar ratio **(a)** C:N (R(C:N)), **(b)** Si:C (R(Si:C)), **(c)** Si:N (R(Si:N)) versus relative variation in specific growth rate (μ:μ_{max}) for *Thalassiosira oceanica* (*To*, HL: open circles, LL: closed triangles) and *Ditylum brightwellii* (*Db*, HL: open squares, LL: closed squares).

these contrasting results (Price, 2005). However, in the same growth conditions, we did not observe a significant interspecific difference in our study. The contrasting results observed on the coupling or decoupling of C and N under Fe limitation may thus be more related to growth conditions (temperature, length of the daily cycle…) than to interspecific differences.

4.2 Biogenic silica and ratios Si:C, Si:N

Most of the studies show an increase in biogenic silica under Fe limitation. We also observed a significant increase in the degree of silicification of Fe-L co-limited *T. oceanica*, but no clear trend under Fe limitation (Fig. 3e). The increase under Fe-L co-limitation may be due to light limitation only. Claquin et al. (2002) indeed showed that light limitation increases the amount of biogenic silica per cell of *Thalassiosira pseudonana*. Two recent studies also showed no change or a weak decrease in cellular biogenic silica of Fe-limited cells of *Chaetoceros dichaeta* (Hoffmann et al., 2007) and some clones of *Pseudonitzschia* (Marchetti and Harrison, 2007), respectively. These results are observed between two values ("low Fe" and "high Fe"), but the effect of Fe on silicification may depend on the degree of Fe limitation (this study; Timmermans et al., 2004). However, there is no significant trend in R(BSi) versus $R(\mu)$ when comparing

different diatoms, either on a per cell (Fig. 5c) or per cell volume basis (data not shown). This suggests interspecific differences in terms of silicification in response to Fe or Fe-L limitation.

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., 2001a). 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).

Although we did not study cell morphology or soluble pools, these hypotheses may also be valid for *D. brightwellii*. Indeed, this species has spines, which may contain a large fraction of biogenic silica (e.g. *C. gracilis*, Rogerson et al., 1986). Timmermans et al. (2001a) observed more/longer spines for the Fe-limited diatoms *C. calcitrans* and *C. brevis* when grown at LL. A decrease in their number or length in *D. brightwellii*, due to Fe and light limitations, may thus affect the BSi content. Besides, Chisholm et al. (1978) showed that for *D. brightwellii*, the intracellular pool of Si may represent up to 50% of total cellular Si, and the size of internal soluble pool can be influenced by environmental variables (Martin-Jézéquel et al., 2000). However, although these mechanisms may explain why we observed a variation in the silicification of diatoms, the underlying processes are not explained. The causal link between iron and silicification has still to be discovered. A few hypotheses can be proposed, based on the silicification process and the possible role of the frustule as a defense mechanism.

It is known that the energy for silicon metabolism is closely linked to respiration (Martin-Jézéquel et al., 2000). Iron limitation can impair respiration in microalgae (Allen et al., 2008; Petroutsos et al., 2009), which may disrupt silicification in diatoms. Another effect might be the control of Fe on the cell cycle via the cellular growth rate. Claquin et al. (2002) indeed showed for light and nutrient (N, P)-limited cells of *T. pseudonana* a relationship between the increased length of the G2 phase (during which Si is assimilated) and the higher degree of silicification under limitation. 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, 2010). 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, 2010). 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.

This difference between the two species may also be related to their ability to escape grazing. 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 better survive the gut passage of copepods (Jensen and Bathmann, 2007).

Under mild Fe limitation ($\mu > 40\%\ \mu_{max}$), we observed an increase in Si:C and Si:N ratios (Fig. 4c–f), which has been noted previously by other studies (see review by Marchetti and Cassar, 2009). We also noted a decoupling between Si, C and N under Fe-L co-limitation, which has been described recently for in situ studies (Hopkinson and Barbeau, 2008; Moore et al., 2007) but not for monospecific laboratory cultures yet. As changes in BSi were lower than in C or N under LL or HL at a given specific growth rate, the differences in Si:C and Si:N between the two irradiances depended mainly on the differences in the C and N contents. Under LL, the higher C content and the lower BSi value (for *T. oceanica*) and higher C and N content (for *D. brightwellii*), compared to HL conditions, induced a lower value of Si:C for *T. oceanica* at a given specific growth rate and a lower value of Si:C and Si:N for *D. brightwellii* at $\mu > 0.6\,d^{-1}$. Besides, under severe limitation, we observed a decrease in these ratios. This pattern was especially clear for *D. brightwellii*. The decrease observed in this species was due to a larger decrease in biogenic silica under Fe limitation (by 60%) than in the cellular N and C content (by 50% and 40%, respectively).

When comparing the relative variation in these ratios versus the relative decrease in μ, both limitations and both species showed very similar patterns (Fig. 6). R(Si:C) and R(Si:N) increased significantly from 100% to ~40% of μ_{max}:

$$R(\mathrm{Si:C}) = 2.11(\pm 0.17) - 1.07(\pm 0.22) \cdot R(\mu);$$
$$r^2 = 0.48, \quad p < 0.00001, \quad n = 28$$

and

$$R(\mathrm{Si:N}) = 2.40(\pm 0.22) - 1.53(\pm 0.29) \cdot R(\mu);$$
$$r^2 = 0.52, \quad p < 0.00001, \quad n = 28$$

For values of μ:μ_{max} below 40%, R(Si:N) tends to decrease down to values close to 1, i.e. close to the value at μ_{max}:

$$R(\mathrm{Si:N}) = 0.05(\pm 0.59) + 4.93(\pm 1.70) \cdot R(\mu);$$
$$r^2 = 0.75, \quad p < 0.01, \quad n = 10$$

A decrease in R(Si:C) is also significant for μ:μ_{max} between 20% and 40% (i.e. if the two lowest growth rates are not taken into account):

$$R(\mathrm{Si:C}) = -0.65(\pm 0.74) + 6.09(\pm 2.12) \cdot R(\mu);$$
$$r^2 = 0.58, \quad p < 0.05, \quad n = 8$$

Such a decrease has never been observed yet. Our results are difficult to compare with in situ Fe fertilization data, among other things because of shifts in the phytoplanktonic community towards large cells after Fe addition, which prevents from comparing μ and μ_{max}. Such shifts were not observed during onboard 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). In that study exploring the impact of Fe limitation on ratios of particulate nutrients, 34 out of 44 stations presented some form of Fe limitation, and BSi:PON and BSi:POC were generally found to decrease in Fe amended samples compared to the control (Firme et al., 2003). However, out of 25 stations that were considered Fe-limited, where no change in phytoplankton size classes occurred after Fe addition, and where elemental composition was measured, BSi:PON and/or BSi:POC ratios were similar in both treatments at 3 stations, and lower in the control at 5 stations. These results thus present interesting similarities with ours, and more studies, both in vitro and in situ, should be conducted to further investigate the link between variations in the elemental composition and variations in the specific growth rate.

4.3 Oceanographic relevance

Results indicating that diatoms increase their Si:N ratio under Fe limitation led to the assumption that (i) Fe increases the degree of silicification of diatoms and that (ii) more silicified, Fe limited diatoms would sink faster and that their frustule would be better preserved when reaching the seafloor, with implications for the use of opal as a paleoproxy (Hutchins and Bruland, 1998; Takeda, 1998; Boyle, 1998). In biogeochemical models which consider the cycling of major nutrients such as C, N, P or Si, it is thus usually assumed that diatom Si content, Si:N and Si:C ratios increase under limiting conditions, and that biogenic silica is efficiently exported below the mixed layer depth because of a lower remineralisation rate than organic C, N or P (e.g. Aumont et al., 2003; Fasham et al., 2006; Moore et al., 2004). This general mechanism fuels the so-called "silica pump" in systems like the Southern Ocean or the Equatorial Pacific (Dugdale et al., 1995). However, in the Southern Ocean, the drawdown of silicic acid occurs during the diatom spring bloom, when limitations (e.g. iron and light) are relieved. In the Ross Sea for example, large silicic acid drawdown and subsequent export of biogenic silica to the deep ocean are concomitant with the diatom bloom (SO-JGOFS AESOPS program, Landry et al., 2002; Nelson et al., 2002; Sigmon et al., 2002). In the Indian sector of the Southern Ocean, in situ observations and results from a coupled physical-biogeochemical model also suggest that more than 80% of the annual C and Si export occur between December and March at the end of the spring bloom (Pondaven et al., 1998, 2000). Additionally, the production of fecal pellets and the formation of aggregates, which are both a major source of biogenic matter towards the deep ocean, increase at the end of blooms (Thornton, 2002), and BSi is better preserved in fecal pellets and aggregates (Moriceau et al., 2007). All together, these observations suggest that most of the silica pump occurs during bloom events, under non limiting conditions.

Our results show that a large diatom may be more silicified under conditions of optimal growth than when its specific growth rate is $<\sim 40\%$ of μ_{max} due to Fe and Fe-L limitations (Fig. 3f). If this pattern also holds for other large diatoms, especially HNLC ecologically relevant species, it might reinforce the impact of the spring bloom on the silicon sink. Besides, less silicified, Fe limited diatoms may dissolve more rapidly. Although this pattern has not been documented yet, high dissolution rates of biogenic silica have indeed been reported in Fe-limited systems such as the Southern Ocean (Beucher et al., 2004).

Overall, our results suggest that the decoupling between Si, C and N in surface waters of Fe and Fe-L limited areas may be less straightforward than previously thought. These results may have important implications for the understanding of the biogeochemical cycles and estimates of biogenic matter export.

5 Conclusions

General trends in the elemental composition of Fe limited and Fe-L co-limited diatoms could be determined by taking into account our results and literature data, and by considering the degree of limitation (i.e. reduction in growth rate).

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 whole, these results show that using a constant C:N ratio to infer Si:C from Si:N, as often done for modelling and in situ experiments, may lead to a bias.

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. The mechanisms controlling the silicification process are not fully elucidated yet and a few hypotheses can be proposed to explain the role of iron in silicification. They include (i) the direct effect of Fe on silicon metabolism through the impairment of respiration, (ii) the

indirect control of Fe on the cell cycle via the cellular growth rate.

Variations in Si:C or Si:N seem to be more constrained, at least under mild limitation. For μ:$\mu_{max} > 40\%$, a clear trend is indeed observed, with an increase in Si:N ratio with increasing limitation. Under more severe limitation, Si:N and Si:C tend to decrease. More in vitro, in situ and modelling studies are needed in that range of limitation, in order to further investigate the link between variations in the elemental composition, variations in the specific growth rate, and their impact on the biogeochemical cycles at the ecosystem level.

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