Preference of carbon absorption determines the competitive ability of algae along atmospheric CO$_2$ concentration

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 31500340, 31670548 and 31872032

Abstract
Although many studies have focused on the effects of elevated atmospheric CO$_2$ on algal growth, few of them have demonstrated how CO$_2$ interacts with carbon absorption capacity to determine the algal competition at the population level. We conducted a pairwise competition experiment of Phormidium sp., Scenedesmus quadricauda, Chlorella vulgaris and Synedra ulna. The results showed that when the CO$_2$ concentration increased from 400 to 760 ppm, the competitiveness of S. quadricauda increased, the competitiveness of Phormidium sp. and C. vulgaris decreased, and the competitiveness of S. ulna was always the lowest. We constructed a model to explore whether interspecific differences in affinity and flux rate for CO$_2$ and HCO$_3^-$ could explain changes in competitiveness between algae species along the gradient of atmospheric CO$_2$ concentration. Affinity and flux rates are the capture capacity and transport capacity of substrate respectively, and are inversely proportional to each other. The simulation results showed that, when the atmospheric CO$_2$ concentration was low, species with high affinity for both CO$_2$ and HCO$_3^-$ (HCHH) had the highest competitiveness, followed by the species with high affinity for CO$_2$ and low affinity for HCO$_3^-$ (HCLH), the species with low affinity for CO$_2$ and high affinity for HCO$_3^-$ (LCHH) and the species with low affinity for both CO$_2$ and HCO$_3^-$ (LCLH); when the CO$_2$ concentration was high, the species were ranked according to the competitive ability: LCHH > LCLH > HCHH > HCLH. Thus, low resource concentration is beneficial to the growth and reproduction of algae with high affinity. With the increase in atmospheric CO$_2$ concentration, the competitive advantage changed from HCHH species to LCHH species. These results indicate the important species types contributing to water bloom under the background of increasing global atmospheric CO$_2$, highlighting the importance of carbon absorption characteristics in understanding, predicting and regulating population dynamics and community composition of algae.

KEYWORDS
affinity, algae, bicarbonate, carbon absorption, carbon dioxide, competition, flux rate

TAXONOMY CLASSIFICATION
Biodiversity ecology; Community ecology; Conservation ecology; Global change ecology
1 | INTRODUCTION

With the increase of atmospheric \( \text{CO}_2 \), many plant ecologists have taken an experimental or modeling approach to identify the growth, reproduction and distribution of algae along the gradient of \( \text{CO}_2 \) concentration (Bolton & Stoll, 2013; Brown et al., 2019; Low-Đécarie et al., 2011). However, few studies have shown how the relative concentration of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) in water affect the algal growth and competitive advantage at a population level (Beardall & Raven, 2017; Li et al., 2015; Low-Đécarie et al., 2011; Ma et al., 2019; Pardev et al., 2018; Sandrini et al., 2016; Van de Waal et al., 2011; Verspagen et al., 2014). Some \( \text{CO}_2 \) in the water is hydrolyzed to \( \text{HCO}_3^- \), which changes the pH value of water, and then affects the relative concentration of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) in the water along the gradient of atmospheric \( \text{CO}_2 \) concentration. The preference for \( \text{CO}_2 \) and \( \text{HCO}_3^- \) is different between algal species due to evolution (Litchman et al., 2015; Schippers, Lurling, et al., 2004a; Schippers, Mooij, et al., 2004b). Therefore, studying the changes in the relative concentration of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) in water along the gradient of atmospheric \( \text{CO}_2 \) concentration and the effect of these changes on algal growth and interspecific competition ability is an important perspective for understanding and predicting the changes in population dynamics and community composition of algae under the background of increasing global atmospheric \( \text{CO}_2 \), and therefore an basis for maintaining the health of an aquatic ecosystem.

The carbon absorption of algae includes the capture and transport of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) (Hammer et al., 2019; Xiao et al., 2017). The affinity and flux rates of substrate \( \text{CO}_2 \) and \( \text{HCO}_3^- \) vary among algal species (Reinfelder, 2011; Stojkovic et al., 2013). Affinity refers to the ability of the binding site on the transporter to capture the substrate, while flux rate refers to the maximum transport capacity of the transporter when the binding site is saturated (Lines & Beardall, 2018; Sandrini et al., 2014). Many studies have shown that high affinity is usually accompanied by a low flux rate (Hepburn et al., 2011; Reinfelder, 2011; Stojkovic et al., 2013; Tortell, 2000). When the substrate concentration is low, the species with high affinity perform better; in contrast, when the substrate concentration is high, the species with a high flux rate perform relatively better (Lines & Beardall, 2018; Reinfelder, 2011; Sandrini et al., 2014). Therefore, the two metrics are effectively capturing different aspects of carbon absorption and, subsequently, should profoundly impact the growth and competition of algae along the atmospheric \( \text{CO}_2 \) gradient.

Based on previous studies, we predict that when the \( \text{CO}_2 \) concentration in the atmosphere is low, both \( \text{CO}_2 \) and \( \text{HCO}_3^- \) concentrations in water are low, which is favorable for the growth, reproduction of algae with high affinity for both \( \text{CO}_2 \) and \( \text{HCO}_3^- \), and such species would be competitive dominant (Schippers, Lurling, et al., 2004a; Schippers, Mooij, et al., 2004b). When the atmospheric \( \text{CO}_2 \) concentration is high, the pH of the water is low, and the water has relatively more \( \text{CO}_2 \) and less \( \text{HCO}_3^- \) (Brown et al., 2019; Hasler et al., 2016). In this way, as atmospheric \( \text{CO}_2 \) continues to increase, the increase rate of \( \text{CO}_2 \) in water increases, while the increase rate of \( \text{HCO}_3^- \) decreases, and the content of \( \text{HCO}_3^- \) may even decrease. Therefore, algae with low affinity for \( \text{CO}_2 \) and high affinity for \( \text{HCO}_3^- \) would be competitive dominant when atmospheric \( \text{CO}_2 \) continued to increase.

In this study, a pairwise competition experiment was conducted to investigate changes in the growth and competitive advantage of four species of algae (\textit{Phormidium sp.}, \textit{Scenedesmus quadricauda}, \textit{Chlorella vulgaris} and \textit{Synedra ulna}) when the atmospheric \( \text{CO}_2 \) concentration increased from 400ppm to 760ppm. A model was developed to explore whether interspecific differences in affinity and flux rate for \( \text{CO}_2 \) and \( \text{HCO}_3^- \) between algal species could explain these changes. The purpose is to highlight the importance of carbon preference in algal growth, reproduction, and competition along atmospheric \( \text{CO}_2 \) concentrations, contributing to our understanding of algal population dynamics and community composition along environmental gradients and providing a direction to predict bloom causing species in the context of increasing global atmospheric \( \text{CO}_2 \).

2 | MATERIALS AND METHODS

2.1 | Investigation

To study the response of algal growth and competition to atmospheric \( \text{CO}_2 \) concentration, a three-factor design with 3 replications was used. The factor species were cyanobacteria, \textit{Phormidium sp.; green algae, Scenedesmus quadricauda and Chlorella vulgaris; diatoms, Synedra ulna}. Culture treatments were monoculture and mixture of two species, and therefore the treatments of monoculture and mixture were 4 and 6. The atmospheric \( \text{CO}_2 \) concentration was 400ppm (“low \( \text{CO}_2 \)”) or 760ppm (“high \( \text{CO}_2 \)”). All four kinds of algae were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (http://algae.ihb.ac.cn/), and then cultivated in a biochemical incubator (BJPX-150, Biobase, China) to the required amount (>10⁷ Cells/L), used as the original algae sample.

The medium was configured according to the composition and concentration of BG11. 400ml of medium was placed in a 500ml beaker, and the inoculation density of each algal sample in each beaker was 10⁶ Cells/L. A total of 420 such beakers were divided into 10 groups of 42 beakers each, which were 4 groups of monoculture species and 6 groups of mixture species.

Half of each group of samples (21 samples) were randomly selected and placed in an artificial climate chamber with 400ppm \( \text{CO}_2 \) gas, and the other half was placed in the artificial climate chamber with 760ppm \( \text{CO}_2 \). The artificial climate chamber was connected with a \( \text{CO}_2 \) cylinder, which can adjust the indoor atmospheric \( \text{CO}_2 \) level to the set concentration. Cultures were stirred 3 times a day. Three samples from each group in each climate chamber were randomly selected to measure algal density and water quality every 3days. Other algae samples continued to grow. In this way, each sample was independent. A monoculture treatment was used as a control to study the competitive ability. For example, the number of individuals in monoculture treatment of \textit{Phormidium sp.}, compared to the number of individuals of \textit{Phormidium sp.} in mixture with \textit{S.
quadricula, C. vulgaris and S. ulna, respectively. Such measurements were made seven times in total.

0.1 ml solution was taken from each sample after fully stirred, and then poured into a 0.1 ml, 20mm×20mm counting chamber. The algae density is calculated by the equation

\[ N = n \times \frac{A}{A_c \times V} \]  

(1)

where \( N \) is the algal density; \( n \) is the counted number of algae; \( A \) is the area of counting chamber; \( A_c \) is the area of visual field \( x \) number of visual fields; and \( V \) is the volume of counting chamber.

After the population density in monoculture and mixture experiments were calculated, the competitive ability of each species was calculated by relative neighbor effect (RNE). This method was proposed by Markham and Chanway for the calculation of competition intensity among individuals of higher plants (Markham & Chanway, 1996). After redefining the parameters, the competitive advantage among algae species was estimated from the equation:

\[ \text{RNE} = \frac{P_{+N} - P_{-N}}{X} \]  

(2)

where \( P \) is the algal density in the presence (+\( N \)) and absence (−\( N \)) of neighbors; \( X \) is \( P_{-N} \) when \( P_{-N} \) is greater than \( P_{+N} \); and \( X \) is \( P_{+N} \) when \( P_{+N} \) is greater than \( P_{-N} \). The RNE is positive when the interaction is competitive, and a relatively low RNE indicates competitive dominance.

We used an analysis of variance (ANOVA) followed by Tukey’s honestly significant difference (HSD) test to test the effects of \( CO_2 \) and species on the RNE value. An ANOVA followed by Tukey’s HSD test was used to test the effects of measurement time (length of growth time), interspecific interaction, \( CO_2 \), and species on growth rate of algae. The significance level was set at 0.05. These analyses were performed using SPSS 22.0 (IBM, USA).

2.2 | Model

Our results and previous studies suggested that different species responded differently to increased atmospheric \( CO_2 \) concentrations, even though they belonged to the same taxon (Ji et al., 2017; Sandrini et al., 2016). To explore the mechanism of this difference, a model was developed to simulate whether interspecific differences in carbon absorption capacity determine the response of algal competitive advantage to elevated atmospheric \( CO_2 \) concentration. According to the carbon absorption capacity, algal species can be divided into species with high affinity for both \( CO_2 \) and \( HCO_3^- \) (HCHH); species with high affinity for \( CO_2 \) and low affinity for \( HCO_3^- \) (HCLH); species with low affinity for \( CO_2 \) and high affinity for \( HCO_3^- \) (LCHH); species with low affinity for both \( CO_2 \) and \( HCO_3^- \) (LCLH).

Based on previous studies (Anazawa, 2012; Ji et al., 2017; Lindberg & Collins, 2020; Schippers, Mooij, et al., 2004a; Verspagen et al., 2014), 15 equations were used to construct the model. The environmental conditions set by the model were basically consistent with the experimental conditions.

The \( CO_2 \) in atmosphere enters the water through air-water exchange. The \( CO_2 \) flux across the air–water interface depends on the difference in partial pressure:

\[ f_t = (pCO_{2a} - pCO_{2w}) \times k_0 \times E \]  

(3)

\( f_t \) is the \( CO_2 \) flux per unit area of air–water interface at time \( t \); \( pCO_{2a} \) is the partial pressure of \( CO_2 \) in atmosphere; \( pCO_{2w} \) is the partial pressure of \( CO_2 \) in water; \( k_0 \) is the solubility of carbon dioxide gas, i.e. Henry constant; and \( E \) is the gas change rate.

After \( CO_2 \) enters the medium, the chemical equilibrium which is \( CO_2 + H_2O\rightarrow H_2CO_3=H^++HCO_3^-\rightarrow H^++CO_3^{2-} \) would change, resulting in the decrease of \( pH \) in water. Studies have shown that water \( \Delta p \) will decrease by about 0.01 units for each increase of 1 Pa of \( PCO_2 \), so water \( \Delta p \) is related to the partial pressure of \( CO_2 \) in water:

\[ pH_t = pH_0 - \Delta pCO_{2w} \times B \times 0.01 \]  

(4)

\( pH_t \) and \( pH_0 \) are \( pH \) values at time \( t \) and in initial time, respectively; \( \Delta pCO_{2w} \) is the change in partial pressure of \( CO_2 \) in water; \( B \) is the cushion coefficient.

The concentration of total dissolved inorganic carbon (\( DIC = CO_2 + HCO_3^- + CO_3^{2-} \)) in water changes with the amount of \( CO_2 \) entering the water. At the same time, algal growth will absorb \( CO_3^- \) and \( HCO_3^- \) in water, and algal respiration will release \( CO_2 \). These processes also change the \( DIC \) concentration. Therefore, the variation of \( DIC \) concentration with time can be expressed as:

\[ \frac{dDIC}{dt} = \frac{f_t}{z} - \sum_{i=1}^{n} (u_{1,i} + u_{2,i})X_{i,t} + \sum_{i=1}^{n} r_{i,t}X_{i,t} \]  

(5)

\( z \) is the depth of water column, \( f \) division by \( z \) converts the flux per unit surface area into the corresponding change in \( DIC \) concentration; \( u1 \) and \( u2 \) are uptake of dissolved \( CO_2 \) and \( HCO_3^- \) by the photosynthetic activity of the algae community, respectively (as calculated by Equations 8 and 9); \( r \) is the respiration rate (as calculated by Equation 11); \( X \) is population density of algae (as calculated by Equation 13); \( s \) is the algae species, \( n \) is the number of species, when \( n = 1 \), it means that there is only one species, that is, it simulates the situation of monoculture, and \( s = 1 \); when \( n = 2 \), it means that the simulated situation is mixture culture, and \( s = 1 \) or 2.

According to the equilibrium dissociation of \( DIC (CO_2 + HCO_3^- + CO_3^{2-}) \) components, changes in the concentration of dissolved \( CO_2 \) and \( HCO_3^- \) are described by:

\[ [CO_2] = \frac{[H^+]^2 \times [DIC]}{[H^+]^2 + k_1[H^+] + k_2} \]  

(6)

\[ [HCO_3^-] = \frac{k_1[H^+] \times [DIC]}{[H^+]^2 + k_1[H^+] + k_2} \]  

(7)
$k_1$ and $k_2$ are the equilibrium dissociation constants of $\text{CO}_2$ and $\text{HCO}_3^-$, respectively.

The uptake rate of dissolved $\text{CO}_2$ and $\text{HCO}_3^-$ by the photosynthetic activity of the algae community in Equation 5 depends on the substrate concentration and the affinity and flux rate of species $s$ to the substrate (Here, affinity and flux rates are quantified by half-saturation constant and maximum absorption rate, respectively. Half-saturation constant are the substrate concentrations required to reach half of the maximum absorption rate. The higher half-saturation constant is, the worse the substrate capture ability of the binding site on the transporter is, so it is inversely proportional to affinity. The maximum absorption rate is the substrate absorption rate of species when the binding site on the transporter is saturated, and the maximum absorption rate is proportional to the flux rate), as well as the intensity of light and the carbon contents in the cell:

$$u_{1,s,t} = \frac{u_{1,\text{max},s} \times [\text{CO}_2]_t}{H_{1,s} + [\text{CO}_2]_t} \times P_t \times \frac{1 - Q_{\text{t},s,t}}{Q_{\text{max},s,t}}$$

(8)

$$u_{2,s,t} = \frac{u_{2,\text{max},s} \times [\text{HCO}_3^-]_t}{H_{2,s} + [\text{HCO}_3^-]_t} \times P_t \times \frac{1 - Q_{\text{t},s,t}}{Q_{\text{max},s,t}}$$

(9)

$u_{1,\text{max},s}$ and $u_{2,\text{max},s}$ are the maximum absorption rate of species $s$ to $\text{CO}_2$ and $\text{HCO}_3^-$ respectively; $H_{1,s}$ and $H_{2,s}$ are the half-saturation constants of species $s$ to $\text{CO}_2$ and $\text{HCO}_3^-$ respectively; $P$ is the photosynthetic rate; $Q_t$ is the cellular carbon content; and $Q_{\text{max},s,t}$ is the maximum amount of carbon that can be stored in its cell. The cellular carbon content is proportional to the growth rate and respiration rate:

$$g_{s,t} = g_{\text{max},s,t} \times \frac{Q_{\text{t},s,t}}{Q_{\text{max},s,t}}$$

(10)

$$r_{s,t} = r_{\text{max},s,t} \times \frac{Q_{\text{t},s,t}}{Q_{\text{max},s,t}}$$

(11)

$g_{s,t}$ and $r_{s,t}$ are the growth rate and respiration rate of species $s$, respectively; $g_{\text{max},s,t}$ and $r_{\text{max},s,t}$ are the maximum growth rate and the maximum respiration rate of species $s$, respectively. At the same time, the carbon absorption process of algae increases the amount of carbon in cells, and the growth and respiration of algae consumes carbon in cells, and these processes determine the change of cellular carbon content:

$$\frac{dQ_t}{dt} = u_{1,s,t} + u_{2,s,t} - r_{s,t} - g_{s,t}$$

(12)

With the propagation of algae, the population density becomes larger. The change of the population density of algae over time is as follows:

$$\frac{dX_s}{dt} = g_{s,t}(1 - m)X_s \left(1 - \frac{X_s}{C}\right)$$

(13)

$m$ is the mortality rate, and $C$ is the environmental capacity. After the population density in monoculture and pairwise competition experiments are calculated, the competitive ability of each species is calculated by RNE (Equation 2).

The continuous increasing of population density may cause a self-shading effect that affects light intensity, and the photosynthetic rate at average depth can be expressed as the average of the photosynthetic rate at all depths:

$$P_t = \frac{1}{Z} \int_0^Z P(z, I_z)dz$$

(14)

$I_t$ is light intensity, and the notation $P(I(z))$ indicates that the photosynthetic rate is a function of the local light intensity $I$, which in turn is a function of depth $z$. $P(I)$ and $I(z)$ are represented by the equations:

$$P(I) = \frac{P_{\text{max},I}}{P_{\text{max},I} + I}$$

(15)

$$I(z) = I_0 \exp(-k_{bg}z - k_{e}z)$$

(16)

where $P_{\text{max},I}$ is the maximum photosynthetic rate; $\alpha$ is the slope of the $p(l)$ curve at $l = 0$; $I_0$ is the incident light intensity at the top of the column; $k_{bg}$ is the background turbidity of the medium; and $k$ is the specific light attenuation coefficient of an algae cell.

The ten levels of atmospheric $\text{CO}_2$ concentration were 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 ppm. The concentration of $\text{CO}_2$ in the atmosphere is expected to rise from current levels of 380 ppm to 1000 ppm within the next century (Bulling et al., 2010). In addition, $\text{CO}_2$ in freshwater ecosystems does not only originate from dissolution of atmospheric $\text{CO}_2$ but also from mineralization of organic carbon obtained from terrestrial sources in the surrounding watershed (Verspagen et al., 2014). Therefore, a large range of $\text{CO}_2$ concentration level was set, that is, 200–2000 ppm.

As mentioned above, algae species were classified into four kinds based on their affinity and flux rate for $\text{CO}_2$ and $\text{HCO}_3^-$, and affinity and flux rate were quantified by half-saturation constant and maximum absorption rate, respectively. The corresponding parameter settings of each kind of algae are: HCHH: $H1 = 1$, $H2 = 30$, $u_{1,\text{max}} = 0.2$, $u_{2,\text{max}} = 0.2$; HCLH: $H1 = 1$, $H2 = 1200$, $u_{1,\text{max}} = 0.2$, $u_{2,\text{max}} = 0.4$; LCHH: $H1 = 40$, $H2 = 30$, $u_{1,\text{max}} = 0.4$, $u_{2,\text{max}} = 0.2$; LCLH: $H1 = 40$, $H2 = 1200$, $u_{1,\text{max}} = 0.4$, $u_{2,\text{max}} = 0.4$. Since the concentration of $\text{HCO}_3^-$ in fresh water is generally much higher than that of $\text{CO}_2$, the affinity for $\text{CO}_2$ over $\text{HCO}_3^-$ is assumed to be one order of magnitude higher. The values of other performance parameters of algae and environmental conditions are same among algal species (Table 1). The model was run over 1000 time-steps, such that the algal community stabilized by the end of the run.

R 3.5.1 was used to run the simulation. An ANOVA followed by Tukey’s HSD test was used to test the effects of $\text{CO}_2$ and species on the RNE values. An ANOVA followed by Tukey’s HSD test was used to test the effects of measurement time (length of growth time), interspecific interaction, $\text{CO}_2$, and species on growth rate. The significance level was set at 0.05. The statistical analyses were performed using SPSS 22.0 (IBM, USA).
### RESULTS

In the experiments, the cell density of all algal species increased significantly when the atmospheric CO\(_2\) concentration increased from 400ppm to 760ppm (Table S1; Figure 1). At 400ppm, the algae could be ranked according to cell density: *Phormidium* sp. > *C. vulgaris* > *S. quadricauda* > *Synebra ulna*; at 760ppm, the algae could be ranked according to cell density: *S. quadricauda* > *Phormidium* sp. > *C. vulgaris* > *S. ulna* (Figure 1).

According to the calculation method of RNE, the RNE value of *S. quadricauda* reflected the potential decrease in cell density when *S. quadricauda* cultured with *C. vulgaris*, *Phormidium* sp. and *S. ulna* comparing to *S. quadricauda* cultured alone, and the same behavior mostly occurred when the other three species compete in pairs, except for the potential increase in cell density when *C. vulgaris* cultured with *S. quadricauda* and *S. ulna*, and *Phormidium* sp. cultured with *S. ulna* at 400ppm.

A positive value of RNE reflected the decrease cell density and therefore indicated the interspecific competition; a negative value of RNE reflected the increase cell density and therefore indicated the interspecific facilitation. The RNE values of *S. quadricauda* in mixture with other three species were all positive along the CO\(_2\) gradient, and the same pattern was observed for *S. ulna* in mixture with other three species, *C. vulgaris* in mixture with *Phormidium* sp., *Phormidium* sp. in mixture with *S. quadricauda* and *C. vulgaris*, respectively (Figure 2), indicating that the interaction between *S. quadricauda* and other three species, *S. ulna* and other three species, *C. vulgaris* and *Phormidium* sp., *Phormidium* sp. and *S. quadricauda*, *Phormidium* sp. and *C. vulgaris* were interspecific competition. When *C. vulgaris* in mixture with *S. quadricauda* and *S. ulna*, respectively, and *Phormidium* sp. in mixture with *S. ulna*, the RNE values changed from negative to positive along the CO\(_2\) gradient (Figure 2), indicating that the interaction between *C. vulgaris* and *S. quadricauda*, *C. vulgaris* and *S. ulna*, and *Phormidium* sp. and *S. ulna* was interspecific facilitation.

Differences in RNE among species indicated that at low atmospheric CO\(_2\) concentration (400ppm), the algae were ranked according to the competitive ability: *C. vulgaris* > *Phormidium* sp. > *S. quadricauda* > *Synebra ulna* (Figure 2). When the CO\(_2\) concentration increased to 760ppm, the algae were ranked according to their competitive ability: *S. quadricauda* > *Phormidium* sp. > *C. vulgaris* > *S. ulna* (Table 2; Figure 2).

The simulation results showed that the cell density of all four algae increased significantly with the increase of CO\(_2\) concentration (Table S2; Figure 3). At 400ppm, the algae could be ranked according to cell density: HCHH > HCLH > LCHH > LCLH; at 1200ppm, the algae could be ranked according to cell density: HCLH > HCHH > LCLH > LCHH; at 2000ppm, the algae could be ranked according to cell density: LCHH > LCLH > HCHH > HCLH (Figure 3).

According to the calculation method of RNE, the RNE values of the HCHH species reflected the potential decrease in cell density when the HCHH species grew with other three species comparing to HCHH species grew alone, and the same behavior occurred when the other three species competed in pairs. The RNE values of the four species growing in pairs were all positive on the CO\(_2\) gradient (Figure 4), indicating that the interaction of the four species growing in pairs were interspecific competition. The differences in RNE among species showed that when the CO\(_2\) concentration was low (200–1600ppm), the algae were ranked according to the competitive ability: HCHH > HCLH > LCHH > LCLH; when the CO\(_2\) concentration was high (1800–2000ppm), the algae were ranked according to the competitive ability: LCHH > LCLH > HCHH > HCLH (Table 3; Figure 4).

With the increase of atmospheric CO\(_2\) concentration, the CO\(_2\) concentration in water increased significantly; when the HCHH, HCLH and LCLH species were mixed in pairs, the HCO\(_3^-\) concentration first increased and then decreased, and when the LCHH species and other species are mixed in pairs, respectively, the HCO\(_3^-\) concentration increased significantly (Table 4; Figure 5).

### DISCUSSION

Our study showed that the competitive ability of algae changed differently when CO\(_2\) increased from 400 to 760ppm, and the
FIGURE 1 Effects of time, atmospheric CO$_2$ concentration, and competition on the density of *Phormidium* sp., *Scenedesmus quadricauda*, *Chlorella vulgaris* and *Synedra ulna* over time in the experiments. Figures (a)-(f) are the algal density in the pairwise competition experiments when CO$_2$ concentration was 400 ppm; figures (g)-(l) are the algal density in the pairwise experiments when CO$_2$ concentration was 760 ppm. Standard errors of three replicates are shown.
different changes of competitiveness among algae species along the gradient of atmospheric CO$_2$ concentration was due to the interspecific differences in affinity and flux rate for CO$_2$ and HCO$_3^-$.

These results provide an important perspective for understanding and predicting the changes of population dynamics and community composition of algae under the background of increasing global atmospheric CO$_2$.

The results of experiments showed that when the CO$_2$ concentration increases from 400 to 760 ppm, the competitiveness of S. quadricauda increased, the competitiveness of Phormidium sp. and C.
vulgaris decreased, and the competitive dominant species changed from C. vulgaris to S. quadricauda. Thus, the competitive ability of different algae species responded differently to the increase of atmospheric CO₂ concentration, even though they belonged to the same taxa (both C. vulgaris and S. quadricauda belonged to green algae). Other ecologists have also shown that the competitiveness of
Our simulation results showed that the reason for this difference of competitiveness is that algae have different absorption capacity for CO$_2$ and HCO$_3^-$, that is, different affinity and flux rates for CO$_2$ and HCO$_3^-$. Affinity and flux rate are the capture capacity and transport capacity of substrate, respectively, which are inversely proportional to each other. Low resource concentration is beneficial to the growth and reproduction of algae with high affinity and high resource concentration is beneficial to the growth and reproduction of algae with high flux rate. According to the carbon absorption capacity of algae, algae are divided into four types: HCHH species with high affinity for both CO$_2$ and HCO$_3^-$; HCLH species with high affinity for CO$_2$ and low affinity for HCO$_3^-$; LCHH species with low affinity for CO$_2$ and high affinity for HCO$_3^-$; LCLH species with low affinity for both CO$_2$ and HCO$_3^-$. 

The increase of atmospheric CO$_2$ concentration affected the competitiveness of algae with different carbon absorption capacity by affecting the carbon balance in freshwater ecosystem. When the atmospheric CO$_2$ concentration is low, both the CO$_2$ and HCO$_3^-$ in water are low, then the species with high affinity for both CO$_2$ and HCO$_3^-$ had the highest competitiveness. When atmospheric CO$_2$ increases, CO$_2$ in water increases rapidly, while HCO$_3^-$ increases slowly or even decreases due to the decrease of pH. On this condition, the species with low affinity for CO$_2$ and high affinity for HCO$_3^-$ would be dominant. Thus, with the increase of atmospheric CO$_2$ concentration, the dominant species changed from HCHH species to LCHH species.

Ji et al. investigated the competitive relationship between a harmful cyanobacteria and three green algae at low and high CO$_2$ concentrations. The results showed that two of the green algae were competitively superior to the cyanobacteria at low CO$_2$, whereas the competitive ability of cyanobacteria increased compared to the green algae at high CO$_2$ (Ji et al., 2017). Sandrini et al. showed that the increased CO$_2$ availability will be beneficial for the low affinity but high flux bicarbonate absorption system, and cyanobacteria with this absorption system are likely to become the main component of cyanobacteria bloom in the future (Sandrini et al., 2016). These results imply that the carbon absorption capacity is the root cause for interspecific differences in competitiveness of algae.

Since cyanobacteria bloom has become a major water quality problem in many eutrophic lakes around the world, previous studies mostly focused on the change of competitive advantage between cyanobacteria and eukaryotic algae (Bestion et al., 2018; Huisman et al., 2018; Ji et al., 2017; Ma et al., 2019). The traditional view is that rising CO$_2$ levels will particularly benefit eukaryotic phytoplankton species rather than cyanobacteria because cyanobacteria have developed an efficient CO$_2$ concentration mechanism (CCM) to adapt to the low CO$_2$ environment (Badger & Price, 2003; Huisman et al., 2018; Ma et al., 2019; Wolf et al., 2019). However, with the in-depth study, researchers found that eukaryotic algae also have a complex CCM mechanism to adapt to low CO$_2$ concentration (Giordano et al., 2005; Ji et al., 2017). In addition, recent studies have found that some cyanobacteria have low affinity but high

| Source          | CO$_2$   | HCO$_3^-$ |       |       |
|-----------------|---------|-----------|-------|-------|
|                 | df      | F         | p     | df    | F     | p     |
| Species         |         |           |       |       |
|                 | 5       | 56.20     | <.001 | 5     | 873.15 | <.001 |
| CO$_2$          | 9       | 857.27    | <.001 | 9     | 2392.82 | <.001 |
| Species × CO$_2$| 45      | 27.05     | <.001 | 45    | 127.06 | <.001 |

Note: p < .05 is taken to be significant.
flux bicarbonate absorption system to adapt to the high CO₂ concentration (Sandrini et al., 2014, 2016; Visser et al., 2016). Thus, the carbon absorption capacity of algae is an important attribute to predict its response to elevated CO₂.

In addition to the response of algal growth to atmospheric CO₂ concentration, our model also includes the influence of the photosynthesis and respiration of algae on the change of inorganic carbon concentration in water (Equation 5). The algal communities may influence CO₂ emissions into the atmosphere and thus feedback on the ongoing and future climate change (Lewington-Pearce et al., 2020). However, the interaction between algal growth and CO₂ concentration has not been fully studied. Therefore, the importance of aquatic plants in the global carbon cycle should be considered in future studies on the response of aquatic plants to climate change, to predict the trend of future climate change and the response mechanism of growth of aquatic plants more comprehensively.

5 | CONCLUSION

This study highlights the importance of carbon absorption capacity in understanding, predicting and regulating population dynamics and community composition of algae. According to the carbon absorption capacity, algae species can be classified as HCHH, HCLH, LCHH and LCLH species. Whether cyanobacteria or eukaryotes, HCHH species should be paid more attention at low CO₂ levels; while LCHH species should be paid more attention at high CO₂ levels. These results help understanding algal population dynamics and community composition along environmental gradients, predicting bloom causing species under the background of increasing global atmospheric CO₂, and providing an important basis for maintaining the health of aquatic ecosystem.

AUTHOR CONTRIBUTIONS

Qing Shi Zhou: Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); software (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal). Yang Gao: Data curation (equal); funding acquisition (equal); investigation (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal). Jing Ming Hou: Data curation (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal). Tian Wang: Conceptualization (equal); formal analysis (equal); methodology (equal); software (equal); visualization (equal). Long Tang: Conceptualization (equal); formal analysis (equal); funding acquisition (equal); project administration (equal); validation (equal); writing – original draft (equal); writing – review and editing (equal).

ACKNOWLEDGMENTS

Funding – The National Natural Science Foundation of China (no. 31670548, no. 31872032 and no. 31500340) and the Fundamental Research Funds for Central Universities.

CONFLICT OF INTEREST

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Data available from the Dryad Digital Repository (https://doi.org/10.5061/dryad.d7wm37q40).

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How to cite this article: Zhou, Q. S., Gao, Y., Hou, J. M., Wang, T., & Tang, L. (2022). Preference of carbon absorption determines the competitive ability of algae along atmospheric CO₂ concentration. *Ecology and Evolution*, 12, e9079. https://doi.org/10.1002/ece3.9079