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Examining the effects of elevated CO_2 on the growth kinetics of two microalgae, *Skeletonema dohrnii* (Bacillariophyceae) and *Heterosigma akashiwo* (Raphidophyceae)

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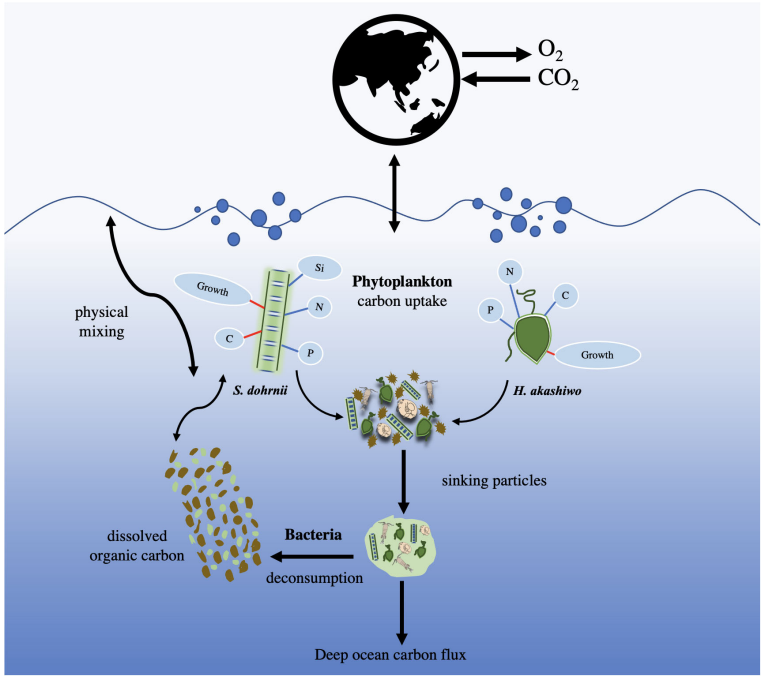
Carbon dioxide (CO_2) serves as the primary substrate for the photosynthesis of phytoplankton, forming the foundation of marine food webs and mediating the biogeochemical cycling of C and N. We studied the effects of CO_2 variation on the Michaelis-Menten equations and elemental composition of *Skeletonema dohrnii* and *Heterosigma akashiwo*. CO_2 functional response curves were conducted from 100 to 2000 ppm. The growth of both phytoplankton was significantly affected by CO_2, but in different trends. The growth rate of *S. dohrnii* increased as CO_2 levels rose up to 400 ppm before reaching saturation. In contrast to *S. dohrnii*, the growth rate of *H. akashiwo* increased with CO_2 increasing up to 1000 ppm, and then CO_2 saturated. In addition, *H. akashiwo* showed a slower growth rate than *S. dohrnii* for all CO_2 concentrations, aside from 1000 ppm, and the Michaelis-Menten equations revealed that the half-saturation constant of *H. akashiwo* was higher than *S. dohrnii*. An increase in CO_2 concentration was seen to significantly affected the POC: Chl-*a* of both *S. dohrnii* and *H. akashiwo*, however, the effects on their elemental composition were minimal. Overall, our findings indicate that *H. akashiwo* had a more positive reaction to elevated CO_2 than *S. dohrnii*, and with higher nutrient utilization efficiency, while *S. dohrnii* exhibited higher carbon fixation efficiency, which is in line with their respective carbon concentrating mechanisms. Consequently, elevated CO_2, either alone or in combination with other limiting factors, may significantly alter the relative relationships between these two harmful algal blooms (HAB) species over the next century.

KEYWORDS

CO2, Skeletonema dohrnii, Heterosigma akashiwo, Michaelis-Menten equations, half saturated constant, carbon fixation



GRAPHICAL ABSTRACT

1 Introduction

The ocean absorbs about 30% of anthropogenic emissions per year (Gattuso et al., 2015), and the pH of the ocean surface has decreased by 0.1 units since the Industrial Revolution compared to pre-industrial levels due to the accumulation of carbon dioxide in the atmosphere brought by human activity, suggesting a 30% rise in acidity (Orr et al., 2005). In addition, the pH of the ocean surface is expected to fall to 7.8 by the end of the century (Gazeau et al., 2013). This would mean a 150-200% increase in acidity. However, microalgae play an extremely important role in ecosystems by using carbon dioxide for photosynthesis to produce carbohydrates, proteins, pigments and lipids, which are powerful consumers of carbon dioxide.

Over the past 70 years, researchers have studied and advanced the cultivation of microalgae. Some factors that impact algal development have been thoroughly researched, such as light irradiation, temperature and nutrient concentration (Fu et al., 2007), while others, such as the effects of pH and CO_2 on microalgal growth, still merit more research. Algae thrive in water, where the CO_2 concentration is significantly lower than in the air, and the diffusion rate of CO_2 in water is only one 10,000th of that in the atmosphere. In order to response to this low CO_2 concentration, algae have developed a specialized CO_2-concentration mechanism (CCM) to elevate the CO_2 concentration inside the cell. The great efficiency of algal photosynthesis relies on CCM at the catalytic site of the carboxylating enzyme Rubisco, thus enhancing CO_2 fixation (Burlacot et al., 2021). CCMs have been developed by several algae species, such as diatoms and dinoflagellates to increase the CO_2 concentrations close to the Rubisco active site. Among these processes is a bicarbonate uptake active transport system (Huertas and Lubian, 1998). For the main carbon-fixing enzyme Rubisco, CO_2 is the preferred carbon substrate in algae. Carbon fixation may be encouraged by increased CO_2 for certain species but not for others since Rubisco efficiency can vary between different groups (Beardall et al., 2009).

Microalgae can be cultured in a variety of ways, such as one-off culture, continuous culture and semi-continuous culture, each of which has its advantages and disadvantages (Hofmann et al., 2019). In the semi-continuous culture process, the frequent addition of fresh medium not only increases the nutrients in the medium, but also decreases the biological density and enhances light transmission. This leads to improved photosynthetic efficiency and growth rate of algal cells, ultimately helping to maintain a favorable growth state for the cells. (Guinotte and Fabry, 2008). Regeneration rate and initial density, as important parameters in semi-continuous culture mode, have important effects on the growth and intracellular biochemical components of microalgae.

Over the next 50 to 100 years, it is projected that greenhouse warming will cause average sea surface temperatures to increase by as much as 5°C. This rise in temperature, coupled with increased precipitation, runoff and ice melting, will likely result in lower surface salinities in many parts of the ocean (Fu et al., 2012). While CO_2, as a major greenhouse gas, gradually increases in air carbon dioxide concentration, leading to a gradual increase in the partial pressure carbon dioxide (pCO_2) of seawater, so the potential impact of ocean acidification (OA) on the growth of marine phytoplankton and the rate of biomineralization has attracted attention. Recent evidence demonstrates that some coastal ecosystems and estuaries are already experiencing significant levels of anthropogenic

acidification (Landsberg, 2002; Anderson et al., 2008; Jeong et al., 2013). Due to the frequent occurrence of harmful algal blooms (HABs) in these ecosystems, it is crucial to study their response to changing CO_2 and/or pH, either individually or in conjunction with other variables. Both *Skeletonema spp* and flagellate *Heterosigma spp* are widely distributed in coastal waters globally and have broad range of thermohaline tolerances (Imai and Itakura, 1999). When the temperature and salinity are suitable, it can proliferate in large numbers to create red tides, which can have negative impacts on marine ecosystems. In China, reports of red tide brought on by *Skeletonema* spp. and *flagellate Heterosigma* spp. offshore seas exist (Qing-Qing et al., 2017; Li et al., 2021). Meanwhile, these common species of harmful algal blooms have been responsible for red tides in numerous countries, leading to widespread fish deaths and substantial economic repercussions (Khan et al., 1997; Zhang et al., 2006). Although there have been some experimental studies of ocean acidification and nutrient changes on harmful algal blooms (Kempton et al., 2008; Sun et al., 2011; Thangaraj and Sun, 2020), very few studies have explored the impact of seven different CO_2 concentrations.

In this experiment, the algae species were taken from Chinese coastal waters. Although it would be more realistic to cultivate them under natural conditions, since these temporal fluctuations are impossible to realistically simulate in laboratory cultures, our experiments used the current temperate offshore summer temperature of 25°C and seven different pCO_2 values. The cell physiological and biochemical reposes to these conditions were then compared with the aim of revealing the possible effects of global change on interspecies competition and dominance in harmful algal bloom events.

2 Materials and methods

2.1 Alga sources and growth conditions

Skeletonema dohrnii was isolated from the central Yellow Sea (China), and *Heterosigma akashiwo* was isolated from Qinhuangdao, a nearshore city at the north Bohai Sea (China) (Thangaraj et al., 2019), and both of them were pre-cultured in f/2 seawater medium and then transferred to the Aquil medium. In the experiment, *Skeletonema dohrnii* were grown in Aquil medium (100 µmol L^{-1} NO_3^-, 100 µmol $L^{-1}SiO_4^{4-}$, 10 µmol $L^{-1}PO_4^{3-}$) (Price et al., 1989). While *Heterosigma akashiwo* were cultured in an enriched artificial medium to prevent nutrient limitation (500 µmol L^{-1} NO_3^-, 100 µmol $L^{-1}SiO_4^{4-}$, 50 µmol $L^{-1}PO_4^{3-}$). Each experiment had three biological replicates. These two algae were both aerated with CO_2 gas and grown under cool white fluorescent light with a 12:12 h light: dark cycle at 25°C and an irradiation of 160 µmol photo $m^{-2}s^{-1}$.

2.2 Experiment design

To reduce contamination, accurate methods were used in this study. Experiments were performed in triplicate 1 L acid-washed and autoclaved conical bottles. The experiments were conducted using a semi-continuous culture method to measure the effect of CO_2 on domestication and steady-state growth (Fu et al., 2008). The pCO_2 of the incubators was controlled at seven different partial pressure levels (100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, 1000 ppm and 2000 ppm) by controlling the inflow of ambient air and pure CO_2 filtered by 0.2 µm. The seawater medium was bubbled at the corresponding CO_2 concentration for at least 24h before the experiment, with continuous aeration, starting from the first day of the experiment to the end of the last day of the experiment. Daily dilutions were made based on growth rate measured from *in vivo* chlorophyll fluorescence using a Turner 10AU fluorometer (Turner Designs), and the dilution volume was adjusted to maintain a constant growth rate and to bring the biomass up to pre-dilution levels. Final sampling took place when steady state growth was achieved for each growth condition, which is defined as no significant difference (less than 10%) in growth rates after at least three consecutive dilutions.

2.3 Cell abundance and growth rate

Daily live microscopic cell counts and *in vivo* fluorescence (measured with a Turner 10AU fluorometer) were used to monitor growth rates. Cell abundance was measured using a hemocytometer counting chamber under an optical microscope. The specific growth rate (μ) was determined as Equation 1:

$$\mu = (lnN_2 - lnN_1)/\Delta t \quad (1)$$

where μ=growth rate (d^{-1}), X_1=cellular density (cells mL^{-1}) at time X_1, X_2 = cellular density (cells mL^{-1}) at time X_2, and Δ t refers to the time period between X_1 and X_2 (d).

The maximum growth rate was estimated by numerically maximizing the equation (Moorhead and Weintraub, 2018). The growth rates of all the species at all the CO_2 levels were fitted to Michaelis–Menten equation as Equation 2:

$$\mu = \mu_{max}S/(K_{1/2} + S) \quad (2)$$

to estimate maximum growth rates (μ_{max}) and half saturation constants ($K_{1/2}$) for CO_2 concentration (S). In the CO_2 curve experiments growth rates for both these autotrophic species were assumed to be zero at 0 ppm CO_2 (Zhu et al., 2017).

2.4 Elemental and Chl-a analysis

To analyze particulate organic carbon (POC) and particulate organic nitrogen (PON), 100 ml culture samples from each of the triplicate bottles were filtered onto precomputed (450°C, 4 h) GF/F glass fiber filter (0.7 µm, 25 mm, Whatman) under low vacuum. POC and PON were then analyzed by a Costech ECS4010 Elemental Analyzer (Costech International S. P. A., Milan, Italy) following the protocol of Fu et al. (2007). Samples (50ml) for particulate organic phosphorus (POP) were obtained in the same manner as described above. Each treatment was filtered onto 0.6 µm polycarbonate filters (GE Healthcare, CA) and dried in an oven at 60 °C overnight for

biogenic silica (BSi) analysis using a 5 mL aliquot of *S. dohrnii* culture. POP was measured as Fu et al. (2005). BSi analysis followed Brzezinski and Nelson (1995). The values of cell quota were derived from the measured values divided by manual cell counts on the last day of experiment (when the cultures are harvested).

For chlorophyll-*a* (Chl-*a*) determination, the cultures were from each treatment replicate filtered onto GF/F filters (0.7 µm, 25 mm, Whatman) and extracted with 90% acetone at −20°C for 24 h for analysis (Maat et al., 2014). The Chl-*a* concentration was then determined by using the Turner-Designs Trilogy fluorometer (model 10-AU, TurnerBioSystems, Sunnyvale, CA, USA) and calculated based on the formula of Parsons (Welschmeyer, 1994).

2.5 Sinking rate

The sinking rate of phytoplankton was measured using the SETCOL method (Bienfang, 1981), which involves a vertical sedimentation column. Three water outlets were sealed, and the algal liquid was poured into the column, filling it completely. The column was then sealed and placed in the dark at a consistent temperature for 3 hours. The volume of algal liquid in each layer was measured, then filtered onto a GF/F filter and kept at -20°C for the final determination.

2.6 pH and dissolved inorganic carbon measurement

The pH in each bottle was monitored every day using a microprocessor pH-meter (pH S210-K, Switzerland), calibrated with pH 7 and 10 buffer solutions. CO_2 equilibration was also verified using dissolved inorganic carbon (DIC) measurements. The dissolved inorganic carbon (DIC) content was determined using a total organic carbon analyzer (TOC-VCPN, Shimadzu, Japan). The carbon dioxide partial pressure content in solution was calculated with the help of CO_2 sys (Pierrot and Lewis, 2011) to verify the carbon dioxide gradient of the experimental treatments (data not shown).

2.7 Statistical analysis

In this study, SPSS21.0 and Prism9 was used for statistical analysis and one-way ANOVA was carried out to evaluate the effects of pCO_2 on various indicators of algae. Tukey HSD multiple comparisons were used to examine differences between treatment groups. The measured data were presented as mean values ± standard deviation (SD). The value of $p<0.05$ was considered a statistically significant difference.

3 Results

3.1 Growth profiles of *S. dohrnii* and *H. akashiwo*

The cell growth of *S. dohrnii* and *H. akashiwo* were affected significantly under elevated pCO_2 as shown in Figure 1. The growth rate of *S. dohrnii* increased as CO_2 concentration rose from 100 ppm to 400 ppm, achieving saturation at 500 ppm and 1000 ppm and the maximum growth rate was 1.50 d^{-1} (Figure 1A). However, the growth rate of *S. dohrnii* at 2000 ppm was decreased when compared to the growth rate at 400 ppm ($p<0.05$).

The growth rate of *H. akashiwo* was considerably impacted by CO_2 concentration at 25°C ($p<0.05$). Similar to the trend of *S. dohrnii*, the growth rate of *H. akashiwo* was limited at low CO_2 concentrations, and unlike *S. dohrnii*, *H. akashiwo* grew continuously as the CO_2 concentration climbed from 100 to 1000 ppm until becoming saturated at 1000 ppm and 2000 ppm (Figure 1B). The maximum growth rate was 1.13 ± 0.20 d^{-1} at 1000 ppm, which was close to the theoretical growth rate of 1.03 d^{-1} fitted by Michaelis-Menten equation (Table 1). Although the growth rate of *S. dohrnii* and *H. akashiwo* both decreased slightly at 2000 ppm, the decrease of *S.dornii* was more remarkable than *H. akashiwo* when relative to 400 ppm. This inhibition may be attributed to the decrease in pH at 2000 ppm when the cells require haoadditional energy to maintain intracellular pH homeostasis.

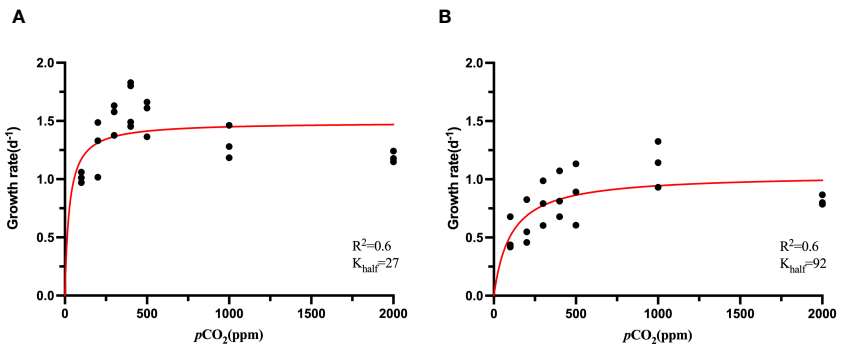


FIGURE 1
CO_2 functional response curves of showing specific growth rate (and fitted curves) across a range of CO_2 concentration from ~100 to ~2000 ppm of *S. dohrnii* **(A)** and *H. akashiwo* **(B)** at 25°C. (Confidence interval 95%).

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TABLE 1 Comparison of the curve fitting results for maximum growth rate (d^{-1}) and half-saturation constants ($K_{1/2}$), calculated from the CO_2 functional response curves of *S. dohrnii* and *H. akashiwo* at 25°C.

Species	Maximum growth rate (d^{-1})	$K_{1/2}$ ppm CO_2
S. dohrnii	1.50 ± 0.09	26.74 ± 16.90
H. akashiwo	1.03 ± 0.09	91.65 ± 37.18

Values represent the means and errors are the standard errors from fitting.

3.2 Cell quotas and elemental ratios

Generally, as is shown in the Figure 2, there was no significant (p>0.05) variation in the values of the cell quota of N (Q_N) and the cell quota of P (Q_P) (Figures 2B, C), while increasing CO_2 resulted in the cell quota of C (Q_C) of *S. dohrnii* varied considerably (p<0.05, Figure 2A). Specifically, Q_C in *S. dohrnii* at 100 ppm, 400 ppm, and 500 ppm was significantly lower than that at 200ppm and 1000ppm, and it was also significantly higher at 200 ppm, 300 ppm and 1000 ppm than that at 400 ppm and 2000 ppm(p<0.05).

In comparison with *S. dohrnii*, the Q_C of *H. akashiwo* varied under different carbon dioxide settings, and Q_C, Q_P and Q_N of *H. akashiwo* reached the maximum value at 300 ppm (Figures 2D–F). However, this shift trend was not significant (p>0.05). Besides, with increasing CO_2 concentration, the Q_C in *H. akashiwo* showed a pattern of increasing, then decreasing, and then increasing again with increasing CO_2 concentration. Likewise, Q_N displayed a trend of increasing, then decreasing, and then increasing again. Variations in CO_2 concentration affected Q_N in much the same manner as Q_C (Figure 2B).

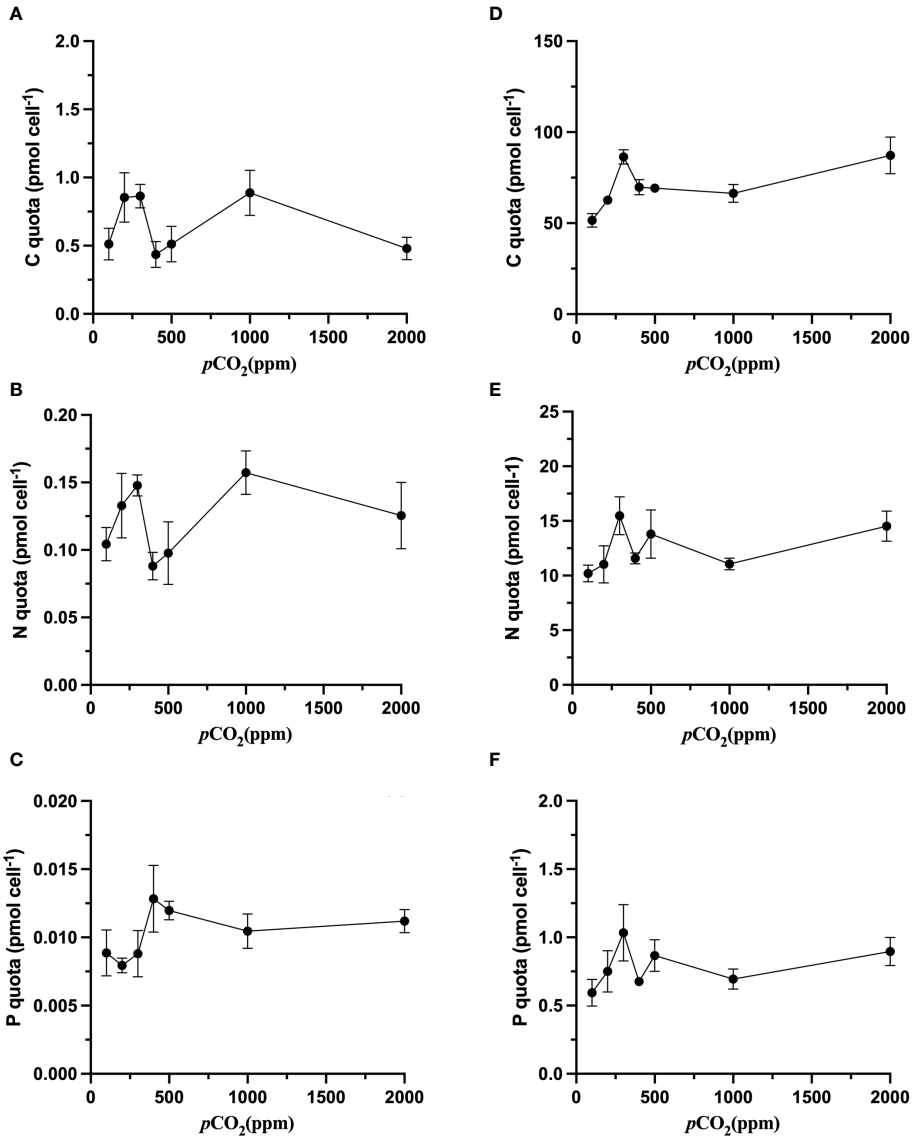

FIGURE 2
The effects of CO_2 on the C quota (pmol $cell^{-1}$), N quota (pmol $cell^{-1}$), P quota (pmol $cell^{-1}$) of *S. dohrnii* **(A–C)** and *H. akashiwo* **(D–F)** in each of the seven CO_2 treatments. Error bars denote standard deviations of averaged results from three replicate bottles. The scales of the y-axes are very different.

The response of cellular elemental composition to carbon dioxide is analyzed at four levels: low carbon dioxide partial pressures (100 ppm, 200 ppm, 300 ppm), current carbon dioxide partial pressures (400 ppm and 500 ppm), end-of-century carbon dioxide partial pressure (1000 ppm), and a very high partial pressure (2000 ppm). The C: N of *S. dohrnii* was not substantially altered when the carbon dioxide concentration increased ($p>0.05$, Figure 3A). However, the C: P and C: Si ratios were altered considerably ($p<0.05$, Figures 3B, C). The C: N of *S. dohrnii* at 200, 300 and 1000 ppm were significantly higher than that at 400, 500 and 2000 ppm ($p<0.05$). The ratio of C: Si of *S. dohrnii* were lower at present air pressure and very high carbon dioxide levels, but no significant difference compared to low pCO_2.

Contrary to the *S. dohrnii*, the C: N ratios of *H. akashiwo* were not significantly changed in different CO_2 treatments ($p>0.05$, Figure 3D). The C: P of the cells of *H. akashiwo* were significantly higher ($p<0.05$) than those of *S. dohrnii* at 100 ppm, 400 ppm, 500 ppm and 2000ppm (Figure 3E). Higher C: P was founded in the cells of *H. akashiwo* compared to *S. dohrnii*, implying that their contributions to the oceanic carbon, nitrogen and phosphorus cycles are different.

3.3 POC: Chl-*a* ratios

The POC: Chl-*a* ratios of *S. dohrnii* were influenced by changing carbon dioxide concentration (Figure 4). The POC: Chl-*a* ratios of

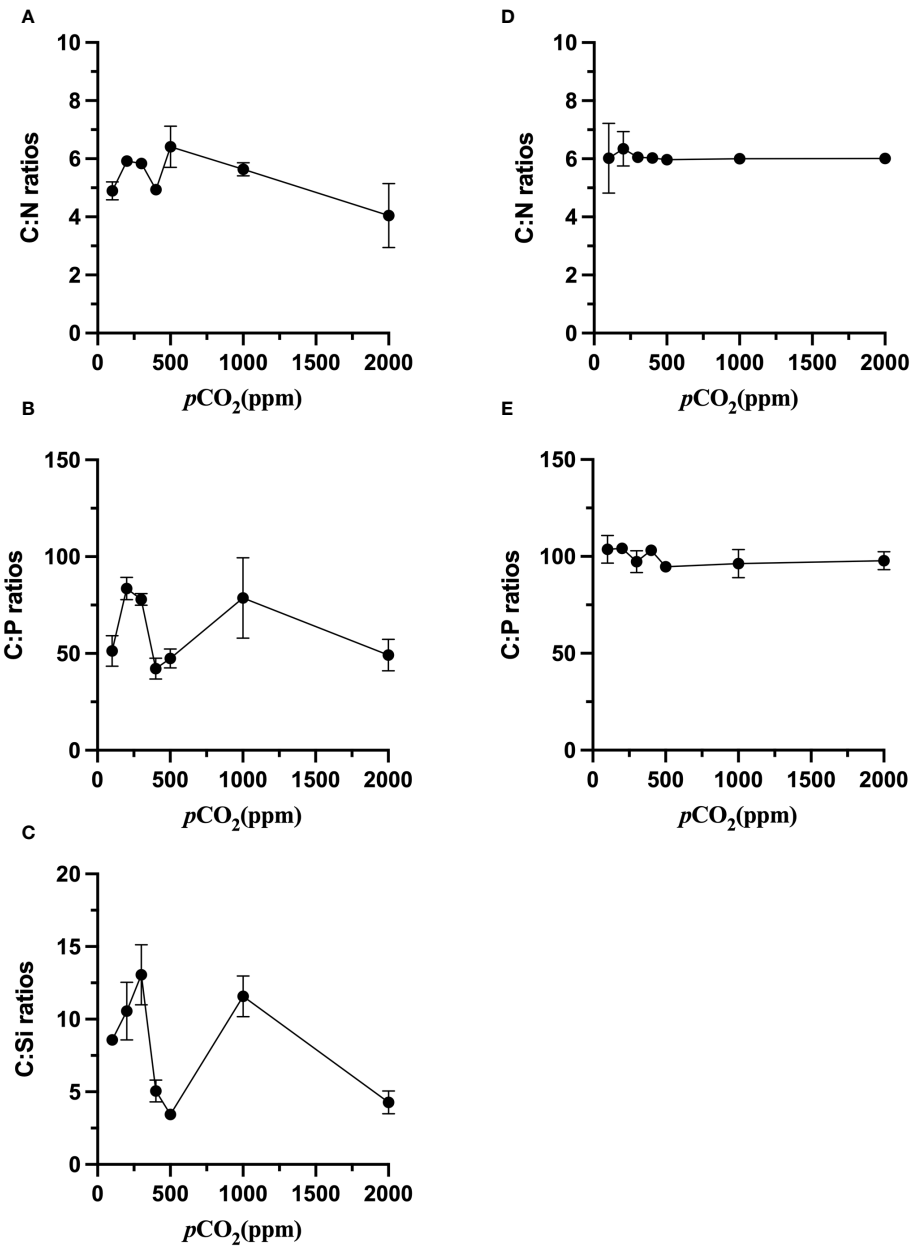

FIGURE 3
Elemental ratios of *S. dohrnii* **(A–C)** and *H. akashiwo* **(D, E)** in the seven CO_2 treatments. Error bars denote standard deviations of averaged results from three replicate bottles.

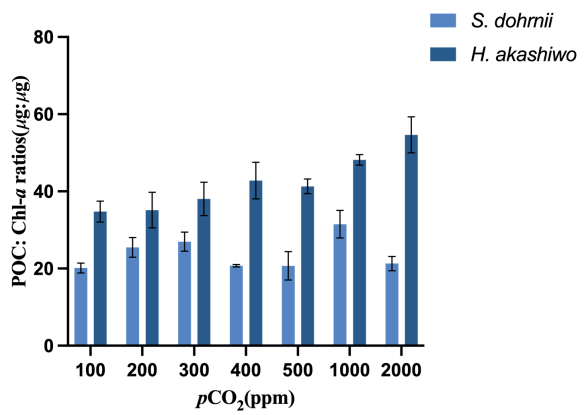

FIGURE 4
POC: Chl-*a* ratios of *S. dohrnii* and *H. akashiwo* in each of the seven CO_2 treatments. Error bars denote standard deviations of averaged results from three replicate bottles.

S.dohrnii were significantly higher at 1000 ppm than 100 ppm, 400 ppm, 500 ppm and 2000 ppm(p<0.05). Carbon dioxide concentration also had a significant effect (p<0.05) on POC: Chl-*a* ratios of *H. akashiwo*. The POC: Chl-*a* of *H. akashiwo* tended to increase with increasing carbon dioxide and was significantly higher at a very high carbon dioxide partial pressure (2000 ppm) than at low carbon dioxide partial pressures (100, 200, 300 ppm) and current carbon dioxide partial pressures (400 ppm and 500 ppm). Besides, the POC: Chl-*a* ratios of *H. akashiwo* were higher than *S. dohrnii* at all the CO_2 levels tested.

3.4 Sinking rate

As the CO_2 concentration increased from 100 ppm to 500 ppm, the sinking rate of *S. dohrnii* tended to increase, when it came to 1000 ppm, the sinking rate began to decrease. The sinking rate of *S. dohrnii* at 2000 ppm was significantly (p<0.05) higher than that at 100ppm (Figure 5A). The sinking rate of *H. akashiwo* was negative at all carbon dioxide concentration gradients from 100 ppm to 2000 ppm, which indicated that *H. akashiwo* did not sink but ascend under the experimental conditions. Furthermore, as the carbon dioxide concentration increases from 100 ppm to 400 ppm, the ascent rate of *H. akashiwo* gradually increases. However, as the carbon dioxide concentration rises from 400 ppm to 2000 ppm, the ascent rate of *H. akashiwo* decreases continuously (Figure 5B). The ascent rate of *H. akashiwo* exhibits a pattern of initially increasing and then decreasing with the carbon dioxide concentration, reaching its maximum value of 4.67cm h^{-1} at then carbon dioxide concentration of 400ppm. The ascent of this algal species is significantly lower at both low carbon dioxide concentration of 100 ppm and high carbon dioxide concentration of 2000ppm compared to the ascent rate at 400ppm (p<0.05).

4 Discussion

Our research showed that *S. dohrnii* and *H. akashiwo* reacted in different ways when exposed to different CO_2 levels, as indicated by their growth rate and elemental composition.

According to the study, the growth rate of *S. dohrnii* was limited when the CO_2 concentration decreased to below 400 ppm, which is crucial due to the fact that CO_2 levels usually drop to extremely low levels during algal blooms in coastal waters (Jef et al., 2018). *S. dohrnii* may benefit from higher summertime water temperature, particularly during later algal blooms when the pCO_2 of the water is low. Under the current atmospheric CO_2 level, the growth rate of *S. dohrnii* has reached a saturation point. According to our findings, the growth rates of *H. akashiwo* will likely be stimulated by elevated CO_2 within the range predicted for the next century, while the growth rate of *S. dohrnii* will remain unchanged. As ocean acidification (OA) continues, it is possible that the current advantage of *S. dohrnii* will diminish and *H. akashiwo* will begin to benefit more in the future. This could result in an increase in the frequency and severity of red tides caused by *H. akashiwo*. It is possible that the red tide is shifting from being mainly diatom dominated to being mainly dinoflagellate dominated. In turn, this could alter the phytoplankton community composition off the coast of China, which could have an effect on the food web and the carbon and nutrient biogeochemical cycling.

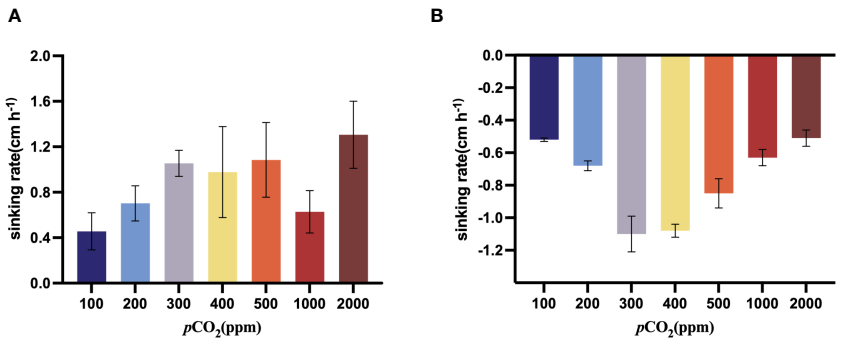

FIGURE 5
Sinking rate (cm/h) of *S. dohrnii* **(A)** and *H. akashiwo* **(B)** in the seven CO_2 treatments. Error bars denote standard deviations of averaged results from three replicate bottles.

In practice, experimental evolution often uses growth rate or competitive ability as a measure of fitness for selection experiments conducted in semi-continuous (batch) culture settings, or population carrying capacity as a measure of fitness in continuous culture (Collins et al., 2014). Consistent with earlier research, the results show that CO_2 from current levels to end-of-century levels has minimal effects on various phytoplankton species (Joel, 1999; Wu et al., 2014; Hutchins and Fu, 2017). According to our study, the growth rate of *S. dohrnii* was not raised at 400 ppm, and our experimental results agreed fairly well with the notion that the growth and photosynthesis of *Skeletonema costatum* in the current atmospheric CO_2 concentrations have reached saturation state (Gao et al., 2019). As per the model, the growth rate of *H. akashiwo* is predicted to increase by 40% as the CO_2 levels transition from the current level to 700 ppm (Schippers et al., 2004). This hypothesis was also well supported by our experimental findings which showed that raising CO_2 to 1000 ppm would result in a 32.6% increase in *H. akashiwo* growth rate compared to the current CO_2 levels.

Growth limitation by CO_2 in marine cyanobacteria, green algae and diatoms has been reported from both field and lab work (Ashida et al., 2003: Fu et al., 2007). As numerous species of the phytoplankton community have an effective carbon-concentrating mechanism that allows them to evade the CO_2 limit in lower levels of pCO_2, changes in carbon dioxide levels have minimal effect on them (Reinfelder, 2011). Phytoplankton utilize ribulose bisphosphate carboxylase-oxygenase (Rubisco) in order to fix CO_2. The half-saturation constant of Rubisco for CO_2 is approximately 20~70μM (Badger et al., 1998), while the soluble CO_2 in seawater is only 10~15μM. To overcome this, phytoplankton have developed strategies such as the use of HCO_3^- and Carbon-Concentrating Mechanisms (CCMs) to deal with the insufficient CO_2 levels (Giordano et al., 2005). The CCMs consist of an active transport system for bicarbonate uptake and bicarbonate dehydration catalyzed by intracellular carbonic anhydrase (CA) to form CO_2 (Fu et al., 2008; Garcia et al., 2011). When the CO_2 concentration in the environment increases, the inorganic carbon uptake mechanism of algae is activated, and the CCMs are down-regulated, leading to a decrease in the ability of algal cells to utilize HCO_3^-, so that the energy originally used for the transfer and utilization of HCO_3^- is reduced and converted to photosynthesis, thus improving photosynthetic efficiency and promoting the growth of algae (Qiu and Gao, 2002; Ashida et al., 2003).

Variations in Rubisco efficiencies can be seen between different microalgae (Tortell, 2000). *H. akashiwo* does not possess the CCMs making it highly dependent on CO_2 for inorganic carbon (Badger et al., 1998). The lack of CCMs in *H. akashiwo* would prevent it from directly or indirectly absorbing HCO_3^-, so the ability to uptake CO_2 is the only source of energy may help to explain why increased CO_2 stimulates the growth of this alga (Hansen, 2002).

Through photosynthesis, algae synthesize organic matter, and this can cause a shift in the C, N and P composition and content of the algal cell. Our findings demonstrate that a rise in CO_2 levels had an effect on the cell quota of C (Q_C) and N (Q_N) of *S. dohrnii* and *H. akashiwo*. The cell quota of C of *S. dohrnii* was 80% lower at 2000ppm than that at 1000ppm and there were no significant differences in Q_N. While the Q_C, Q_N of *H. akashiwo* were more sensitive to the changes of pCO_2. One explainable reason is that the disturbance of seawater can dramatically alter the partial pressure of CO_2, but causes only relatively small changes in HCO_3^- availability, and *H. akashiwo* does not have the potential to take up HCO_3^- directly or to utilize it indirectly by using extracellular carbonic anhydrase (Nimer et al., 1999; Hansen, 2002). Thus, it may be reasonable that cell number and elemental composition are affected by changes in CO_2 in *H. akashiwo*. A future increase in atmospheric pCO_2 concentration would have the same effect. The Q_C and Q_N of *H akashiwo* were both increased by elevated CO_2. Although the biochemical composition of both species was not determined in this study, it is not clear whether the changes in Q_C and Q_N in *H. akashiwo* under CO_2 enrichment was caused by an increase in carbohydrate or protein synthesis. Consequently, if the red tides transition from *S. dohrnii* to *H akashiwo*, there will be an elevated presence of carbon and nitrogen in biogeochemical cycling. Besides, it is noteworthy that *S. dohrnii* but not *H akashiwo*, had an increased Q_N under elevated CO_2 over 400ppm.This finding may suggest that the *S. dohrnii* may be more vulnerable to nitrogen limitation under future environmental conditions. Thus, the current competitive advantage that *S. dohrnii* enjoys under elevated CO_2 could potentially be negated in estuaries where N is the limiting nutrient for bloom development.

Current study reveals that phytoplankton are quite are variable. Changes in growth rate and CO_2 availability can occur with no change in elemental composition (both strains of *T. weissflogii* and *T. oceanica*), or with large differences in elemental stoichiometry (*T. pseudonana*, *D. salina*) (King et al., 2015). The effects of pCO_2 variation on the elemental ratios of *H. akashiwo* were minimal relative to those of *S. dohrnii.* In this experiment, the effect of CO_2 concentration changes on the elemental composition of the cells of *H. akashiwo* was slight, but there was a significant difference on C: Chl-*a*. In general, a lower POC: Chl-*a* ratio indicates higher carbon fixation efficiency in plants (McGrath and Lobell, 2013). This implies that plants more effectively convert photosynthetic products (such as glucose) into organic carbon compounds, rather than wasting energy in the photosynthesis process. This high-efficiency carbon fixation enables plants to be more productive in terms of growth and production. In the case of *H. akashiwo*, a higher ratio suggests higher nutrient utilization efficiency, while *S. dohrnii* exhibits higher carbon fixation efficiency. The fluctuating POC: Chl-*a* ratio in *S. dohrnii* might imply a more complex or variable physiological response to CO_2 levels, which might be influenced by other environmental or internal factors not represented in this data. Some results showed that elevated CO_2 concentration increases the C: N ratio or C: P ratio of planktonic algae (Engel et al., 2005), especially in the low trophic state (Li et al., 2012). However, it has also been shown that changes in CO_2 concentration did not affect elemental chemical composition (Qiu and Gao, 2002). Olischlaeger et al. (2014) previously studied the changes in C: N of algae in response to high CO_2 concentrations, and the results showed that at the same temperature, the changes in algal C: N caused by high CO_2 concentrations were not significant. The increased carbon and nitrogen contents for *H. akashiwo* grown under elevated CO_2 could be due to increased carbon fixation and a higher uptake of NO_3^- (Bi et al., 2012). In the future, the effect of

CO_2 on phytoplankton elemental composition must be taken into account together with other environmental parameters, such as nutrition and light availability, which can also have a significant impact on stoichiometry (Finkel et al., 2010).

The study of phytoplankton sinking rate is crucial for a thorough understanding of the effectiveness of carbon sinks since direct carbon sedimentation by phytoplankton is an essential pathway for oceanic carbon sinks (Sun, 2011). The rate of sedimentation of marine phytoplankton is affected by a variety of factors, including the physiological condition of the phytoplankton, lack of nutrients, and disruption of the ocean.

When the algae are in good physiological condition, the cells are active and the sinking rate is slow, and vice versa (Wang et al., 2022). Diatoms are a class of unicellular or multicellular algae rich in silicon, and as an important structural protein, the silicophilic proteins play an important role in the silicification process (Martin-Jézéquel et al., 2000). The high sedimentation rate of *S. dohrnii* at 500 ppm may be attributed to the high content of Si in the algal cells at this time, and the pro-silica proteins on the surface of the algal cells are able to combine with the silicate in the environment to promote the deposition and polymerization of silicate, which ultimately causes the diatom cells to form a hard siliceous shell on their exterior, thus accelerating its sedimentation rate. Research has revealed that a decrease in pH values will slow down the dissolution of silicon (Si) in sinking particulate matter and ocean acidification will cause a 17 ± 6% increase in the silicon (Si) to nitrogen (N) elemental ratio in sinking biogenic material (Taucher et al., 2022). Moreover, the silicophilic proteins in diatoms may also be involved in a variety of other biological processes, such as cell adhesion, signaling, and so on (Martin-Jézéquel et al., 2000). Phytoplankton with spines and horns in their cell structure or with flagella generally typically have a lower sinking rate, whereas *H. akashiwo* has two unequal flagella with some swimming ability, which explains why *H. akashiwo* prefers to ascent rather than sink(Tobin et al., 2013).

The two species of harmful algal blooms inhabiting the same environment reacted differently to predicted global changes, suggesting that the impact of increasing CO_2 levels will be specific to certain taxa or even individual species. It is likely that *H. akashiwo* will grow more quickly than *S. dohrnii* in the coming decades, providing it with an advantage in interspecific competition. Larger diatoms (diameter >40 µm) were more likely to be stimulated by future increases in CO_2 availability, and the increase in growth rate by pCO_2 was size-dependent. Smaller diatom species showed a 5% increase in growth rate, while the largest diatom species showed a 30% increase in growth rate (Wu et al., 2014). Changes in cell nutrition ratios and quotas caused by CO_2 further point to a connection between continuous eutrophication, which has already been linked to HAB blooms and global change variables.

The assimilation of inorganic carbon by phytoplankton through light energy is an important basis of the ocean's material cycle and energy flow, and their conversion of dissolved CO_2 to the particulate state is a vital biological process of the "biological pump". The amount of CO_2 that is taken up by the oceans from the atmosphere depends on the biological pump, as well as on the effects of ocean acidification. The reaction of phytoplankton to ocean acidification and other environmental factors is determined by species characteristics, cell size and adaptability to the environment; hence, differences in the impacts of ocean acidification on the physiological processes of phytoplankton will inevitably lead to changes in the composition of the phytoplankton community. Therefore, in order to accurately predict the effects of future alterations in temperature and CO_2 levels, the interactive effects of other variables such as precipitation, stratification, light, and nutrient inputs on the physiology of phytoplankton.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

JQ: Data curation, Formal analysis, Software, Visualization, Writing – original draft. MJ: Investigation, Writing – review & editing. JS: Writing – review & editing, Methodology, Project administration, Supervision, Validation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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