Calcium concentration regulates interspecific interaction of algae along the nutrient gradient

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Abstract

Although many studies have focused on the influence of abiotic stress gradients on biological interactions, few studies explained the effects of both resource and non-resource stress on the facilitation. We conducted a controlled experiment to study the effects of nutrient stress on the interspecific between *Phormidium corium* and *Scenedesmus quadricauda* along calcium gradient. The results showed that when the calcium concentration was high, the competitive interaction changed into positive interaction along the nutrient stress. In the stress environment, the population density is low and the encounter rate is low. Moreover, facilitation may improve the harsh environment and make it more beneficial to the beneficiary species. When the calcium concentration was low, the facilitation reached the peak under medium resource stress. This phenomenon may be caused by the interaction between salt stress and nutrient. When calcium stress was high, it may not be conducive to the absorption of nutrients by algae. While the facilitation effect may be strongest in the medium stress environment due to the relatively sufficient resource. These results emphasized the importance of non-resource stress in interspecific relationships, pointed out a new direction for the study of facilitation, and provided theoretical guidance for the prevention of freshwater blooms.

Highlights

Calcium concentration affected the response of interspecific relationship of algae to nutrient stress.

When calcium stress was low, the relationship between P. corium and S. quadricauda changed from competitive interaction to facilitation with the increase of nutrient stress gradient.

When the calcium stress was high, the facilitation reached the peak under medium nutrient stress.

Introduction

Ecologists generally believe that abiotic stress and biological interactions (competition and facilitation) are important factors affecting biological distribution, thus it is the basic goal of ecologists to study how biological interaction responds to the changes of abiotic stress gradient (Bennett et al. 2015; Chaieb et al. 2020; Qi et al. 2018). Previous studies mostly focused on the relative importance of negative interaction (competition) in community construction (Abbas et al. 2015; Mellard et al. 2012; Sauter et al. 2021; Tilman 1990). However, positive interaction (facilitation) is gradually considered to be an important mechanism of community construction by affecting species diversity and ecosystem function (Adams et al. 2022; He et al. 2013; Maestre and Cortina 2004). Stress gradient hypothesis (SGH) predicts that with the increase of stress gradient, competitive interaction turns into positive interaction due to the amelioration of environmental stress or consumer defense (Bertness and Callaway 1994). Although many empirical and model studies supported SGH theory, there are still a few researches that are inconsistent with this theory (Holmgren and Scheffer 2010; Kawai and Tokeshi 2007; Maestre and Cortina

2004). Previous studies mostly considered only one stress factor, and most of them were resource stress, ignoring the common effects of resource and non-resource stress gradient on biological interaction.

We predict that the response of biological interaction to resource stress gradient may be regulated by non-resource factors. When non-resource factors are sufficient, biological interaction is only affected by resource stress. At this time, the response of biological interaction to resource stress gradient should consistent with the predictions of SGH theory, that is, with the increase of resource stress gradient, competitive interaction turns into positive interaction. When the degree of non-resource stress is high, the species interaction is jointly affected by two stress factors. At this time, with the increase of resource stress gradient, the interspecific relationship of species becomes more complex, and the facilitation of species along stress gradient may be unimodal.

Cyanobacterial blooms have become a major water quality problem in many eutrophic lakes worldwide. They can produce a variety of toxins, causing liver, digestive and neurological diseases (Ji et al. 2017). Thus, the understanding of the impact of environmental conditions on the interspecific relationship between cyanobacteria and eukaryotic phytoplankton is of great significance. Nutrient is the important resource factor for algae growth (Mellard et al. 2012; Zhang et al. 2020). Many studies have been carried out on the response of algal competition to nutrient gradient. Bacillariophyta demonstrate a stronger affinity for nitrate nitrogen absorption, while *Microcystis* shows some preference for ammonium/nitrate nitrogen (Fogg 2001). However, few studies pay attention to the impact of non-resource stress gradient on algal interspecific relationship. Some studies have found that salt stress has an interactive effect with nutrient factors, which affects nutrient absorption (Adenan et al. 2013; Garcia-Sanchez et al. 2000; Sordet et al. 2014; Takami et al. 2012).

However, studies on the effects of salinity stress on algal community composition mostly focused on the role of sodium ions. Many inland fresh waters have high calcium hardness, but the freshwater ecological effect of calcium concentration change has not been paid attention to (Chen et al. 2015). In inland waters, the calcium hardness is usually higher in summer and autumn due to the more active biological activity and organic matter decomposition, as well as increased water evaporation. Conversely, it is relatively lower in winter and spring, as the biological activity and organic matter decomposition in the water are relatively lower, while precipitation increases (Shi et al. 2012). The calcium concentration in water is closely related to the seasonal growth of species. The change of calcium concentration can not only directly cause the change of water hardness, but also affect the physiological characteristics of algae, such as osmotic potential, activities of photosynthetic enzyme and respiratory enzyme, antioxidant enzyme activities, and even affect the interspecific competitiveness by affecting the resource capture rate of algae, and thus affect the community composition of algae. Therefore, studying the effects of calcium concentration and nutrient on algae competition is of great significance for maintaining the health of freshwater ecosystem.

In this study, a controlled experiment was conducted to study the effects of total nitrogen concentration (the oligotrophic, mesotrophic and eutrophic condition was 0.83, 8.26 and16.51 mg/L, respectively) on

the interspecific relationship between *Phormidium corium* and *Scenedesmus quadricauda* under different calcium concentration (the low and high calcium concentration was 10 and 100 mg/L, respectively). *Phormidium corium* and *Scenedesmus quadricauda* are commonly found Cyanophyta and green algae, respectively, in inland water bodies. Studying the interspecific relationship between these two algae is of great significance for maintaining the structure of algal communities and preventing water blooms (Garg et al. 2002; Gaysina et al. 2019). The purpose is to understand and predict the response of biological interaction to abiotic stress, refine SGH theory, and provide theoretical guidance for the prevention and control of freshwater bloom.

Materials and Methods

To study the response of algal growth and competition to total nitrogen and calcium concentrations, a three-factor design with 3 replications was used. The factor species were Cyanophyta, *Phormidium corium*; Chlorophyta, *Scenedesmus quadricauda*. Culture treatments were monoculture and mixture of two species. The calcium concentration was regulated by $CaCl_2{\cdot}2H_2O$, and the nutrient concentration was regulated by $NaNO_3$.

Algae were purchased from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (http://algae.ihb.ac.cn/) and cultured to the required number (> 10^7 Cells/L) in a biochemical incubator (BJPX-150, Biobase, China) for use as original algae samples. The two types of algae were transferred into conical flasks under aseptic conditions, and the bottle mouths are sealed with sealing film. They were cultured for 5 days in a light culture chamber, with BG11 as the culture medium, culture temperature of 25°C, light intensity of 2000lx, and light-dark cycle ratio of 12h:12h.

Other nutrients in the medium are sufficient for maintaining the photosynthesis and growth of algae. The medium was configured according to the composition and concentration of BG11 (0.04 g/L K_2HPO_4, 0.1 g/L $MgSO_4{\cdot}7H_2O$, 0.04 g/L $CaCl_2{\cdot}2H_2O$, 0.006 g/L Citric acid, 0.006 g/L Ammonium ferric citrate, 0.001 g/L $EDTANa_2$, 0.1 g/L Na_2CO_3, 1 mL/L A5 trace element solution) to ensure adequate nutrient and trace elements. 0.1mL solution was removed from each sampling and some might evaporate. In order to keep the water level relatively constant as well as maintain a constant supply of nutrients into the system, we injected nutrients back into the system.

400mL medium was placed in a 500mL beaker, and the inoculation density of each algae sample in each beaker was 2×10^4 Cells/L. A total of 54 beakers were used: 18 beakers of monoculture *P. corium*, 18 beakers of monoculture *S. quadricauda*, and 18 beakers of *P. corium* in mixture with *S. quadricauda*. All the beakers were placed in phytotron for 15 days. Control the lighting to 4000 lux, temperature to 25°C, and apply a 0.45μm filter on the lid of the beaker to prevent excessive loss of moisture through evaporation. Then the nutrient and calcium treatment were applied. Shake manually 1–2 times per day.

54 beakers of materials were divided into 6 groups, each including three beakers of monoculture *P. corium*, three beakers of monoculture *S. quadricauda* and three beakers of *P. corium* in mixture with *S.*

quadricauda. Each group was treated with different total nitrogen and calcium levels: the oligotrophic, mesotrophic and eutrophic condition was 0.83, 8.26 and16.51 mg/L, respectively; the low and high calcium concentration was10 and 100 mg/L, respectively.

After full stirring, 0.1ml solution from each sample were taken and injected into 0.1ml 20mm×20mm counting chamber. The field view method was used to calculate the density of algae, and the formula was as follows:

$$\mathrm{N} = \mathrm{n} \times \frac{A}{A_c \times V}$$

1

where N is the density of algae, cells/L; n is the number of algal cells counted, cells; A is the area of the counting chamber, mm^2; A_c is the counting area, that is, field area × field number, mm^2; V is the volume of the counting chamber, L.

The difference form of Lotka-Volterta competition model is used to calculate the mixed culture of two algae species, and the formula is as follows:

$$\frac{\mathrm{N_{a_n}\text{-}N_{a_{n\text{-}1}}}}{\mathrm{t_n\text{-}t_{n\text{-}1}}} = \frac{\mathrm{r_a N_{a_{n\text{-}1}}\left(K_a\text{-}N_{a_{n\text{-}1}}\text{-}\alpha N_{b_{n\text{-}1}}\right)}}{\mathrm{K_a}} \quad \frac{\mathrm{N_{b_n}\text{-}N_{b_{n\text{-}1}}}}{\mathrm{t_n\text{-}t_{n\text{-}1}}} = \frac{\mathrm{r_b N_{b_{n\text{-}1}}\left(K_b\text{-}N_{b_{n\text{-}1}}\text{-}\beta N_{a_{n\text{-}1}}\right)}}{\mathrm{K_b}}$$

2

where Na_n and Nb_n are the density of algae *a* and algae *b* at time t_n in mixture treatment, respectively; Na_{n-1} and Nb_{n-1} are the density of algae *a* and algae *b* at time t_{n-1} in mixture treatment, respectively; r_a and r_b are the intrinsic growth rates of algae *a* and algae *b* in monoculture treatment, respectively; K_a and K_b are the maximum environmental capacity of algae *a* and algae *b* in monoculture treatment, respectively; α is the competition inhibition parameters of algae *b* to algae *a*; β is the competition inhibition parameters of algae *a* to algae *b*.

Logistics regression test was used to fit the growth curve of algae in monoculture and pairwise competition experiments at each nutrient and calcium level. Repeated measures ANOVA followed by a Tukey's HSD test was used to analyze the effects of calcium concentration and nutrient on growth and competitiveness of *P. corium* and *S. quadricauda*. The significance level was set at 0.05. These analyses were performed using SPSS 22.0 (IBM, USA).

Results

In monoculture experiment, under all culture conditions, the cell density of two algae species increased significantly with time ($P < 0.05$), and the calcium concentration, nutrient concentration and the interaction of the two factors had significant effects on the cell density of the two algae (Table 1; Fig. 1).

When the nutrient concentration was low, the cell density of *S. quadricauda* was higher than that of *P. corium* regardless of the high or low calcium concentration; when the nutrient concentration is medium and high, the cell density of *P. corium* was higher than that of *S. quadricauda* whether the calcium concentration was high or low (Fig. 1).

Table 1
Summary of repeated measurements variance of the effects of time, calcium concentration and nutrient concentration on cell density of *Phormidium corium* and *Scenedesmus quadricauda* in monoculture experiments

Algal species	Effects	Source	*d.f.*	F	*p*
Phormidium corium	Within-subject	Time	12	16574.27	< 0.001
		Time*calcium	12	1192.77	< 0.001
		Time*nutrient	24	4016.08	< 0.001
		Time* calcium * nutrient	24	769.50	< 0.001
	Between-subject	Calcium	1	2834.67	< 0.001
		nutrient	2	10363.86	< 0.001
		Calcium * nutrient	2	1439.34	< 0.001
Scenedesmus quadricauda	Within-subject	Time	12	1831.77	< 0.001
		Time*calcium	12	85.90	< 0.001
		Time*nutrient	24	267.19	< 0.001
		Time* calcium * nutrient	24	31.59	< 0.001
	Between-subject	Calcium	1	1050.22	< 0.001
		nutrient	2	2374.29	< 0.001
		Calcium * nutrient	2	273.72	< 0.001

In monoculture experiment, the growth rates of *P. corium* and *S. quadricauda* decreased with time. When the nutrient concentration was low, the growth rate of both species dropped to around zero or even

negative in early time; when the nutrient concentration was medium, the growth rates of both species dropped to about zero on day 14; when the nutrient concentration was high, the growth rate of both species is basically always greater than zero (Fig. 2).

In mixture experiment, under all culture conditions, the cell density of the two algae increased significantly with time ($P < 0.05$), and the calcium concentration, nutrient concentration and the interaction of the two factors had significant effects on the cell density of the two algae (Table 2; Fig. 3). In addition, when the nutrient concentration was low, the final cell density of *S. quadricauda* was higher than that of *P. corium* whether the calcium concentration was high or low; when the nutrient concentration was medium and high, regardless of the high or low calcium concentration, the cell density of *P. corium* was higher than that of *S. quadricauda* (Fig. 3).

Table 2
Summary of repeated measurements variance of the effects of time, calcium concentration and nutrient concentration on cell density of *Phormidium corium* and *Scenedesmus quadricauda* in mixture experiments

Algal species	Effects	Source	*d.f.*	F	*p*
Phormidium corium	Within-subject	Time	12	112656.40	< 0.001
		Time*calcium	12	6812.98	< 0.001
		Time*nutrient	24	24584.87	< 0.001
		Time* calcium * nutrient	24	2525.00	< 0.001
	Between-subject	Calcium	1	9161.97	< 0.001
		Nutrient	2	31994.37	< 0.001
		Calcium * nutrient	2	3016.49	< 0.001
Scenedesmus quadricauda	Within-subject	Time	12	71941.65	< 0.001
		Time*calcium	12	9818.95	< 0.001
		Time*nutrient	24	13389.24	< 0.001
		Time* calcium * nutrient	24	2761.84	< 0.001
	Between-subject	Calcium	1	5941.04	< 0.001
		Nutrient	2	18893.06	< 0.001
		Calcium * nutrient	2	2316.56	< 0.001

In mixture experiment, the growth rates of *P. corium* and *S. quadricauda* decreased with time. When the nutrient concentration was low, the growth rate of both species dropped to around zero or even negative in early time; when the nutrient concentration was medium and high, the growth rates of both species dropped to about zero on day 14 (Fig. 4).

In monoculture experiment, the final cell density of *P. corium* and *S. quadricauda* increased significantly with the increase of nutrient concentration at low and high calcium concentration (Table 3; Fig. 5).

Table 3
Summary of two–way ANOVA of the effects of calcium concentration and nutrient concentration on final cell density of *Phormidium corium* and *Scenedesmus quadricauda* in monoculture and mixture experiments

Algal species	Culture method	Source	*d.f.*	F	*p*
Phormidium corium	Monoculture	Calcium	1	3232.61	< 0.001
		Nutrient	2	9770.69	< 0.001
		Calcium * nutrient	2	1097.71	< 0.001
	Mixture	Calcium	1	16180.17	< 0.001
		Nutrient	2	35822.41	< 0.001
		Calcium * nutrient	2	8979.84	< 0.001
Scenedesmus quadricauda	Monoculture	Calcium	1	97.90	< 0.001
		Nutrient	2	859.74	< 0.001
		Calcium * nutrient	2	41.98	< 0.001
	Mixture	Calcium	1	23166.80	< 0.001
		Nutrient	2	20362.34	< 0.001
		Calcium * nutrient	2	8583.83	< 0.001

In mixture experiment, at low calcium level, the cell density of *P. corium* and *S. quadricauda* increased significantly with the increase of nutrient level. The cell density of *P. corium* at oligotrophic, mesotrophic and eutrophic conditions were 18.7×10^4, 623×10^4 and 1100×10^4 cells/ml, respectively. In comparison, the cell density of *P. corium* in mesotrophic and eutrophic condition was 3231.55% and 5782.35% higher than that in the oligotrophic condition, respectively (Table 3; Fig. 5). The cell density of *S. quadricauda* in oligotrophic, mesotrophic and eutrophic conditions were 44×10^4, 403×10^4 and 555×10^4 cells / ml, respectively. In comparison, the cell density of *S. quadricauda* in mesotrophic and eutrophic condition was 815.91% and 1161.36% higher than that in the oligotrophic condition, respectively (Table 3; Fig. 5).

In mixture experiment, under the condition of high calcium, the cell density of *P. corium* and *S. quadricauda* first increased and then decreased with the increase of nutrient level. The cell density of *P. corium* under oligotrophic, mesotrophic and eutrophic conditions were 41.1×10^4, 437×10^4 and 403×10^4 cells/mL, respectively. In comparison, the cell density of *P. corium* in mesotrophic and eutrophic condition was 963.26% and 880.54% higher than that in the oligotrophic condition, respectively (Table 3; Fig. 5). The cell density of *S. quadricauda* in oligotrophic, mesotrophic and eutrophic conditions were 44.8×10^4, 220×10^4 and 131×10^4 cells / ml, respectively. In comparison, the cell density of *S. quadricauda* in mesotrophic and eutrophic condition was 8391.07% and 192.41% higher than that in the oligotrophic condition, respectively (Table 3; Fig. 5).

At low calcium level, with the increase of nutrient level, the competitive inhibition parameters of *P. corium* increased significantly, while the competitive inhibition parameters of *S. quadricauda* increased first and then decreased (Table 4; Fig. 6). Under oligotrophic and mesotrophic conditions, the competitive inhibition parameters of *S. quadricauda* to *P. corium* were less than 0, and this competitive parameters under eutrophication condition were greater than 0, indicating that *S. quadricauda* could facilitate the growth of *P. corium* at low and medium nutrient concentrations, while *S. quadricauda* would inhibit the growth of *P. corium* at high nutrient concentration, and the lower the nutrient concentration, the stronger the facilitation effect was (Table 4; Fig. 6). Under oligotrophic and eutrophication conditions, the competitive inhibition parameters of *P. corium* to *S. quadricauda* were less than 0, while in mesotrophic condition, this competitive inhibition parameter was greater than 0, which indicated that *P. corium* would facilitate the growth of *S. quadricauda* at low and high nutrient concentrations, and the facilitation effect was larger at low nutrient concentration, while the growth of *S. quadricauda* was inhibited by *P. corium* at medium nutrient concentration (Table 4; Fig. 6).

Table 4
Summary of two–way ANOVA of the effects of calcium concentration and nutrient concentration on competition inhibition parameters of *Phormidium corium* and *Scenedesmus quadricauda* in mixture experiments

Algal species	Source	*d.f.*	F	*p*
Phormidium corium	Calcium	1	11319.19	< 0.001
	Nutrient	2	2319.05	< 0.001
	Calcium * nutrient	2	1779.14	< 0.001
Scenedesmus quadricauda	Calcium	1	6663.43	< 0.001
	Nutrient	2	13389.24	< 0.001
	Calcium * nutrient	2	1909.02	< 0.001

At high calcium level, the competitive inhibition parameters of both *P. corium* and *S. quadricauda* increased significantly with the increase of nutrient level (Table 4; Fig. 6). Under oligotrophic condition, the competitive inhibition parameter of *S. quadricauda* to *P. corium* was less than 0, while this competitive parameters under mesotrophic and eutrophication conditions were greater than 0, and the competition inhibition parameter value was the largest in eutrophication condition, indicating that *S. quadricauda* could facilitate the growth of *P. corium* at low nutrient concentrations, while *S. quadricauda* would inhibit the growth of *P. corium* at medium and high nutrient concentration, and the inhibition effect increased with the increase of nutrient concentration (Table 4; Fig. 6). Under oligotrophic conditions, the competitive inhibition parameters of *P. corium* to *S. quadricauda* were less than 0, while in mesotrophic and eutrophication conditions, this competitive inhibition parameter was greater than 0, and the competition inhibition parameter value was the largest in eutrophication condition, which indicated that *P. corium* would facilitate the growth of *S. quadricauda* at low nutrient concentration, while the growth of *S.*

quadricauda was inhibited by *P. corium* at medium and high nutrient concentrations, and the inhibition effect increased with the increase of nutrient concentration (Table 4; Fig. 6).

Discussion

Our results showed that when the concentration of calcium was high, the competitive interaction changed to facilitative interaction with the increase of nutrient stress; when the concentration of calcium was low, the facilitation effect among species increased first and then decreased with the increase of nutrient stress. These results provide a new direction for the refinement of SGH theory and studies on facilitation between species.

The stress gradient hypothesis (SGH) was firstly proposed by Bertness and Callaway (1994), emphasizing the importance of positive interaction in ecology (Bertness and Callaway 1994). In the past two decades, facilitation has become a common research topic and its definition is to improve the growth of beneficiary species without damaging the facilitation species (Brooker et al. 2008; Bruno et al. 2003; Bulleri et al. 2011; Callaway et al. 2002; Chaieb et al. 2020). For example, facilitation is considered to play an important role in biodiversity-ecosystem function and community composition (Cardinale et al. 2002), and positive interspecific interactions have also been shown to affect evolution and coevolution (Kikvidze and Callaway 2009; Lawrence et al. 2012; Lortie 2007). SGH theory hypothesized that with the increase of abiotic stress gradient, the importance of facilitative interaction within community increased relative to competitive interaction (He et al. 2013). If so, in theory, ecosystem function can be maintained within a certain degree even if the environment changes seriously. However, SGH theory was still a controversial theory because many empirical tests support this theory, while some studies showed that it has the greatest facilitation effect under medium stress level, that is, the "Hump hypothesis" (Piccardi et al. 2019; Qi et al. 2018; Rius and McQuaid 2009; Tashiro et al. 2013; Travis et al. 2006; Zhang et al. 2020).

In the mixed culture experiment, there have been differences in competitive advantage among species within the culture time. Different types of algae exhibit different preferences for the uptake and utilization of nutrients. Nutrient concentration also affects the absorption and utilization of nutrients by phytoplankton. Some studies suggest that certain species of blue-green algae, such as Microcystis, exhibit a preference for nitrogen, while the chloroplasts of green algae have a relatively high preference for phosphorus. Therefore, an increase in nitrogen concentration may be more favorable for the growth of blue-green algae, leading to oxygen depletion, food web disruption, toxin production, and suppression of competing groups in freshwater systems.

Our results showed that when the nutrient concentration was low, the growth rate of *S. quadricauda* was the highest under all calcium ions; when the nutrition was sufficient, the growth rate of *P. corium* was the highest whether the calcium concentration was high or low (Fig. 1). This means that *S. quadricauda* has a high tolerance to resource stress; and the resource capture rate of *P. corium* is high. Because interspecific competition tends to favor whichever species grows fastest under the conditions used (Riegman et al., 1996), in mixed cultures, when the nutrient concentration was low, *S. quadricauda* was

dominant; when the nutrient concentration was high, the *P. corium* was dominant (Fig. 3). The excellent competitors with fast growth speed and high capture rate of all resources occupy the high trophic habitat, but their high tolerance to resource demand enable them to survive in the low trophic habitat. When the nutrient concentration was low, the interspecific relationship between *P. corium* and *S. quadricauda* was promoted in the mixed culture. This may be due to the mechanism that under nutrient limitation, the organic nutrients released by the decaying cells of facilitation species can improve the nutritional level of the environment, so as to promote the growth of beneficiary species.

Our results showed that when the calcium concentration was high, that is, the algae species were only affected by nutrient stress, the change of interspecific relationship between the two algae along the stress gradient accorded with SGH theory. There are two main explanations for this result. First, in stressful environment, individuals are unlikely to interact with each other due to density-dependence, because low population density means that the encounter rate is low. In addition, the resources are relatively sufficient, so the interspecific competitive relationship is weak. When the stress is low, the abundance is high and the resources are limited. Therefore, the interspecific competition will be strong (Bertness and Ewanchuk 2002; Callaway and Callaway 2007). Secondly, if facilitation may improve the harsh environment and make it more beneficial to the beneficiary species, which may enable the beneficiary species to survive in the environment that could not exist previously (Bertness and Callaway 1994). Therefore, compared with competitive interaction, facilitative interaction will increase.

When the calcium concentration is low, the interspecific relationship of algal species was affected by two factors. Currently, the change of interspecific relationship along resource stress gradient conforms to the "Hump hypothesis", that is, the facilitation effect reached the peak under medium resource stress. This phenomenon may be caused by the interaction between salt stress and nutrient (Park et al. 2020). For example, the absorption of NO_3^- across the plasma membrane is considered to be the membrane depolarization induced by NO_3^- and the influx of NO_3^- depending on pH value. However, the hydrolysis of salt ions will change the pH of the water, and then destroy the H^+ pump of algae and influence the difference in intracellular and extracellular pH values, resulting in the change of cell membrane potential, and finally affect the absorption of NO_3^- (Garcia-Sanchez et al. 2000; Sordet et al. 2014; Takami et al. 2012). High calcium stress may be detrimental to the absorption of nutrients by algae, resulting in the fierce competition for limited resources in the harsh environment (Garcia-Sanchez et al. 2000; Koropatkin et al. 2007; Holmgren and Scheffer 2010). However, the resource was relatively sufficient in the medium stress environment, thus the facilitation effect may be strongest.

Our research focused on the regulatory effect of calcium concentration on the response of interspecific relationship of algae to nutrient stress, and proposed a possible explanation for this phenomenon. However, the general mechanism of the regulation of non-resource factors on the response of interspecific relationship to resource stress needs to be further studied. Moreover, the effects of other factors caused by changes of calcium and nutrient concentration on algal growth and competition also need to be further studied.

Conclusion

Calcium concentration affected the response of interspecific relationship between *P. corium* and *S. quadricauda* to nutrient stress. In the absence of calcium stress, the relationship between *P. corium* and *S. quadricauda* changed from competitive interaction to facilitation with the increase of nutrient stress gradient; in the presence of calcium stress, the facilitation reached the peak under medium nutrient stress. This study provides a new direction for resolving the controversy between the SGH theory and the " Hump hypothesis ", i.e., the response of interspecific relationships to resource stress is influenced by non-resource levels. These results emphasized the joint action of multiple stress factors, especially the importance of non-resource stress in the study of interspecific relationship, pointed out a new direction for the refinement of SGH theory and studies on facilitation, and provided theoretical guidance for the prevention and control of freshwater bloom.

Declarations

Author contributions

QingShi Zhou: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Validation; Visualization; Writing - original draft; Writing - review & editing. **JingMing Hou**: Conceptualization; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Software; Supervision; Visualization; Writing - original draft; Writing - review & editing. **Yang Gao**: Conceptualization; Data curation; Formal analysis; Software; Supervision; Validation; Visualization; Writing - original draft. **Tian Wang**: Investigation; Resources; Software; Supervision; Validation; Visualization; Writing - review & editing. **BingYao Li**: Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software. **ZhanPeng Pan**: Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization. **XueLiang Sun**: Resources; Software; Supervision; Validation; Visualization. **ChangHui Duan**: Software; Supervision.

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Competing Interest declaration

The authors declare no competing interests.

Data Availability statements

The datasets generated during and analyzed during the current study are available in the Dryad repository, https://doi.org/10.5061/dryad.866t1g1sf.

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Figures

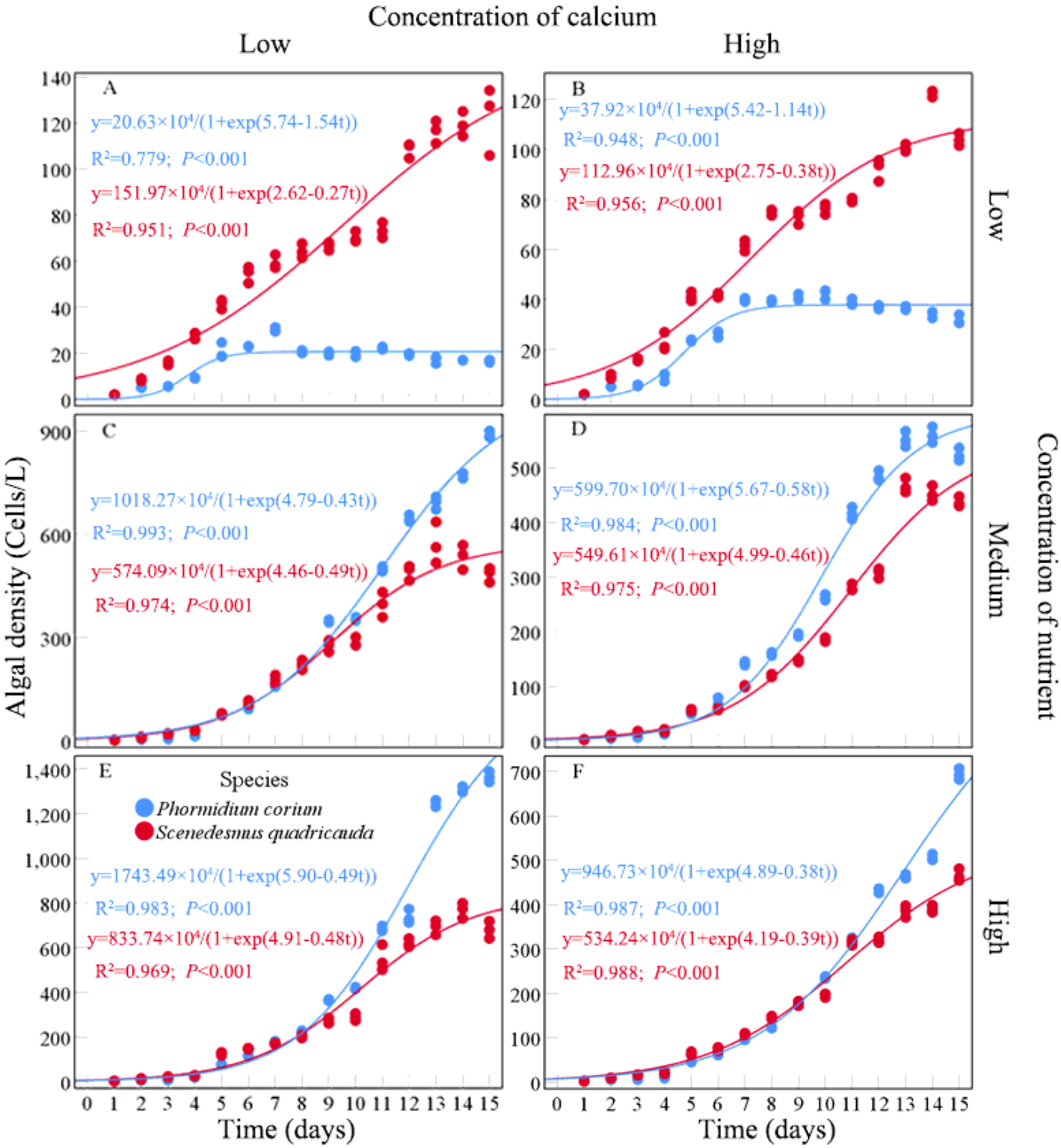

Figure 1

Growth curves of two algal species (*Phormidium corium*and *Scenedesmus quadricauda*)in monoculture experiments at three nutrient concentration levels and two calcium concentration levels (A–low nutrient concentration and low calcium concentration; B–low nutrient concentration and high calcium concentration; C–medium nutrient concentration and low calcium concentration; D–medium nutrient concentration and high calcium concentration; E–high nutrient concentration and low calcium concentration; F–high nutrient concentration and high calcium concentration).

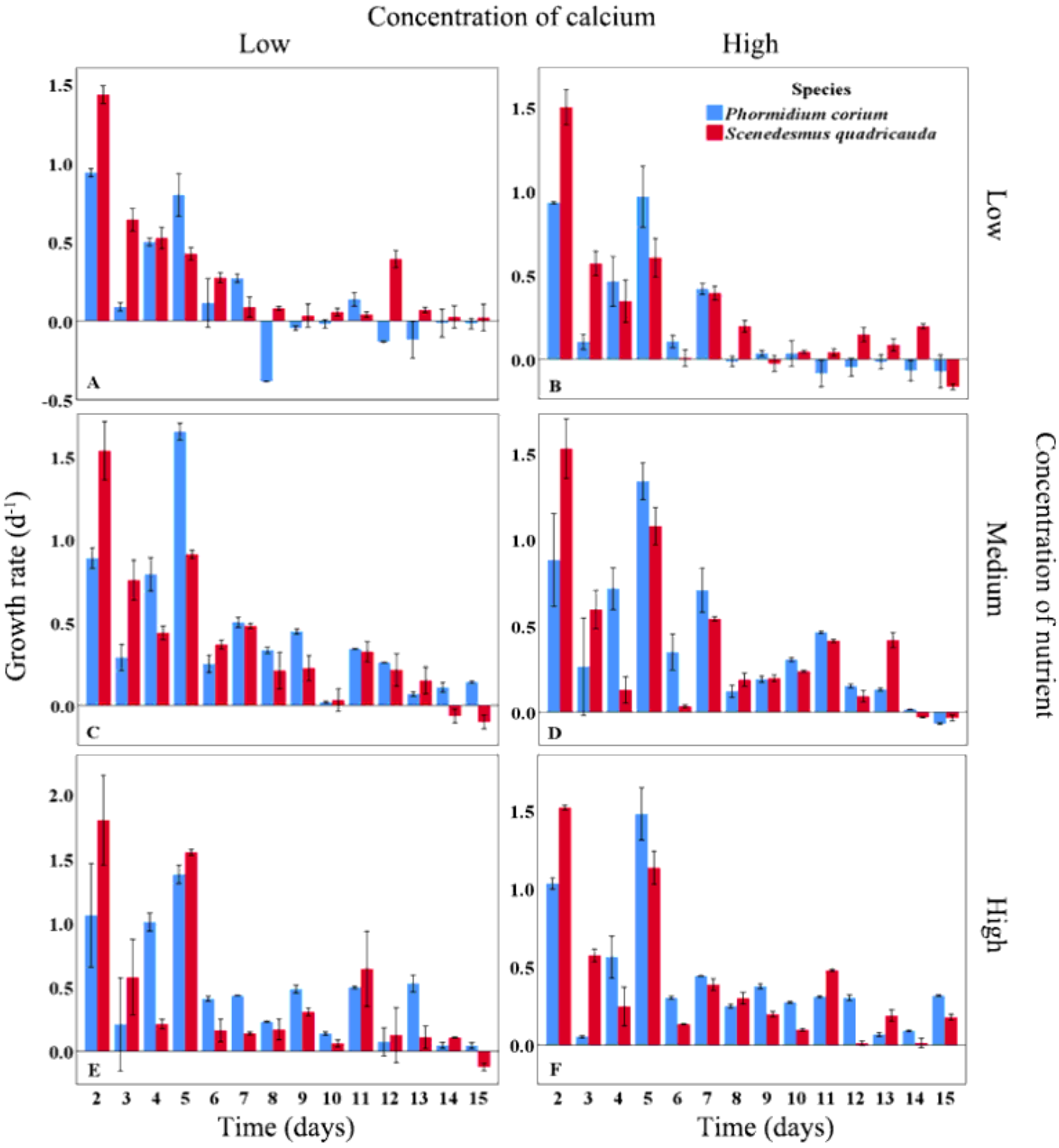

Figure 2

Growth rates of two algal species (*Phormidium corium* and *Scenedesmus quadricauda*) in monoculture experiments at three nutrient concentration levels and two calcium concentration levels (A–low nutrient concentration and low calcium concentration; B–low nutrient concentration and high calcium concentration; C–medium nutrient concentration and low calcium concentration; D–medium nutrient concentration and high calcium concentration; E–high nutrient concentration and low calcium concentration; F–high nutrient concentration and high calcium concentration).

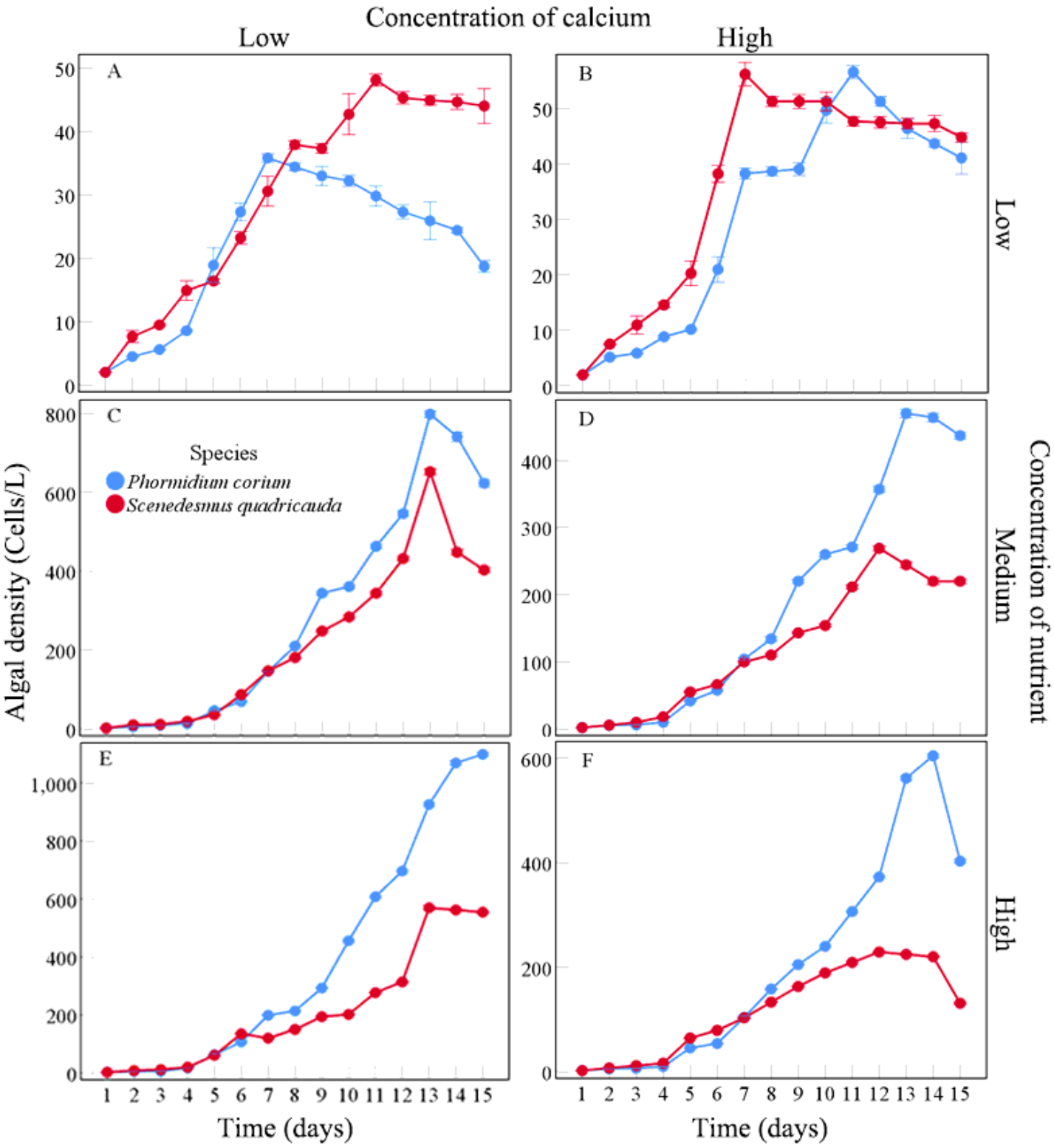

Figure 3

Growth curves of two algal species (*Phormidium corium* and *Scenedesmus quadricauda*) in mixture experiments at three nutrient concentration levels and two calcium concentration levels (A–low nutrient concentration and low calcium concentration; B–low nutrient concentration and high calcium concentration; C–medium nutrient concentration and low calcium concentration; D–medium nutrient

concentration and high calcium concentration; E–high nutrient concentration and low calcium concentration; F–high nutrient concentration and high calcium concentration).

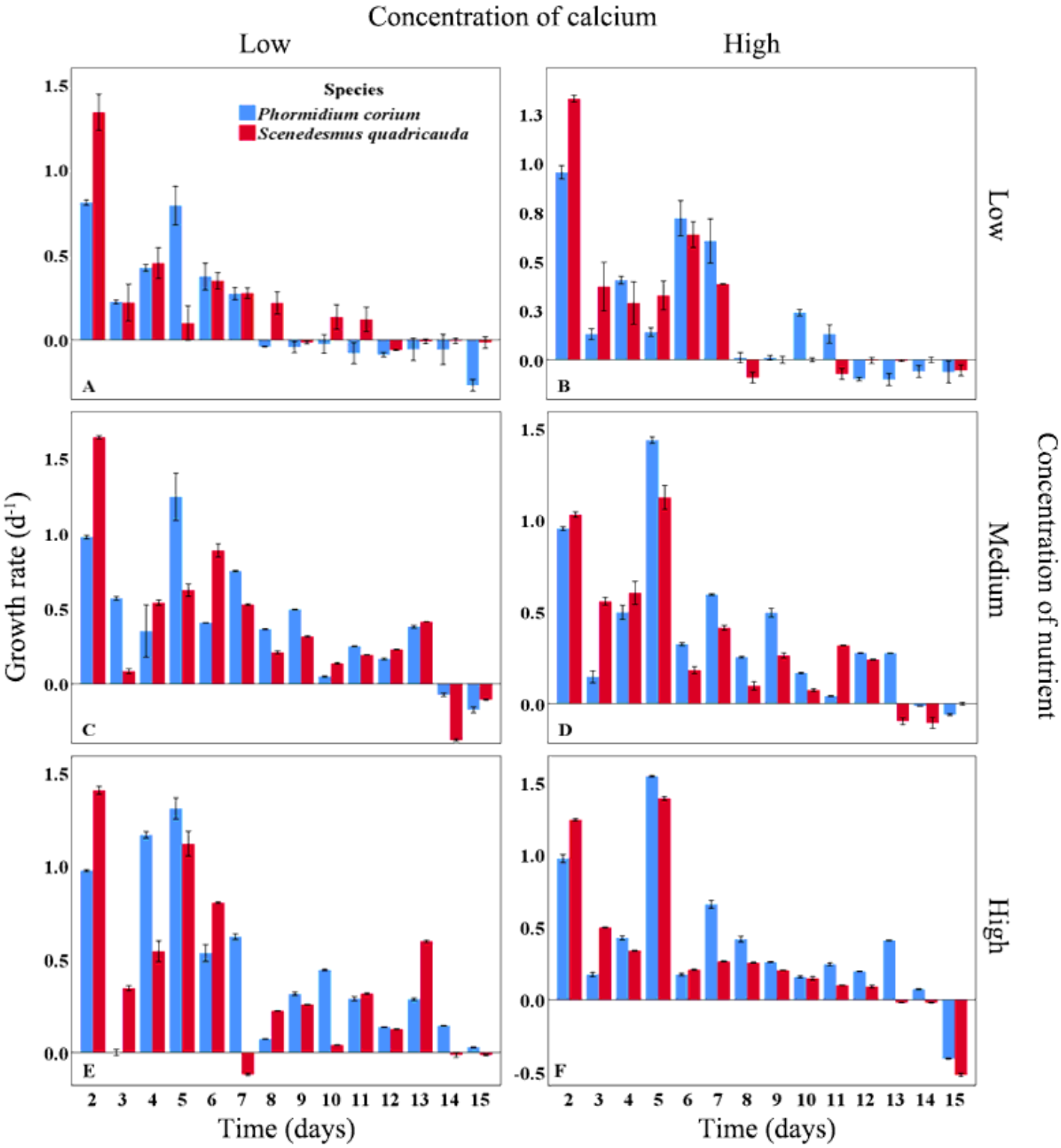

Figure 4

Growth rates of two algal species (*Phormidium corium* and *Scenedesmus quadricauda*) in mixture experiments at three nutrient concentration levels and two calcium concentration levels (A–low nutrient

concentration and low calcium concentration; B–low nutrient concentration and high calcium concentration; C–medium nutrient concentration and low calcium concentration; D–medium nutrient concentration and high calcium concentration; E–high nutrient concentration and low calcium concentration; F–high nutrient concentration and high calcium concentration).

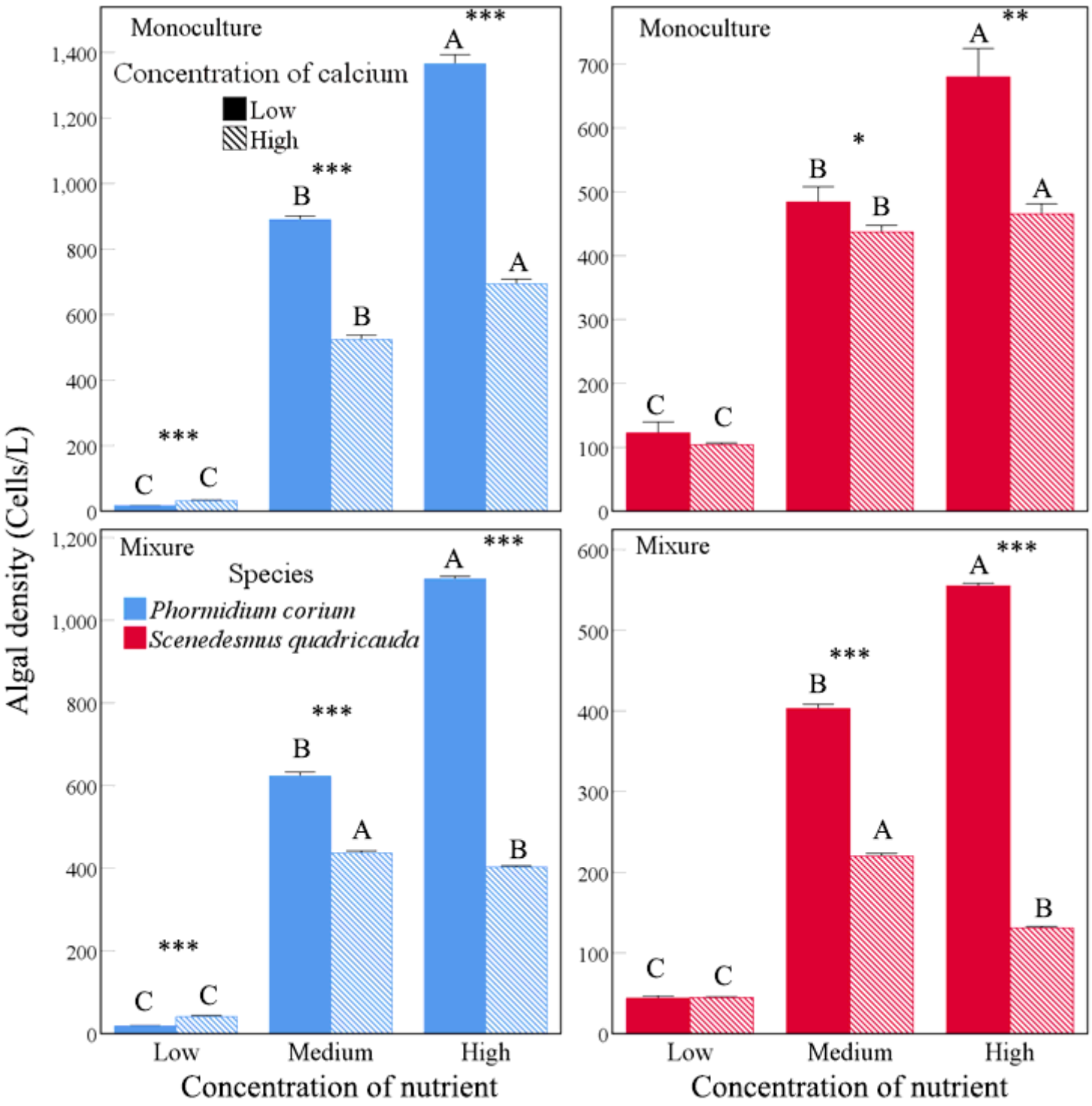

Figure 5

Effects of the nutrient concentration and calcium concentration on the final cell density of two algal species (*Phormidium corium* and *Scenedesmus quadricauda*) in monoculture (top) and mixture (bottom)

experiments. Capital letters indicate significant differences in nutrient concentration; asterisks indicate significant differences among calcium concentration.

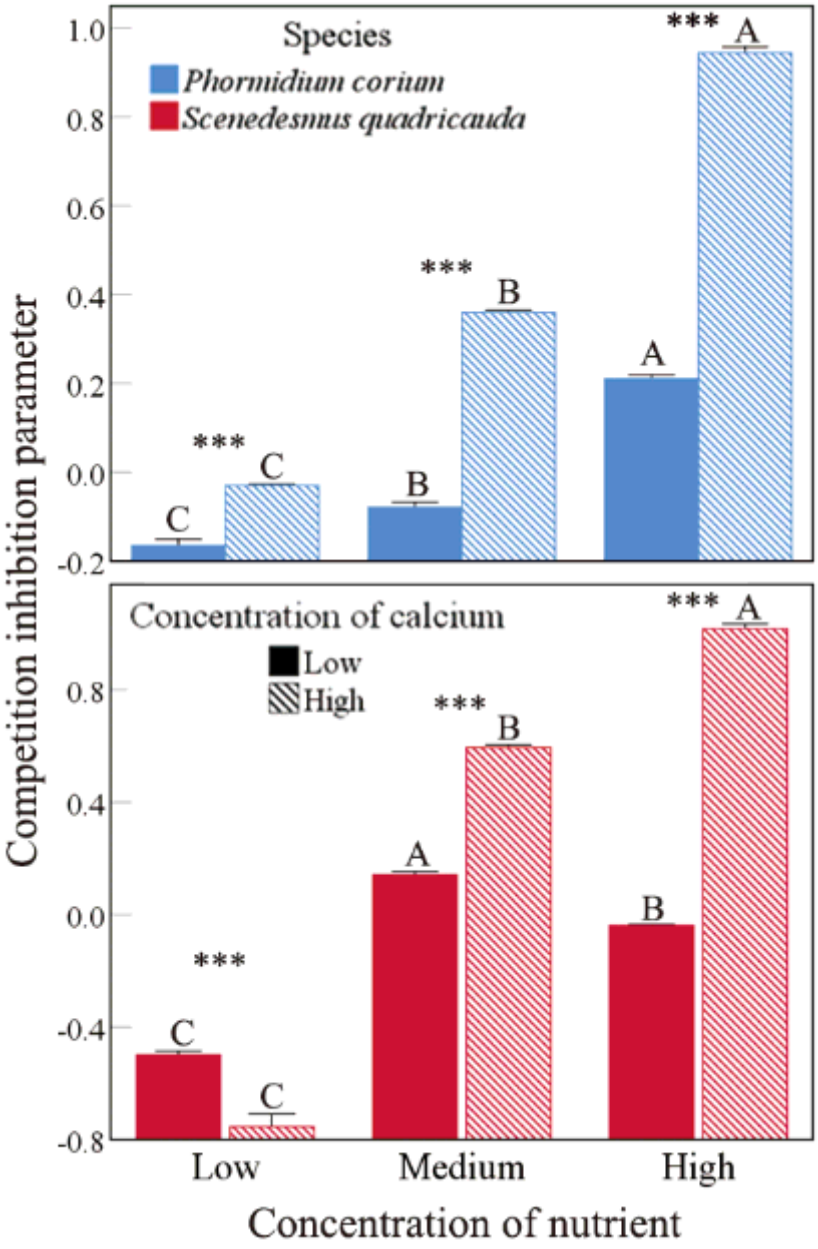

Figure 6

Effects of the nutrient concentration and calcium concentration on the competition inhibition parameters of two algal species (*Phormidium corium* and *Scenedesmus quadricauda*) in mixture experiments. Capital letters indicate significant differences in nutrient concentration; asterisks indicate significant differences among calcium concentration.