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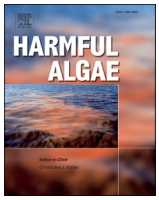

Original Article

# A novel strategy of bloom forming cyanobacteria *Microcystis* sp. in response to phosphorus deficiency: Using non-phosphorus lipids substitute phospholipids

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ABSTRACT

Despite significant reductions in phosphorus (P) loads, lakes still experience cyanobacterial blooms. Little is known regarding cellular P regulation in response to P deficiency in widely distributed bloom causing species such as *Microcystis*. In this study, we investigated changes in P containing and non-P lipids contents and their ratios concomitantly with the determinations of expression levels of genes encoding these lipids in cultural and field *Microcystis* samples. In the culture, the content of phosphatidylglycerol (PG) decreased from 2.1 μg $g^{-1}$ in P replete control to 1.2 μg $g^{-1}$ in P-deficient treatment, while non-P lipids, like sulfoquinovosyldiacylglycerol (SQDG) and monogalactosyldiacylglycerol (MGDG), increased dramatically from 13.6 μg $g^{-1}$ to 142.3 μg $g^{-1}$, and from 0.9 μg $g^{-1}$ to 16.74 μg $g^{-1}$, respectively. The expression of the MGDG synthesis gene, *mgdE*, also increased under low P conditions. Significant positive relationships between soluble reactive phosphorus (SRP) and ratios of P-containing lipids (PG) to non-P lipids, including SQDG, MGDG and digalactosyldiacylglycerol (DGDG) ($P < 0.05$) were observed in the field investigations. Both cultural and field data indicated that *Microcystis* sp. might increase non-P lipids proportion to lower P demand when suffering from P deficiency. Furthermore, despite lipid remodeling, photosynthetic activity remained stable, as indicated by comparable chlorophyll fluorescence and *Fv/Fm* ratios among cultural treatments. These findings suggested that *Microcystis* sp. may dominate in P-limited environments by substituting glycolipids and sulfolipids for phospholipids to reduce P demand without compromising the photosynthetic activity. This effective strategy in response to P deficiency meant a stricter P reduction threshold is needed in terms of *Microcystis* bloom control.

## 1. Introduction

*Microcystis* sp. is one of the most important species responsible for harmful algal blooms, posing serious threats to public health and the environment (Chia et al., 2018; Jochimsen et al., 1998; Lee et al., 2018; Pimentel and Giani, 2014; Qian et al., 2019; Su et al., 2016). Phosphorus (P) is an essential nutrient for its growth, directly influencing the scale and severity of algal blooms (Huang et al., 2016; Schindler et al., 2016). In natural water bodies, dissolved phosphorus (DP) mainly consists of dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) (Li et al., 2015a; Zhang et al., 2018). Among them, orthophosphate (Pi) is the main type of DIP that can be directly utilized by algae (Huang et al., 2019; Muscarella et al., 2014) and is the preferred form for *Microcystis* sp. (Zhang et al., 2021, 2020). It is generally believed that high concentrations of bioavailable P favor the growth of *Microcystis* sp. (Conley et al., 2009; Ren et al., 2020; Yang et al., 2014). However, *Microcystis* sp. can also survive and maintain relatively slow growth levels in P-limited environments, sometimes even reaching exponential growth phases (Cai and Tang, 2021; Hu and Zhang, 2019), and may lead to *Microcystis* sp. blooms (Yuan et al., 2019). This indicates that *Microcystis* sp. has a well-developed low-P adaptation mechanism that requires further investigation.

Existing studies have shown that *Microcystis* sp. can respond to P limitation genetically by upregulating the expression of P transport

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protein genes (Harke et al., 2012). At the same time, alkaline phosphatase (AP) hydrolyzes DOP into DIP for utilization by *Microcystis* sp., enabling good growth under low-P conditions (Dyhrman et al., 2007; Li et al., 2015b). Moreover, *Microcystis* sp. can engage in luxury uptake to accumulate phosphorus as polyphosphate (poly-P) (Shi et al., 2003; Wan et al., 2019; Wang et al., 2022). Additionally, P deficiency can activate P-independent metabolic pathways in *Microcystis* sp. to restore cellular balance and alleviate metabolic stress (Peng et al., 2017, 2018). Simultaneously, phytoplankton also have the ability to reduce their cellular P demand by up to 50% in P-limited environments (Bertilsson et al., 2003; Geider and La Roche, 2002; Krauk et al., 2006). Although *Microcystis* sp. possesses diverse strategies for coping with low P conditions, it remains unclear whether *Microcystis* sp. is capable of reducing its intracellular P demand under P limitation and how this reduction is achieved.

P in phytoplankton primarily exists in nucleic acids and phospholipids (Van Mooy and Devol, 2008; Van Mooy et al., 2006). Under P-limited conditions, the lipid composition of phytoplankton thylakoid membranes undergoes changes. The content of phospholipid phosphatidylglycerol (PG) decreases, while the content of non-P lipids monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyldiacylglycerol (SQDG) increases (Awai et al., 2007; Endo et al., 2016; Van Mooy et al., 2006). Additionally, the ratio of MGDG to DGDG within phytoplankton cells also decreases (Xu and Miao, 2020). However, this mechanism has not been reported in *Microcystis* sp., and further investigation is needed to determine whether changes in thylakoid lipid composition affect photosynthesis.

In this study, *Microcystis* sp. blooms samples were collected from Guanqiao Pond, Qingling Lake, and Donghu Lake in Wuhan city. The P concentration in the water and the lipid composition (PG, DGDG, SQDG, MGDG) in the cells were measured. In addition, a cultural experiment was conducted, and the expression levels of key lipid synthesis genes (*pgs*A, *sqd*B, *mgd*E) under different P stress conditions were quantified using real-time fluorescence quantitative PCR. An effective P response strategy in *Microcystis* sp. gave light in understanding why *Microcystis* sp. dominates phytoplankton assemblages when levels of P are often low. The findings of this research will provide important theoretical and scientific evidence for understanding the formation of P-limited *Microcystis* sp. blooms in lakes and offer new insights for the prevention and control of *Microcystis* sp. blooms.

## 2. Materials and methods

### 2.1. Field sample collection and analysis

Sample collection was conducted in Wuhan City from June to December 2021, including surface water samples (0–50 cm) of the Guanqiao (GQ) Pond in June to December, Qingling (QL) Lake in July and August, and Donghu (DG) Lake in August (Fig. 1). The GQ pond is an abandoned aquaculture body of water that experiences frequent algal blooms throughout the year, with algal densities generally between $10^7$ and $10^9$ Cells $L^{-1}$. QL Lake and DG Lake are large shallow lakes that experience *Microcystis* sp. algal blooms in the summer, with sampling algal densities ranging between $10^6$ and $10^8$ Cells $L^{-1}$. Each water sample collected was 500 mL in volume and intended for biological and biochemical analysis. Additionally, 500 mL of *Microcystis* sp. algal bloom water samples were enriched using zooplankton nets. For cell density estimation, Lugol solution was used to preserve the samples, and cell counts were performed using an Olympus BX 41 microscope (Olympus Corporation, Japan) following the method described by Bowe (Bowe, 2002), aiming to determine the *Microcystis* sp. algae cell density. The enriched *Microcystis* sp. algal bloom samples were transported back to the laboratory and subjected to freeze-drying. Subsequently, the lipid content of the samples was analyzed using LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) (Yoon et al., 2012b).

### 2.2. Microcystis aeruginosa cultures

Experiments were conducted using the *Microcystis aeruginosa* strain FACHB 1322 obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB-collection, Wuhan, China). Cultures were cultivated in Blue Green (BG)-11 medium under a bank of fluorescent lights delivering a light intensity of approximately 100 μmol photons $m^{-2}$ $s^{-1}$, following a 12:12 light/dark cycle at 21 °C. Prior to the start of the experiment, exponentially growing *Microcystis aeruginosa* cells were collected by centrifugation at 3000 rpm for 10 min. The cells were then washed three times with phosphate-free BG11 medium and subsequently cultured in phosphate-free BG11 medium for 48 h to deplete the cellular P stores to a low level (Wang et al., 2018). Subsequently, the culture was inoculated into three different phosphate concentrations of BG11 medium for cultivation (Rippka et al., 1979). The phosphate concentrations were 0 mg $L^{-1}$, 0.03 mg $L^{-1}$, and 0.05 mg $L^{-1}$.

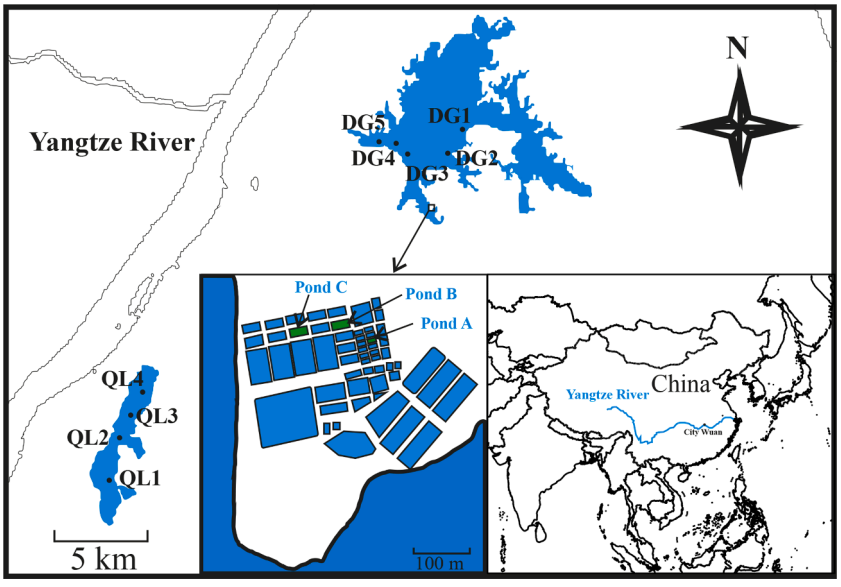


**Fig. 1.** Distribution of sampling points for field samplesv(GQ: Guanqiao Pond, QL: Qingling Lake, DG:Donghu Lake).

### 2.3. Determination of SRP, cell density, and specific growth rate

SRP concentrations were determined according to Murphy and Riley (Murphy and Riley, 1962). Samples for cell density estimation were preserved with Lugol's solution and counted with an Olympus BX 41 microscope (Olympus Corporation, Japan) (Bowe, 2002). The specific growth rate (μ), defined as the time required for cell density to double, was calculated using the following equation: $\mu = 1/N*dN/dt$, Where (t) represents the culture duration, and (N) denotes the cell density.

### 2.4. Determination of intracellular P and sulfur(S)

Enriched *Microcystis* sp. cells are lyophilized, and two sets of samples for intracellular P and S are prepared. The intracellular P content is determined using a total phosphorus measurement method applied to sediment (Ruban et al., 1999). Intracellular S is quantified by incinerating the sample in the high-temperature combustion chamber of an elemental analyzer (Vario macro cube), converting S into sulfur dioxide ($SO_2$) gas. The generated gas undergoes a series of purification and separation steps before the pure $SO_2$ is introduced into a detector for concentration analysis (Jones, 2001).

### 2.5. The fluorescence of Chl a and photochemical efficiency of photosystem II

Chl a fluorescence of *Microcystis aeruginosa* samples was conducted using a plant efficiency analyzer (Pocket PEA, Hansatech Instruments Ltd., UK). Prior to fluorescence measurements, each sample was acclimated in darkness for 30 min. After acclimation, a saturating light pulse of 3500 μmol(quanta) $m^{-2}$ $s^{-1}$ was applied for 1 second, effectively closing all reaction centers, and fluorescence parameters were measured.

### 2.6. Lipid extraction and determination

The lipid extraction method used in this study was based on Yoon's protocol with some modifications. Five milligrams of freeze-dried algal powder were weighed and mixed with 6 mL of methanol:chloroform (20:10, v:v) solvent at room temperature (Yoon et al., 2012a). The mixture was vigorously vortexed for 1 hour using a vortex shaker. Then, 3 mL of a mixture of 1 mol/L potassium chloride and 0.2 mol/L phosphoric acid solution was added to remove protein impurities. The mixture was vortexed thoroughly. After centrifugation at 1000 × g for 5 min at 4 °C, the organic phase was collected, and the organic solvent was evaporated using a nitrogen blower. The lipid content was determined using LC-MS/MS. Chromatographic conditions were performed using an ACQUITY Ultra Performance Liquid Chromatograph (Waters Corporation, USA) coupled with Xevo TQ-S, separated by a BEH C18 column (2.1 mm × 50 mm, 1.7 μm; Waters Corporation, USA), and analyzed using Multiple Reaction Monitoring (MRM) scanning mode. The mobile phases in positive ion mode were A: methanol: acetonitrile: ultrapure water (19:19:2, v/v/v) + 10 mmol/L ammonium acetate + 0.1% formic acid; B: isopropanol + 10 mmol/L ammonium acetate + 0.1% formic acid. The gradient elution program was set as follows: 0 min, 10% B; 1 min, 10% B; 6 min, 25% B; 10 min, 60% B; 10.1 min, 10% B; 13 min, 10% B. In negative ion mode, the mobile phases were A: methanol: ultrapure water (85:15, v/v) + 10 mmol/L ammonium acetate; B: isopropanol + 10 mmol/L ammonium acetate. The gradient elution program was set as: 0 min, 20% B; 1 min, 20% B; 8 min, 40% B; 9 min, 80% B; 11 min, 80% B; 11.1 min, 20% B; 14 min, 20% B. The flow rate was 0.2 mL/min, with an injection volume of 1 μL per sample (Li et al., 2014).

The standards MGDG 16:3/18:3 and DGDG 18:3/18:3 were purchased from Matreya LLC, USA, while PG 16:0/18:1 and SQDG 16:0/18:3 were obtained from Avanti Polar Lipids, UK.

### 2.7. RNA extraction and quantitative PCR

Take 50 ml of algae solution and centrifuge at 4000 rpm at 4°C for 10 min. Discard the supernatant and transfer the sample into a pre-cooled mortar. Grind the sample into a powder, add an appropriate amount of RNAex, and mix well. Centrifuge at 12,000 g at 4°C for 5 min, then collect the supernatant for subsequent RNA extraction. RNA extraction is carried out using the kit with catalog number AG21101 following the provided procedure, and reverse transcription into cDNA is performed using the kit with catalog number AG11728 according to the steps outlined.Triplicate reactions were performed using Applied Biosystems Power SYBR® Green PCR Master Mix as follows: 10 μL SYBR Green Master Mix, 0.4 μL forward and reverse primers, 1 μL 1:10 dilution of cDNA, and nuclease-free water to achieve a final volume of 20 μL. The quantitative PCR (qPCR) program for most gene targets consisted of an initial denaturation step at 95 °C for 10 min, followed by 45 cycles of 95 °C for 15 s, 62 °C for 30 s, and 72 °C for 30 s. This was followed by an additional step of 95 °C for 15 s and 60 °C for 30 s in an Applied Biosystems 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, California, USA). However, the rpoC1 RNA polymerase gene, used as the housekeeping gene, had a lower annealing temperature of 53 °C (Ginn et al., 2010).To monitor for genomic DNA or PCR contamination, no reverse transcriptase controls and no template controls were included for each sample and primer pair. Additionally, dissociation curves were performed for each reaction as a quality control measure. The fold changes in gene expression were calculated using the Relative Expression Software Tool (REST 2009), which considers differences in reaction efficiency during the fold change calculation (http://www.gene-quantification.de/download.html) (Pfaffl et al., 2002). For each experiment, a single time point sample was used as the calibrator for fold change calculations. The amplification efficiencies of the primer pairs ranged from 95% to 105% with an $r^2$ value of 0.98 or higher (Table 1).

### 2.8. Statistical analysis

In this study, correlation analyses were visualized using SigmaPlot 14.0 and the "ggplot2" package in R software v4.2.2 (including the correlations between lipids and intracellular stoichiometry with SRP). Bar charts and line graphs were created using Origin 2021.

## 3. Results

### 3.1. Microcystis sp. cell density in the field

Field results showed that *Microcystis* blooms occurred in GQ Pond from June to November (Fig. 2a), QL Lake in July and August (Fig. 2b), and DG Lake in August (Fig. 2c). In the GQ pond, the SRP (soluble

**Table 1**
Amplification primers used in PCR, RT-qPCR, and sequencing reactions.

| Gene | | Amplification primers 5′–3′ |
|---|---|---|
| *pgs*A | CDP-diacylglycerol–glycerol-3-phosphate 3-phosphatidyltransferase | (F) GCGACATAAAAGGGGCTAATTGGTG (R) GAAGGCAAGGGAGCGATTAAGAGAG |
| *sqd*B | sulfolipid (UDP-sulfoquinovose) biosynthesis protein | (F) AGTGTACGGTAAAGGTGGTCAAACG (R)CGGATTGAGCAGGATTGGCGATC |
| *mgd*E | monogalactosyl diacylglycerol synthase 1 | (F) CTCGCAAGGAAGTCTGGGTGAATAC (R) AGCATCTAAACGACGCAGGGTAATC |
| 16S | Ribosomal RNA | (F)GCGATGGTCGTCCCCTAGAGG (R) CCTTGTTGTAAGCGTCGGATGATTC |
| *rpoC1* | RNA polymerase housekeeping gene | (F)CCTCAGCGAAGATCAATGGT (R)CCGTTTTTGCCCCTTACTTT |

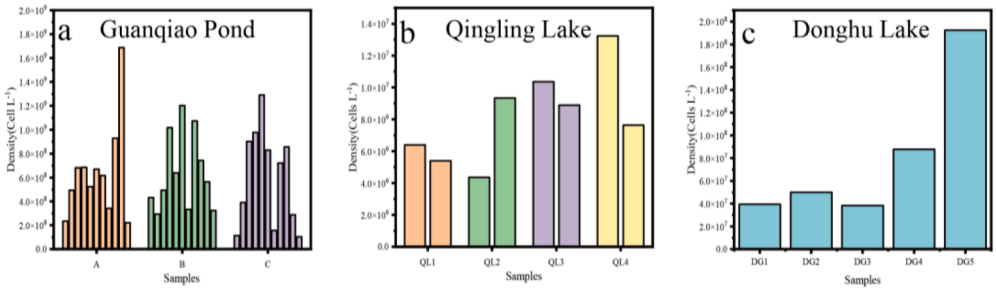


**Fig. 2.** *Microcystis* sp. cell density in the field.

reactive phosphorus concentration ranges from 0.02 to 0.96 mg $L^{-1}$. In QL Lake, the SRP concentration ranges from 0.0064 to 0.16 mg $L^{-1}$, and in DG Lake, the SRP concentration ranges from 0.04 to 0.16 mg $L^{-1}$. The *Microcystis* sp. density in the water ranged from $4.4 \times 10^6$ to $1.7 \times 10^9$ cells/L (S1). Despite the low P concentration in the water bodies, *Microcystis* sp. algae density remains high.

### 3.2. Relationship between SRP concentration and lipids contents in Microcystis sp

To explore the relationship between variations in different lipids and P stress. In terms of phospholipids, utilizing particulate P to represent intracellular P content in *Microcystis*, we observed a significant positive correlation between PG and both PP and SRP ($P < 0.05$) (Fig. 3a and b). Regarding sulpholipids, there was a significant positive correlation between DIN:SRP and intracellular S and SQDG content in *Microcystis* ($P < 0.05$) (Fig. 4a and b), along with a significant positive correlation between SRP and PG:SQDG ($P < 0.05$) (Fig. 4c). In the case of glycolipids, we found a significant positive correlation between DIN:SRP and MGDG ($P < 0.05$) (Fig. 5a), with a positive correlation trend observed for DGDG but not statistically significant ($P > 0.05$) (Fig. 5c). Moreover, SRP demonstrated significant positive correlations with both PG:MGDG and PG:DGDG($P < 0.05$) (Fig. 5b and d), while showing a significant negative correlation with MGDG:DGDG($P < 0.05$) (Fig. 5e).

We defined the following categories based on SRP concentrations: SRP < 0.02 mg $L^{-1}$ as low P, 0.02 < SRP < 0.18 mg $L^{-1}$ as moderate P, and SRP > 0.18 mg $L^{-1}$ as high P. When dividing SRP concentrations into low, medium, and high levels for correlation analysis, a significant negative correlation was observed between SRP and DGDG, MGDG, and SQDG when SRP concentrations were at low levels (SRP < 0.025 mg $L^{-1}$). When SRP levels are moderate to high, the negative correlation between MGDG and DGDG is not significant, and there is even a positive trend between SQDG and SRP (Fig. 6).

### 3.3. Cell density, specific growth rate chlorophyll fluorescence in cultures

SRP concentrations in the three treatments (0 mg $L^{-1}$ P, 0.03 mg $L^{-1}$ P, 0.05 mg $L^{-1}$ P) remained relatively stable during the 40-day cultivation around the set concentrations (Fig. 7a). Higher SRP concentrations were associated with increased cell density in the *Microcystis* sp. (Fig. 7b). Furthermore, the P-deficient treatment exhibited a significantly lower growth rate compared to the other two treatment groups (Fig. 7c).

On the final day of cultivation, the results indicate that the *Fv/Fm* values were equivalent for the 0 mg $L^{-1}$ P and 0.03 mg $L^{-1}$ P treatment groups, while the 0.05 mg $L^{-1}$ P treatment exhibited the highest *Fv/Fm* value. The chlorophyll a fluorescence rise kinetics curve (OJIP) remained consistent across all three treatment (Fig. 8).

### 3.4. The lipid content and gene expression in cultures

The lipid content within the *Microcystis aeruginosa* cells was determined on the final day of cultivation. Among the three treatment groups, the content of PG decreased with decreasing SRP concentration (Fig. 9a), while the content of MGDG and SQDG increased with decreasing SRP concentration (Fig. 9b and c). The qPCR results indicate that as the SRP concentration decreases, the expression level of the monogalactosyldiacylglycerol synthase gene (*mgd*E) increases, while the expression levels of the phosphatidylglycerol synthase gene (*pgs*A) and sulfoquinovosyldiacylglycerol synthase gene (*sqd*B) exhibit an initial increase followed by a decrease. Moreover, in the 0 mg $L^{-1}$P treatment group, the expression level of the *mgd*E gene is significantly higher than that of the *pgs*A and *sqd*B genes (Fig. 10).

## 4. Discussion

Freshwater blooms caused by the cyanobacterium *Microcystis* sp., are the most widespread harmful algal blooms globally (Ho et al., 2019). P is an essential nutrient for living organisms and is often a limiting nutrient in freshwater ecosystems (Conley et al., 2009; Ho et al., 2019; Huang et al., 2019; Muscarella et al., 2014; Ren et al., 2020). The P utilization strategies of *Microcystis* sp. have been extensively studied, but the underlying biochemical and molecular mechanism of *Microcystis* sp. bloom under low ambient P condition is still unclear. In current studies, field observations have shown that *Microcystis* sp. blooms occurred under low

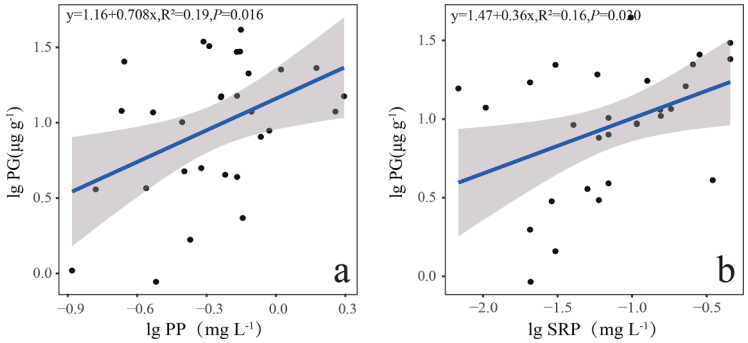


**Fig. 3.** Correlation of PG with SRP(a) and PP(b).

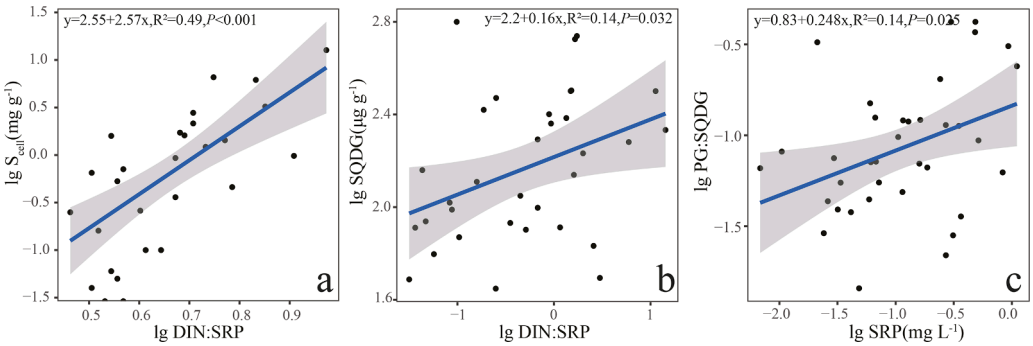


**Fig. 4.** The correlation between DIN:SRP and intracellular S(a) and SQDG content(b), as well as the correlation between SRP and PG:SQDG(c).

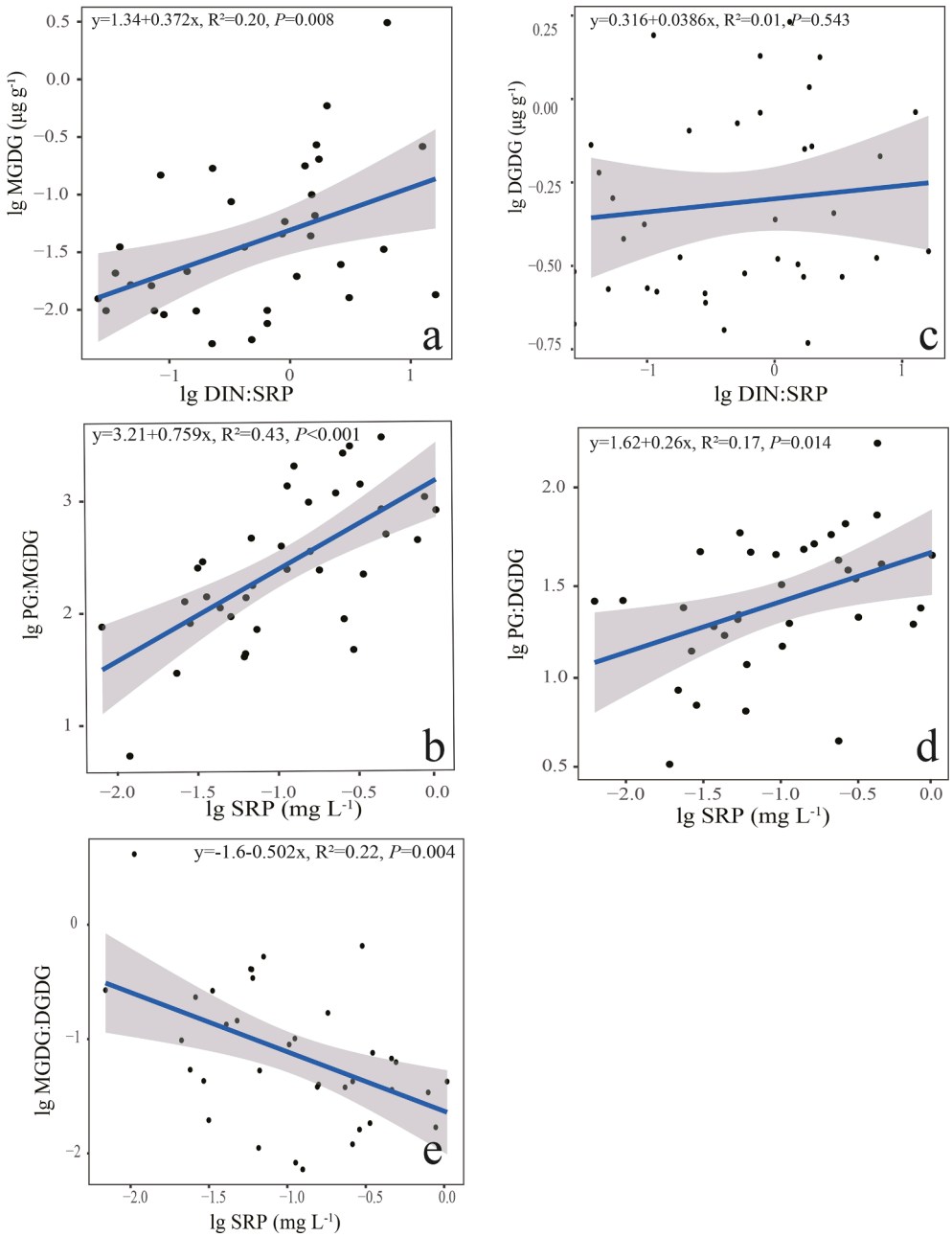


**Fig. 5.** The correlation between DIN:SRP and the content of MGDG(a) and DGDG(c), as well as the correlation between SRP and PG:MGDG(b), PG:DGDG(d) and MGDG:DGDG(e).

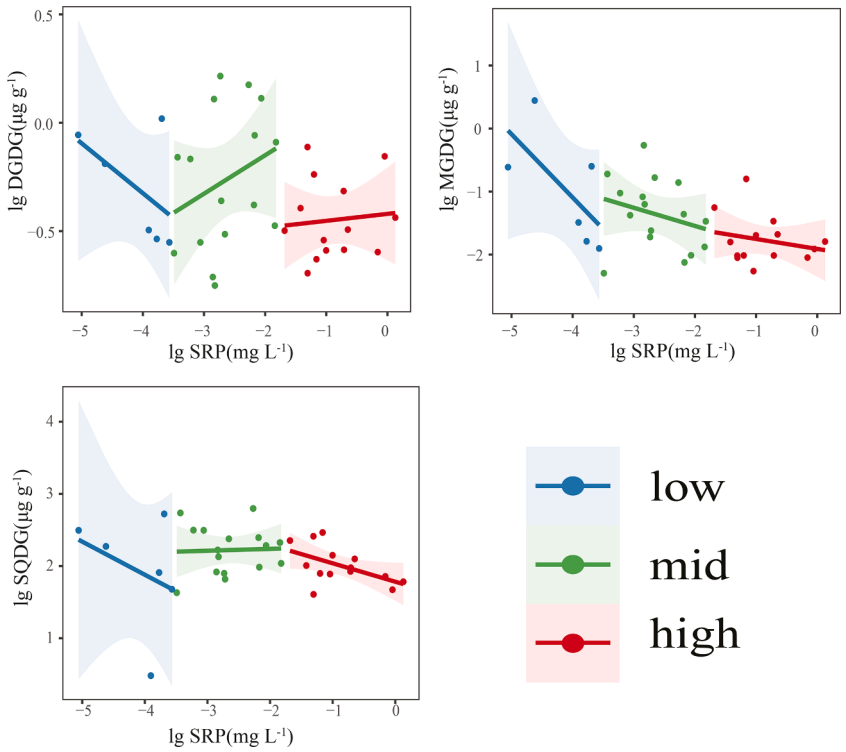


**Fig. 6.** Correlation of SRP with MGDG (a), DGDG (b), and SQDG (c).
(low: SRP < 0.02 mg $L^{-1}$; mid: 0.02 < SRP < 0.18 mg $L^{-1}$; high: SRP > 0.18 mg $L^{-1}$)

P conditions. Here, we found a previously unrecognized response of *Microcystis* sp. to low P supply, such as the utilization of non-P lipids as a substitute for phospholipids, thereby reducing cellular P demand. Most notably, when this substitution occurs, *Microcystis* sp. maintained comparable photosynthetic activity inspite a reduction in specific growth rate. These findings was further supported by biochemical and molecular studies conducted in *Microcystis* sp. cultures. These results collectively indicated that *Microcystis* sp. employed a non-P lipids substitution strategy, allowing it to cope with low Pconditions without sacrificing its activity.

Our research reveals that *Microcystis* sp. can substitute phospholipids with non-P lipids, reducing cellular P demand without compromising photosynthetic activity, even when SRP levels drop below 0.02 mg $L^{-1}$ and specific growth rates decline. This adaptation is corroborated by the consistent findings of decreased cellular P quotas and phospholipid PG content in both lab cultures and field samples of *Microcystis* sp., as P concentrations diminish (Fig. 9d). Cyanobacteria, including *Microcystis* sp., commonly increase inorganic P uptake and transport, produce extracellular phosphatases for organic P hydrolysis, and reduce P-containing biochemicals as part of their response to P scarcity (Harke et al., 2012; Van Mooy et al., 2006; Wan et al., 2019). Additionally, these organisms modulate membrane transport protein expression and enzyme activities to lower intracellular P quotas (Saxton et al., 2012; Wan et al., 2019), with variations observed among individual cells (Wei et al., 2022). Thus, *Microcystis* sp. maintains robust cell densities in P-depleted waters by effectively managing their intracellular P resources, indicating a sophisticated survival strategy that contributes to their resilience and bloom formation capability.

PG is an indispensable phospholipid with photosynthetic functions in plants and cyanobacteria (Weier et al., 2005). Both PG and SQDG serve as the two negatively charged membrane lipids in microalgae, suggesting potential functional similarities within the cell (Kobayashi, 2016). Further previous investigations have revealed the crucial role of SQDG in substituting PG, especially under PG deficiency conditions, such as P starvation, in *Arabidopsis* (Yoshihara et al., 2021). Similarly, the marine microalga *Prochlorococcus* has been found to substitute SQDG for PG under P-limited conditions (Van Mooy et al., 2006). In this study, a similar strategy was identified in *Microcystis* sp.. Field experiments demonstrated that as P concentrations decreased, cellular sulfur (S) content increased (Fig. 4a). Furthermore, the positive correlation between PG and SRP while negative relationship between SQDG and SRP indicated the potential of *Microcystis* sp. to replace PG with SQDG. Our culture experiments supported this observation, showing a decrease in PG content and an increase in SQDG content as P concentrations decreased.

Similar results were observed for glycolipids substitution in *Microcystis* sp.. For example, MGDG showed a positive correlation with DIN: SRP ratio ($P < 0.01$), and DGDG displayed a positive correlation trend (not significant) in field blooming samples. Moreover, the significant positive correlations between lipids ratio and SRP concentrationssuggested MGDG and DGDG substitution for PG in *Microcystis* sp. under P deficiency. This observations aligns with previous observations in plant cells and marine phytoplankton where DGDG and MGDG levels increased under P-deficient conditions (Bertilsson et al., 2003; Shemi et al., 2016). The induction of key gene expression involved in glycolipid conversion during P deficiency further supported the substitution mechanism (Dörmann and Benning, 2002). In our culture experiments, the expression of the glycolipid synthesis gene *mgd*E was enhanced in *Microcystis* sp. when the P concentration in the medium was low. Interestingly, DGDG and its synthesis key ecoding gene were not identified. A possible explanation for these observations might be that *Microcystis* sp. could overcome P stress by using glycolipid to replace PG and predominantly utilize SQDG or MGDG rather than DGDG, which have rarely been reported in *Microcystis* sp. so far.

PG is a critical photosynthetic phospholipid in both plants and cyanobacteria, essential to the photosynthetic apparatus (Domonkos et al., 2008; Weier et al., 2005). The lipid triumvirate of MGDG, DGDG, and SQDG forms the backbone of the thylakoid membranes, underpinning chloroplast functionality (Domonkos et al., 2008). sp.It was reported that despite an increase in SQDG compensating for reduced PG in

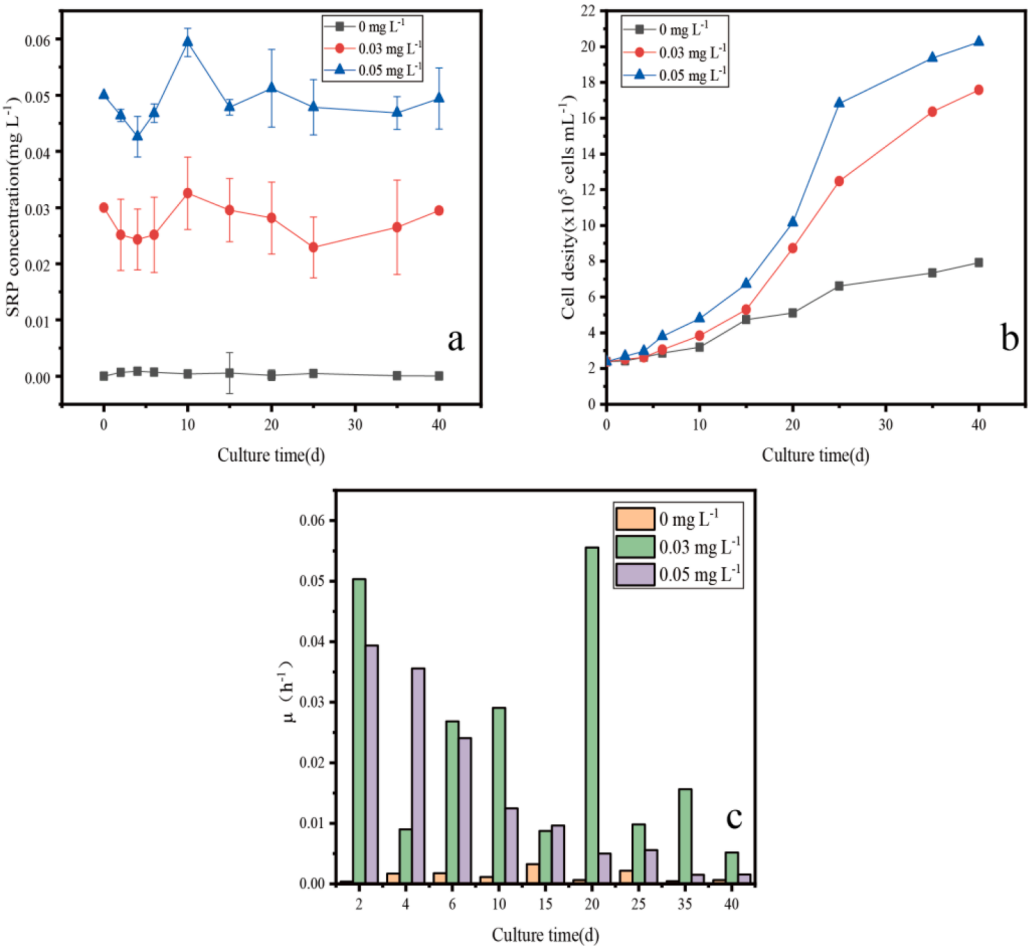


**Fig. 7.** SRP concentration (a), algal cell density (b), and specific growth rate (c) of FACHB 1322.

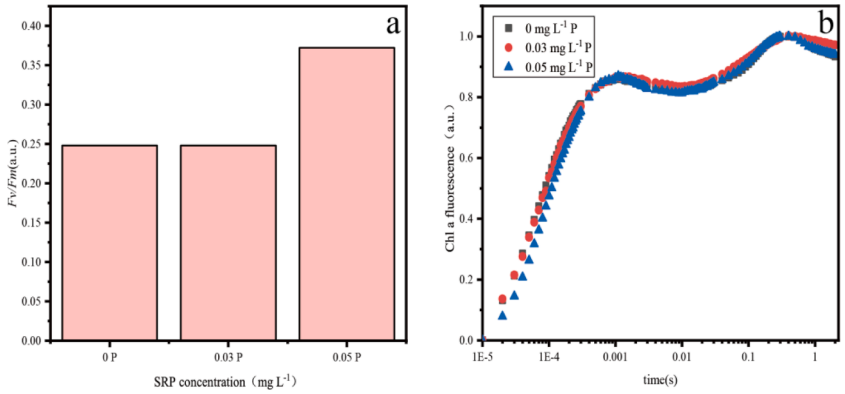


**Fig. 8.** The PSII photochemical efficiency (a) and chlorophyll fluorescence induction kinetics (OJIP) curve (b) of FACHB 1322.

*Synechococcus* sp., photosynthetic efficiency did not recover (Bogos et al., 2010). Our research painted a different picture for *Microcystis* sp.. Varied treatments seemed to exert no discernible effect on the OJIP curves or photosynthetic activity. This observation coincided with an increase in the MGDG:DGDG ratio as SRP levels declined, hinting at lipid remodeling mechanisms. The balance between MGDG and DGDG is documented as being vital for photosynthetic stability and efficiency (Dörmann and Benning, 2002), where a heightened MGDG:DGDG ratio correlates with improved light utilization (Peng et al., 2019) and confers environmental stress resilience, linked to salt tolerance and cold resistance (Hirayama and Mihara, 1987; Nishida and Murata, 1996). Therefore, wehypothesized that the escalated MGDG:DGDG ratio was a compensatory mechanism that preserved photosynthetic efficiency and enhanced the survival prospects of *Microcystis* sp.. Furthermore, our controlled experiments indicated that a deficiency in P led to a decreased specific growth rate when compared with other treatments. This suggests that while lipid remodeling may preserve cellular photosynthetic activity, it does not mitigate the reduction in cell proliferation rate.

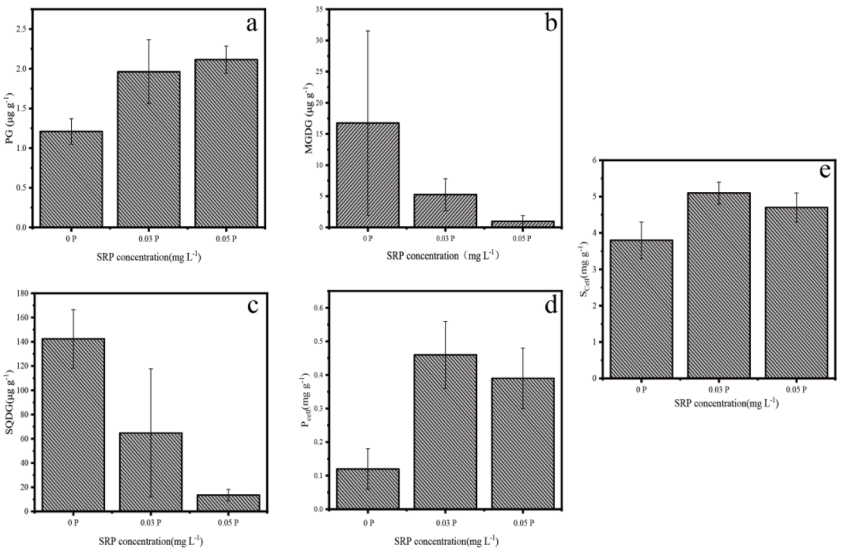


**Fig. 9.** Content of PG (a), MGDG (b), SQDG (c), $P_{cell}$(d) and $S_{cell}$(e) in different treatment of FACHB 1322.

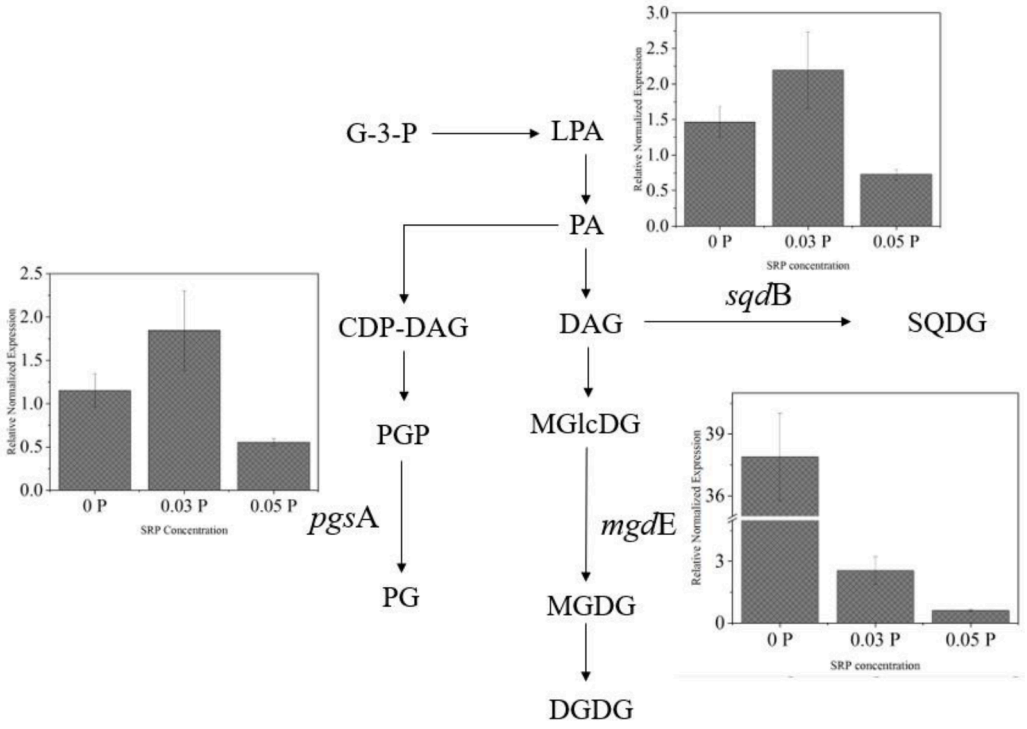


**Fig. 10.** Under phosphate stress conditions, the non-P lipids substitution for the phospholipid pathway and differential expression of lipid synthesis genes.

## 5. Conclusion

In conclusion, our research has demonstrated a strong correlation between the gene regulation of phospholipids and non-P lipids in *Microcystis* sp. and the ambient phosphorus levels. We observed a decrease in phospholipid content and a corresponding increase in non-P lipids as environmental phosphorus concentrations declined. This adaptive response, where non-P lipids substitute for phospholipids, ensures the maintenance of biological functions and photosynthetic activity under phosphorus scarcity. The consistency of our findings across both field and laboratory settings underscores the robustness of this adaptation strategy. Future studies should aim to explore the intricacies of this mechanism and identify the critical thresholds at which it operates, possibly extending the research to include other dominant freshwater algal species. Our findings suggest that *Microcystis* sp. may leverage its effective low-phosphorus response to gain a competitive edge, which could explain the limited success in controlling cyanobacterial blooms through phosphorus reduction. Therefore, we advocate for a more stringent phosphorus reduction threshold to effectively manage *Microcystis* sp. blooms.

## CRediT authorship contribution statement

**Zhenghan Liu:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Lingling Wan:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition. **Jingjie Zhang:** Writing – review & editing, Visualization, Investigation. **Dong Bai:**

Supervision, Methodology, Investigation. **Chunlei Song:** Funding acquisition, Conceptualization. **Yiyong Zhou:** Formal analysis, Conceptualization. **Hong Shen:** Investigation. **Xiuyun Cao:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2024.102694.

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