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Abstract

The interaction between iron availability and the phytoplankton elemental composition was investigated during the in situ iron fertilization experiment EIFEX and in laboratory experiments with the Southern Ocean diatom species *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*. Contrary to other in situ iron fertilization experiments we observed an increase in the bPSi : POC, bPSi : PON, and bPSi : POP ratios within the iron fertilized patch during EIFEX. This is possibly caused by a relatively stronger increase in diatom abundance compared to other phytoplankton groups and does not necessarily represent the amount of silicification of single diatom cells. In laboratory experiments with *F. kerguelensis* and *C. dichaeta* no changes in the POC : PON, PON : POP, and POC : POP ratios were found with changing iron availability in both species. BPSi : POC, bPSi : PON, and bPSi : POP ratios were significantly lower in the high iron treatments compared to the controls. In *F. kerguelensis* this is caused by a decrease in cellular bPSi concentrations and therefore possibly less silicification. In *C. dichaeta* no change in cellular bPSi concentration was found. Here lower bPSi : POC, bPSi : PON, and bPSi : POP ratios were caused by an increase in cellular C, N, and P under high iron conditions. We therefore assume that iron limitation does not generally increase silicification of diatoms and that changes in the bPSi : POC, bPSi : PON, and bPSi : POP ratios under iron fertilization in the field are caused by a variety of different mechanisms. These results imply that the effect of iron on nutrient uptake is more complex than hitherto assumed.

1 Introduction

Recent studies showed that the canonical Redfield ratio of 106 : 16 : 1 for C : N : P is not a general stoichiometric optimum for all marine phytoplankton species but rather represents an average of species specific ratios, which can differ extremely from the Redfield ratio (Ho et al., 2004; Klausmeier et al., 2004; Quigg et al., 2003; Twining et

al., 2004). It is reported that while the POC : PON ratio is often close to the Redfield ratio, diatoms in general have lower than the Redfield PON : POP and POC : POP ratios (Ho et al., 2004; Quigg et al., 2003). This is supported by observations from the Southern Ocean (SO) where waters dominated by diatoms have lower PON : POP and POC : POP ratios compared to waters dominated by the haptophyte *Phaeocystis antarctica* (Arrigo et al., 2002; Arrigo et al., 1999).

Beside these differences in the elemental composition of different phytoplankton classes, nutrient availability can influence stoichiometry of individual species. As a possible explanation for deviations from the Redfield ratio the trace metal iron is discussed. Iron is needed in the nitrogen metabolism of phytoplankton cells as it is essential in the enzymes for nitrate reduction, nitrate and nitrite reductase. Therefore in High Nutrient Low Chlorophyll (HNLC) regions like the SO, where iron limits phytoplankton growth, higher POC : PON and lower PON : POP ratios compared to the Redfield ratio may be expected. However, as the POC : PON ratio is rather constant independent of phytoplankton species (Ho et al., 2004; Quigg et al., 2003) and iron concentration (Greene et al., 1991; Price, 2005), while the POC : POP and PON : POP ratios are much more variable, intracellular phosphate seems to be more influenced by iron availability. The exact mechanisms how the elemental composition of phytoplankton is influenced by iron and why there are large species specific differences remain unknown.

Besides the impact on POC, PON, and POP composition, one important effect of iron limitation on the elemental stoichiometry is an increase in the bPSi : POC, bPSi : PON, and bPSi : POP ratio of diatoms (Hutchins and Bruland, 1998; Price, 2005; Takeda, 1998; Timmermans et al., 2004; Twining et al., 2004). It has been generally assumed that this is caused by an increase in cellular silicate concentrations rather than a decrease in cellular C, N, and P. However, data on the cellular elemental composition of SO diatoms under different iron availabilities are rare and not always a decrease of cellular bPSi with increasing iron availability is observed (Takeda, 1998). It is therefore not certain that iron fertilization generally decreases frustule silicification and the resulting effects on grazing protection, sinking rates, and remineralization have to be considered

in relation to the species specific response to iron availability.

In this study we examined the effect of iron deplete and replete growth conditions on the elemental composition of two Antarctic diatom species *Fragilariopsis kerguelensis* and *Chaetoceros dichaeta*. These species were selected because of their important contribution to the diatom biomass in the SO community. Further, they represent two different degrees of silicification with *F. kerguelensis* being stronger silicified compared to *C. dichaeta*. These results were compared to size fractionated bPSi : POC, bPSi : PON, and bPSi : POP ratios during the in situ iron fertilization experiment EIFEX.

Aim of this study is to investigate the effect of iron on silicification of two important SO diatom species and thus to help interpreting changes in the bPSi : POC, bPSi : PON, and bPSi : POP ratio observed in field experiments.

2 Material and methods

A detailed description of the phytoplankton community structure and of the total POC, PON, POP, and bPSi concentrations during EIFEX is given by Hoffmann et al. (2006). Additionally the POC : PON, POC : POP, and PON : POP ratios of the total plankton community, the >20 μm, 2–20 μm, and the <2 μm size fraction and the total bPSi : POC ratios are presented. In the manuscript at hand we supplement this data set with POC, PON, POP, and bPSi concentrations as well as the bPSi : POC, bPSi : PON, and the bPSi : POP ratios of the >20 μm and the <20 μm size fraction during EIFEX since bPSi was only measured in these size classes. These data are completed with results from laboratory experiments, performed with the SO diatom species *F. kerguelensis* and *C. dichaeta*.

F. kerguelensis and *C. dichaeta* were isolated on board RV "Polarstern" during the SO iron fertilization experiment EIFEX. Isolation procedure, cultivation conditions, and trace metal clean handling were the same as described in Hoffmann et al. (2007). Two experiments with three iron treatments and three replicates each were carried out. In one treatment no iron was added to the culture media, in the other two high iron treat-

ments 100 and 1000 nM Fe were added. In these treatments free iron concentrations were 1.55 nM Fe' (all inorganic Fe species) and 15.5 nM Fe', respectively, estimated after Timmermans et al. (2001). We additionally grew *F. kerguelensis* without iron and EDTA addition, to investigate if this chelator has an effect on growth and stoichiometry. Culture conditions and treatment labels are listed in Table 1.

Chlorophyll measurements, cell counts, and determination of the photosynthetic efficiency Fv/Fm were performed as described in Hoffmann et al. (submitted to Biogeosciences).

Size fractionated samples for POC, PON, POP, and bPSi measurements during EIFEX were taken as described by Hoffmann et al. (2006). POC, PON, POP, and bPSi samples from the laboratory experiments were not size fractionated. However, the filters used and sample storage was the same as for the EIFEX samples.

POC and PON measurements of the EIFEX and *F. kerguelensis* samples as well as all POP, bPSi, and HPLC pigments measurements were carried out as described by Hoffmann et al. (2006). The POC and PON content of the *C. dichaeta* cultures was analyzed using an Euro EA-CN IRMS elemental analyzer linked to Finnegan Delta Plus radio isotope mass spectrometer as described by Carman and Fry (2002). Acetanelid and peptone were used as standards.

Growth rates were calculated as $\mu=(t_2-t_1)^{-1}\cdot \ln\left(N_2/N_1\right)$ where μ is the net growth rate d^{-1} and N_1 and N_2 are the cell concentrations at t_1 and t_2 respectively. For statistical analysis Students t-test was used. Differences found are reported as significant in the text if $p<0.05$.

3 Results

3.1 Size fractionated particulate organic matter during EIFEX

During the in situ iron fertilization experiment EIFEX, the particulate organic matter (POM) showed a different behavior in the >20 μm and the <20 μm size fractions inside

the iron fertilized patch (Fig. 1). Almost no changes were found in the >20 μm size fraction during the first 16 days of the experiment followed by a 4.1, 1.9, 4.1, and 1.4 times increase in bPSi, POC, PON, and POP concentrations respectively (Fig. 1a). In the <20 μm size fraction no changes in POC, PON, and POP concentrations were found while bPSi concentrations increased continuously during the whole 37 days of the experiment by a factor of 2.5 (Fig. 1b).

The bPSi : POC, bPSi : PON, and bPSi : POP ratios of the total biomass increased 2.1, 1.3, and 2.6 times respectively during the experiment inside the fertilized patch, while no trend was observed outside the patch (Table 2). Separation of the total biomass in >20 μm and <20 μm size fractions shows that the same trends were found in the molar ratios of both size classes (Fig. 2). The bPSi : POC, bPSi : PON, and bPSi : POP ratios increased continuously in the <20 μm size fraction from 0.1, 0.9, and 14.8 at the start of the experiment to 0.4, 2.3, and 40.0 at day 37 inside the fertilized patch. Outside the patch the values were lower in the second half of the experiment. In the >20 μm fraction the elemental ratios did not increase steadily. The values were 0.3 (bPSi : POC), 3.0 (bPSi : PON), and 14.5 (bPSi : POP) in the beginning and decreased within the first 16 days of the experiment, followed by a large increase at day 21 to 0.7, 3.4, and 35.5. During the rest of the experiment the values stayed at an elevated level.

3.2 Growth parameters in the laboratory experiments

In *F. kerguelensis* and *C. dichaeta* cultures, iron fertilization resulted in a significant increase in maximum growth rate, chlorophyll concentrations, and photosynthetic efficiency (Fv/Fm) compared to the non fertilized treatments (Fig. 3 and Table 3). In *F. kerguelensis* growth rates, Chl concentrations, and Fv/Fm were not statistically different (t-test; p=0.3–0.6) in both low iron treatments A and B. Iron addition resulted in a distinct increase in each of these parameters with higher values at 15.5 nM Fe' compared to 1.55 nM Fe'. Mean chlorophyll concentrations were 0.8 μg l^{-1} at the beginning of the experiment for all treatments. The chlorophyll concentrations increased

to 15.4 $\mu g\,l^{-1}$ in 34 days without iron and EDTA addition (Fig. 3a). Under EDTA addition Chl concentrations were slightly lower (8.1 $\mu g\,l^{-1}$). The addition of 1.55 nM Fe' resulted in a significant increase in the chlorophyll concentrations compared to both treatments without iron addition (t-test; p=0.003 and 0.0007). After 34 days Chl concentrations reached a value of 76 $\mu g\,l^{-1}$, which is a 95 times increase. Higher iron concentrations of 15.5 nM Fe' additionally increased Chl concentrations to a value of 96.4 $\mu g\,l^{-1}$. Fv/Fm values of *F. kerguelensis* were between 0.15–0.29 at the beginning of the experiment. These low values indicated that the start culture was iron limited before the experiment. However, the variance here and in the low iron treatments throughout the experiment was relatively high, possibly caused by the very low biomass. Without iron addition maximum Fv/Fm values of 0.29 were reached. Under iron addition Fv/Fm values increased to 0.44 (treatment C) and 0.6 (treatment D) (Table 3). Maximum growth rates roughly doubled from 0.10 and 0.12 in the low iron treatments to 0.18 and 0.2 in the high iron treatments (Table 3).

In *C. dichaeta* Chl concentrations in both high iron treatments increased rapidly about 30 times to 96.1 and 97.0 $\mu g\,l^{-1}$ in 25 days (Fig. 3b). Thereafter concentrations leveled off and highest values were reached at day 36 with 113.9 $\mu g\,l^{-1}$ (treatment C) and 112.8 $\mu g\,l^{-1}$ (treatment D). In the low iron treatment Chl concentrations increased only 11.6 times until day 25. However, growth continued throughout the experiment reaching concentrations almost as high as the high iron treatments of 87.3 $\mu g\,l^{-1}$ at day 42. Fv/Fm values were between 0.19 and 0.21 at the beginning of the experiment and increased more than two times reaching maximum values of 0.54 (treatment C) and 0.56 (treatment D) after iron addition (Table 3). Under iron limitation maximum Fv/Fm value was 0.32. Maximum growth rate in the low iron treatment was 0.22 (Table 3). Unlike *F. kerguelensis*, we found no difference between the addition of 1.55 and 15. nM Fe'. In both high iron treatments the maximum growth rate was 0.31.

3.3 Elemental ratios in the laboratory experiments

In both species no significant changes in the POC : PON, POC : POP, and PON : POP ratio were found between the high and low iron treatments and the different growth periods (Fig. 4 and 5). However, all elemental ratios were below the Redfield ratio of C : N : P 106 : 16 : 1. *C. dichaeta* and *F. kerguelensis* had mean POC : PON ratios of 5.8 and 5.5, which is lower than the Redfield ratio of 6.6. More severe are the differences in the POC : POP and PON : POP ratios which are 44.6 and 7.7 in *C. dichaeta* and 17.8 and 3.3 in *F. kerguelensis* respectively. Here the difference from the Redfield POC : POP ratio of 106 is 58% (*C. dichaeta*) and 83% (*F. kerguelensis*). The deviation from the Redfield PON : POP ratio of 16 is 52% (*C. dichaeta*) and 80% (*F. kerguelensis*). In both species high iron concentrations resulted in lower bPSi : POC, bPSi : PON, and bPSi : POP ratios compared to the low iron treatments. At day 34 (*F. kerguelensis*) and day 31 (*C. dichaeta*) the ratios were roughly twice as high in the low iron treatment compared to the high iron treatments.

3.4 Cellular composition

Chl per cell increased in all high iron treatments in both species. Cellular chlorophyll concentrations of *F. kerguelensis* and *C. dichaeta* were about two times higher in the high iron treatments (Table 3). Interestingly the cellular elemental composition shows different strategies of the two species both resulting in lower bPSi : POC, bPSi : PON, and bPSi : POP ratios under high iron concentrations. Mean cellular C, N, and P concentrations of all treatments were 18.9, 3.6, and 1.0 pmol cell^{-1} in *F. kerguelensis* showing no significant changes due to iron concentrations (t-test; p=0.1–0.9). However, bPSi concentrations per cell were significantly higher in both low iron treatments compared to the high iron treatments (t-test; P=0.035 and 0.039). In contrast to that no significant change in the cellular bPSi concentration in *C. dichaeta* was found (mean of 0.7 pmol cell^{-1}; t-test; p=0.2–0.9), while cellular C, N, and P concentrations were about twice as high under high iron concentrations.

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4 Discussion

4.1 Deviation from the Redfield ratio

The elemental composition of diatoms is known to be extremely variable between different species (Sarthou et al., 2005). A general observation from laboratory experiments (Ho et al., 2004; Price, 2005; Quigg et al., 2003) and field studies (Arrigo et al., 1999; Coale et al., 2004; Hoffmann et al., 2006) is that the mean POC : PON ratio of diatoms is close to the Redfield ratio of 6.6 and shows only minor changes due to environmental conditions. In agreement to that we found mean POC : PON ratios of 5.5 for *F. kerguelensis* and 5.8 for *C. dichaeta* that showed no significant changes with iron concentration. The PON : POP and POC : POP ratios of diatoms are generally lower than the Redfield ratio (Fu et al., 2005; Ho et al., 2004; Quigg et al., 2003; Sarthou et al., 2005) and are much more susceptible to changes in nutrient supply. For *Thalassiosira weissflogii* a higher accumulation of P and resulting lower PON : POP and POC : POP ratios are reported under iron limiting conditions in laboratory experiments (Price, 2005). It remains unknown if this is due to a luxury uptake and storage of P or if specific physiological processes under iron limitation force the cell to a higher P usage. However, the elemental PON : POP and POC : POP ratios of both species tested in this study were not affected by the iron concentration of the growth medium (Figs. 4 and 5). Similar findings are reported for the PON : POP ratio *C. dichaeta* and *Nitzschia* sp. (Takeda, 1998) and *Phaeodactylum tricornutum* (Greene et al., 1991). Takeda (1998) showed that in vitro iron fertilization can lead to opposed changes in the ratio of NO_3^- : PO_4^{3-} consumption depending on the oceanic region. While he found an increase in the ratio of NO_3^- : PO_4^{3-} consumption in the SO, no changes were found in the Subarctic North Pacific and a decrease is reported from waters of the Equatorial Pacific. Hoffmann et al. (2006) report a wide variability between the PON : POP and POC : POP ratios of the different phytoplankton size classes after iron fertilization in the SO. While both ratios increased from far below Redfield values to close to Redfield values

in the microplankton, the opposite trend was observed in the nanoplankton. Here start values close to the Redfield ratio decreased to far lower values after iron fertilization.

Although we found no changes in the relative elemental composition of both species and in the absolute cellular C, N, and P concentrations in *F. kerguelensis*, total cellular C, N, and P concentrations roughly doubled with higher iron concentration in *C. dichaeta* (Table 3). This effect may partly be caused by a change in cell volume. We found an increase in the cell volume of *C. dichaeta* by a factor of 1.3 with higher iron concentrations (data not shown) that was similar to those reported by Hoffmann et al. (2007). However, this increase in cell volume would only result in cellular C, N, and P concentrations of 2.7, 0.5, and 0.06 pmol cell^{-1} respectively and can therefore not explain all of the observed increase (compare Table 3). Additionally a higher C, N, and P accumulation must have taken place in this species under high iron concentrations. It can be speculated that increased C uptake due to higher photosynthetic activity and higher nitrate uptake under high iron concentrations are responsible for this observation. To our knowledge no physiological effect is known that could explain increased P uptake under high iron concentrations. No change in cell size of *F. kerguelensis* was found during this experiment, which is consistent with findings reported by Timmermans et al. (2004) for the same species. These authors showed that the cellular nutrient consumption ratios of four SO diatom species grown under different iron concentrations differ extremely and showed no collective trend. Cellular N consumption increased in *Actinocyclus* sp., *Thalassiosira* sp., and *C. pennatum* with increasing iron concentration, while no changes were found in *F. kerguelensis*. In agreement with our observations, none of the species tested by Timmermans et al. (2004) showed higher cellular P uptake under iron limitation.

These results show that luxury P consumption, as reported by Price (2005) for *T. weissflogii*, is not a general mechanism controlled by iron availability, but rather a species specific reaction. It has been recently shown that phytoplankton cells absorb P on their cell surfaces in an amount of 14 % to 57 % of total cellular P (Fu et al., 2005; Sanudo-Wilhelmy et al., 2004). The measured PON : total POP and POC : total POP

ratios might therefore generally be lower than the truly intracellular stoichiometry and thus may falsify our interpretation of the nutritional status of the cells. When corrected for the surface bound P Fu et al. (2005) describe an increase in the C : intracellular P and N : intracellular P ratios of a factor of 1.2 to 2 in all species tested, including one Southern Ocean *Chaetoceros* species. It can only be speculated what the effect of these observations on the proposed POP export may be. It is possible that sinking cells would lose a lot of the surface bound P during sinking due to microbial uptake. This could possibly decrease the amount of total P exported to the deep sea greatly. These uncertainties lead us to the suggestion to use PON : POP and POC : POP ratios with great caution in terms of nutrient drawdown ratios and for biogeochemical modeling. The general observation that the POC : PON ratio is less affected by environmental conditions and generally closer to the Redfield ratio makes it a far better proxy for these purposes.

4.2 Impact of iron on silicification

A collective observation from in situ iron fertilization experiments is that the growth of diatoms, especially large species, is stimulated to a greater degree than other phytoplankton groups. In these experiments, besides the increase in cell counts and chlorophyll concentrations, the drawdown of nitrate in iron fertilized waters is often used to follow the biomass development. As iron is an essential component in the enzymes responsible for nitrogen uptake, nitrate and nitrite reductase, it was not surprising to see that nitrate uptake was greatly enhanced by iron fertilization. More remarkable was the observation that higher iron availability decreased the bPSi : PON ratio in bottle incubation experiments in all HNLC regions (Brzezinski et al., 2003; De La Rocha et al., 2000; Franck et al., 2003; Franck et al., 2000; Hutchins and Bruland, 1998; Martin and Fitzwater, 1988; Takeda, 1998; Watson et al., 2000). This phenomenon is not only caused by increasing N uptake but also by lower cellular bPSi concentrations (Hutchins and Bruland, 1998; Takeda, 1998) and thus also affected the bPSi : POC and bPSi : POP ratios. Because of the relative increase in bPSi under iron limitation, Boyle (1998)

suggested that iron-limited diatoms grow thicker and thus heavier silica shell, which sink faster to the sea floor and are less remineralized. This is thought to be a reason for the high accumulation rates of diatom frustules in SO sediments despite relatively low bPSi production in the euphotic zone, known as the "opal paradox" (Nelson et al., 1995; Tréguer et al., 1995).

Contrary to other in situ iron fertilization experiments, which showed decreasing bPSi : PON and bPSi : POC ratios, respectively (Boyd et al., 2005; Coale et al., 2004; Gall et al., 2001), the bPSi : POC, bPSi : PON, and bPSi : POP ratios of the total biomass increased 1.8 to 2.6 times during EIFEX (Table 2). This is caused by a stronger increase of bPSi concentrations compared to POC, PON, and POP concentrations, which can be explained by a relative increase in the diatom abundance and a shift towards diatom species that are stronger silicified compared to others (Hoffmann et al., 2006).

In the >20 μm size fraction concentrations of bPSi, POC, PON, and POP increased inside the fertilized patch (Fig. 1a). Here again increasing bPSi : POC, bPSi : PON, and bPSi : POP ratios result from a stronger enhancement in bPSi concentrations compared to the other parameters. As this size fraction was dominated by diatoms from the beginning (Hoffmann et al., 2006), this observation can only be caused by a shift in the diatom community structure towards heavily silicified species. In the <20 μm size faction no increase in POC, PON, and POP concentrations was found, while bPSi concentrations increased steadily throughout the experiment (Fig. 1b). In this size fraction cell counts and HPLC pigment data show a shift from a haptophyte dominated towards a diatom dominated phytoplankton community (Hoffmann et al., 2006). This resulted in increasing bPSi concentrations, while POC related biomass showed no changes. Changes in species composition during EIFEX may have been more pronounced compared to other in situ iron fertilization experiments. Thus the increasing bPSi : POC, bPSi : PON, and bPSi : POP ratios during EIFEX may not necessarily contradict the theory of weaker silicification with iron fertilization, however, species specific differences and reactions of the heterotrophic biomass may falsify the picture.

To further understand these mechanisms and explain the differences between EIFEX and other experiments, we performed laboratory experiments with two SO diatoms *F. kerguelensis* and *C. dichaeta*, which were both important species during EIFEX, and determined cellular POC, POP, PON, and bPSi concentrations. The bPSi : POC and bPSi : PON ratios of both species were relatively close to those found in the field, while the bPSi : POP ratios were lower in both cultures (Figs. 4, 5 and Table 2). As described above the cellular P pool is more complex than the C and N pools due to the existence of surface bound P. The surface bound P concentration is dependent on the amount of surface bound Mn (Sanudo-Wilhelmy et al., 2004), which is explained by the adsorption of P to cell-surface-bound Mn hydroxides and oxides. In the SO Mn concentrations are known to be very low (Martin et al., 1990). It is therefore possible that in this region surface bound P concentrations and thus total cellular P concentrations are lower compared to our laboratory experiments, where all trace metals, including Mn, were added in excess. This could possibly explain the higher Si : P ratios observed during in the field during EIFEX. This assumption is further supported by the observation that C : P and N : P ratios of all size classes during EIFEX reported by Hoffmann et al. (2006) were higher compared to those found in *F. kerguelensis* and *C. dichaeta* in this study (Figs. 4 and 5).

Our laboratory experiments show that increased cellular bPSi concentrations under iron limitation are not a general phenomenon of all diatom species. In both species tested we observed higher bPSi : POC, bPSi : PON, and bPSi : POP ratios under low iron availability. However, while bPSi concentrations per cell were significantly increased under iron limitation in *F. kerguelensis*, no changes were found in *C. dichaeta* (Table 3). In the latter species the increase in the bPSi : POC, bPSi : PON, and bPSi : POP ratios is caused by lower cellular C, N, and P concentrations as described above. Analysis of Si consumption per cell led to similar results showing higher cellular Si accumulation under low iron concentrations in *Actinocyclus* sp., *Thalassiosira* sp., and *F. kerguelensis*, but no significant change in cellular Si accumulation in *Corethron pennatum* (Timmermans et al., 2004). We suggest that species specific changes in the

elemental composition as well as the reactions of other phytoplankton groups and the heterotrophic biomass conceal the effect of iron on diatom silicate uptake in the field. Changes in the bPSi : POC, bPSi : PON, and bPSi : POP ratios with iron fertilization should thus be carefully interpreted in terms of diatom silicification.

The reason for changes in nutrient uptake and storage under changing iron availability is, with the exception of nitrate, not well understood. Fe limitation directly decreases the uptake rates of SO diatoms for silicic acid (Brzezinski et al., 2005; De La Rocha et al., 2000; Franck et al., 2003; Franck et al., 2000). However, it is generally accepted that increased Si uptake is caused by an increased duration of the cell wall synthesis phase. Si uptake is closely related to the G_2+M phase of the cell cycle. Nutrient (N, Fe), light, or temperature limitation that prolong this phase lead to higher silicification in diatoms (see review in Martin-Jézéquel et al., 2000). Despite lower uptake rates under Fe limitation the increased period available for Si uptake resulted in higher silicification of diatom frustules. However, our results and those of previous studies show that this phenomenon is not valid for all diatom species. As we observed that bPSi : POC, bPSi : PON, and bPSi : POP ratios were not affected by the changes in the cellular concentrations, these mechanisms will be of less importance for analysis of nutrient budgets. However, they can possibly affect the sinking behavior as well as the remineralization of frustules in the sediments. As *in situ* iron fertilization experiments are performed with the aim to increase the uptake of atmospheric CO_2 and carbon export to the deep sea, impact of iron on sedimentation and remineralization is of great interest. Higher sinking rates were observed for iron limited cells of *Actinocyclus* sp. (Muggli et al., 1996) and of *F. kerguelensis*, *Nitzschia* sp., and *Navicula* sp. during the in situ iron fertilization experiment SOIREE (Waite and Nodder, 2001). This phenomenon is thought to be a mechanism for the preservation of a seed population or could possibly give the cells access to higher nutrient concentrations in deeper waters (De La Rocha et al., 2000). Additionally it can be speculated that a stronger silicification of the frustules could increase the cell wall stability and therefore provide a better grazing protection under unfavorable growth conditions. It is also possible that iron limitation decreases

the ability of diatoms to maintain buoyancy. Usually diatom cells control buoyancy by the selective exchange of heavier and lighter ions in the vacuole, the so-called ionic pump, which requires a lot of energy (Anderson and Sweeney, 1978). Iron limitation might reduce the efficiency of energy-producing pathways needed by the cells to maintain buoyancy (Sarthou et al., 2005). However, diatoms are known to dominate the phytoplankton community in turbulent waters (Harris, 1986). It is therefore not likely that a successful phytoplankton group such as diatoms suffer from permanent stress to maintain cell buoyancy in regions like the SO, which are characterized by low iron concentrations and extremely deep mixing throughout the year. We therefore assume that increased sinking rates are mainly caused by a stronger silicification. However, the largest diatom cells during SOIREE, *Trichotoxon* sp. and *Thalassiothrix* sp., showed only little changes in sinking rates (Waite and Nodder, 2001). These species specific differences may result from different impacts of iron on silicification as shown here and in previous studies.

The sediments of the SO mainly consist of diatom frustules of *Fragilariopsis kerguelensis*. This species is heavily silicified and has shown to increase silicification under low iron conditions in laboratory experiments including this study. Therefore, high accumulation rates of silica frustules in the sediment are probably mainly caused by strong silicification in this species under iron limitation. We therefore suggest that a decrease in silicification and lower sinking rates of *F. kerguelensis*, as observed by Waite and Nodder (2001), could decrease the dominant contribution of this species to the Si export under long-term iron fertilization in the SO. Less silicified frustules could additionally be more affected by grazing and remineralization and thus decrease carbon export as well. However, the observation that the cellular Si content of *C. dichaeta* was not dependent on the iron availability, as well as the observation that *Trichotoxon* sp. and *Thalassiothrix* sp. did not change their sinking rates (Waite and Nodder, 2001), imply that other, also important SO diatom species, would not be affected. If all of those species would increase their cellular C concentrations similar to our observations of *C. dichaeta*, this could even increase the carbon export with iron fertilization.

In conclusion we suggest that changes in the bPSi : C, bPSi : N, and bPSi : P ratios with changing iron availability should be carefully interpreted in terms of nutrient export. Changes in the phytoplankton community structure as well as sinking rates and grazing protection of dominant species might be of greater importance for biomass export and remineralization.

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Table 1. Iron and EDTA concentrations in the different treatments of the two laboratory experiments.

Species	Treatment			
	A	B	C	D
F. kerguelensis	no Fe addition no EDTA	no Fe addition 10 μM EDTA	1.55 nM Fe' 10 μM EDTA	15.5 nM Fe' 10 μM EDTA
C. dichaeta	–	no Fe addition 10 μM EDTA	1.55 nM Fe' 10 μM EDTA	15.5 nM Fe' 10 μM EDTA

Table 2. Molar ratios of bPSi : POC, bPSi : PON, and bPSi : POP of the total biomass during the iron fertilization experiment EIFEX in- and outside the iron fertilized patch. * Hoffmann et al. (2006).

Day	bPSi : POC*	bPSi : PON	bPSi : POP
inpatch	μmol : μmol	μmol : μmol	μmol : μmol
0	0.24	1.52	17.19
11	0.23	1.39	15.46
16	0.29	1.89	16.99
22	0.44	2.46	28.84
26	0.40	2.18	27.16
29	0.38	1.99	21.88
34	0.40	2.12	25.15
37	0.50	2.80	45.51
outpatch			
0	0.24	1.52	17.19
12	0.36	2.03	19.18
18	0.27	1.45	15.65
27	0.21	1.08	12.24
35	0.37	2.01	22.64

Table 3. Cellular elemental composition and chlorophyll concentration, growth rate and maximum Fv/Fm of *F. kerguelensis* at day 34 in the two low iron treatments A and B and the high iron treatments C and D and of *C. dichaeta* at day 31 in the low iron treatment B and the high iron treatments C and D. Values marked with * were significantly different (t-test; $p<0.05$) between high and low iron treatments. Values marked with ° were only significantly different between treatment B and C.

				Elemental composition			
Treatment	μmax d^{-1}	Fv/Fm max	Chl pg $cell^{-1}$	C pmol $cell^{-1}$	N pmol $cell^{-1}$	P pmol $cell^{-1}$	Si pmol $cell^{-1}$
F. kerguelensis	*	*	*				*
A	0.12±0.01	0.29±0.08	1.9±0.8	17.4±4.1	2.9±0.5	0.7±0.2	14.2±0.5
B	0.10±0.01	0.29±0.05	2.3±0.9	15.7±1.3	2.5±0.2	0.7±0.1	12.0±1.0
C	0.18±0.0	0.44±0.06	3.6±0.8	21.5±2.6	4.0±0.3	1.0±0.2	9.0±1.2
D	0.20±0.01	0.60±0.05	4.5±0.9	19.3±6.3	3.7±1.3	1.0±0.2	9.1±1.3
C. dichaeta	*	*	*	°	°	°	
B	0.22±0.01	0.32±0.00	0.4±0.2	2.0±0.03	0.4±0.0	0.05±0.0	0.6±0.1
C	0.31±0.03	0.54±0.02	1.1±0.2	6.1±0.7	1.1±0.1	0.1±0.03	0.7±0.1
D	0.31±0.02	0.56±0.02	0.8±0.1	3.5±0.7	0.6±0.1	0.08±0.02	0.6±0.2

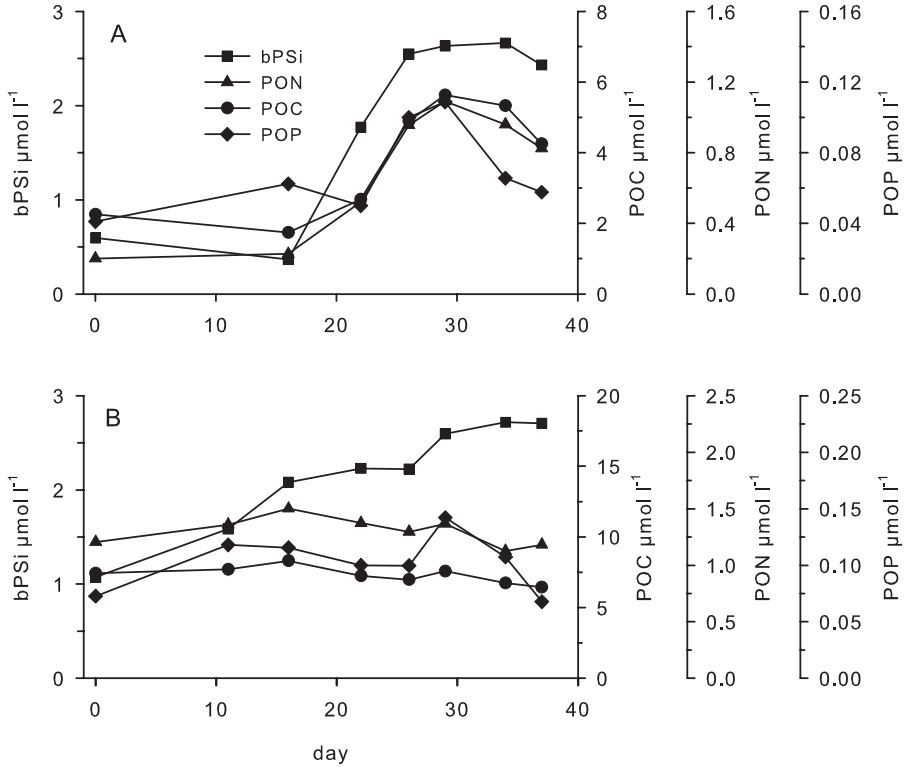

Fig. 1. Concentrations of bPSi, POC, PON, and POP in the >20 μm size fraction **(a)** and in the <20 μm size fraction **(b)**.

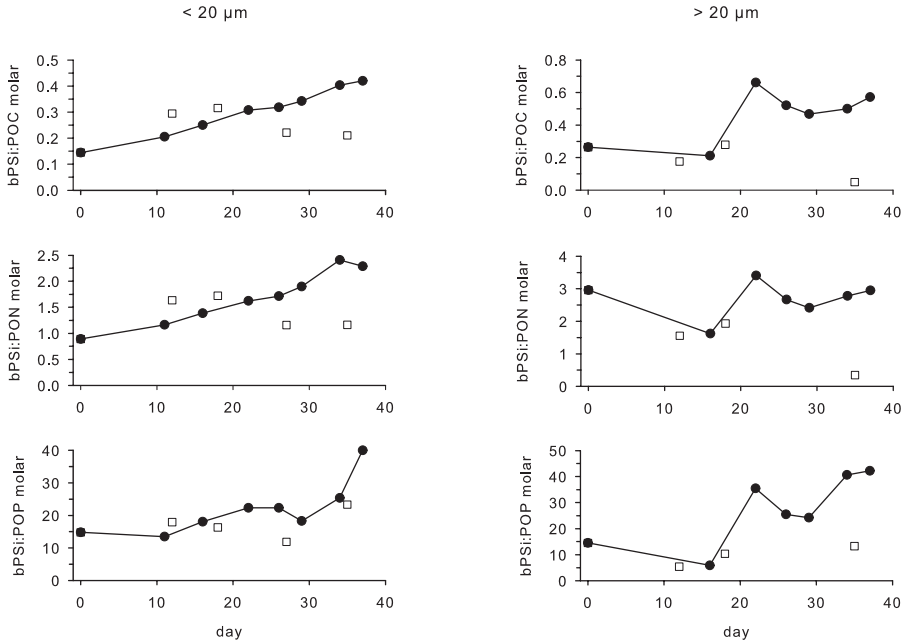

Fig. 2. Molar ratios of the <20 μm and the >20 μm size fraction inside the iron fertilized patch (dark circles) and outside the iron fertilized patch (open squares).

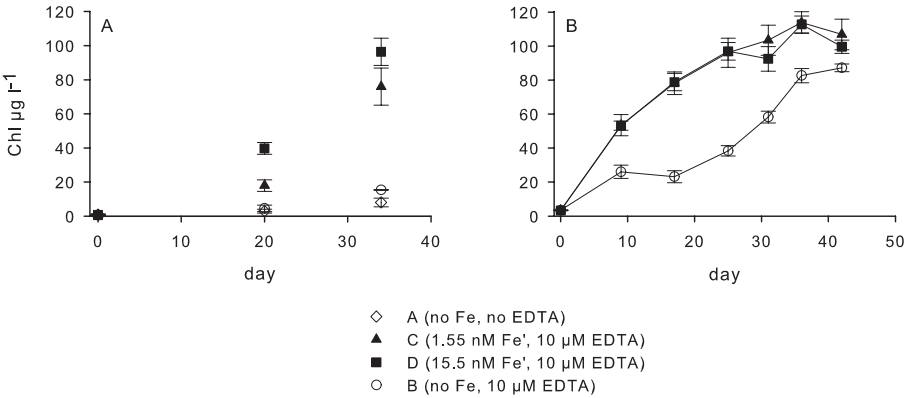

Fig. 3. Chlorophyll concentrations in *F. kerguelensis* **(a)** and *C. dichaeta* **(b)** at the different iron concentrations.

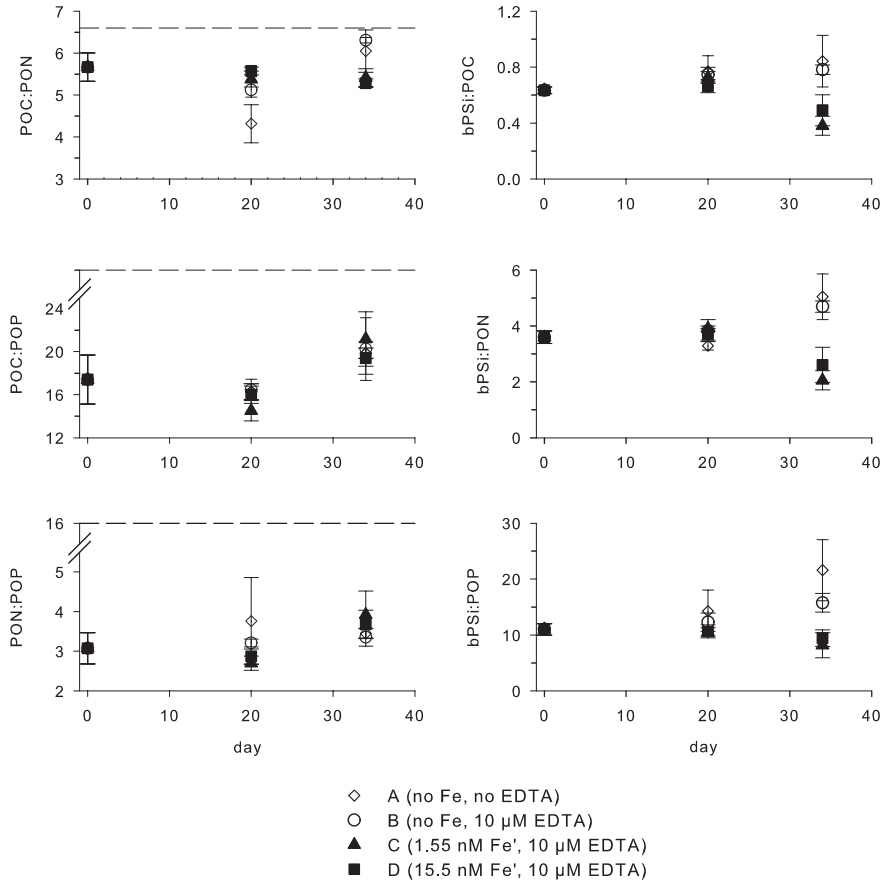

Fig. 4. Molar elemental ratios in *F. kerguelensis* grown under different iron and EDTA concentrations.

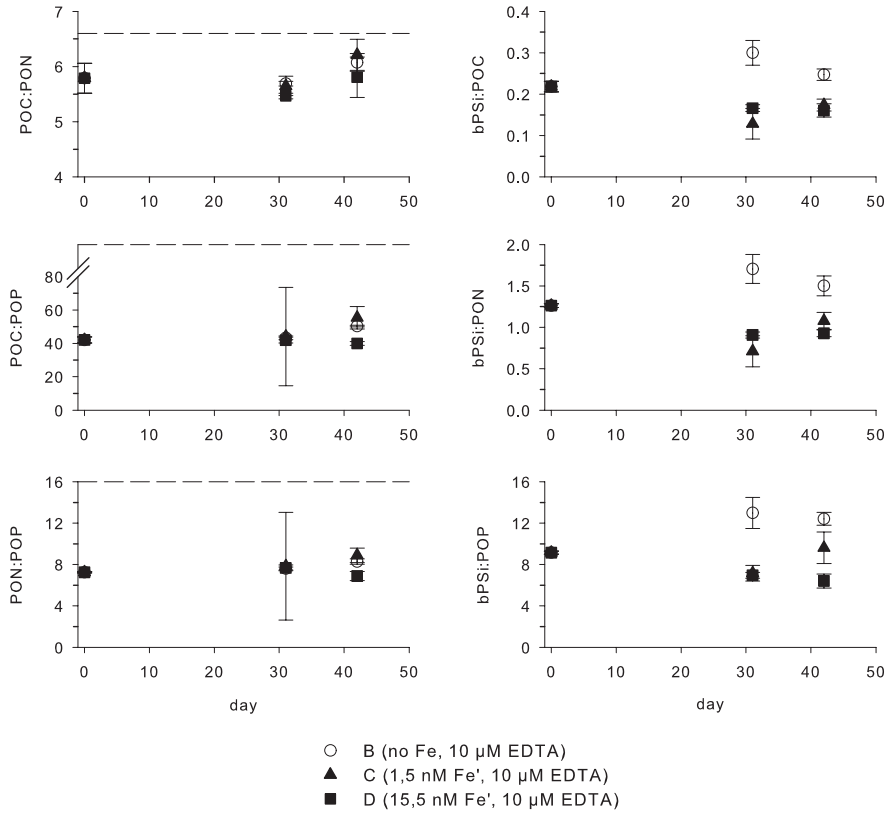

Fig. 5. Molar elemental ratios in *C. dichaeta* grown under different iron and EDTA concentrations.