EFFECTS OF \( \text{PCO}_2 \) AND IRON ON THE ELEMENTAL COMPOSITION AND CELL GEOMETRY OF THE MARINE DIATOM *PSEUDO-NITZSCHIA PSEUDELICATISSIMA* (BACILLARIOPHYCEAE)\(^1\)

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*Running head:* Effects of pH and Fe on diatom
ABSTRACT

Partial pressure of CO₂ ($p_{\text{CO}_2}$) and iron availability in seawater show corresponding changes due to biological and anthropogenic activities. The simultaneous change in these factors precludes an understanding of their independent effects on the ecophysiology of phytoplankton. In addition, there is a lack of data regarding the interactive effects of these factors on phytoplankton cellular stoichiometry, which is a key driving factor for the biogeochemical cycling of oceanic nutrients. Here, we investigated the effects of $p_{\text{CO}_2}$ and iron availability on the elemental composition (C, N, P and Si) of the diatom *Pseudo-nitzschia pseudodelicatissima* (Hasle) Hasle by dilute batch cultures under 4 $p_{\text{CO}_2}$ (~200, ~380, ~600, and ~800 µatm) and 5 dissolved inorganic iron ($\text{Fe}^\prime$; ~5, ~10, ~20, ~50, and ~100 pmol L$^{-1}$) conditions. Our experimental procedure successfully overcame the problems associated with simultaneous changes in $p_{\text{CO}_2}$ and $\text{Fe}^\prime$ by independently manipulating carbonate chemistry and iron speciation, which allowed us to evaluate the individual effects of $p_{\text{CO}_2}$ and iron availability. We found that the C:N ratio decreased significantly only with an increase in $\text{Fe}^\prime$, whereas the C:P ratio increased significantly only with an increase in $p_{\text{CO}_2}$. Both Si:C and Si:N ratios decreased with increasing $p_{\text{CO}_2}$ and $\text{Fe}^\prime$. Our results indicate that changes in $p_{\text{CO}_2}$ and iron availability could influence the biogeochemical cycling of nutrients in future oceans with high CO₂ levels, and, similarly, during the time course of phytoplankton blooms. Moreover, $p_{\text{CO}_2}$ and iron availability may also have affected oceanic nutrient biogeochemistry in the past, as these conditions have changed markedly over the Earth’s history.

Key index words: carbon dioxide, cell size, diatom, elemental composition, iron, nutrients, ocean acidification

Abbreviations: BSi, biogenic silica; CCMs, carbon concentration mechanisms; CV, cell volume; DIC, dissolved inorganic carbon; Fe', dissolved inorganic iron; $k_{\text{H}}$, half-saturation constant for growth; $p_{\text{CO}_2}$, partial pressure of CO₂; PN, particulate nitrogen; POC, particulate organic carbon; PP, particulate phosphate; SA, surface area;
TA, total alkalinity; \( V_A \), valve aspect ratio; \( x_{\text{CO}_2} \), concentration of \( \text{CO}_2 \); \( \alpha \), initial slope of Monod kinetics; \( \mu_{\text{max}} \), maximum specific growth rate

**INTRODUCTION**

The dissolution of \( \text{CO}_2 \) that is primarily emitted from anthropogenic activities causes the partial pressure of \( \text{CO}_2 \) (\( p_{\text{CO}_2} \)) to increase and the pH to decrease in surface oceans. Ocean pH has decreased by \(~0.1\) unit since preindustrial times and will continue to decrease as long as fossil fuels are burned without significant efforts to reduce the atmospheric \( \text{CO}_2 \) (Doney et al. 2009). The rate of pH decline during the Anthropocene (beginning in the late 18th century; Crutzen 2002) is probably considerably more rapid than that which occurred over the past several tens of millions of years (Doney et al. 2009). Concomitant with ocean acidification, the ferrous to ferric iron composition (Millero et al. 2009) and the conditional stability constant of iron-ligand complexes (Shi et al. 2010) could increase in the future as atmospheric \( \text{CO}_2 \) rises. In addition to increasing atmospheric \( \text{CO}_2 \), other human perturbations, such as land use and \( \text{SO}_x \) and \( \text{NO}_x \) emissions, will further alter iron distribution and bioavailability in the open ocean (Mahowald et al. 2009). Therefore, ocean acidity and iron availability will show corresponding changes in future high-\( \text{CO}_2 \) oceans. Based on this finding, experiments that use natural seawater will not be able to distinguish the impact of carbonate chemistry or iron bioavailability on phytoplankton ecophysiology.

A critical challenge is to understand how the rapid decline in pH during the Anthropocene era affected phytoplankton ecophysiology. However, the atmospheric \( \text{CO}_2 \) concentrations during the Quaternary period (\(~1.8\) million years ago to the present; Gradstein et al. 2004) have been close to their lowest level (180–390 ppm; Doney et al. 2009) during the past 60 million years (\(<\sim4000\) ppm; Pearson and Palmer 2000). Most marine phytoplankton groups had already evolved prior to the decline in the levels of \( \text{CO}_2 \) in the atmosphere and oceans (Falkowski et al. 2004). Therefore, diatoms, which are the predominant primary producers in the present oceans (Falkowski et al. 2004), and many other algae, have adapted to the low \( \text{CO}_2 \) conditions by developing \( \text{CO}_2 \) concentration mechanisms (CCMs). These mechanisms elevate the substrate
concentration around the enzyme Rubisco, which is involved in CO$_2$ fixation (Hopkinson et al. 2011; Reinfelder 2011; and references therein). The upregulation of CCMs may incur substantial energy and nutrient costs. Therefore, an increase in pCO$_2$ may result in decreased CCMs cost, resulting in the enhanced growth of diatoms and other algae (Hutchins et al. 2009; Hopkinson et al. 2010). This physiological plasticity alters the composition of biochemical constituents such as the components of CCMs and can modify the cellular elemental composition associated with macromolecular stoichiometry (Geider and La Roche 2002).

Previous studies have reported that an increase in seawater pCO$_2$ alters the physiology and cellular elemental composition or nutrient consumption ratio of diatoms (Burkhardt et al. 1999, Sun et al. 2011), dinoflagellates, raphidophytes (Fu et al. 2008), cyanobacteria (Fu et al. 2007), and plankton communities in a mesocosm enclosure (Riebesell et al. 2007). For example, high pCO$_2$ conditions accelerated photosynthesis of many phytoplankton species (e.g. Rost et al. 2003, Sun et al. 2011) and the N$_2$-fixation rates of N$_2$-fixing cyanobacteria (e.g. Hutchins et al. 2007, Levitan et al. 2007). Other studies using unialgal cultures showed that the C:P ratio increased, and the Si:C ratio decreased, in diatoms, dinoflagellates, raphidophytes, and cyanobacteria with increasing pCO$_2$ in seawater (Fu et al. 2007, Fu et al. 2008, Sun et al. 2011). However, the C:N ratio was relatively unaffected by changes in seawater pCO$_2$. It should be noted that most of these studies were conducted under conditions with abundant macronutrients and trace elements, such as iron. However, although it is widely recognized that the primary productivity is limited by iron in large areas of the ocean (de Baar 1994, Saito et al. 2008), the interactive effects of pCO$_2$ and iron on the elemental composition of phytoplankton have not been examined.

Iron is an essential trace element for phytoplankton growth because of its role in key metabolic processes such as photosynthesis, respiration, and nitrate and nitrite assimilation (Raven et al. 1999). The iron found in oceanic regions is mainly derived from continental sources; however, iron has an extremely low solubility in oxic surface seawater (<~0.1 nmol L$^{-1}$; Kuma et al. 1996). Therefore, the phytoplankton, particularly diatoms, in the oceanic regions located far from iron sources are iron-limited (de Baar...
In addition, the iron concentration varies spatiotemporally by one to two orders of magnitude due to water mass exchange and biological uptake in the western subarctic Pacific (Sugie et al. 2010a, Nishioka et al. 2011). Therefore, phytoplankton need to adapt and survive in a fluctuating iron environment (Sugie and Kuma 2008, Sugie et al. 2011). Changing iron availability results in changes in the elemental composition of the diatoms; specifically, the cellular Si:N ratio increases as iron bioavailability decreases (e.g. Takeda 1998). The following mechanisms have been suggested for this increase in Si:N ratio: (i) an increase in silicification or resting spore formation (e.g. Sugie et al. 2010b); (ii) an increase in surface area (SA) to cell volume (CV) ratio (Marchetti and Harrison 2007); (iii) a reduction in cellular N content (e.g. Takeda 1998); and (iv) a response to the high Si:N ratio or high Si concentration of the extracellular environment (Kudo 2003, Finkel et al. 2010a). A recent study reported that the relationship between elemental composition and the bioavailable iron concentration is not always linear (Bucciarelli et al. 2010). Therefore, culture experiments should be conducted over a wide range of iron concentrations to improve our understanding of the stoichiometry of phytoplankton as it relates to changes in iron availability. In addition, simultaneous measurements of the C, N, P, and Si composition of diatoms have rarely been conducted despite their importance.

In the present study, we describe a new method for evaluating the individual effects of $pCO_2$ and iron availability on marine phytoplankton ecophysiology. We investigated the interactive effects of $pCO_2$ and iron on the elemental compositions (C, N, P and Si) and cell geometry of the diatom *Pseudo-nitzschia pseudodelicatissima* (Hasle). *Pseudo-nitzschia* species are ubiquitous, even in iron-depleted oceanic environments (Hasle 2002, de Baar et al. 2005). Therefore, species of the genus *Pseudo-nitzschia* are among the most suitable diatoms for examining the interactive effects of $pCO_2$ and iron in order to understand the biogeochemical cycling of nutrients in high-CO$_2$ oceans.

**MATERIALS AND METHODS**
Diatom strain and culture conditions. Seawater for the culture medium was collected from Onjuku, Chiba, Japan (35°18′N, 140°38′E). Salinity of the seawater was 34.2. Initially, the seawater was filtered through a 0.22 µm cartridge filter (Advantech Co. Ltd., Tokyo, Japan). Macronutrients were then added to the filtered seawater and the seawater was aged for ~1 month in an acid-washed 50 L polypropylene carboy, to precipitate dissolved iron, excess to its solubility, as conducted previously (Sugie et al. 2010b). Stock solutions of macronutrient were passed through a Chelex 100 resin (Bio-Rad, CA, USA) to remove trace metals, as described by Price et al. (1988/89). The filtered seawater was then passed through a 0.1 µm filter (Merck Millipore, MA, USA) to sterilize it and to eliminate particulate iron prior to use. The background iron concentration of the filtered seawater was 0.47 nmol L\(^{-1}\), as measured by flow-injection with chemiluminescence detection (Obata et al. 1993).

Seawater for the isolation of \(P. \) pseudodelicatissima was collected from Harima Nada, Seto Island Sea, Japan (34°77′N, 134°70′E) in 2009. The experiment was conducted within 1.5 years after isolation. A single cell was isolated using a capillary pipette and rinsed several times with 0.1 µm filtered seawater. Although, the strain was not completely axenic, bacterial contamination was minimized by the use of sterile techniques and serial transfer during exponential growth. To identify the species, the diatom cell was cleaned according to the method described by Nagumo (1995), and the cleaned frustule was observed using a scanning electron microscope. Species identification was performed according to Hasle and Syvertsen (1997). The strain was maintained in modified Aquil medium (Price et al. 1988/89) at 20°C under Neolumisuper fluorescent light at 100 µmol photons m\(^{-2}\) s\(^{-1}\) (FLR40S•W/M, Mitsubishi Electric Osram Ltd., Yokohama, Japan), measured using QSL radiometer (Biospherical Instrument Inc., CA, USA), and 12h light:12 h dark. The light intensity was measured at the center of the culture bottle. The modified Aquil medium was composed of 0.1 µm filtered seawater, ~100 µmol L\(^{-1}\) NO\(_3^−\), ~6 µmol L\(^{-1}\) PO\(_4^{3−}\), ~150 µmol L\(^{-1}\) Si(OH)\(_4\), and Aquil metals chelated with 100 µmol L\(^{-1}\) of EDTA. Because 100 µmol L\(^{-1}\) of EDTA can out-compete any natural ligand that may be present in the medium (Gerringa et al., 2000), the iron (including background iron) in the culture medium should be in equilibrium with EDTA.
The iron, other trace metals, and EDTA stock solutions were mixed in 1 L polycarbonate culture bottles before the addition of 1 L of modified Aquil medium. All equipment used in the culture experiment was acid-washed (soaked for at least 24 h in either 1 or 4 mol L\(^{-1}\) HCl solution; 1 mol L\(^{-1}\) HCl was used for polycarbonate bottles) followed by rinsing thoroughly with Milli-Q water (>18.0 MΩ cm\(^{-1}\), Merck KGaA, Darmstadt, Germany). Preparation and sampling for all experiments were conducted in a class 1000 clean room and at a class 100 clean bench, respectively, to avoid inadvertent trace metal contamination.

**Experimental design.** Carbonate chemistry during the culture experiment was manipulated by injecting controlled dry air with a specific CO\(_2\) concentration (\(x_{\text{CO}_2}\)) (Nissan Tanaka Corp., Saitama, Japan) directly into the culture bottles at a flow rate of ~10 mL min\(^{-1}\). The injected air was passed through a 0.2 µm in-line filter to avoid contamination from the gas cylinder or lines and humidified by passing the gas through Milli-Q water. The \(x_{\text{CO}_2}\) of the injected air was set at 171, 386, 614, and 795 ppm, corresponding to the glacial minimum, present, and two possible future CO\(_2\) conditions, respectively (Table 1). For each CO\(_2\) condition, five concentrations of dissolved inorganic iron species (Fe\(^{\prime}\)), representing ~50–100% of the maximum growth rate of *P. pseudodelicatissima* \((\mu_{\text{max}} = \sim 1.9\ \text{d}^{-1})\), were used: 3.5, 7.0, 18, 30 and 70 pmol L\(^{-1}\). The concentrations correspond to pFe\(^{\prime}\) (= \(-\log_{10}[\text{Fe}^{\prime}]\)) of 11.5, 11.1, 10.8, 10.5, and 10.2, respectively (Table 1). The Fe\(^{\prime}\) concentration was calculated according to Sunda and Huntsman (2003). When calculating Fe\(^{\prime}\) concentrations, background iron was included with the added iron (see above). Because the iron-EDTA buffer system is pH sensitive (Sunda and Huntsman 2003), the defined Fe\(^{\prime}\) concentrations were obtained by recalculation using pH values that were calculated from the dissolved inorganic carbon (DIC) and total alkalinity (TA) data. The DIC and TA were measured at the start and end of the experiment (Table 1). To achieve steady state and equilibrium of the carbonate chemistry and iron-EDTA system, \(x_{\text{CO}_2}\) controlled air was bubbled into the modified Aquil medium at a flow rate of ~30 mL min\(^{-1}\) for 3–4 days before the addition of the diatom cells. Experiments were conducted in duplicate bottles maintained under the same temperature and light conditions as the stock cultures.
Prior to initiating the culture experiment, *P. pseudodelicatissima* cells were acclimated to the four CO₂ conditions stated above, under high (70 pmol L⁻¹) or low (4.0 pmol L⁻¹) Fe' conditions. The acclimation period was 9 days, corresponding to ~20 and ~10 cell divisions for the high and low Fe' conditions, respectively. In the culture experiment, cells acclimated under high Fe' conditions were used for the two higher Fe' treatments, while cells acclimated under low Fe' conditions were used for the three lower Fe' treatments. Approximately 50–100 cells mL⁻¹ were added to each medium at the beginning of the experiment. Cells were cultured by dilute batch culture and were harvested at less than 5% of the carrying capacity of the modified Aquil medium. The diatoms were cultured in the experimental media for 4–6 days while they were still in exponential growth, a period that corresponded to between the 7th and 10th cell division under experimental conditions.

**Growth rate, cell size and geometry.** Growth was monitored daily using a Multisizer 4 Coulter Counter (Beckman Coulter Inc., CA, USA) to calculate the specific growth rate. Because *P. pseudodelicatissima* forms chains, we measured the biovolume of each sample at least three times. Specific growth rates were calculated from the linear regression between the natural log of the biovolume and time (day). The maximum specific growth rate (μ_max) and half saturation constant for growth (k_μ) were obtained by nonlinear fitting of the growth rate and Fe' data to the Monod equation (e.g., Sarthou et al. 2005). The initial slope of the growth rate to Fe' curve (α) was calculated as μ_max divided by k_μ (e.g. Healey 1980). In addition, we calculated the net elemental (E) uptake rate (ρ) per unit SA to account for the effect of the difference in cell size on nutrient uptake ability as follows; ρESA = Q_E × μ ÷ SA, where Q_E represents cell quota of C, N, P, or Si. The maximum ρESA (ρESA-max) and the half saturation constant for net uptake rate (k_ρ) values against Fe' concentration were obtained using a method similar to that for specific growth rate. The ρESA-max and k_ρ represent the maximum possible nutrient uptake ability per unit SA and the sensitivity of nutrient uptake transporter sites against the Fe' concentrations (i.e., uptake affinity), respectively. At the end of the culture experiment, a small amount of each of the samples was fixed with neutralized formalin (~1% final volume) to measure the cell number and geometry. The cell number was
counted four times per sample using a Fuchs-Rosenthal hemacytometer (Erma Inc., Tokyo, Japan) at ×200 magnification using a differential interference contrast equipped microscope (Olympus Corp., Tokyo, Japan). The apical length and transapical or pervalver lengths of up to 20 cells from one of the duplicate bottles were measured to calculate the CV and SA by using digital images of the cells and an objective micrometer at ×400 fold magnification (Sun and Liu 2003). Geometric calculations of the CV and SA were performed according to the equation suggested by Marchetti and Harrison (2007). The valve aspect ratio was calculated by dividing the apical length by the transapical or pervalver length of the cell.

**Chemical analyses.** The DIC and TA were measured at the start and end of the experiment using a potentiometric Gran plot method with dilute HCl (0.1 mol L$^{-1}$; Wako Co. Ltd., Osaka, Japan) and a total alkalinity analyzer (Kimoto electric Co. Ltd., Osaka, Japan), as described by Edmond (1970). However, as the EDTA began absorbing protons below pH ~4, the titration data below pH 4 were eliminated from the Grand plot. The stability of the titration analysis was checked using DIC reference material (KANSO Co. Ltd., Osaka, Japan), which the DIC value was traceable to the certified reference materials supplied by Andrew Dickson, University of California, San Diego, USA. The analytical errors were <0.1% for DIC (~1.1 µmol kg$^{-1}$) and TA (~1.4 µmol kg$^{-1}$). At the end of the culture period, macronutrients were measured using a QuAAtro-2 continuous flow analyzer (Bran+Luebbe, SPX Corp., NC, USA). At the end of the experiment, cells were harvested on a precombusted GF/F filter for particulate organic carbon (POC), particulate nitrogen (PN), and particulate phosphorus (PP) analysis. Cells were harvested on a polycarbonate membrane filter (pore size, 0.8 µm) for biogenic silica (BSi) analysis. Filter samples for POC and PN were freeze-dried, and the concentrations were measured using a CHN analyzer (Perkin Elmer Inc., MA, USA). PP was measured using a spectrophotometer (Hitachi High-Teck Corp. Tokyo, Japan) after high temperature combustion and acid hydrolysis of the filters as described by Solórzano and Sharp (1980). For BSi analysis, the filter was digested by heating to 85°C for 2 h in 0.5% Na$_2$CO$_3$ solution (Paasche, 1980). After neutralizing with 0.5 mol L$^{-1}$ HCl, the silicic acid concentration was measured using a QuAAtro-2 continuous flow analyzer. All data for...
POC, PN, PP and BSi concentrations were corrected by subtracting values obtained from appropriate filter blanks. Cellular elemental concentrations (C, N and P) were calculated by dividing POC, PN, or PP concentrations by cell density and CV. The SA normalized Si as an indicator of frustule thickness was calculated by dividing BSi concentration by cell density and SA.

Statistics. Data trends obtained under different pCO₂ and iron conditions were evaluated using F-tests, and regression coefficients were evaluated using t-tests. The regression formula was chosen to achieve the highest accuracy (i.e., F value and correlation coefficient). Data for fitting the Monod equation were calculated using Origin software (version 8.0, OriginLab Corp., MA, USA) with a non-linear method. Multi-regression analyses were conducted using PASW statistics software (version 17.0, SPSS Inc., IL, USA). Significant results are reported at the 95% confidence level.

RESULTS

Medium conditions. At the beginning of the experiment, seawater pCO₂ was close to steady state with the xCO₂ of the bubbled air in the three higher CO₂ bottles (412 ± 8, 609 ± 11 and 769 ± 11 µatm), whereas a slightly higher value than the expected steady state value was observed in the lowest xCO₂ treatment (251 ± 17 µatm) (Table 1). The corresponding pH values (represented as mean ± range of duplicate bottles) for the 171, 386, 614, and 795 ppm xCO₂ treatments were 8.22 ± 0.02, 8.05 ± 0.01, 7.90 ± 0.01, and 7.81 ± 0.01, respectively. During the course of the experiment, the DIC decreased due to phytoplankton growth that exceeded DIC addition by bubbling. The decrease in DIC was greater in treatments with high Fe’ conditions and 171 ppm xCO₂ treatments than that in low Fe’ conditions and higher xCO₂ treatments because photosynthesis and the bubbling of low xCO₂ air simultaneously depressed DIC. At the end of the culture period, the pCO₂ had decreased by ~30–300 µatm, while the pH had increased by 0.03–0.20 units, depending on the extent of phytoplankton growth (Table 1). Further, during the experiment, the Fe’ changed due to the increase in pH. The change in Fe’ ranged from ~15% under the low Fe’ conditions to 100–140% under the high Fe’ conditions (Table 1). We used the means of the initial and final pCO₂ and Fe’ values for
the subsequent data analysis. The macronutrient levels remained sufficient [~100 µmol L\(^{-1}\) \(\text{NO}_3^-+\text{NO}_2^-\); ~5.5 µmol L\(^{-1}\) \(\text{PO}_4^{3-}\); and ~145 µmol L\(^{-1}\) \(\text{Si(OH)}_4\)] for phytoplankton growth at the end of the experiment.

**Growth rate, cell size, and geometry.** The specific growth rate increased with Fe\(^{'}\) concentration from ~1.0 to ~2.0 d\(^{-1}\) (Fig. 1A). Multi-regression analysis indicated that the specific growth rate was strongly correlated with Fe\(^{'}\) but not with \(\text{pCO}_2\), within the investigated ranges (Table 2). The \(\mu_{\text{max}}\) values for the 171, 386, 614 and 795 ppm \(\times\text{CO}_2\) treatments calculated using the Monod equation were 1.81 ± 0.03, 1.89 ± 0.03, 2.06 ± 0.04, and 1.98 ± 0.05, respectively. The \(k_\mu\) values for the four \(\times\text{CO}_2\) treatments were 1.48 ± 0.18, 3.64 ± 0.35, 4.97 ± 0.39 and 4.23 ± 0.55 pmol Fe\(^{'}\) L\(^{-1}\), respectively. The initial slope of the Monod regression (\(\alpha\)) was highest for the 171 ppm \(\times\text{CO}_2\) treatment and decreased with increasing \(\text{pCO}_2\) (Fig. 2). The CV showed a gradual, although significant, increase with increasing Fe\(^{'}\) and decreasing \(\text{pCO}_2\) (Fig. 1B, Table 2). CV was positively correlated with specific growth rate (Fig. 1C) and varied with the length of the transapical or pervalver axis but not with the length of the apical axis (Fig. 1D, E). Therefore, \(\text{SA}/\text{CV}\) was tightly regulated by the valve aspect ratio (\(\text{VARatio}\); Fig. 1F). The lengths of the apical axis and transapical or pervalver axis were not significantly influenced by variations in \(\text{pCO}_2\). \(\text{VARatio}\) and \(\text{SA}/\text{CV}\) significantly increased with increasing \(\text{pCO}_2\) and decreased with increasing Fe\(^{'}\) concentrations (Table 2). Further, although *P. pseudodelicatissima* cells were acclimated to only two Fe\(^{'}\) regimes, they appeared to be fully acclimated to the experimental conditions because the results indicated gradual changes in the growth rate, cell size, and geometry with respect to the Fe\(^{'}\) variation.

**Cellular C, N, P, and Si.** The highest intracellular (In\(_{\text{cell}}\)) C and N concentrations and the SA normalized BSi concentration (Si/SA), were measured at 10.6–10.2 pFe\(^{'}\) (25–63 pmol L\(^{-1}\)), when the growth rates were 80–95% of \(\mu_{\text{max}}\) (Fig. 3). The empirical equation for each element (C, N, P, and Si) was obtained from the data in Figure 3 as follows:

\[
\text{In}_{\text{cell}} \ [\text{C}] \ (\text{mol} \ L^{-1}) = -863 + (166 \times \text{pFe}^{'} ) - (7.86 \times \text{pFe}^{'}^2) \ (F_{2,37} = 21.9, \ p < 0.001) \quad (1)
\]

\[
\text{In}_{\text{cell}} \ [\text{N}] \ (\text{mol} \ L^{-1}) = -134 + (26.1 \times \text{pFe}^{'} ) - (1.24 \times \text{pFe}^{'}^2) \ (F_{2,37} = 35.7, \ p < 0.001) \quad (2)
\]
\[
\text{In}_{\text{cell}} [P] (\text{mmol L}^{-1}) = (-7.35 \times 10^{-3}) + (1464 \times p\text{Fe}') - (70.9 \times p\text{Fe}'^2) + (0.101 \times p\text{Fe}' \times p\text{CO}_2) - (1.21 \times p\text{CO}_2) (F_{4,35} = 18.2, p < 0.001) \tag{3}
\]

\[
[\text{Si}]/\text{SA} (\text{mmol m}^{-2}) = -112 + (21.4 \times p\text{Fe}') - (1.03 \times 10^{-3} \times p\text{CO}_2) - (1.00 \times p\text{Fe}'^2) (F_{3,36} = 28.6, p < 0.001) \tag{4}
\]

The \text{In}_{\text{cell}} C, N, and P concentrations and \text{Si}/\text{SA} graphs had quadric surfaces with respect to the pFe'. However, changes in \text{pCO}_2 linearly affected only the \text{In}_{\text{cell}} P and \text{Si}/\text{SA} concentrations while changes in \text{pCO}_2 were not significantly associated with \text{In}_{\text{cell}} C and N concentrations. \text{Si}/\text{SA} decreased significantly with increasing \text{pCO}_2 (t = -5.8, p < 0.001, df = 39). In contrast, \text{In}_{\text{cell}} P concentration increased significantly with decreasing \text{pCO}_2 (t = -2.9, p = 0.006, df = 39).

In general, the highest \rho_{\text{ESA}} was detected for the 171 and 386 ppm x\text{CO}_2 treatments under high Fe' conditions, whereas the lowest \rho_{\text{ESA}} was measured for the high-CO\text{O}_2 and low-Fe' conditions (Fig. 4). All regression coefficients, with the exception of \rho_{\text{NSA}} for \text{pCO}_2 were significant (Table 2); the \rho_{\text{CSA}}, \rho_{\text{PSA}}, and \rho_{\text{SiSA}} increased with increasing Fe' concentration and decreasing with increasing \text{pCO}_2 (Fig. 4A, C, D). In contrast, \rho_{\text{NSA}} was affected only by iron availability and increased with increasing Fe' (Fig. 4B, Table 2). The maximum \rho_{\text{ESA}} (\rho_{\text{ESA-max}}) and half saturation constant for the \rho_{\text{ESA}} (k_{\rho}) as a function of Fe' were determined by fitting the data with the Monod model (Table 3). The highest \rho_{\text{ESA-max}} values of \rho_{\text{CSA}}, \rho_{\text{PSA}}, and \rho_{\text{SiSA}} were obtained for the 171 ppm x\text{CO}_2 treatment, whereas those for the 614 and 795 ppm x\text{CO}_2 treatments were similar. The \rho_{\text{ESA-max}} of \rho_{\text{NSA}} was not affected by \text{pCO}_2 variation (Table 3). The highest \alpha values of \rho_{\text{CSA}}, \rho_{\text{NSA}}, and \rho_{\text{PSA}} were obtained for the 171 ppm x\text{CO}_2 treatment, whereas the other three x\text{CO}_2 treatments had similar \alpha values (Table 3).

*Elemental composition.* The cellular C:N ratio significantly increased from ~5.9 to ~6.5 as Fe' concentration decreased, while the coefficient for \text{pCO}_2 was not statistically significant (Fig. 5A, Table 2). In contrast, the cellular C:P ratio significantly increased with increasing \text{pCO}_2, but not with Fe' concentration (Fig. 5B, Table 2). The average cellular C:P ratios (mean \pm 1SD of ten replicates) for the 171, 386, 614, and 795 ppm x\text{CO}_2 treatments were 98 \pm 9.0, 119 \pm 22, 136 \pm 31, and 139 \pm 15, respectively (Fig. 5B). The cellular N:P ratio was positively correlated with \text{pCO}_2 and Fe' and ranged from...
~15 in the low $p$CO$_2$ and Fe$^+$ conditions to ~26 in the high $p$CO$_2$ and Fe$^+$ conditions (Fig. 5C, Table 2). The cellular Si:N and Si:C ratios decreased significantly as $p$CO$_2$ and Fe$^+$ concentration increased (Fig. 5D, E, Table 2). The cellular Si:P ratio was positively correlated with $p$CO$_2$, but negatively correlated with Fe$^+$ (Fig. 5F, Table 2).

**DISCUSSION**

We demonstrated that the elemental composition and cell geometry of the marine diatom *P. pseudodelicatissima* are influenced by variations in $p$CO$_2$, Fe$^+$ or a combination of both factors. In addition, we found that the elemental composition of cells changed linearly with $p$CO$_2$ and log$_{10}[Fe^+]$. It is important to distinguish between the effects of these factors when evaluating the results obtained using natural phytoplankton communities particularly under iron-limited conditions. This is because carbonate chemistry and iron bioavailability can change simultaneously in natural seawater (Millero et al. 2009; Shi et al. 2010). These simultaneous changes preclude understanding of their independent effects on phytoplankton ecophysiology. By manipulating the carbonate chemistry and iron speciation independently, we overcome this problem and evaluated the individual effects of $p$CO$_2$ and iron availability.

_Growth rate, cell size and geometry._ We found that the specific growth rate and the theoretical maximum specific growth rate ($\mu_{\text{max}}$) were not affected by $p$CO$_2$. These results are similar to those of a recent study of eight phytoplankton species belonging to four phyla (Berge et al. 2010). Trimborn et al. (2008) reported that *Pseudo-nitzschia multiseries* has a highly efficient CCMs and the activity of the CCMs may increase in response to a decrease in DIC availability. Because the use of CCMs may consume a substantial part of the energy for growth (Hopkinson et al. 2010, 2011), the increase in CO$_2$ availability may benefit phytoplankton (Hutchins et al. 2009). Shi et al. (2010) reported that the cellular iron requirements (Fe:C ratio) of model diatom species (*Thalassiosira pseudonana*, *Thalassiosira weissflogii*, and *Phaeodactylum tricornutum*) seem to increase under low CO$_2$ (160 ppm) conditions possibly because of the need to upregulate CCMs. CCMs are energy-using processes that require ATP. If this ATP is supplied by PSI cyclic photophosphorylation (cf. Raven 1999, Allen 2003, Beardall et al.
the high Fe content of the PSI and the associated cyclic electron transport pathway may increase the iron requirement of diatoms when CCMs are upregulated under conditions of low CO$_2$ availability. Further, our results show that iron uptake affinity ($\alpha$) increased with decreasing $p$CO$_2$ (Fig. 2). This supports the idea that the energy demand for the development of the CCMs increases when $p$CO$_2$ decreases (Beardall et al. 2005, Young and Beardall 2005, Hopkinson et al. 2011). In addition, we observed a relatively high uptake affinity and maximum uptake rate for C and nutrients by *P. pseudodelicatissima* cells grown under the lowest $p$CO$_2$ condition. The ability of the cells to develop high affinity iron transport and elevated C and nutrient uptake under conditions of low CO$_2$ availability may partly overcome the less favorable growth conditions.

Interestingly, the cell volume (CV) of *P. pseudodelicatissima* increased significantly as $p$CO$_2$ decreased. Theoretically, CV increases under substrate replete conditions (e.g. Thingstad et al. 2005, Finkel et al. 2010b). In the present study, CV decreased as iron availability decreased as observed elsewhere (Marchetti and Harrison 2007, Sugie and Kuma 2008). Furthermore, CV changed due to the changes in transapical or pervalver axis length rather than apical length, i.e., the V$_A$ ratio was affected by Fe’ and $p$CO$_2$ variation. These findings are in accordance with the previous study of six *Pseudo-nitzschia* strains that indicated that V$_A$ ratio increased with decreasing iron availability (Marchetti and Harrison, 2007). However, the larger CV observed under low $p$CO$_2$ conditions is apparently a competitive disadvantage in CO$_2$-stressed environments. In the present study, the $p$E$_{SA}$ and its affinities were highest under low $p$CO$_2$ conditions, which can offset the growth disadvantage of a large CV. Therefore, the growth rate of *P. pseudodelicatissima* may not be affected by $p$CO$_2$ variations. Tortell et al. (2008) reported that the relative abundance of *Pseudo-nitzschia subcurvata* to *Chaetoceros* spp. (subgenus *Hyalochaete*) increased with a decrease in CO$_2$ (100 ppm $x$CO$_2$ bubbled). That finding partly supports our observation that *Pseudo-nitzschia* species can maintain their growth rates under low $p$CO$_2$ conditions.

*Elemental composition.* We demonstrated that the cellular elemental composition varied significantly under different $p$CO$_2$ and Fe’ conditions. With a few exceptions,
such as *Heterosigma akashiwo* (Raphidophyceae, Fu et al. 2008), phytoplankton C:N ratios are generally not affected by $pCO_2$ variation (Burkhart et al. 1999, Sun et al. 2011), as observed in the present study. The constant C:N ratio of diatoms grown under different $pCO_2$ conditions suggests that the coupling of C and N metabolism is not affected by $pCO_2$ variations. In contrast, we found that the C:N ratio decreased with increasing iron availability. Bucciarelli et al. (2010) reported that the C:N ratio of the diatom *Thalassiosira oceanica* decreased with increasing in iron-limitation, but they were unable to detect a decreasing trend when evaluating compiled published data for 14 diatom species. Factors that alter the C:N ratio are related to growth conditions (e.g., temperature and light conditions) and show interspecific differences (Price 2005, Bucciarelli et al. 2010). In phytoplankton, iron-limitation leads to nitrogen co-limitation (Milligan and Harrison 2000) because iron is a cofactor of nitrate and nitrite reductases (Raven et al. 1999). The ratio of the iron coefficient in the regression in $\rho_{CSA}$ to that in $\rho_{NSA}$ was ~3.4 (Table 2), which is lower than the corresponding C:N ratio, suggesting a rapid decrease in N uptake activity relative to C uptake activity in response to a decrease in iron availability. Therefore, we conclude that a decrease in iron availability causes an increase in the C:N ratio of *P. pseudodelicatissima*.

When the $pCO_2$ was increased from ~200 to ~750 µatm, the C:P ratio increased by approximately 40% because $\rho_{PSA}$ decreased faster as $pCO_2$ increased than did $\rho_{CSA}$ (Fig. 4, Table 3). The elevation of the C:P ratio in diatoms and other phytoplankton under high $pCO_2$ conditions has previously been observed only under iron-replete conditions (e.g., Fu et al. 2008: *Prorocentrum minimum*, and King et al. 2011: *Attheya* sp.). Note that the contribution of extracellularly adsorbed phosphate was not affected by $pCO_2$ variation as examined using unialgal culture of the diatom *Chaetoceros* subgenus *Hyalochaete* (Sugie unpublished data). Within the intracellular fraction, the P-rich macromolecules responsible for cellular elemental compositions are RNA, DNA, and phospholipids (Geider and La Roche 2002). Specifically, the cellular RNA content increases as the growth rate of the diatom increases (Elser et al. 2003, Leonardos and Geider 2004). Our results indicate that the apparent nutrient uptake rates and iron uptake affinity were high under low $pCO_2$ conditions, but that the specific growth rate was not
significantly affected. Shi et al. (2010) reported that cadmium carbonic anhydrase, which is a key component of CCMs, was upregulated at 160 ppm $x$CO$_2$ relative to 275–950 ppm $x$CO$_2$. It can be assumed that $P$. pseudodelicatissima is able to increase its RNA synthesis under low $p$CO$_2$ conditions to upregulate nutrient and Fe uptake transporter proteins and CCMs, such as carbonic anhydrase, resulting in a relatively low C:P ratio. In the present study, the C:P ratio was not affected by iron availability (Table 2). In contrast, Price (2005) reported that the C:P ratio of the diatom $T$. weissflogii increased with increasing iron concentration; however, this trend was unclear at 50–100% of $\mu$:$\mu_{\text{max}}$. Young and Beardall (2005) reported that a decrease in iron availability increased the activity of CCMs in $D$. tertiolecta (Chlorophyceae), suggesting that iron-limitation increases the C:P ratio through an increase in RNA synthesis. However, the upregulation of CCMs in response to iron-limitation appears to be small compared to that in response to a decrease in $p$CO$_2$ (e.g. Burkhardt et al. 2001, Trimborn et al. 2009). However, the very limited information from very different taxa makes it difficult to determine the effect of iron availability on the C:P ratio. The upregulation of CCMs may require ATP, for which the C:P ratio is 10:3; nevertheless, the contribution of cellular P derived from ATP appears to be much lower than that of other P-rich cellular constituents (Geider and La Roche 2002). The C:P ratio of phytoplankton was previously reported to be affected by phosphate and light availability (e.g. Diehl et al. 2005). However, we believe that carbonate chemistry also contributes substantially to variation in the canonical C:P value of 106 (Redfield et al. 1963). Furthermore, the atmospheric CO$_2$ concentration has changed dramatically over the geological time scale (Pearson and Palmer 2000; Doney et al. 2009). Thus, we hypothesize that the observed change in C:P value in response to $p$CO$_2$ variation of seawater plays a key role in the biogeochemical cycling of oceanic P.

We found an increasing trend in the N:P ratio with increasing $p$CO$_2$. We predict that the N:P ratio of phytoplankton will increase in the future with increasing $p$CO$_2$ in oceans with high-CO$_2$. This, in turn, will lead to an increase in P availability in P-limiting oligotrophic environments, that will result in an increase of N$_2$ fixation (c.f. Moutin et al. 2008), the rate of which is significantly enhanced by an increase in $p$CO$_2$.
(Hutchins et al. 2007, Levitan et al. 2007). However, the new production will decrease in N-limiting environments, where the iron concentration is sufficiently high to allow exhaustion of nutrients (e.g. Sugie et al. 2010a). These N-limiting environments are high productivity regions, including the majority of the coastal regions (Tyrrell and Law 1997, Wong et al. 2002). Unlike the C:P ratio, the N:P ratio was significantly affected by iron availability. As discussed above, iron availability has a greater effect on N assimilation than on C assimilation. The elevation of the N:P ratio in response to an increase in iron availability has been measured in unialgal culture of Antarctic diatom species (Timmermans et al. 2004, Timmermans and van der Wagt 2010). Changes in iron availability due to pH variations (Millero et al. 2009; Shi et al. 2010), which are difficult to predict, may modulate the trend in the N:P ratio observed in the present study.

We found that the Si:C, Si:N, and Si:P ratios varied according to the variations in $pCO_2$ and iron availability (Fig. 5). This finding supports the suggestion by Claquin et al. (2002) that the Si and other nutrient assimilation processes are uncoupled. Iron availability was previously reported to affect cellular Si content or the ratio of Si to other nutrients (e.g. Bucciarelli et al. 2010, Sugie et al. 2010b and references therein). However, there is very little information available about the effect of $pCO_2$ on silicification of diatoms. Previous studies using natural plankton communities detected no significant effect of $pCO_2$ change on Si dynamics (Feng et al. 2009, 2010). Sun et al. (2011) reported that the Si:C ratio and the Si cell quota of the diatom *P. multiseries* decreased with an increase in $pCO_2$ from ~220 to ~730 µatm, but were not significantly different between ~400 and ~730 µatm $pCO_2$. Si:C and Si:N ratios tend to decrease with increasing $pCO_2$, as observed in the present study; however, the possible mechanisms underlying the decrease in diatom Si content are largely uncertain. Milligan et al. (2004) reported that the intracellular Si efflux and frustule dissolution rates of the diatom *T. weissflogii* were higher under high $pCO_2$ conditions (~750 µatm) than under low $pCO_2$ condition (~100 µatm). Accordingly, further studies to determine the effects of carbonate chemistry on silicon dynamics are required. Moreover, under natural conditions, iron bioavailability and speciation will change with pH (Millero et al. 2009, Shi et al. 2010), and the iron concentration oscillates seasonally due to physical and biological dynamics.
These factors are critical for controlling the dynamics of diatomaceous Si (Takeda 1998, Sugie et al. 2010b); however, it is difficult to predict the direction of future changes in iron availability. To enable predictions of the future environment, sufficient data regarding variations in $p$CO$_2$ and iron availability in the past and present must be obtained.

**Oceanographic relevance.** The cellular elemental composition is primarily changed through substrate limitation or depletion (Diehl et al. 2005, Marchetti and Harrison 2007, Sugie et al. 2010b). In the present study, the cellular elemental composition varied with changes in carbonate chemistry, and DIC is apparently a non-limiting substrate for growth. Our results indicate that $p$CO$_2$ and iron availability could influence the biogeochemical cycling of nutrients in future high-CO$_2$ oceans in a manner similar to that observed for phytoplankton blooms and in the geologic past (e.g. Pearson and Palmer 2000, Morel 2008). However, future iron bioavailability is difficult to predict because of the uncertainty regarding the precise chemical properties of iron-binding ligands. Understanding the changes in the binding affinity of iron ligands and photoreactivity of iron-ligand complexes in response to declining pH are important issues to predict the bioavailability of iron in future high-CO$_2$ oceans. The present study provides a new method for evaluating the individual effects of $p$CO$_2$ and iron availability on phytoplankton ecophysiology. This method, in combination with natural plankton incubations, should provide a useful means for assessing the interactive effects of $p$CO$_2$ and iron.

Even though *Pseudo-nitzschia* is a cosmopolitan genus (Hasle 2002), *P. pseudodelicatissima* strain used in the present study was isolated from a coastal region. Marchetti and Harrison (2007) reported that iron-limitation induced trends in the elemental composition of several *Pseudo-nitzschia* species were consistent between coastal and oceanic isolates. However, Berge et al. (2010) suggested that oceanic species might be more sensitive to variations in $p$CO$_2$ and pH. To evaluate future nutrient biogeochemistry in oceans with high CO$_2$, studies of the interactive effects of ocean acidification and iron availability using other phytoplankton species and natural plankton communities are required. In particular, the elemental compositions and nutrient
drawdown ratios of natural plankton communities have rarely been shown to be affected
by $p$CO$_2$ variations (e.g. Feng et al. 2009, 2010), except for dissolved organic carbon
(DOC) production under nutrient-depleted conditions (Yoshimura et al. 2010) and
transparent exopolymer particle and DOC production in a mesocosm enclosure in
southern Norway (Riebesell et al. 2007). In order to clarify the overall trends in changes
in C and other nutrients biogeochemistry in the oceans in response to changes in
carbonate chemistry, we need to resolve the discrepancies between the data for natural
phytoplankton communities and those for unialgal cultures.

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(in Japanese)


Figure legends

Figure 1. Change in (A) specific growth rate (µ) and (B) cell volume (CV) against Fe' concentration (−log\(_{10}[\text{Fe'}]\) = pFe') and pCO\(_2\) and relationships between (C) µ and CV, (D) CV and apical axis (A\(_{ax}\)), (E) CV and transapical or pervalver axis (TP\(_{ax}\)), and (F) valve aspect ratio (VA\(_{ratio}\): A\(_{ax}\) divided by TP\(_{ax}\)) and surface area (SA) to CV ratio of *Pseudo-nitzschia pseudodelicatissima* grown under various pCO\(_2\) and iron conditions. Open circles in (A) and (B) represent scatter diagram of mean of the beginning and end of pFe (x-axis) and pCO\(_2\) (y-axis) values. Data and error bars in (D) to (F) represent mean and ± 1SD (n = 20). Solid and dotted lines in (C), (E) and (F) represent linear regression of the data (mean value) and 95% CL of the regression, respectively. The regression formulae are: (C) CV = 20.9 (± 5.0) × µ + 42.3 (± 8.0) (\(F_{1,18} = 17.4, p = 0.001, R^2 = 0.49\)), (E) TP\(_{ax}\) = 0.016 (± 0.000) × CV + 1.06 (± 0.04) (\(F_{1,18} = 1008, p < 0.001, R^2 = 0.98\)), (F) SA/CV = 0.171 (± 0.007) × VA\(_{ratio}\) − 0.185 (± 0.070) (\(F_{1,18} = 513, p < 0.001, R^2 = 0.97\)).

Figure 2. The initial slope (α) of the regression of the specific growth rate against Fe' concentration calculated by fitting the Monod model.

Figure 3. Change in intracellular concentrations of (A) C (mol L\(^{-1}\)), (B) N (mol L\(^{-1}\)) and (C) P (mmol L\(^{-1}\)) and (D) Si content per surface area (mmol m\(^{-2}\)) of *Pseudo-nitzschia pseudodelicatissima* against pCO\(_2\) and −log\(_{10}[\text{Fe'}]\) variations. Open circles are the same representation as in Fig. 1A.

Figure 4. Change in net uptake rate (ρ) of the nutrients per unit surface area (SA). (A) ρ\(_{CSA}\) (mol m\(^{-2}\) d\(^{-1}\)), (B) ρ\(_{NSA}\) (mol m\(^{-2}\) d\(^{-1}\)) and (C) ρ\(_{PSA}\) (mmol m\(^{-2}\) d\(^{-1}\)) and (D) ρ\(_{SiSA}\) (mol m\(^{-2}\) d\(^{-1}\)) of *Pseudo-nitzschia pseudodelicatissima* against pCO\(_2\) and −log\(_{10}[\text{Fe'}]\) variations. Open circles are the same representation as in Fig. 1A.

Figure 5. Change in the cellular elemental composition of *Pseudo-nitzschia pseudodelicatissima* against pCO\(_2\) and −log\(_{10}[\text{Fe'}]\) variations. (A) C:N, (B) C:P, (C) N:P, (D) Si:N, (E) Si:C, and (F) Si:P ratio. Open circles are the same representation as in Fig. 1A.
Table 1. Medium conditions at the start and end of the experiment. Treatment was represented as the CO2 concentration of bubbled air (ppm) and Fe level from low (Fe1) to high (Fe5) condition. Data represent mean ± range of the duplicate bottles.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Measured TA (µmol kg(^{-1}))</th>
<th>Measured DIC (µmol kg(^{-1}))</th>
<th>Calculated (p\text{CO}_2) (µatm)</th>
<th>Calculated pH (total scale)</th>
<th>Calculated Fe' (pmol L(^{-1}))</th>
<th>Measured TA (µmol kg(^{-1}))</th>
<th>Measured DIC (µmol kg(^{-1}))</th>
<th>Calculated (p\text{CO}_2) (µatm)</th>
<th>Calculated pH (total scale)</th>
<th>Calculated Fe' (pmol L(^{-1}))</th>
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<tbody>
<tr>
<td>171-Fe1</td>
<td>2335 ± 1.2</td>
<td>1959 ± 6.8</td>
<td>241.3 ± 8.1</td>
<td>8.24 ± 0.01</td>
<td>3.1 ± 0.2</td>
<td>2328 ± 0.1</td>
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<td>2326 ± 3.5</td>
<td>1878 ± 3.0</td>
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<td>2317 ± 2.3</td>
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<td>2315 ± 0.9</td>
<td>2037 ± 13</td>
<td>372.0 ± 23</td>
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<td>2091 ± 9.4</td>
<td>469.8 ± 25</td>
<td>8.00 ± 1.02</td>
<td>64 ± 5.4</td>
</tr>
<tr>
<td>-Fe5</td>
<td>2337 ± 1.9</td>
<td>2182 ± 0.9</td>
<td>744.5 ± 3.6</td>
<td>7.83 ± 0.00</td>
<td>65 ± 0.5</td>
<td>2324 ± 1.0</td>
<td>2113 ± 0.2</td>
<td>531.9 ± 2.9</td>
<td>7.95 ± 0.00</td>
<td>105 ± 1.0</td>
</tr>
</tbody>
</table>
Table 2. Change in specific growth rate, cell volume (CV), surface area (SA) to CV ratio, valve aspect ratio (VA<sub>ratio</sub>; apical axis divided by transapical or pervalver axis length), net C, N, P and Si uptake rate (ρ) per SA, and elemental compositions against pCO<sub>2</sub> and pFe (−log<sub>10</sub>[Fe']) variations during the course of the experiment. Listed are the constant (a) and the coefficients (b and c) of regression equation of \( y = a + b \times p\text{CO}_2 + c \times p\text{Fe}' \). Asterisks represent the significance level of the constant and coefficients (t-test, df = 39 except for CV of df = 19); *: \( p < 0.05 \), **: \( p < 0.01 \). n.s.: not significant coefficient. The insignificant parameter (pCO<sub>2</sub> or pFe) was eliminated from the multi-regression analysis.

<table>
<thead>
<tr>
<th></th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>Significance of the regression</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth rate and cell size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>μ (d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>7.57**</td>
<td>n.s.</td>
<td>−0.562**</td>
<td>( F_{1,38} = 452, p &lt; 0.001, R^2 = 0.82 )</td>
</tr>
<tr>
<td>CV (µm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td>197**</td>
<td>−1.90×10&lt;sup&gt;−2&lt;/sup&gt;*</td>
<td>−10.58**</td>
<td>( F_{2,17} = 15.0, p &lt; 0.001, R^2 = 0.60 )</td>
</tr>
<tr>
<td>SA/CV (µm&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>6.46×10&lt;sup&gt;−2&lt;/sup&gt; 2.25×10&lt;sup&gt;−4&lt;/sup&gt;*</td>
<td>0.150**</td>
<td>( F_{2,17} = 20.9, p &lt; 0.001, R^2 = 0.68 )</td>
<td></td>
</tr>
<tr>
<td>VA&lt;sub&gt;ratio&lt;/sub&gt;</td>
<td>−0.992</td>
<td>1.11×10&lt;sup&gt;−3&lt;/sup&gt;*</td>
<td>0.909**</td>
<td>( F_{2,17} = 22.3, p &lt; 0.001, R^2 = 0.69 )</td>
</tr>
<tr>
<td><strong>Net elemental uptake rate per unit SA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ρCSA (mol m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>87.8**</td>
<td>−3.99×10&lt;sup&gt;−3&lt;/sup&gt;*</td>
<td>−6.76**</td>
<td>( F_{2,37} = 89.0, p &lt; 0.001, R^2 = 0.82 )</td>
</tr>
<tr>
<td>ρNSA (mol m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>25.1**</td>
<td>n.s.</td>
<td>−1.99**</td>
<td>( F_{1,38} = 133, p &lt; 0.001, R^2 = 0.77 )</td>
</tr>
<tr>
<td>ρPSA (mmol m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>681**</td>
<td>−0.129**</td>
<td>−47.5**</td>
<td>( F_{2,37} = 53.5, p &lt; 0.001, R^2 = 0.73 )</td>
</tr>
<tr>
<td>ρSi&lt;sub&gt;SA&lt;/sub&gt; (mol m&lt;sup&gt;−2&lt;/sup&gt; d&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>12.8**</td>
<td>−1.77×10&lt;sup&gt;−3&lt;/sup&gt;*</td>
<td>−0.859**</td>
<td>( F_{2,37} = 34.4, p &lt; 0.001, R^2 = 0.63 )</td>
</tr>
<tr>
<td><strong>Elemental composition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C:N</td>
<td>0.272</td>
<td>n.s.</td>
<td>0.554**</td>
<td>( F_{1,38} = 143, p &lt; 0.001, R^2 = 0.78 )</td>
</tr>
<tr>
<td>C:P</td>
<td>86.1**</td>
<td>8.28×10&lt;sup&gt;−2&lt;/sup&gt;*</td>
<td>n.s.</td>
<td>( F_{1,38} = 20.0, p &lt; 0.001, R^2 = 0.33 )</td>
</tr>
<tr>
<td>N:P</td>
<td>49.8**</td>
<td>1.38×10&lt;sup&gt;−2&lt;/sup&gt;*</td>
<td>−3.37**</td>
<td>( F_{2,37} = 14.5, p &lt; 0.001, R^2 = 0.41 )</td>
</tr>
<tr>
<td>Si:N</td>
<td>−2.77**</td>
<td>−3.47×10&lt;sup&gt;−4&lt;/sup&gt;*</td>
<td>0.398**</td>
<td>( F_{2,37} = 139, p &lt; 0.001, R^2 = 0.88 )</td>
</tr>
<tr>
<td>Si:C</td>
<td>−3.97×10&lt;sup&gt;−2&lt;/sup&gt;−5.34×10&lt;sup&gt;−5&lt;/sup&gt;*</td>
<td>1.75×10&lt;sup&gt;−2&lt;/sup&gt;*</td>
<td>( F_{2,37} = 61.0, p &lt; 0.001, R^2 = 0.76 )</td>
<td></td>
</tr>
<tr>
<td>Si:P</td>
<td>−19.7</td>
<td>9.96×10&lt;sup&gt;−3&lt;/sup&gt;*</td>
<td>3.86*</td>
<td>( F_{2,37} = 6.60, p = 0.004, R^2 = 0.22 )</td>
</tr>
</tbody>
</table>
Table 3. Maximum rate (\(E_{SA-max}\)), half saturation constants for dissolved inorganic Fe (Fe'; \(k_p\)) and initial slope (\(\alpha\)) of net C, N, P and Si uptake (\(\rho\)) per unit surface area (SA) which were calculated by fitting the data to Monod equation against Fe' concentrations at each \(xCO_2\) treatment. Data represent mean ± standard error (\(n = 10\)).

<table>
<thead>
<tr>
<th>Injected air (xCO_2) (ppm)</th>
<th>(\rho E_{SA-max}) (mol m(^{-2}) d(^{-1}))</th>
<th>(k_p) (pmol Fe' L(^{-1}))</th>
<th>(\alpha)</th>
<th>Significance of Monod model</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\rho_{CSA})</td>
<td>171</td>
<td>19.5 ± 0.6</td>
<td>4.8 ± 0.7</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>19.1 ± 0.5</td>
<td>5.7 ± 0.7</td>
<td>3.33</td>
</tr>
<tr>
<td></td>
<td>614</td>
<td>17.3 ± 1.1</td>
<td>5.0 ± 1.4</td>
<td>3.45</td>
</tr>
<tr>
<td></td>
<td>795</td>
<td>18.1 ± 0.8</td>
<td>5.6 ± 1.1</td>
<td>3.24</td>
</tr>
<tr>
<td>(\rho_{NSA})</td>
<td>171</td>
<td>5.3 ± 0.2</td>
<td>4.9 ± 0.8</td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>5.6 ± 0.3</td>
<td>6.1 ± 1.2</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>614</td>
<td>4.8 ± 0.3</td>
<td>5.0 ± 1.5</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>795</td>
<td>5.1 ± 0.3</td>
<td>5.6 ± 1.2</td>
<td>0.92</td>
</tr>
<tr>
<td>(\rho_{PSA})</td>
<td>171</td>
<td>0.19 ± 0.01</td>
<td>3.6 ± 1.0</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>0.19 ± 0.02</td>
<td>8.2 ± 2.8</td>
<td>0.023</td>
</tr>
<tr>
<td></td>
<td>614</td>
<td>0.12 ± 0.01</td>
<td>3.6 ± 1.2</td>
<td>0.031</td>
</tr>
<tr>
<td></td>
<td>795</td>
<td>0.12 ± 0.00</td>
<td>4.7 ± 0.6</td>
<td>0.026</td>
</tr>
<tr>
<td>(\rho_{SiSA})</td>
<td>171</td>
<td>4.1 ± 0.1</td>
<td>3.3 ± 0.6</td>
<td>1.22</td>
</tr>
<tr>
<td></td>
<td>386</td>
<td>3.6 ± 0.2</td>
<td>2.6 ± 0.8</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>614</td>
<td>3.3 ± 0.3</td>
<td>3.4 ± 1.5</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>795</td>
<td>3.1 ± 0.2</td>
<td>3.2 ± 0.9</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Figure 1

(A) $\mu$

(B) CV

(C) 171 ppm

(D) 386 ppm

(E) 626 ppm

(F) 795 ppm

Cell volume ($\mu m^3$)

Apical axis ($\mu m$)

Growth rate ($d^{-1}$)

Surface area / cell volume ratio

Valve aspect ratio

Transapical axis ($\mu m$)

Cell volume ($\mu m^3$)
Figure 2

The graph illustrates the relationship between CO₂ concentration (ppm) and the parameter α. The CO₂ concentrations presented are 171, 386, 614, and 795 ppm, corresponding to values of α ranging from 1.0 to 0.25.
Figure 3
Figure 5

(A) C:N

(B) C:P

(C) N:P

(D) Si:N

(E) Si:C

(F) Si:P