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Effects of temperature on the growth and competition between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* with different phosphorus availabilities

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ABSTRACT

The frequent outbreak of harmful algal blooms (HABs) is one of the most serious outcomes of eutrophication, and temperature plays a critical role in the species succession; therefore, a better understanding of the impact of temperature is essential for mitigating HABs. In this study, a broadly distributed cyanobacterium *Microcystis aeruginosa* and a *Chlorophyta* species *Chlorella pyrenoidosa* were mono-cultured and co-cultured at three different temperatures (15°C, 25°C and 35°C) with different phosphorus (P) availability, to explore algal growth patterns and competition results. In the mono-cultures, *M. aeruginosa* had a poor adaptation for its growth at low temperatures and it grew better at higher temperatures, but *C. pyrenoidosa* had flexibility for its growth in a wider range of temperatures and it showed the best growth at 25°C. However, high P availability could alleviate the negative effects of low temperatures on *M. aeruginosa* growth. Meanwhile, algal P utilization behaviors could have a close relationship with their growth patterns at different temperatures, when higher P availability enhanced the growth advantages of two species at the optimal temperature in the mono-cultures. In the co-cultures, the mutually beneficial effects of two algal species for their growth were observed at low temperatures. But at warmer temperatures, the growth of *M. aeruginosa* was promoted and the growth of *C. pyrenoidosa* was inhibited when compared to monocultures. The superior P utilization of *M. aeruginosa* and its allelopathic effects on *C. pyrenoidosa* could be the main causes. Consistent with the batch experiment in flasks, water temperature in different seasons had a great impact on the growth strategies of algal species, and P enrichment affected algal succession in the natural waters. Overall, our results indicated the significant interactive effects of temperature and P availability on algal growth, and the strong competitive advantages of *M. aeruginosa* in eutrophic waters at high temperatures.

Keywords: *Microcystis aeruginosa*; Algal growth; Temperature; Phosphorus availability; Phosphorus utilization; Competition

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1. Introduction

Eutrophication has negatively affected aquatic ecosystems over the last several decades [1,2], and the frequent outbreak of harmful algal blooms (HABs) is one of the most serious outcomes of eutrophication [3,4]. Globally, cyanobacteria often stand out as the main contributor of HABs and *Microcystis* spp. are often dominant bloom species in many eutrophic waters [5,6]. Meanwhile, HABs are characterized by the variation of dominant species in different seasons and non-cyanobacteria species could enjoy advantages at certain times. For example, *Microcystis* and *Chlorella* were the dominant species in Lake Taihu and their growth advantages often fluctuated wildly in different seasons [7,8]. In Lake Chao, *Chlorophyta* and *Bacillariophyta* species were dominant in spring while *Cyanophyta* species became dominant in summer [9]. Since cyanobacteria generally cause more serious environmental problems [10,11], a better understanding of the seasonal succession of cyanobacteria is essential to explain and control HABs.

Water temperature is the most representative seasonal index that affects the growth, metabolism, reproduction, and survival of living organisms, as well as the interactions among species [12]. Scholars have carried out numerous studies focusing on the effects of temperature on algae during the outbreak period of HABs. For example, Reynolds [13] considered temperature as the determining factor for species succession, when cyanobacteria grew faster at higher temperatures and the growth of many other species was negatively affected at temperatures above 25°C. Gomes and Lürling [14] also reported that temperature could affect the metabolism of cyanobacteria and higher temperature often increased its competitive ability against other algal species. However, a growing number of studies have revealed that the variations of temperature did not necessarily result in the succession of algal species, when the interactions between temperature and other environmental factors could be more important [15,16].

Nitrogen (N) and phosphorus (P) loadings in the water are the key indicators of eutrophication and their dynamics changed significantly during seasons [17], leading to a growing body of researches on the impact of temperature at different nutrient status. For example, the input of N and P in Lake Taihu did not result in the occurrence of cyanobacterial blooms in winter [18], but the total algal biomass and the superiority of cyanobacteria greatly increased with high N and P loadings in spring and summer [19]. Rigosi et al. [20] and Elliott [21] pointed that the interaction of global warming and eutrophication to promote cyanobacterial blooms highly depended on the trophic state. However, these studies have mainly reported experimental results and the specific mechanisms are often contradictory. In addition, these studies mainly examined algal growth and its response in mono-culture systems, and it remains unclear whether the coexistence of species changes the combined effects of temperature and nutrients.

In the present study, we investigated the growth and P utilization properties of a typical cyanobacterium and a *Chlorophyta* species at different temperatures with mono-cultures and co-cultures, together with an incubation experiment in an annular flume. The main goals were to (i) explore the relationship between algal P utilization and growth patterns at different temperatures, (ii) study the effects of P availability on the growth and competition of two species at different temperatures.

2. Materials and methods

2.1. Algal cultures and experimental setup

2.1.1. Mono-cultures and co-cultures of two strains

Microcystis aeruginosa (FACHB 905) and *Chlorella pyrenoidosa* (FACHB 5) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences (FACHB). For the two algal species, *M. aeruginosa* is a dominant genus during the outbreaks of HABs, and *C. pyrenoidosa* was selected because of its common distribution and frequent co-existence with cyanobacteria in many Chinese Eutrophic Ecosystems [22]. Two strains were pre-cultured and activated separately by periodically transferring 5–10 mL of growing cultures to fresh standard BG_{11} medium containing dipotassium phosphate (K_2HPO_4, 7.11 mg P L^{-1}) in Erlenmeyer flasks [23]. Pre-culture was performed under sterile conditions and the flasks were placed at 25°C under 40 μmol photons m^{-2} s^{-1} PAR (photosynthetically active radiation) with cool white fluorescent lamps (light/dark regime of 12 h: 12 h) in the illuminated incubator [24].

After pre-culture, the exponentially growing algae were inoculated into 500 mL flasks containing 300 mL of modified BG_{11} medium for the normal experiment. For mono-cultures, the initial cell density of *M. aeruginosa* and *C. pyrenoidosa* was 1.0×10^6 cells mL^{-1} (with the optical density value at 680 nm [OD_{680}] of 0.020 and 0.025, respectively, Fig. S1), which approximated the cell number at the beginning of HABs in most eutrophic lakes in China [25]. Meanwhile, the initial chlorophyll-a (chl-a) concentration was 52.48 and 48.13 μg L^{-1}, respectively, which was close to the situation in the natural waters. Three temperatures were used in the mono-cultures for different treatments, including low temperature at 15°C (LT treatment), the moderate temperature at 25°C (MT treatment) and high temperature at 35°C (HT treatment). Meanwhile, different initial P availabilities in the modified BG_{11} medium were set for each treatment, including Low-P conditions (0.5 mg P L^{-1} with K_2HPO_4), Moderate-P conditions (1.0 mg P L^{-1} with K_2HPO_4) and High-P conditions (2.5 mg P L^{-1} with K_2HPO_4). Since the high content of P in the standard BG_{11} medium was too high and it could hamper the implementation of our experiment objectives, P supplies were intentionally decreased in the modified BG_{11} medium, when K_2HPO_4 was substituted with KCl to maintain the same potassium ion (K^+) concentrations.

In the co-cultures, *M. aeruginosa* was co-cultured with *C. pyrenoidosa* in flasks in the illuminated incubator with the inoculation ratio of 1:1, and the cell density of each strain was about 0.5×10^6 cells mL^{-1} (with OD_{680} of 0.010 and 0.013, respectively). The initial chl-a content of *M. aeruginosa* and *C. pyrenoidosa* was half of that in the mono-cultures. Cell sizes of two algal species in our study were similar and the same initial cell density could achieve similar biomass at the beginning of the experiment. The culture medium and

culture methods of co-cultures were as described above for monoculture experiments.

Moreover, to investigate algal allelopathic property towards the other species, the filtered growth medium for each strain was added into the fresh growth medium for the other strain, when the mono-culture was adopted and the initial P availability was set at 2.5 mg P L^{-1}. More specifically, during the mono-culturing of two species, 100 mL of the growth medium for each strain at the exponential period was filtered through 0.2 μm mixed cellulose ester filters (Whatman), and the resulting filtered medium (0, 10 and 20 mL) was added into fresh medium (300 mL) for separate inoculation with the other strain. Three temperatures were also set during the incubation.

A schematic diagram of the experiment setup is shown in Fig. S2.

2.1.2. Cultures of phytoplankton community in the flume

Water in Meiliang Bay, Lake Taihu containing natural phytoplankton assemblages was collected in April (spring), August (summer) and October (autumn) of 2018, three seasons that were both characterized by the vigorous growth of algae with different water temperatures in this area (Fig. S3, Table S1). In each season, the raw water samples or P-spiked water samples with K_2HPO_4 (the final total P content in the water was 2.5 mg P L^{-1}) were incubated in an annular flume at *in-situ* temperatures.

The configuration and size of the flume could be seen in Fig. S3, and the detailed methodology could be seen in Supplementary Information (SI).

2.2. Analytical methods of parameters

2.2.1. Algal growth and photosynthetic efficiency

Subsamples were regularly taken for determining cell density in the mono-cultures and co-cultures. Cells were both enumerated by using a flow cytometer (CytoFLEX S, Beckman Coulter Co., Ltd., USA), when *M. aeruginosa* and *C. pyrenoidosa* were clearly differentiated by their autofluorescence [26]. The algal growth rate was determined as follows: $\mu = (\ln N_2 - \ln N_1)/(t_2 - t_1)$, where N_1 and N_2 was the cell density on days t_1 and t_2, respectively. The maximum μ during the whole incubation period was defined as μ_{max}, which was an important index to indicate algal growth state.

A Phyto-PAM fluorometer (Hein Walz, Germany) was adopted to determine the effective quantum yield (F_v/F_m) of algal species in the mono-cultures and co-cultures. The Phyto-PAM fluorometer has been increasingly used to detect algal photosynthetic indices in both laboratory experiments and natural waters, and F_v/F_m could effectively indicate the function of algal photosynthesis apparatus [27–29].

To eliminate the interference of species-specific property and examine the changes of algal growth with different culture methods, μ_{max} and F_v/F_m of *M. aeruginosa* and *C. pyrenoidosa* in the co-cultures were monitored and compared with those in the mono-cultures. Moreover, the percentage changes μ_{max} or F_v/F_m were determined as follows: $K = (G_m - G_c)/G_m$, where G_m was data in the mono-cultures and G_c was data in the co-cultures. For better comparison, F_v/F_m of two species during the exponentially growing phase on Day 7 was used for calculation and comparison.

In the flume experiment, the growth of the phytoplankton community was estimated by directly measuring chlorophyll-a (chl-a) in the water using the ethanol extraction method [30]. Meanwhile, the different phyla of algal species were identified and quantified at ×400 magnification by using a light microscope (Axioskop 40; Zeiss, Germany) with the reference compiled by Hu and Wei [31].

2.2.2. Kinetic parameters of P uptake

According to Fu et al. [32], the kinetic parameters of P uptake by two species were determined using classical Michaelis–Menten regression equations in mono-cultures at different temperatures (LT, MT, HT treatments), and the parameters were compared to investigate algal short-term P utilization behaviors (Table 2). The Michaelis–Menten equation could effectively describe algal uptake of typical nutrients in the surrounding environment [33,34] and the detailed method could be seen in SI.

2.2.3. P accumulation property of algae

According to Van Moorleghem et al. [35], cellular P quotas of two species were determined in the mono-cultures during the incubation to investigate their long-term P accumulation properties. Subsamples of *M. aeruginosa* and *C. pyrenoidosa* cultures were regularly collected, which were then filtered through 0.2 μm mixed cellulose ester filters (Whatman) and added to colorimetric tubes. The filters with retained cells were directly rinsed with ultrapure water and digested at 120°C and 150 kPa for 30 min. The total accumulated P by *M. aeruginosa* or *C. pyrenoidosa* was calculated and cellular P quota was estimated based on the corresponding cell density (fg $cell^{-1}$) [36].

Subsequently, the luxury uptake coefficient of P (Q_c) of two species was calculated as follows: $Q_c = Q_{max}/Q_{min}$, where Q_{max} and Q_{min} were the maximum and the minimum cellular P quota during the experiment. According to previous studies [37,38], Q_c could be used to estimate the allocation strategy and utilization efficiency of accumulated P by two species, when higher Q_c of algae could lead to more times of cell division and higher cell density.

2.2.4. P content in the water

P content in the water was determined using the molybdenum blue method [39] using a segmented flow AutoAnalyzer 3 (SEAL, Germany). The errors caused by the manual operation were avoided to the greatest extent and the detection resolution greatly increased (0.12 ug P L^{-1}).

2.2.5. Cellular release of K^+ and dissolved organic carbon

To explorer, the integrity of *M. aeruginosa* and *C. pyrenoidosa*, the release rate of K^+ by cells during the incubation period was determined and dissolved organic carbon (DOC) content in the algal cultures was also measured (Fig. S4). The details are shown in SI.

2.2.6. Antioxidant responses of algae and scanning electron microscopy images

Before and after different treatments on Day 2, the generation of reactive oxygen species (ROS) by *M. aeruginosa* and *C. pyrenoidosa* and algal superoxide dismutase (SOD) activity were determined (Fig. S5). Moreover, the scanning electron microscopy (SEM) images of mono-cultured *M. aeruginosa* on Day 4 were conducted in different treatments (Fig. S6). The details are shown in SI

2.3. Statistical analysis

All vessels were sterilized via autoclaving at 121°C and 150 kPa for 30 min before use, and solutions were prepared using ultrapure water and analytical-grade chemicals. In this study, experiments were conducted in triplicate and means ± standard deviations were calculated. A parametric three-way repeated-measures analysis of variance (RM-ANOVA) was adopted to determine the effects of temperature, P conditions and species (*M. aeruginosa* and *C. pyrenoidosa*) on the cell density, μ_{max}, F_v/F_m, cellular P quota, and Q_c in the mono-cultures (Table S2). On the other hand, a parametric three-way RM-ANOVA was adopted to determine the effects of P conditions, species and sampling time on the cell density, μ_{max} and F_v/F_m for each treatment in the co-cultures (Table S3), and a parametric three-way RM-ANOVA was adopted to determine the effects of temperature, P conditions and species on the percentage changes of μ_{max} and F_v/F_m (Table S4). In addition, a two-way RM-ANOVA was adopted to determine the effects of temperature and filtrate of algal cultures on the other species. A three-way ANOVA was used to determine the effects of seasons, P-spiking and incubation time on the proportion of different algal species. All data were tested for the normality and variance assumptions of the parametric ANOVA, and no data transformation was needed. If the interaction factor was significant at $p < 0.05$, a one-way ANOVA followed by Tukey's test was used to determine where differences lie. Moreover, one-way ANOVA was used to determine the effects of temperature on the kinetic parameters of P uptake by *M. aeruginosa* or *C. pyrenoidosa*, and a student's *t*-test was used to determine the differences between the two species (Table 1). All statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Growth of two species in mono-cultures

3.1.1. Cell density of two species

In the mono-cultures, *M. aeruginosa* grew faster after Days 4–5 and the maximum cell density was observed at the end of incubation (Fig. 1a). Meanwhile, P availability in the water affected the effects of temperature on its growth. Under Low-P conditions, *M. aeruginosa* grew slowly ($\mu_{max} < 0.35$ d^{-1}) and it could not keep alive in the LT treatment, when cell density began to decrease after Day 2. However, the negative effects of low temperature on *M. aeruginosa* decreased under the Moderate-P and High-P conditions, when sustainable algal growth could be observed in the LT treatment. The growth of *M. aeruginosa* was better in the HT treatment at different temperatures, but the difference only became significant ($p < 0.05$) under High-P conditions.

The growth patterns of *C. pyrenoidosa* were similar to those of *M. aeruginosa* in mono-cultures, but the growth rates of *C. pyrenoidosa* were higher (μ_{max} = 0.52–1.35 d^{-1}; Fig. 1b). The significant interactions ($p < 0.05$) also existed between temperature and P availability. Under Low-P conditions, algal cell density increased from the beginning in both three treatments and μ_{max} exceeded 0.50 d^{-1} with similar values. With increasing P availability, algal growth rates increased and the best growth was observed in the MT treatment ($p < 0.05$).

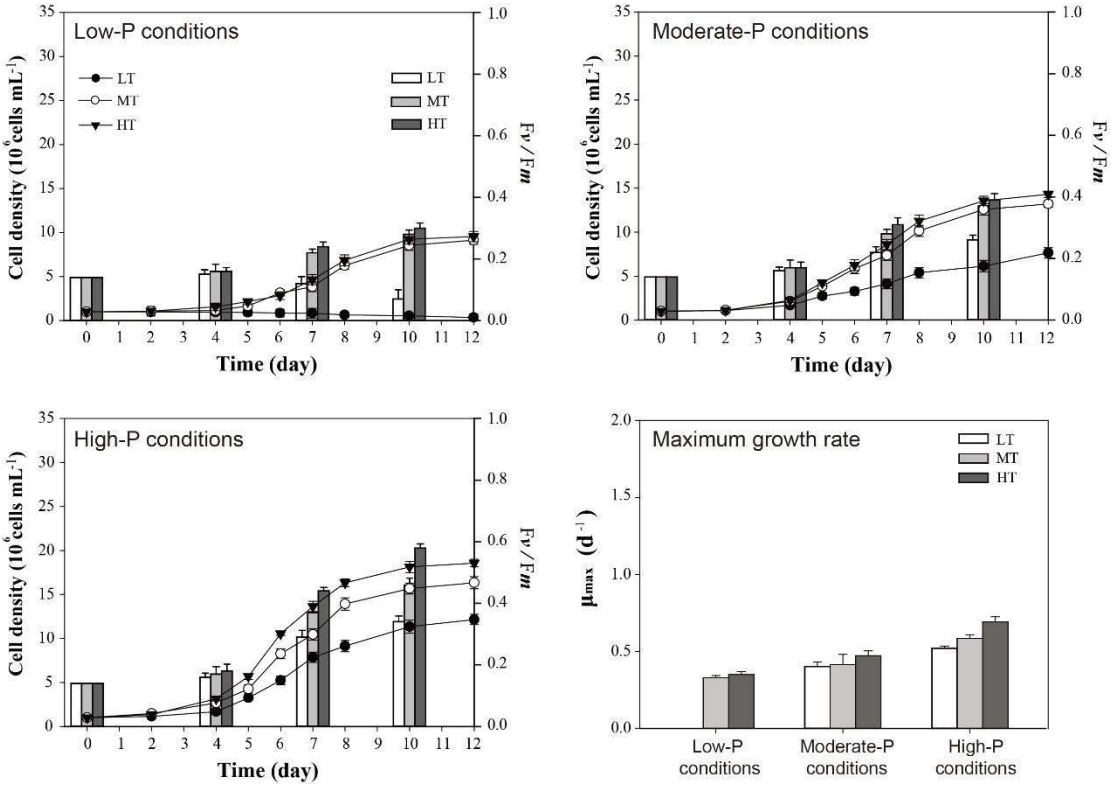
3.1.2. Photosynthetic efficiency of two species

The variations of algal photosynthetic efficiency were consistent with cell density. Briefly, F_v/F_m of *M. aeruginosa* gradually increased over time and it was enhanced with increasing P availability ($p < 0.05$). Moreover, F_v/F_m of *M. aeruginosa* increased with higher temperature, but the superiority of F_v/F_m in HT treatment was only significant ($p < 0.05$) under High-P conditions. Meanwhile, F_v/F_m of *C. pyrenoidosa* also gradually increased during the incubation and it was also promoted with higher P availability in the same treatment ($p < 0.05$). Compared to *M. aeruginosa*, F_v/F_m of *C. pyrenoidosa* was higher and the superiority of F_v/F_m in MT treatment became significant ($p < 0.05$) under Moderate-P and High-P conditions.

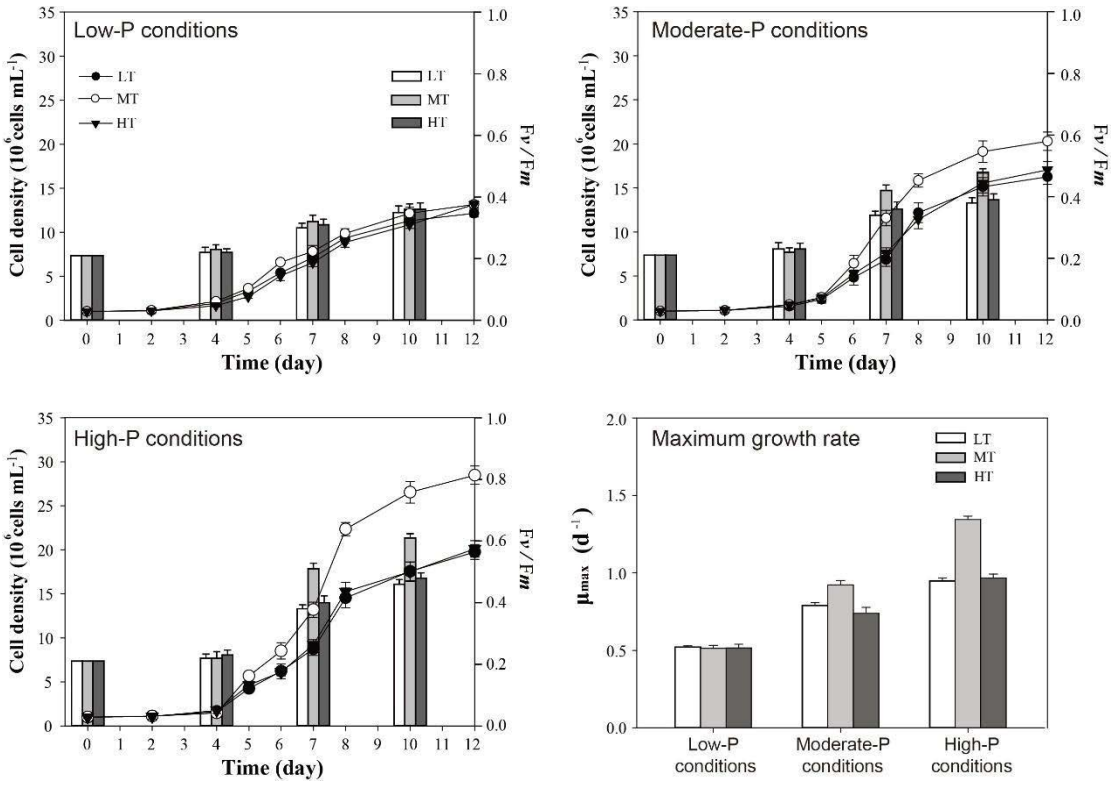
Table 1
Kinetic parameters for P uptake by two species at different temperatures

Treatment	V_{max}		K_m	
	μg [mg dw]$^{-1}$ h^{-1}		μg L^{-1}	
	Microcystis aeruginosa	*Chlorella pyrenoidosa*	*Microcystis aeruginosa*	*Chlorella pyrenoidosa*
LT	7.24(±0.11)^{a}	9.68(±0.34)^{a}*	595.9(±10.15)^{c}*	469.4(±6.25)^{a}
MT	12.21(±0.10)^{b}*	10.35(±0.30)^{a}	476.3(±8.24)^{b}	475.3(±7.88)^{a}
HT	13.86(±0.12)^{c}*	10.84(±0.29)^{a}	448.3(±9.72)^{a}	484.3(±8.33)^{a}*

*indicates significantly higher V_{max} or K_m values between *Microcystis aeruginosa* and *Chlorella pyrenoidosa* at $p < 0.05$; indicators (a–c) represent results of one-way ANOVA test using ranking method, V_{max} or K_m values with different letters for the same algal species are significantly different at $p \leq 0.05$ according to one-way ANOVA.



(a)



(b)

Fig. 1. Cell density (line and scatter) and photosynthesis efficiency (vertical bar) of (a) *Microcystis aeruginosa* and (b) *Chlorella pyrenoidosa* in the mono-cultures at different temperatures with different P availabilities.

3.2. Structural integrity of algal cells

Except for the LT treatment under Low-P and Moderate-P conditions, the release of K^+ by *M. aeruginosa* were both less than 5% before Day 6 (Fig. 2), which indicated the damages on cell membranes of *M. aeruginosa* at low temperatures and the relative integrity of cells in other cases [40]. In comparison, the release of K^+ by *C. pyrenoidosa* was both less than 5% before Day 6 in three treatments under different P conditions before Day 6. Meanwhile, compared to the large-scale cytoclasis of algal cells and the significantly increased DOC content in the cultures after ultrasonic disruption ($p < 0.05$), DOC contents in the *M. aeruginosa* cultures in the LT treatment under Low-P and Moderate-P conditions increased significantly on Day 1 (Fig. S4). In comparison, DOC contents in the *C. pyrenoidosa* cultures both remained relatively constant on Day 1 ($p > 0.05$). The damages on cell membranes of *M. aeruginosa* were also confirmed by SEM images (Fig. S6). It was shown that the surfaces of *M. aeruginosa* cells were rough and damaged in the LT treatment under Low-P conditions, and the negative effects were alleviated with higher P availability in the water.

3.3. Antioxidant responses of algae

Results of Fig. S5 showed that, compared to the initial values, ROS generation by *M. aeruginosa* and its SOD activity on Day 2 remained relatively constant in the MT and HT treatments ($p > 0.05$). However, ROS generation by *M. aeruginosa* on Day 2 greatly increased in the LT treatment ($p < 0.05$), and ROS content was higher with lower P availability in the culture medium. Meanwhile, SOD activity of *M. aeruginosa* on Day 2 in the LT treatment increased with higher P availability in the culture medium. In comparison, ROS generation by *C. pyrenoidosa* and its SOD activity on Day 2 did not change significantly in the three treatments ($p > 0.05$).

3.4. Kinetic parameters for P uptake in mono-cultures

Results showed that P uptake by *M. aeruginosa* was affected by temperature and the uptake rates increased at higher temperatures (Fig. 3, $p < 0.05$). However, P uptake by *C. pyrenoidosa* had no significant responses to temperature ($p > 0.05$).

By comparing the kinetic parameters of two species, V_{max} of *M. aeruginosa* was significantly lower and K_m of *M. aeruginosa* was higher in the LT treatment (Table 1; $p < 0.05$), indicating the faster P uptake and a better affinity to P for *C. pyrenoidosa*. With the temperature increasing, V_{max} of *M. aeruginosa* was enhanced significantly and its K_m decreased significantly ($p < 0.05$). By contrast, V_{max} and K_m of *C. pyrenoidosa* exhibited similar values at different temperatures ($p > 0.05$). As a result, V_{max} of *M. aeruginosa* was significantly higher ($p < 0.05$) and K_m of the two species was comparable in the MT treatment ($p > 0.05$). In the HT treatment, V_{max} of *M. aeruginosa* was also significantly higher and its K_m was lower ($p < 0.05$), indicating the faster P uptake and a better affinity to P for *M. aeruginosa*.

3.5. Cellular P quota in the mono-cultures

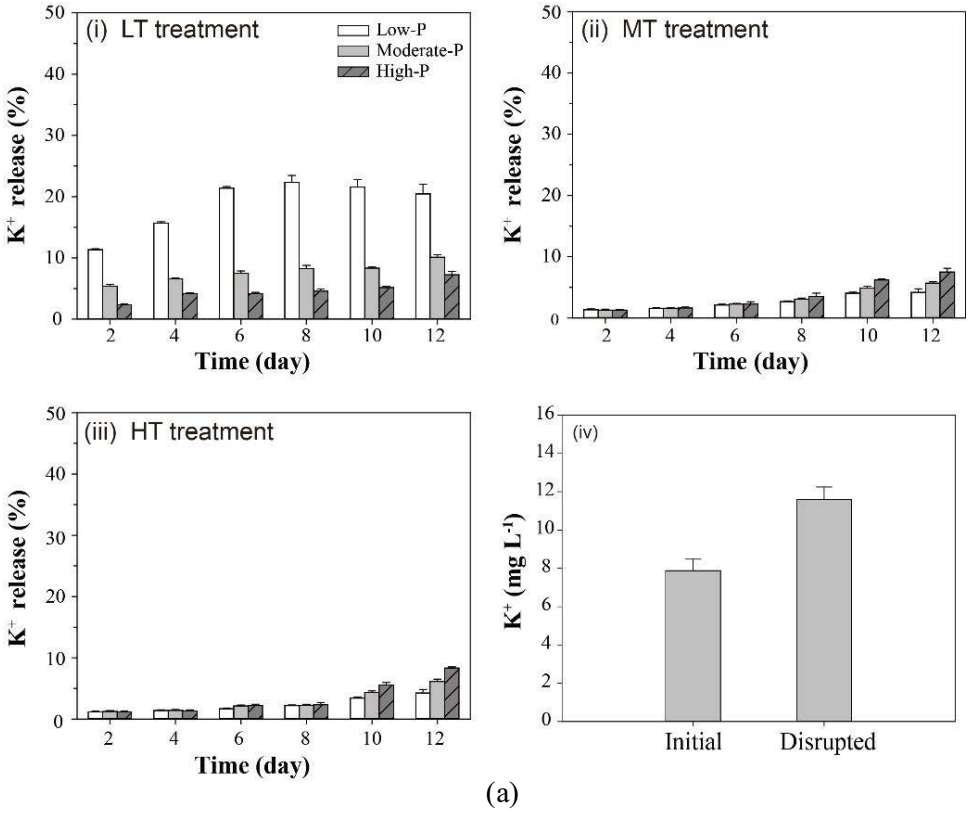
In our study, the cellular P quota of the two species both quickly increased and reached the maximum values on Day 2, followed by a slow decrease during Days 2–12 (Fig. 4). Meanwhile, temperature and P availability had significant interactive effects on the cellular P quota of two species ($p < 0.05$). In different treatments, the cellular P quota of two species increased with higher P availability in the culture medium. At different temperatures, *M. aeruginosa* exhibited the highest cellular P quota in the HT treatment ($p < 0.05$) but the cellular P quota of *C. pyrenoidosa* did not differ significantly ($p > 0.05$). By comparing two species, the cellular P quota of *C. pyrenoidosa* was superior in the LT treatment and it was at a disadvantage in the MT and HT treatments. In particular, *M. aeruginosa* hardly accumulated any P in the LT treatment under Low-P conditions. In addition, Q_c of *M. aeruginosa* was higher at higher temperatures and it reached 11.24 in the HT treatment, when Q_c of *C. pyrenoidosa* was higher in the MT treatment ($p < 0.05$).

3.6. Algal growth and competition in the co-cultures

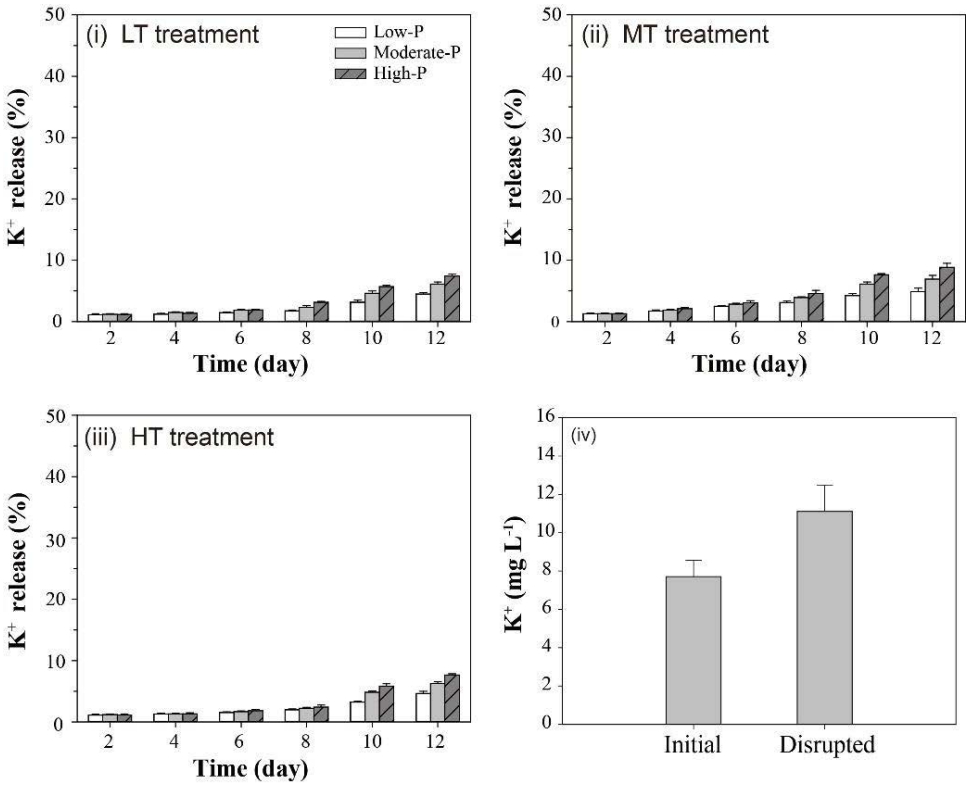
In the LT treatment, *M. aeruginosa* and *C. pyrenoidosa* both grew continuously in the co-cultures and *C. pyrenoidosa* was always the dominant species, when higher P availability promoted the growth rates and photosynthetic efficiency of two species ($p < 0.05$; Fig. 5). In addition, the mutually beneficial effects between two species for their growth were observed in the co-cultures, when μ_{max} and F_v/F_m of two algal species both increased significantly compared with those in the mono-cultures ($p < 0.05$; Table 2). Especially, cell density of *M. aeruginosa* gradually increased under Low-P conditions (5.54×10^6 cells mL^{-1} on Day 14) and its μ_{max} reached 0.36 d^{-1}. However, the percentage changes of μ_{max} and F_v/F_m of the two species both decreased gradually with increasing P availability in the culture medium.

In the MT treatment, *M. aeruginosa* also grew constantly but *C. pyrenoidosa* died after Days 9–10. *C. pyrenoidosa* was superior in the early stage but the biomasses of the two species were similar at the end of incubation ($p > 0.05$). Compared with those in the mono-cultures, μ_{max} and F_v/F_m increased for *M. aeruginosa* ($p < 0.05$) but decreased for *C. pyrenoidosa* ($p < 0.05$), indicating that *M. aeruginosa* growth was promoted whereas *C. pyrenoidosa* growth could be depressed in the co-cultures. In addition, the reductions of μ_{max} and F_v/F_m of *C. pyrenoidosa* increased significantly ($p < 0.05$) with higher P availability in the culture medium.

In the HT treatment, the algal growth patterns were very similar to those in the MT treatment, when μ_{max} and F_v/F_m of *M. aeruginosa* increased ($p < 0.05$) whereas μ_{max} and F_v/F_m of *C. pyrenoidosa* decreased ($p < 0.05$) comparing with mono -cultures. Moreover, compared with the MT treatment, μ_{max} and F_v/F_m of *C. pyrenoidosa* showed a greater decline in the HT treatment (22.5%–47.8% and 12.9%–40.4%, respectively, Table 2), and the maximum density of *C. pyrenoidosa* was lower under the same P conditions ($p < 0.05$). Especially, two species had similar cell densities before Day 8 under High-P conditions and *M. aeruginosa* was distinctly dominant during the following incubation ($p < 0.05$). Consequently, *M. aeruginosa* might have a greater advantage in the HT



(a)



(b)

Fig. 2. The release rate of K^+ by (a) *Microcystis aeruginosa* and (b) *Chlorella pyrenoidosa* cells in different treatments (LT, MT and HT) under High-P, Moderate-P, and High-P conditions.

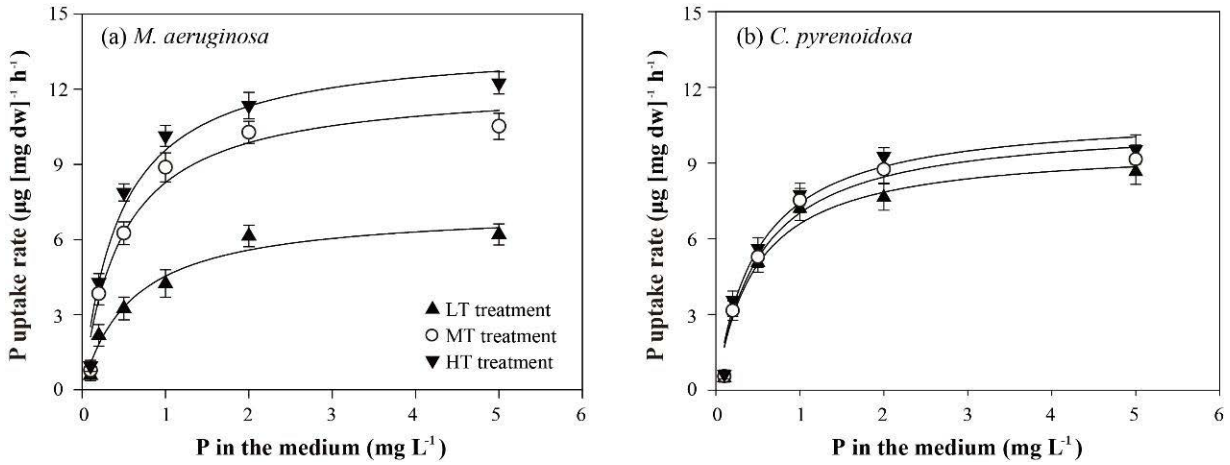


Fig. 3. P uptake rates as a function of ambient P concentrations at different temperatures by (a) *Microcystis aeruginosa* and (b) *Chlorella pyrenoidosa*.

treatment and could exhibit a stronger inhibitory effect on *C. pyrenoidosa* growth.

3.7. Algal allelopathic effect towards the other species

Results in Fig. 6 showed that the filtrate of *M. aeruginosa* cultures had significant inhibition effects on *C. pyrenoidosa* growth (11.8%–53.5%; $p < 0.05$), and the inhibition was stronger with more filtrate in its culture medium. Moreover, the inhibition effects of the filtrate of *M. aeruginosa* cultures on *C. pyrenoidosa* growth increased with higher temperatures. In contrast, *M. aeruginosa* growth did not change significantly ($p > 0.05$) with the addition of the filtrate of *C. pyrenoidosa* cultures.

3.8. Species succession in the flume experiment

In the experiment with raw water, algal biomass measured as chl-a both increased gradually before Day 9 and decreased afterwards (Fig. 7a), probably due to the nutrient deficiency. However, species succession of phytoplankton assemblages in three seasons differed significantly ($p < 0.05$) during the 15-d incubation. In autumn, *Chlorophyta*, *Cyanophyta* and *Bacillariophyta* were the main groups at the beginning, but the proportion of *Cyanophyta* decreased gradually as the experiment progressed (3.3% on Day 15), when *Chlorophyta* and *Bacillariophyta* became absolute dominant at the later stage. In spring, the growth of *Bacillariophyta* was suppressed and the dominant groups were *Chlorophyta* and *Cyanophyta*, when the proportion of *Chlorophyta* was higher during the experiment (24.6%–54.7%). In summer, the main groups were also *Chlorophyta* and *Cyanophyta* during the whole experiment, but the proportion of *Cyanophyta* became significantly higher after Day 6 (>45.0%).

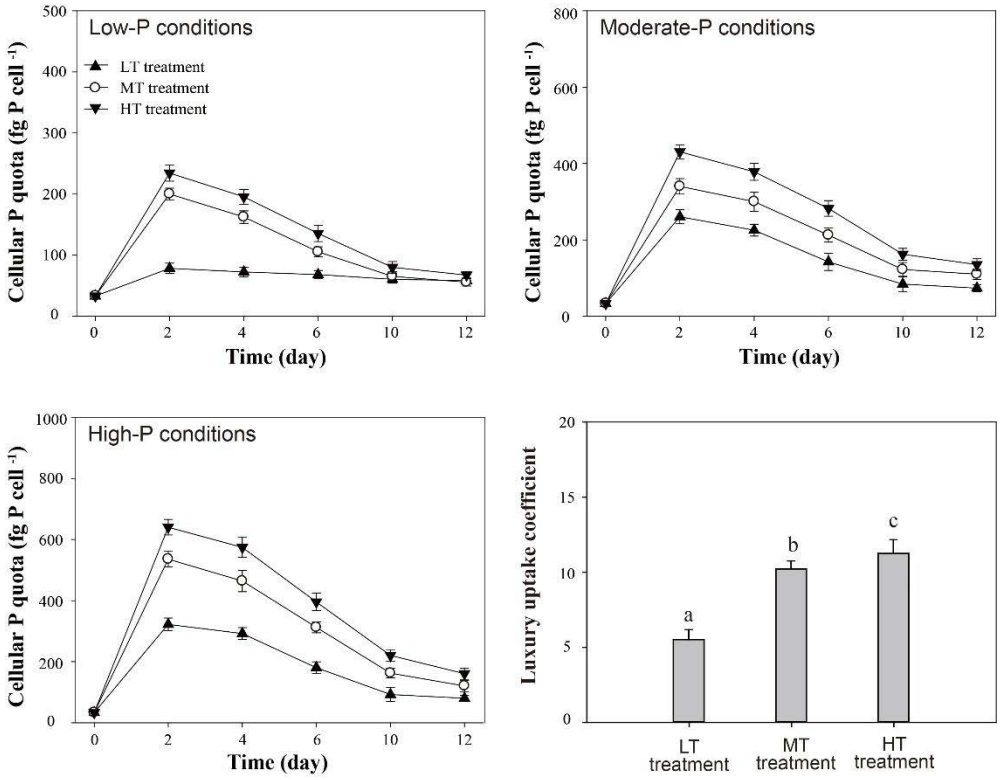
In comparison, sustained growth of phytoplankton was observed in the P-spiked water and species succession of phytoplankton assemblages also differed significantly among seasons ($p < 0.05$), when high P availability changed the proportion of dominant groups (Fig. 7b). In autumn, *Cyanophyta* grew normally and the dominant groups were *Chlorophyta*, *Cyanophyta* and *Bacillariophyta* during the whole experiment. In spring, the dominant groups were also *Chlorophyta* and *Cyanophyta* during the experiment, but the proportion of *Cyanophyta* was significantly higher after Day 9 (>44.3%; $p < 0.05$). In summer, the predominance of *Cyanophyta* greatly increased in P-spiked water ($p < 0.05$) and its proportion exceeded 60% at the later stage.

4. Discussion

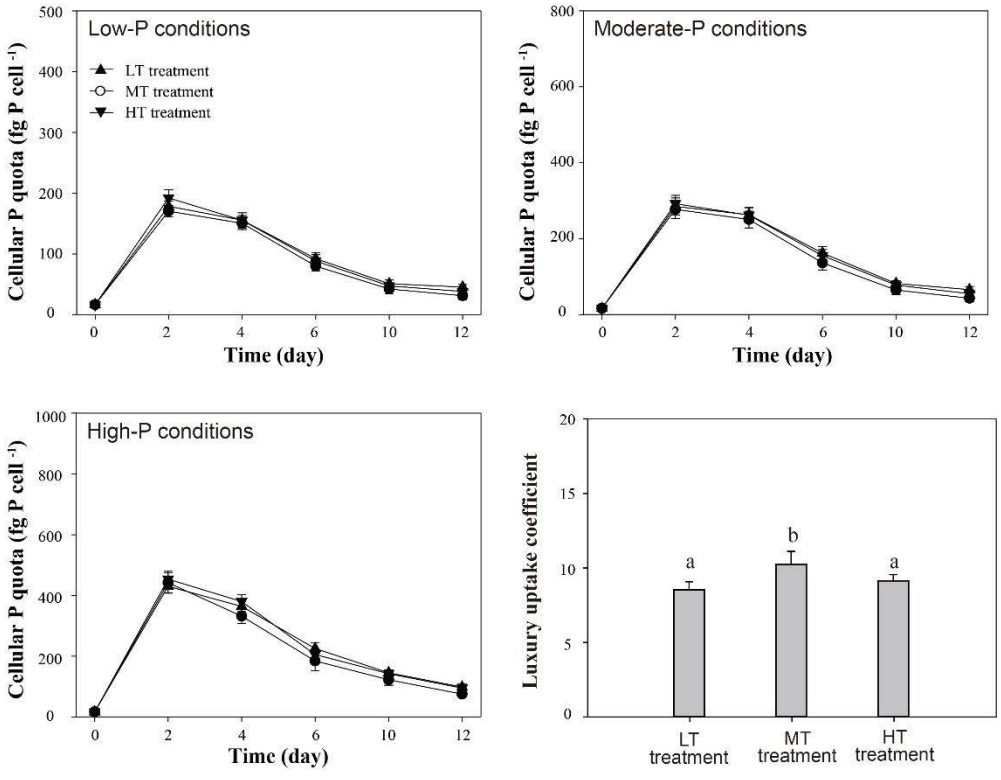
4.1. Algal physiological responses in the mono-cultures

Since P is an essential element for organisms, higher P availability both increased the growth rates and maximum cell densities of two species in the mono-cultures. The growth patterns of the two species were similar under the same conditions but *C. pyrenoidosa* often had the higher μ_{max} and biomass in the mono-cultures. It was probably due to excess energy cost for microcystin production by *M. aeruginosa* [41]. It was also consistent with Lürling et al. [42] who studied dozens of cyanobacterial and *Chlorophyta* species and pointed out that, *Chlorophyta Chlamydomonas reinhardtii* was the fastest-growing species within the tested temperature range (20°C–35°C). In our study, *M. aeruginosa* had a poor adaptation for its growth at low temperatures and it grew better at higher temperatures. However, *C. pyrenoidosa* had flexibility for its growth in a wider range of temperatures, which could maintain a considerable photosynthetic efficiency and cell density in the LT treatment. Confirmed by the cellular release of K^+ and DOC, SEM analysis and algal antioxidant responses, this was probably related to the cell membrane damages of *M. aeruginosa* cells by excessive ROS at low temperatures, which was unfavorable to the photosynthesis and some other physiological processes of cyanobacteria [13,43]. For example, low temperature could decrease the cellular permeability of cyanobacteria, which hindered algal transport of polysaccharides and other essential substances [44,45].

However, P is the substrate in the photophosphorylation of algae to produce adenosine triphosphate (ATP) [46], and P could also directly influence the synthesis of nucleotides and other essential substances [47]. As a

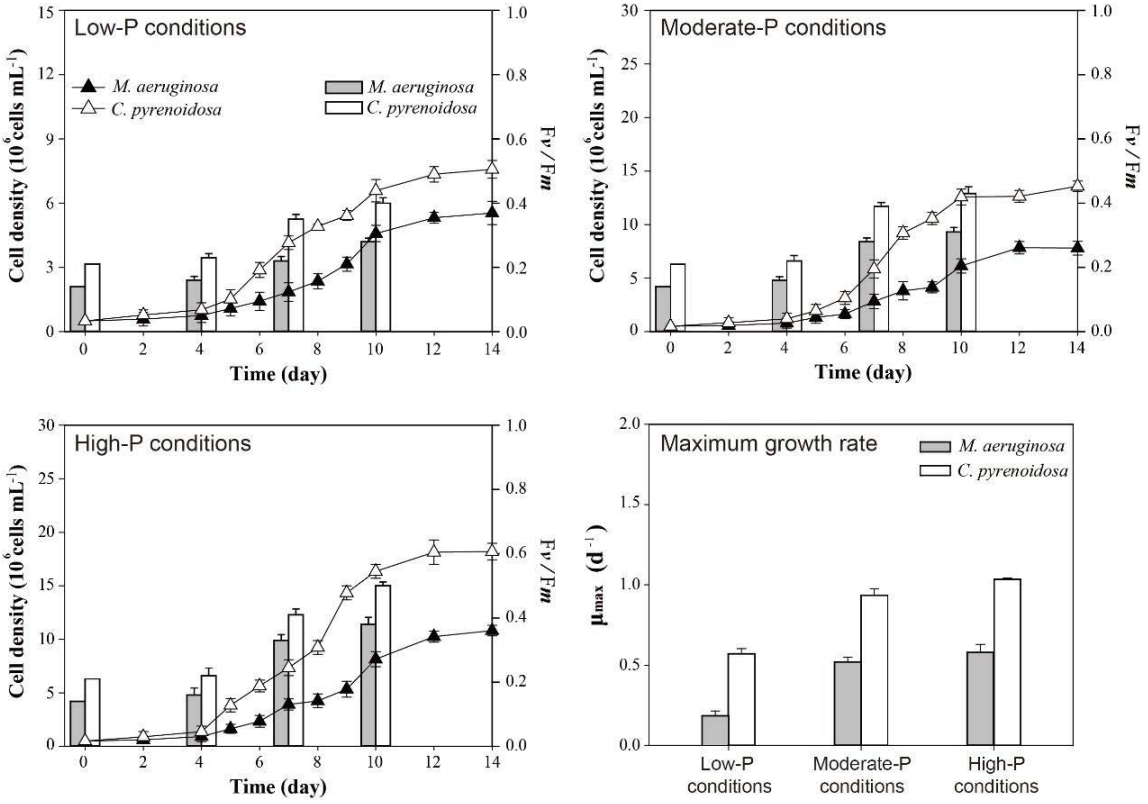


(a)

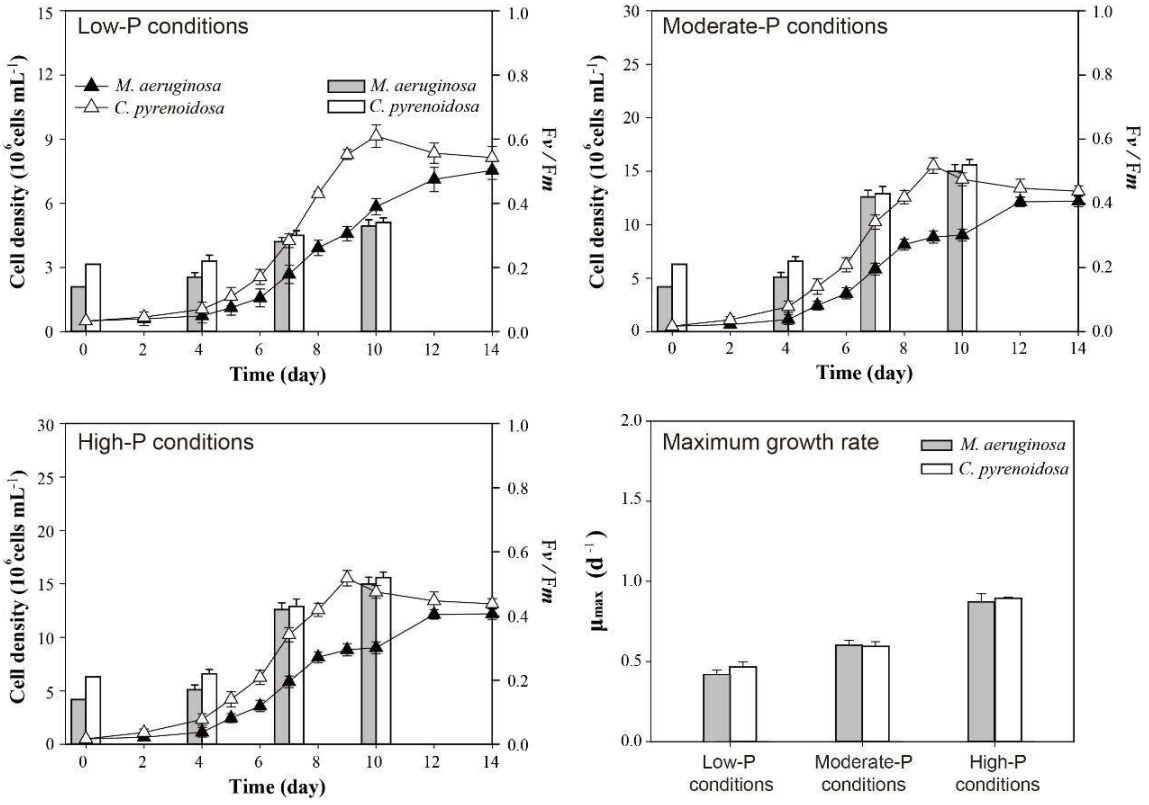


(b)

Fig. 4. Cellular P quota of (a) *Microcystis aeruginosa* and (b) *Chlorella pyrenoidosa* in the mono-cultures at different temperatures with different P availabilities.



(a)



(b)

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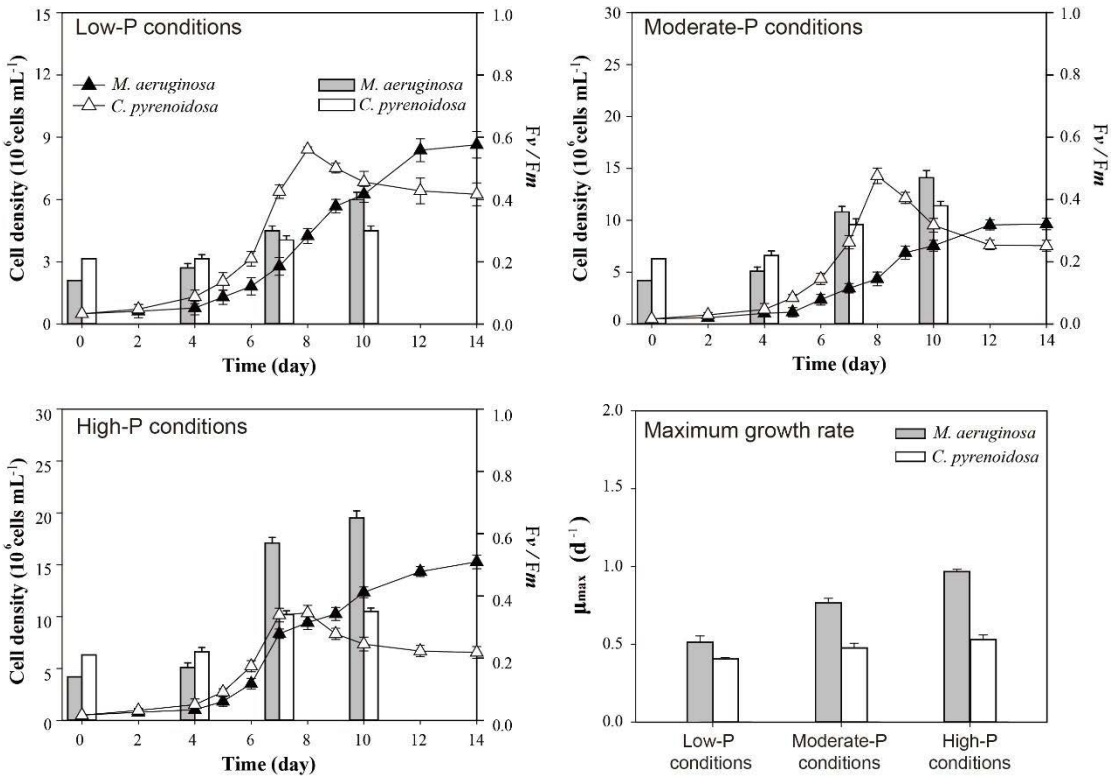


(c)

Fig. 5. Cell density (line and scatter) and photosynthesis efficiency (vertical bar) of two species in the co-cultures at different temperatures with different P availabilities.

Table 2
Algal maximum growth rate and photosynthetic efficiency (F_v/F_m on Day 7) in the co-cultures and the percentages change of two parameters comparing with those in the mono-cultures showing in parentheses

Treatment		Low-P conditions		Moderate-P conditions		High-P conditions	
		μ_{max}	F_v/F_m	μ_{max}	F_v/F_m	μ_{max}	F_v/F_m
LT	*Microcystis aeruginosa*	0.36 (\)	0.22 (+83.3%^{c})	0.54 (+57.4%^{b})	0.28 (+27.3%^{b})	0.58 (+22.6%^{a})	0.33 (+13.8%^{a})
	Chlorella pyrenoidosa	0.62 (+20.4%^{c})	0.35 (+16.7%^{c})	0.91 (+15.0%^{b})	0.39 (+14.7%^{b})	1.02 (+7.4%^{a})	0.41 (+7.9%^{a})
MT	*Microcystis aeruginosa*	0.42 (+27.3%^{a})	0.28 (+27.3%^{c})	0.63 (+77.0%^{c})	0.30 (+17.9%^{b})	0.83 (+54.9%^{b})	0.42 (+13.5%^{a})
	Chlorella pyrenoidosa	0.48 (–8.2%^{a})	0.30 (–7.4%^{a})	0.62 (–32.8%^{b})	0.36 (–16.3%^{b})	0.82 (–39.0%^{c})	0.43 (–32.1%^{c})
HT	*Microcystis aeruginosa*	0.51 (+45.7%^{a})	0.30 (+25.0%^{b})	0.80 (+93.7%^{b})	0.36 (+16.1%^{a})	0.95 (+47.3%^{a})	0.57 (+29.6%^{c})
	Chlorella pyrenoidosa	0.40 (–22.5%^{a})	0.27 (–12.9%^{a})	0.47 (–36.5%^{b})	0.32 (–24.2%^{b})	0.51 (–47.8%^{c})	0.34 (–40.4%^{c})

+ indicates increase and – indicates decrease; indicators (a–c) represent results of one-way ANOVA test using the ranking method, percentage change values with different letters for the same algal species in the same treatment are significantly different at $p \leq 0.05$ according to one-way ANOVA.
\: not applicable

result, algal P utilization behaviors could be closely linked with its adaptation capability to environmental stresses, such as the dark anaerobic conditions, UV radiation, etc. [43,48]. In the LT treatment, the uptake rate of P and algal affinity to P was extremely low for *M. aeruginosa*, which resulted in inefficient utilization of P. With higher P availability, the acquired P by *M. aeruginosa* increased greatly and algal self-repair capability probably increased owing to the roles of SOD in ROS scavenging (Figs. S5 and S6; [49]), which resulted in a stronger adaptation with a considerable growth at low temperatures. The outbreak of cyanobacterial blooms was often observed in summer in Chinese eutrophic lakes, but its duration increased greatly in recent years and vigorous growth of cyanobacteria in some regions algae could even happen in January [18]. Based on our result, eutrophication and the accompanying increases of P loadings in water could be an underlying cause.

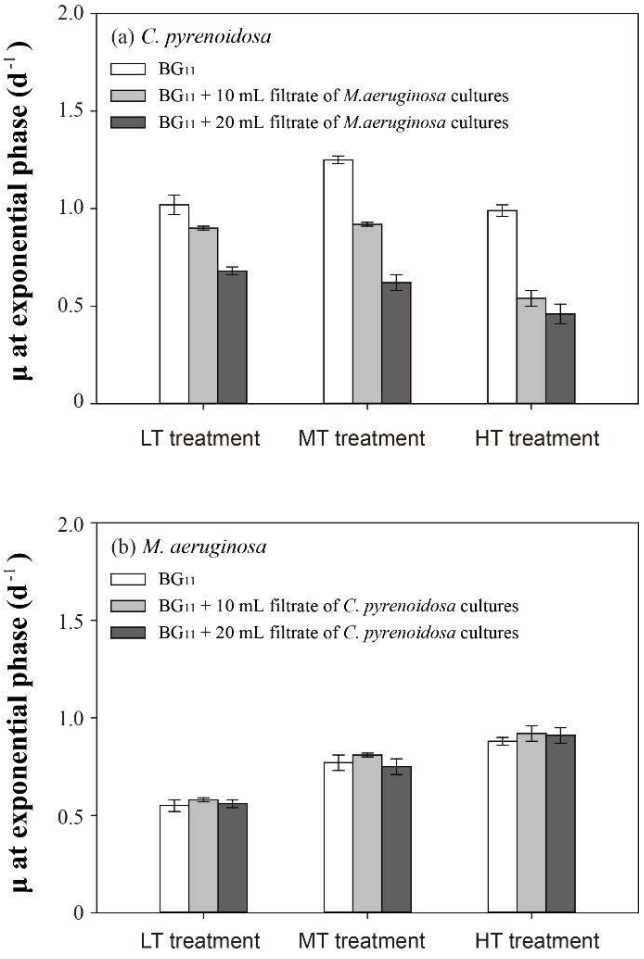


Fig. 6. Effects of the filtrate of cultures on the growth of the other species.

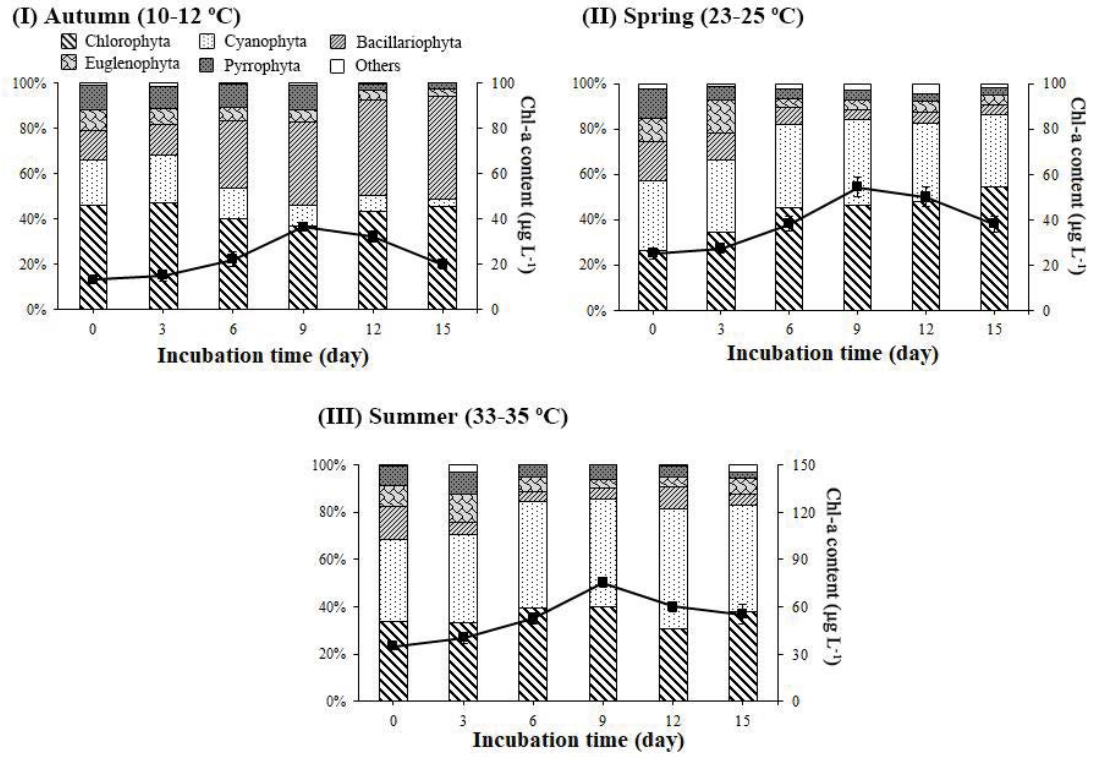
Ren et al. [24] suggested that algal growth patterns under different conditions were attributed to the synergistic effects of P utilization behaviors and its resource allocation strategy. Results in our study indicated that P utilization of *M. aeruginosa* was promoted at higher temperatures, including the P uptake rate, algal affinity to P and accumulation ability of P. It was partly related to the enhancement of algal metabolism processes, which could provide more energy for the active transport of P by cells [50]. Meanwhile, the highest Q_c of *M. aeruginosa* in the HT treatment could result in a best P utilization efficiency, when *M. aeruginosa* could proliferate faster with the same amount of P. It was in agreement with those reported previously. For example, marine phytoplankton required less phosphate-rich ribosomes to produce the required proteins at higher water temperatures [51]. El-Shehawy et al. [52] reported that cyanobacteria could suffer less damages at higher temperatures and climate warming changed the promoting effects of nutrients on cyanobacterial dominance. The synergistic effects of P availability and temperature on cyanobacteria growth were also observed in our study when the growth advantages of *M. aeruginosa* in the HT treatment increased with higher P availability. It was consistent with Rigosi et al. [20] and Brookes and Carey [53] that, nutrients were much more important than temperature in oligotrophic lakes and higher nutrient levels could increase the sensitivity of cyanobacteria to increased temperatures. In contrast, regardless of similar P utilization behaviors in three treatments, *C. pyrenoidosa* showed the highest photosynthetic efficiency and optimal growth in the MT treatment, which was consistent with Q_c. Similarly, the superiority of *C. pyrenoidosa* growth in the MT treatment was also enhanced with higher P availability.

Some scholars have pointed out N/P ratio has significant effects on the growth and succession of algal species, when a high N/P ratio was often beneficial to cyanobacterial growth [54,55]. It was consistent with the results of our study. Another possible reason was that N supply was sufficient in the modified BG_{11} medium in our study when two algal species demanded a large amount of P to enable the utilization of a large supply of both N and P simultaneously to meet greater biomass [56]. As a result, the growth advantages of *M. aeruginosa* in the HT treatment and the growth advantages of *C. pyrenoidosa* in the MT treatment were magnified with more P supplied and lower initial N/P ratios in the culture medium.

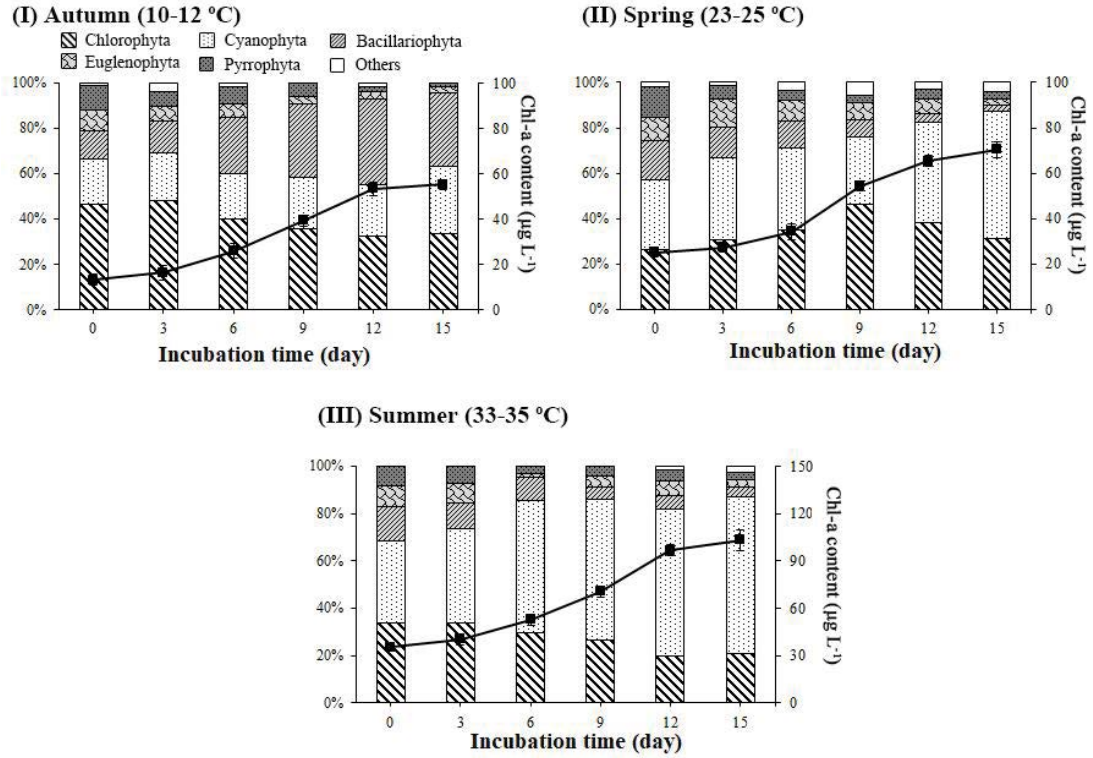
4.2. Competition and interaction between two species

Based on field investigations, HABs are often accompanied by reduced diversity of species and dominance of cyanobacteria [17,57]. For instance, during the outbreak of HABs in the middle and lower reaches of the Yangtze River, *M. aeruginosa* was often the dominant *Cyanophyta* species and *C. pyrenoidosa* was usually the dominant species of *Chlorophyta* [58,59]. However, as mentioned above, *C. pyrenoidosa* grew faster than *M. aeruginosa* in the mono-cultures under the same conditions and it was contrary to the consensus that *Cyanophyta* was the better competitor [60,61]. Our study indicated that the individual effects of temperature might not be the sole determinant for species succession when the combined effects of temperature and P availability in the water on algal competition and interaction could be more decisive.

M. aeruginosa and *C. pyrenoidosa* both persistently grew in the LT treatment and the mutual beneficial effects between two species for their growth were observed in the co-cultures. It was consistent with previous studies that, competition of species did not necessarily result in the negative effects on algae [5,62]. Since *C. pyrenoidosa* exhibited the better P utilization comparing with *M. aeruginosa* at low temperatures, including the faster P uptake rate, a higher affinity to P and a stronger P accumulation ability, the available P for *M. aeruginosa* and *M. aeruginosa* growth were supposed to be further inhibited in the LT treatment in the co-cultures. This unexpected result was probably because of the bioactive secondary metabolites produced by *C. pyrenoidosa*, such as polysaccharide, vitamins and antibacterial compounds [63]. Considering the patterns of *M. aeruginosa* growth in the mono-cultures, the coexistence of cyanobacteria with other species might be able to alleviate its poor adaptation during the period of low temperature and low P levels. In comparison, *M. aeruginosa* could gradually utilize P for its adaptation at low temperatures and maintain its normal growth under Moderate-P and High-P conditions, such as increasing algal



(a) Raw water



(b) P-spiked water

Fig. 7. Species succession of phytoplankton in the ecological flume experiment with (a) raw water and (b) P-spiked water from Meiliang Bay in Lake Taihu.

SOD activity to scavenge excess ROS (Fig. S5). One possible reason was that dissolved inorganic phosphorus (DIP) is the substrate in the photophosphorylation processes of cyanobacteria to produce ATP, which was involved in producing proteins, transporting electrons, and some other processes [46]. Besides, DIP directly impacts the synthesis of nucleotides and other essential substances for cyanobacteria [43]. As the cell density of *M. aeruginosa* increased under Moderate-P and High-P conditions, two species tended to compete for other ecological resources in the water, and the negative allelopathic effects of *M. aeruginosa* emerged [64,65], leading to decreased promotion of co-cultures on *C. pyrenoidosa* growth. The negative effects of *M. aeruginosa* cultures on *C. pyrenoidosa* in the LT treatment further confirmed the allelopathic effects of *M. aeruginosa*.

At higher temperatures, *M. aeruginosa* growth was promoted but *C. pyrenoidosa* growth was inhibited in the co-cultures. This phenomenon was most obvious in the HT treatment, when *M. aeruginosa* grew fastest (0.51–0.95 d^{-1}) and became dominant at the later stage. On one hand, *M. aeruginosa* exhibited the better P utilization in the MT and HT treatments, which could favor its competitive advantages. Rascon et al. [12] also reported that, the shift of phytoplankton toward smaller cyanobacterial species could be driven by its higher competitive ability for nutrient utilization at higher temperatures. On the other hand, the negative allelopathic effects of *M. aeruginosa* on *C. pyrenoidosa* were stronger at higher temperatures. This phenomenon was observed in several other studies [5,66]. For example, rising temperatures could increase microcystin production by *M. aeruginosa*, which might negatively influence the metabolism of other species [67]. With higher P availability in the water, *M. aeruginosa* growth was faster and the allelopathic effects probably resulted in enhanced negative effects on *C. pyrenoidosa*. As a result, the concurrence of HT treatment and high P availability led to the greatest advantage of *M. aeruginosa* in the co-cultures in this study. It was consistent with Kim et al. [56] that, low N/P ratio was often favorable for the development of cyanobacterial blooms at high N levels.

4.3. Implication of experimental results

Features of HABs differed greatly in different regions, and a systematic analysis of the growth strategies of algal species was essential to explain HABs in natural waters. Results in the flume experiment were highly consistent to those in the batch experiment, confirming the generality and validity of our research. Specifically, while *Chlorophyta* showed considerable growth over a wide range of temperatures, temperatures and P enrichment interactively affected the intensification of *Cyanophyta* growth.

Scholars explored the theory of organisms to utilize ecological resources and had extended it to indicate algal growth strategy [68,69], which identified two incompatible strategists. While *r-strategist* usually show a fast intrinsic growth and utilize nutrient for its rapid reproduction, *k-strategist* often had a strong acquisition ability for nutrient and mainly used nutrient for maintaining survival. Our results indicated that the growth strategies of algal species were not constant and temperature had a great impact. At low temperatures, *Chlorophyta* embodied characteristics of both *r-strategist* and *k-strategist*, when it showed a faster growth and had superior P utilization behaviors. In comparison, *Cyanophyta* played as the *k-strategist* and mainly utilized P for its basic survival. As a result, *Chlorophyta* and *Cyanophyta* could coexist in autumn in the flume, when P enrichment only alleviated the negative effects of low temperature on *Cyanophyta* and did not change the predominance of *Chlorophyta*. However, *Chlorophyta* could play as the fast-growing *r-strategist* in spring and summer, which sustained a high growth rate until reaching a plateau or collapsing. Meanwhile, *Cyanophyta* acted as the *k-strategist* and it could survive steadily for a longer period. As a result, the alternating dominance of *Chlorophyta* and *Cyanophyta* was observed in spring and summer, and P enrichment enhanced the advantages of *Cyanophyta* in the flume.

It was generally considered that nutrients were more important than temperature for explaining algal biomass and the cyanobacterial dominance [70]. However, our experiment with the annular flume indicated that, P was important but through its interaction with temperature and not individually in eutrophic lakes. Therefore, P remediation might offset the stimulatory effect of increasing temperature on the growth of cyanobacteria [20]. On the other hand, since N source was not sufficient in the flume experiment, P enrichment resulted in the lower N/P ratio and enabled algal utilization of a larger supply of both N and P simultaneously [56]. As a result, the proportion of *Cyanophyta* was promoted by P enrichment with low N/P ratios in different seasons, as many N-fixing cyanobacteria could satisfy their own N requirements.

Enhanced eutrophication is a common phenomenon in aquatic ecosystems, and the response of phytoplankton is the fundamental driver for algal growth patterns and their succession [17]. Our study reported the P utilization behaviors and growth strategies of *M. aeruginosa* and *C. pyrenoidosa* in mono-cultures and co-cultures, and investigated algal growth patterns in a flume. However, more studies are needed to clarify algal allelopathic effects and the influences of temperatures.

5. Conclusions

- *M. aeruginosa* had a poor adaptation for its growth at low temperatures and it grew better at higher temperatures. *C. pyrenoidosa* showed a flexibility for its growth in a wider range of temperatures and it grew better at moderate temperatures. However, high P availability alleviated the negative effects of low temperatures on *M. aeruginosa*, and led to a stronger adaptation for its growth.
- In the mono-cultures, P utilization of *M. aeruginosa* was promoted at higher temperatures, and its growth advantages in the HT treatment increased with higher P availability in the culture medium. In contrast, P utilization behaviors of *C. pyrenoidosa* were similar in three treatments and higher P availability in the culture medium also enhanced its growth advantages in the MT treatment.
- The combined effects of temperature and P availability on two species could greatly affect their competition results in the co-cultures. The mutual beneficial effects between two species for their growth were observed,

but the growth of *M. aeruginosa* was promoted and *C. pyrenoidosa* was negatively affected in the MT and HT treatments, which was caused by algal P utilization properties and allelopathic effects.

- The growth strategies of algal species were not constant and temperature had a great impact on species succession. In autumn with low temperatures, *Chlorophyta* showed the characteristics of both *r-strategist* and *k-strategist*. However, in spring and summer with warmer temperatures, *Chlorophyta* played as the fast-growing *r-strategist* and *Cyanophyta* acted as the *k-strategist*. Meanwhile, the proportion of *Cyanophyta* was promoted with P enrichment and low N/P ratios.

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Supplementary information

S1. Cultures of phytoplankton community with natural waters in a flume

To explore the generality and validity of our results in a bath experiment based on mono-cultures and co-cultures, *in-situ* water from Meiliang Bay, Lake Taihu containing phytoplankton assemblages were collected and incubated in an annular flume. Meiliang Bay is one of the regions where harmful algal blooms broke out seriously [S1] and this area has been suffered from cyanobacteria for a long time [S2].

In April (spring), August (summer) and October (autumn) of 2018, seasons were both characterized by the vigorous growth of algae with different water temperatures in this area (Fig. S3). The main physicochemical indexes in three seasons could be seen in Table S1. At each season, water samples were directly collected and diverted into 25 L polyethylene darkened carboys. Then, the carboys were placed at 4°C and transported to the laboratory for incubation in an annular flume, with a water depth of about 40 cm. Two blast blowers were used above the water surface with a work/rest regime of 12 h:12 h, and the mean water velocity was about 0.5 m s^{-1}, which was the prevailing water velocity in Lake Taihu [S3,S4]. The mean water velocity was determined by an acoustic doppler velocimeter [S5]. In different seasons, the incubation was maintained at *in-situ* temperatures with the water bath (10°C–12°C in autumn, 23°C–25°C in spring, 33°C–35°C in summer) to ensure uniform heat treatment of the incubation system (Fig. S3). Meanwhile, the P-spiked water with 2.5 mg P L^{-1} was also incubated in the annular flume and the other incubation conditions were as described above.

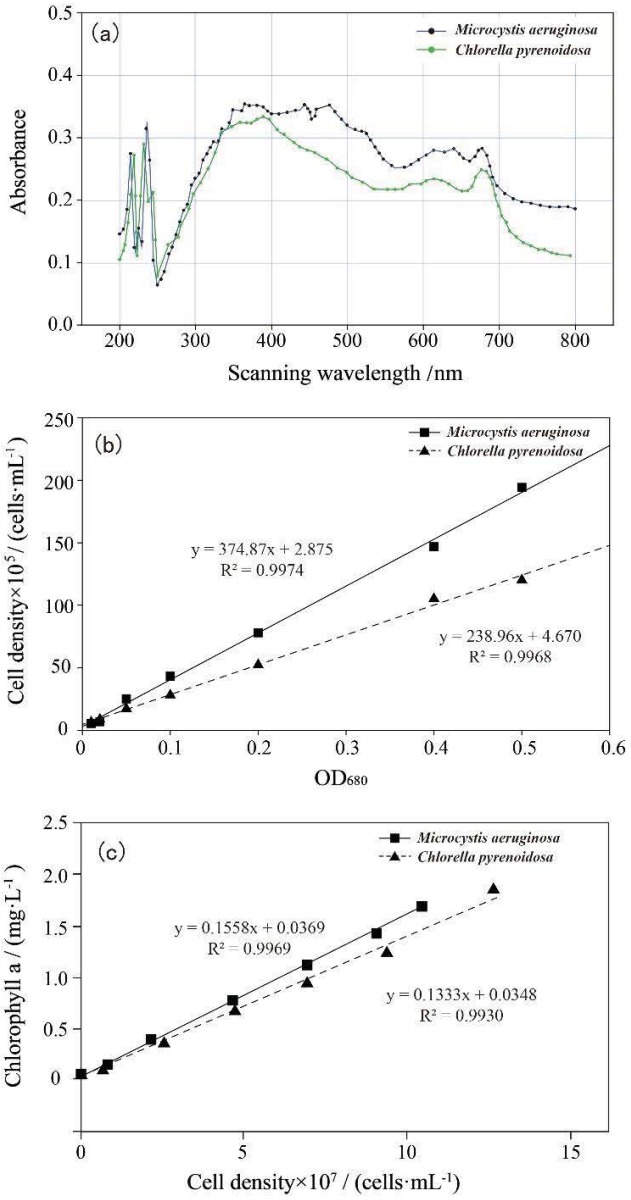

Fig. S1. (a) Absorbance of two algal species during the exponentially growing phase, (b) correlation between OD_{680} and cell density, and (c) correlation between cell density and chlorophyll-a (chl-a) content.

The incubation lasted for 15 d and the growth of the phytoplankton community was estimated by directly measuring chlorophyll-a (chl-a) using the ethanol extraction method [S6]. Meanwhile, the different phyla of algal species were identified and quantified at ×400 magnification using a light microscope (Axioskop 40; Zeiss, Germany) with the reference compiled by Hu and Wei [S7].

S2. Kinetic parameters of P uptake by two species

After pre-cultures and enlarge cultivation, *Microcystis aeruginosa* and *Chlorella pyrenoidosa* cells were rinsed with ultrapure water and immediately transferred into 50 mL colorimetric tubes with modified BG_{11} culture medium containing K_2HPO_4 at initial concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 mg L^{-1}. Tubes were placed in the illumination incubator at different temperatures and incubated under 40 μmol photons m^{-2} s^{-1} PAR for 1 h.

Cell density in all treatments did not change significantly after 1 h of incubation, and P uptake rate of two species was calculated by directly measuring the disappearance of P from the culture medium. The maximum uptake rate (V_{max}, μg [mg dw]$^{-1}$ h^{-1}) and half-saturation constant (K_m, μg L^{-1}) were estimated based on the following equation, where V was the P uptake rate in different tubes and the dry weight (dw) of cells was measured by freeze-drying method.

$$V = \frac{V_{\max}[S]}{K_m + [S]} \quad \text{(S1)}$$

S3. Measurement of the algal release rate of K+ and dissolved organic carbon

As K^+ is often absorbed into the vacuole of algal cells and mainly stored as an enzyme activator, the release of K^+ can be manifested for cellular membrane damages [S8]. In our study, a 10 mL of supernatant sample was regularly taken and filtered through 0.2 μm mixed cellulose ester filters (Whatman) during the 10 d incubation period. Afterwards, the solution was acidified to pH = 2 with HNO_3 and K^+ was measured by inductively coupled plasma mass spectrometry

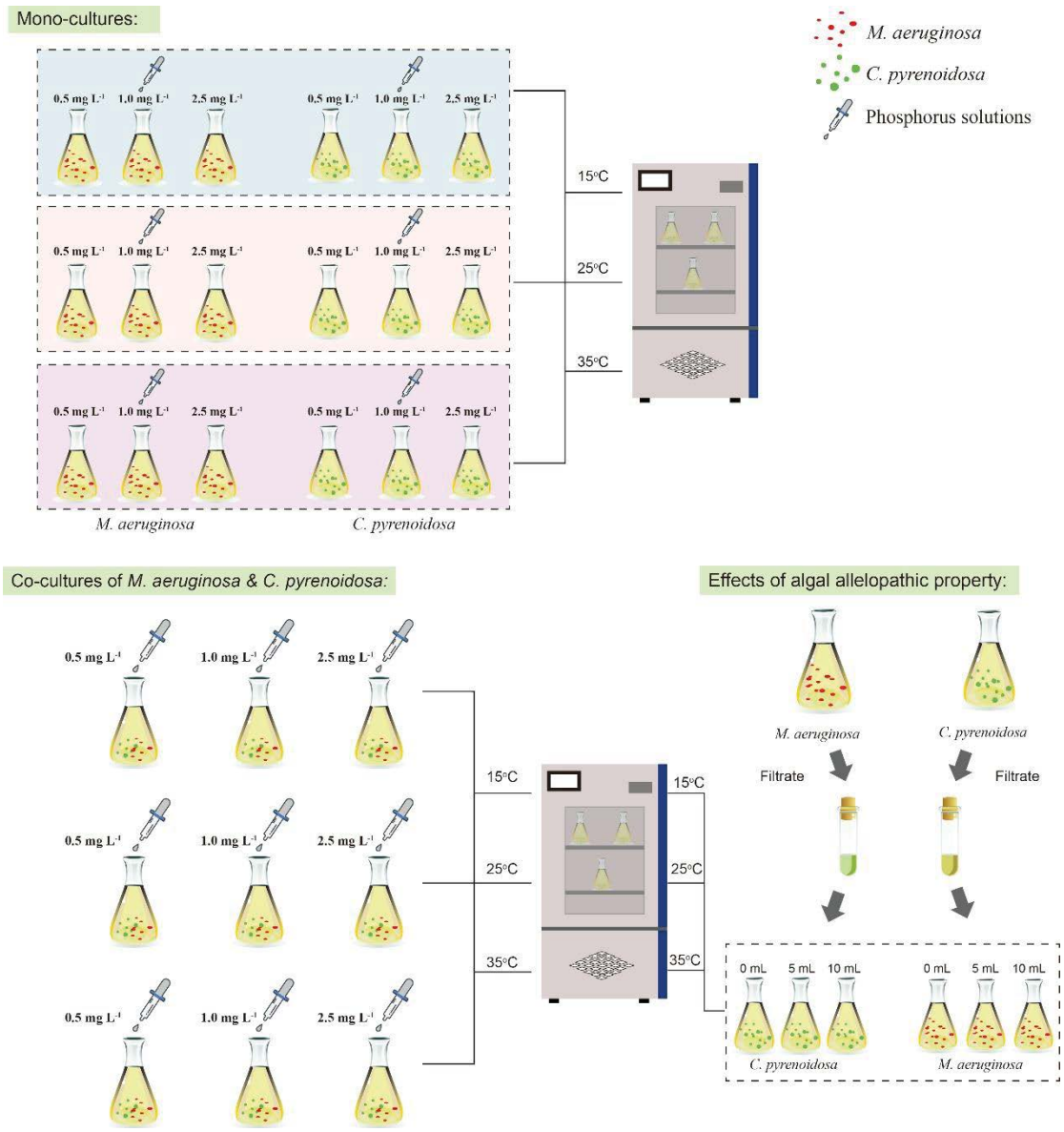

Fig. S2. A schematic diagram of the mono-cultures and co-cultures of two strains.

(IC-PMS) (XII Series, Thermo, USA). The release rate of K^+ by algal cells was calculated using the following equation, where C_0 is the initial K^+ concentration in the algal cultures and C_i is the K^+ concentration of subsamples. Moreover, we have adopted ultrasonic disrupted algal samples to make a comparison.

$$K^+ \text{ Release}(\%) = \frac{(C_i - C_0)}{C_0} \times 100 \quad \text{(S2)}$$

Since the release of intracellular organics matters and an increase of dissolved organic carbon (DOC) could be observed due to the damage of algal cells [S9], the variation of DOC content in algal cultures is also often used as an indicator of the integrity of algal cells. Before and after different treatments on Day 1, 10 mL of a sample of algal cultures was collected and the suspension was centrifuged at 8,000 g for 15 min. Afterwards, the supernatant was filtered through 0.2 μm mixed cellulose ester filters (Whatman) and the filtrate was immediately determined for DOC using a TOC Analyzer (Liquic, Elementar, Germany).

S4. Measurement of reactive oxygen species production and algal superoxide dismutase activity

Before and after different treatments on Day 2, *M. aeruginosa* and *C. pyrenoidosa* cells in the mono-cultures were collected by centrifugation (5,000 g for 10 min, 4°C), washed with phosphate-buffered saline (PBS, 50 mmol L^{-1}, pH 8.0), and suspended in 1.5 mL of PBS solution buffer. The generation of reactive oxygen species (ROS) was monitored using the

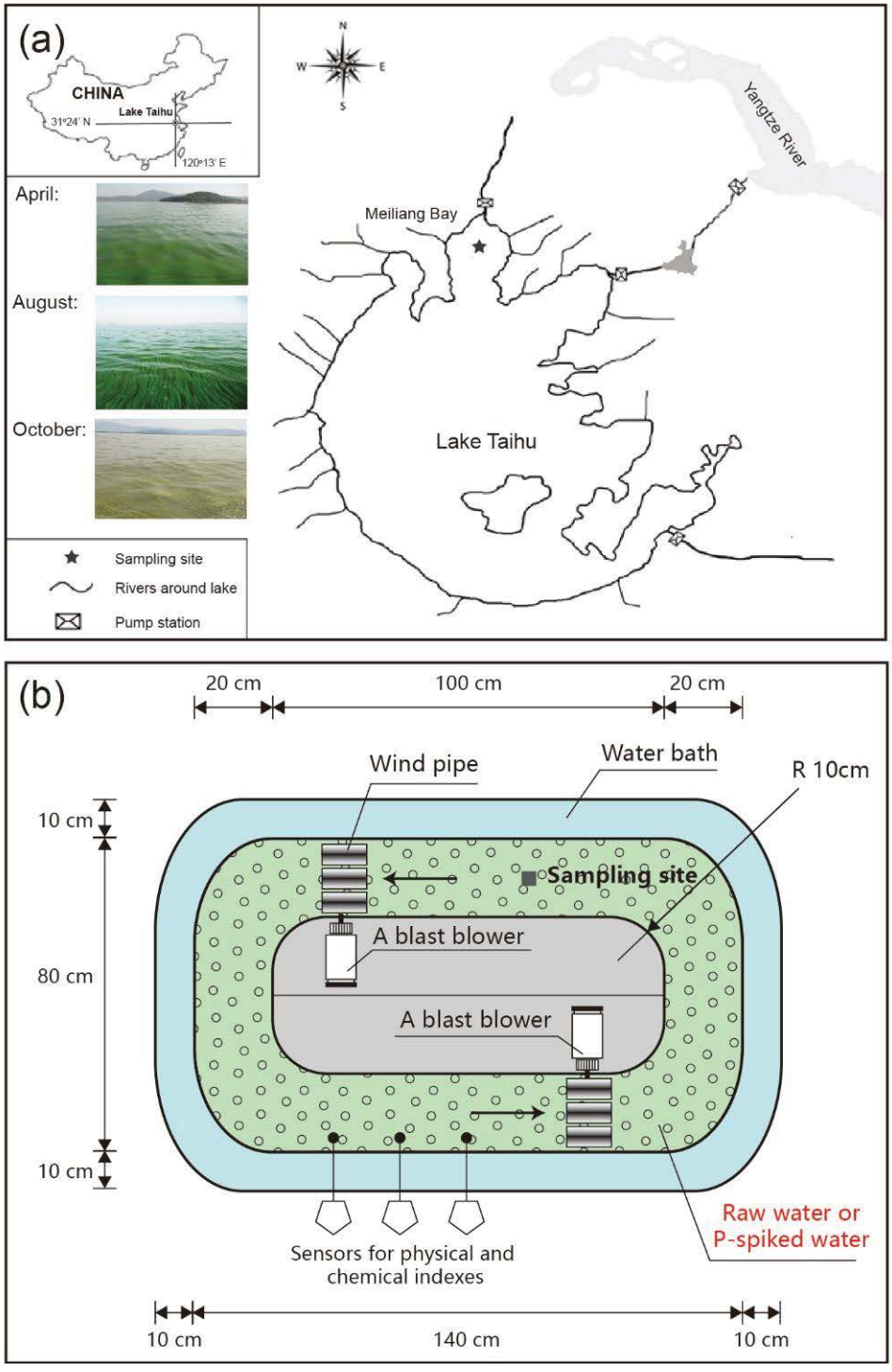

Fig. S3. Cultures of phytoplankton community in an annular flume with natural waters.

ROS-sensitive fluorescence probe 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA; [S10]). Cells of two algal species were incubated with 10 μmol L^{-1} DCFH-DA (Sigma-Aldrich, St. Louis, Missouri, USA), which could diffuse into cells and their acetate groups were cleaved by intracellular esterases to 2′,7′-dichlorodihydrofluorescein (DCFH). Afterwards, the intracellular ROS can oxidize DCFH to the highly fluorescent 2′,7′-dichlorofluorescein (DCF), and the fluorescence intensity is proportional to the ROS content generated by algal cells. After incubation for 15 min at 37°C in the dark,

Table S1
Main parameters of water at the sampling site in three different seasons

Seasons	Chl-a	pH	TP	TDP	TN	NH_4–N	DIP	COD_{Mn}	DOC	Transparency	Turbidity	Velocity	TSS	D_{eu} (PAR)
	(μg L^{-1})			(mg L^{-1})			(μg L^{-1})	(mg L^{-1})		(cm)	(NTU)	(cm s^{-1})	(mg L^{-1})	(m)
Spring (23.2°C–25.4°C)	25.12	7.96	0.163	0.048	3.53	1.24	24.5	5.17	4.92	40.2	30.5	13.8	11.94	1.06
Summer (32.6°C–33.4°C)	35.22	8.44	0.212	0.063	2.68	0.45	15.6	6.55	7.52	35.4	34.6	12.4	14.25	0.92
Autumn (10.3°C–12.4°C)	13.22	7.55	0.174	0.051	2.65	0.48	16.8	4.14	2.68	54.8	29.5	15.4	8.84	1.32

Chl-a: chlorophyll-a content in the water (μg L^{-1});
TP: total phosphorus concentration in the water (mg L^{-1});
TDP: total dissolved phosphorus concentration in the water (mg L^{-1});
TN: total nitrogen concentration in the water (mg L^{-1});
NH_4–N: ammonium nitrogen concentration in the water (mg L^{-1});
DIP: dissolved inorganic phosphorus concentration in the water (μg L^{-1});
COD_{Mn}: chemical oxygen demand in the water (mg L^{-1});
DOC: content of dissolved organic carbon in water (mg L^{-1});
TSS: content of total suspended solid (mg L^{-1});
D_{eu} (PAR): euphotic depth of photosynthetically active radiation (PAR).

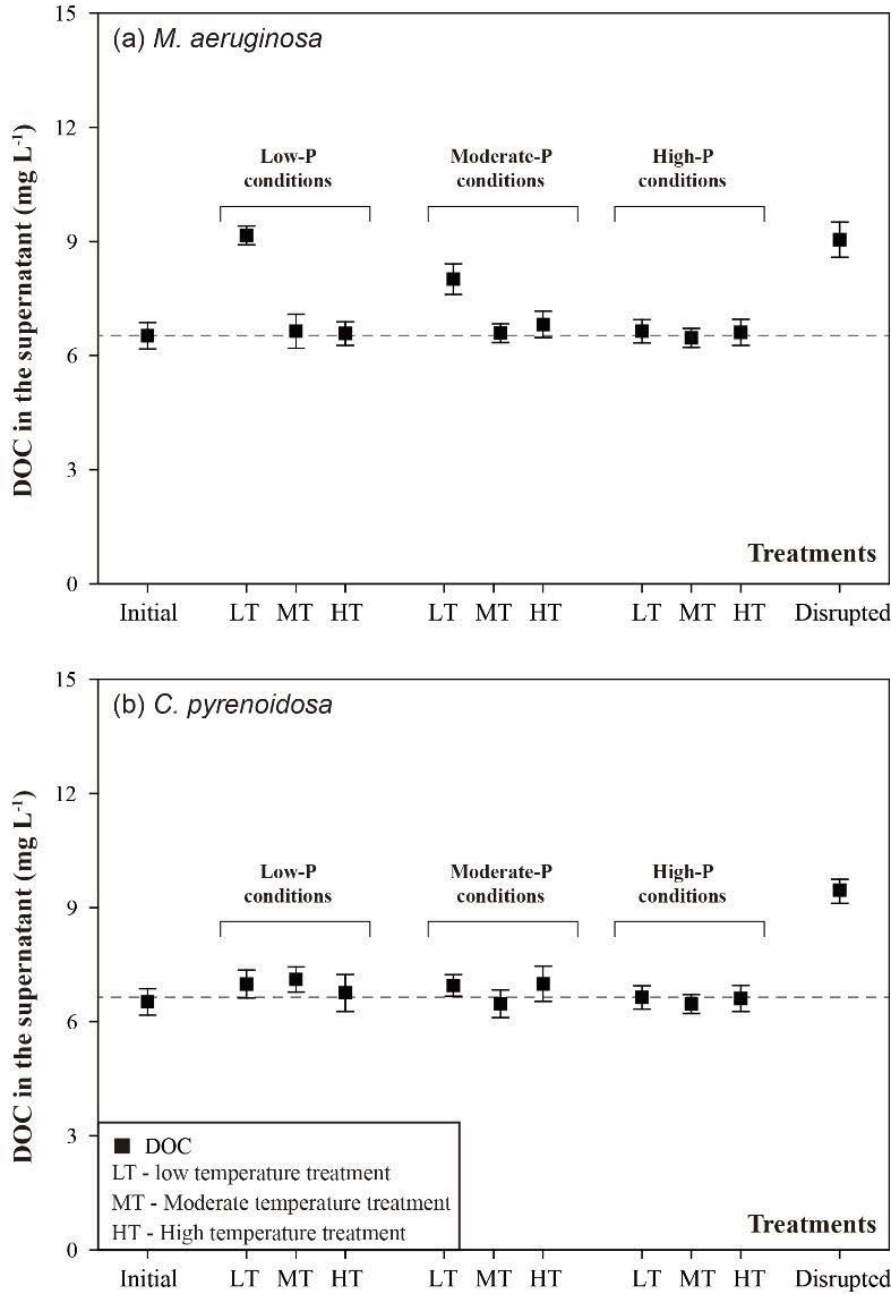

Fig. S4. Variation of DOC content on Day 1 in the supernatant of mono-cultured algal samples in different treatments (LT, MT and HT) under High-P, Moderate-P, and High-P conditions.

algal cells were washed twice with PBS (pH 8.0) and the fluorescence intensity was detected with the excitation at 488 nm and emission at 525 nm [S11].

Meanwhile, *M. aeruginosa* and *C. pyrenoidosa* cells in the mono-cultures were collected by filtrating 10 mL of algal solution using 0.2 μm mixed cellulose ester filters (Whatman) and the collected algal cells were re-suspended in 20 mL of PBS solution (50 mmol L^{-1}, pH 8.0). Then, the cells were disrupted by an ultrasonic cell pulverizer surrounded by ice bags. After centrifugation (5,000 g for 10 min, 4°C), the supernatant was used as crude enzyme solution for detecting superoxide dismutase (SOD) activity with a Total Superoxide Dismutase Assay Kit. The enzyme activity was calculated on the protein content basis using a Bradford Protein Assay Kit. All assay kits used in our study were produced by Jiancheng Bioengineering Institute, Nanjing, China.

S5. Scanning electron microscopy images analysis

To explorer, the microstructure of *M. aeruginosa* in different temperature treatments, scanning electron microscopy (SEM) was conducted for *M. aeruginosa* cells on Day

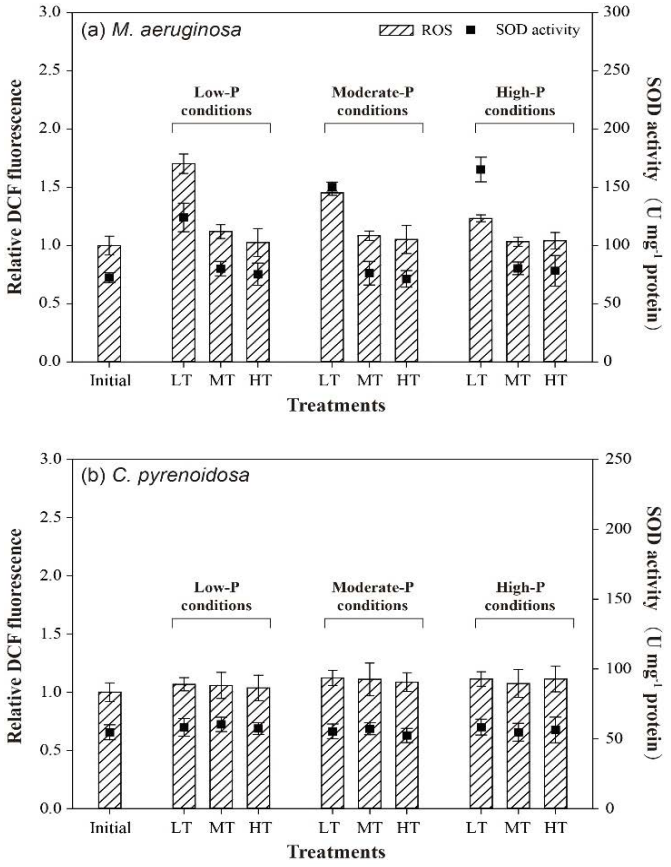

Fig. S5. ROS generation and SOD activity of *Microcystis aeruginosa* and *Chlorella pyrenoidosa* before and after different treatments (Day 2) under Low-P, Moderate-P and High-P conditions.

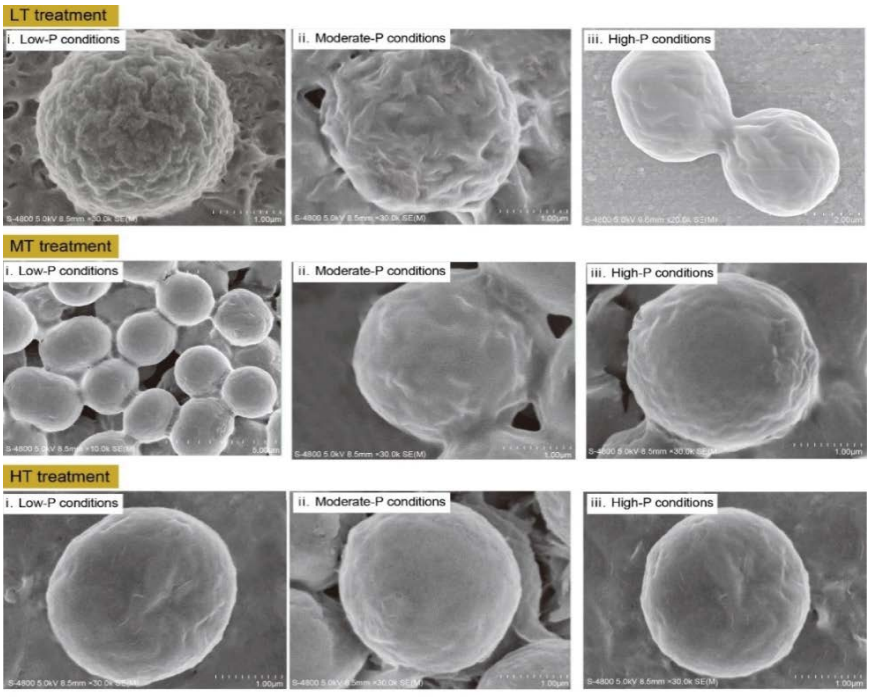

Fig. S6. SEM images of mono-cultured *Microcystis aeruginosa* cells on Day 4 in different treatments under Low-P, Moderate-P and High-P conditions.

Table S3
F-values of three-way ANOVA tests showing the effects of P conditions (Low-P, Moderate-P and High-P), species (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*) and sampling time on the cell density, μ_{max}, and F_v/F_m in the co-cultures

Sources of variation	LT treatment						MT treatment						HT treatment					
	Dependent variables																	
	Cell density		μ_{max}		F_v/F_m		Cell density		μ_{max}		F_v/F_m		Cell density		μ_{max}		F_v/F_m	
	F-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
P conditions	70.24	<0.01	58.16	<0.01	65.48	<0.01	61.28	<0.01	48.35	<0.01	35.18	<0.01	70.18	<0.01	45.26	<0.01	70.35	<0.01
Species	62.35	<0.01	30.64	<0.01	32.14	<0.01	48.12	<0.01	3.26	0.09	7.24	<0.01	42.16	<0.01	30.18	<0.01	51.26	<0.01
Time	81.24	<0.01	NA[a]	NA	60.62	<0.01	95.36	<0.01	NA	NA	65.32	<0.01	101.25	<0.01	NA	NA	48.32	<0.01
Interaction																		
P conditions × Species	10.52	<0.05	12.45	<0.05	7.14	<0.05	18.54	<0.01	0.26	0.74	5.12	0.10	18.54	<0.05	11.32	<0.05	9.35	<0.05
P conditions × Time	11.34	<0.05	NA	NA	6.48	<0.05	10.52	<0.05	NA	NA	10.32	<0.05	9.34	<0.05	NA	NA	10.12	<0.05
Species × Time	7.62	<0.05	NA	NA	10.35	<0.05	16.12	<0.05	NA	NA	1.52	0.15	18.36	<0.05	NA	NA	2.35	0.12
P conditions × Species × time	0.54	0.22	NA	NA	0.75	0.20	0.48	0.23	NA	NA	0.32	0.68	0.49	0.21	NA	NA	1.14	0.24

p indicated the significance of effects of the factors or their interactions;
[a]NA: not available.

Table S2
F-values of three-way ANOVA tests showing the effects of temperature treatments (LT, MT and HT) P conditions (Low-P, Moderate-P and High-P) and species (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*) on the cell density, μ_{max}, F_v/F_m, cellular P quota, and Q_c in the mono-cultures

Sources of variation	Dependent variables									
	Cell density		μ_{max}		F_v/F_m		Cellular P quota		Q_c	
	F-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*	*F*-value	*p*
Treatment	95.34	<0.01	65.40	<0.01	86.35	<0.01	30.15	<0.05	50.28	<0.01
P conditions	80.15	<0.01	101.32	<0.01	74.82	<0.01	164.22	<0.01	NA[a]	NA
Species	74.35	<0.01	20.14	<0.05	3.36	0.22	100.68	<0.01	40.15	<0.05
Interaction										
Treatment × P conditions	20.15	<0.05	12.24	<0.05	18.64	<0.05	18.36	<0.01	NA	NA
Treatment × Species	8.35	<0.05	36.54	<0.01	1.12	0.12	12.14	<0.05	10.22	<0.05
P conditions × Species	5.22	<0.05	7.15	<0.05	0..78	0.26	10.82	<0.05	NA	NA
Treatment × P conditions Species	4.15	0.09	1.58	0.12	0.24	0.58	2.25	0.10	NA	NA

p indicated the significance of effects of the factors or their interactions;
[a]NA: not available.

Table S4
F-values of three-way ANOVA tests showing the effects of temperature treatments (LT, MT and HT), P conditions (Low-P, Moderate-P and High-P) and species (*Microcystis aeruginosa* and *Chlorella pyrenoidosa*) on the percentage changes of μ_{max} and percentage changes of F_v/F_m (Day 7) in the co-cultures

Sources of variation	Dependent variables			
	Percentages changes of μ_{max}		Percentages changes of F_v/F_m	
	F-value	*p*	*F*-value	*p*
Treatment	43.52	<0.01	35.28	<0.01
P conditions	70.15	<0.01	84.32	<0.01
Species	48.64	<0.01	38.26	<0.01
Interaction				
Treatment × P conditions	30.22	<0.05	18.35	<0.05
Treatment × Species	13.42	<0.05	9.84	<0.05
P conditions × Species	11.36	<0.05	8.24	<0.05
Treatment × P conditions × Species	1.25	0.46	0.42	0.53

p indicated the significance of effects of the factors or their interactions.

4 (Fig. S6). Cells were harvested by centrifugation (5,000 g for 10 min, 4°C) and fixed with 2.5% glutaraldehyde solution for 24 h. After mildly rinsing with phosphate-buffered saline, *M. aeruginosa* was dehydrated successively by a series of ethanol solutions in a vacuum drier (50%–100%). Subsequently, the treated cells were mounted on copper stubs, coated with spray gold, and examined through SEM (S-4800, Hitachi, Japan) at 5 kV.

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