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Moderate phosphate supply optimizes lipid productivity and pigment accumulation in the freshwater microalga *Monoraphidium braunii*

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ABSTRACT

Phosphorus availability is a master regulator of microalgal metabolism, yet its dose-dependent effects on the simultaneous optimization of biomass, pigment, protein, and lipid accumulation remain poorly resolved for many industrially relevant species. This study systematically evaluated the effects of a phosphate gradient (0, 2.02, 4.09, 8.18, and 17.14 mg L^{-1}) on growth, biochemical composition, and lipid productivity in *Monoraphidium braunii*. *M. braunii* was cultivated in modified BG-11 medium under controlled laboratory conditions using a batch system for five days. Phosphate deprivation severely suppressed growth and biomass yield, while progressive supplementation supported sustained exponential proliferation and drove a 123% increase in protein content, peaking at 37.5% dry weight at 8.18 mg L^{-1}. Photosynthetic pigments exhibited a characteristic non-linear response, maximizing at 4.09 mg L^{-1} before declining at higher concentrations. Lipid content followed an inverse trajectory, declining by 24% from deprivation to full repletion, yet lipid productivity peaked at 4.09 mg L^{-1} (31.5 mg L^{-1} d^{-1}), a 70% increase over phosphate-deprived cultures revealing that severe nutrient stress elevates cellular lipid fraction at the cost of volumetric output. Collectively, these findings demonstrate that phosphorus availability governs carbon partitioning in *M. braunii*, imposing a fundamental trade-off between lipid enrichment and biomass productivity. Moderate phosphate supply (4.09 mg L^{-1}) simultaneously maximized pigment accumulation and lipid productivity, representing the strategic optimum for integrated biorefinery applications targeting biofuel, nutraceutical, and aquaculture feed co-production.

Keywords: biofuel, lipid productivity, moderate phosphate, phosphate deprivation, photosynthetic pigment.

INTRODUCTION

Microalgae constitute a diverse assemblage of unicellular photosynthetic organisms that contribute significantly to global carbon cycling and oxygen production (Hoque et al., 2025). As a promising renewable resource, microalgae convert solar energy, carbon dioxide, and inorganic nutrients into biomass enriched with commercially valuable lipids, proteins, carbohydrates, and pigments (Olguin et al., 2022). Among these biomolecules, lipids hold particular relevance for biofuel production, nutraceuticals, aquaculture feeds, and pharmaceuticals, positioning lipid productivity optimization as a central research priority in microalgal biotechnology (Georgiou et al., 2024; Yaakob et al., 2021). While numerous cultivation strategies have been investigated to enhance lipid

content including light manipulation and mixotrophic supplementation, nutrient manipulation, particularly phosphorus limitation, has emerged as one of the most effective and economically viable approaches for stimulating lipid biosynthesis (Fakhri et al., 2026; Yang et al., 2018).

Phosphorus represents an essential macronutrient integral to nucleic acid synthesis, energy transfer via adenosine triphosphate (ATP), and membrane phospholipid formation (Bossa et al., 2024). Phosphorus deficiency triggers metabolic reprogramming that favors accumulation of neutral lipids, primarily triacylglycerols (TAGs), as energy-dense storage compounds, frequently at the expense of growth rate and protein biosynthesis (Yaakob et al., 2021). This metabolic shift reflects an adaptive strategy wherein cells redirect carbon flux from growth-related pathways toward lipid storage under nutrient-limited conditions (Maltsev et al., 2023). Empirical evidence substantiates this regulatory role: in *Dunaliella salina*, 50% phosphorus reduction elevated lipid accumulation threefold, while complete deprivation resulted in fourfold increases (Almutairi, 2020). Similarly, *Scenedesmus* sp. accumulated lipids up to 42.5% dry weight under phosphorus stress compared to 22.3% under replete conditions (Yang et al., 2018). However, responses exhibit substantial species-specific variation depending on strain physiology, cultivation regimes, and media composition, necessitating targeted investigations across different taxa.

A central challenge in exploiting phosphorus limitation industrially lies in the inherent trade-off between lipid content and biomass productivity. Severe limitation elevates cellular lipid fraction but simultaneously reduces cell division and photosynthetic efficiency, ultimately depressing volumetric lipid productivity, the product of biomass concentration and lipid content (Yang et al., 2018; Yin-hu et al., 2015). Conversely, phosphorus-replete conditions sustain vigorous growth but suppress lipid accumulation in favor of carbohydrate and protein biosynthesis (Raj et al., 2025). Resolving this trade-off through precise identification of optimal phosphorus concentrations is therefore critical for economically viable, large-scale production (Fakhri et al., 2026).

Monoraphidium braunii, a freshwater green microalga of the family Selenastraceae, has attracted growing attention for its rapid growth kinetics, high photosynthetic efficiency, and capacity to produce carotenoids, proteins, and lipids (Fakhri et al., 2024; Georgiou et al., 2024). Despite these attributes, its physiological and biochemical responses of *M. braunii* to phosphorus limitation particularly regarding lipid biosynthesis remain poorly characterized. This gap is significant, given that phosphorus stress can differentially affect pigment profiles, protein synthesis, and cellular composition in ways that are highly strain-specific (Raj et al., 2025; Yaakob et al., 2021). Elucidating these responses in *M. braunii* is relevant not only for biofuel applications but also for aquaculture feed formulation and integrated biorefinery concepts where a balanced biochemical profile is essential.

This study therefore systematically investigates the effects of varying phosphorus concentrations on growth, biomass productivity, pigment composition, protein content, and lipid accumulation in *M. braunii*. The findings will address the knowledge gap regarding *M. braunii*'s phosphorus response, extend understanding of nutrient-mediated lipid metabolism regulation in green microalgae, and inform rational design of cultivation protocols for industrial application.

MATERIALS AND METHODS

Algal strain and maintenance

Monoraphidium braunii (SAG 48.87) was obtained from the Culture Collection of the University of Göttingen, Germany. Stock cultures were maintained in sterile BG-11 medium under continuous white-LED illumination (200 µmol photon m^{-2} s^{-1}) at 25 °C with gentle aeration until mid-exponential phase. Prior to each experiment, cells were harvested by centrifugation (2000×, 15 min), washed twice with sterile distilled water to eliminate residual nutrients, and resuspended in fresh experimental medium. The inoculum was standardized to an initial cell concentration of 3×10^5 cells mL^{-1}, confirmed by hemocytometer counts and an optical density (OD) at 730 nm of 0.08.

Culture medium and phosphorus treatments

The basal medium was BG-11 prepared with distilled water and analytical-grade reagents ($NaNO_3$, K_2HPO_4, $MgSO_4$, $CaCl_2$, Na_2CO_3, trace minerals, EDTA) (Stanier et al., 1971). Phosphate was supplied solely as K_2HPO_4 at five concentrations: 0, 2.02, 4.09, 8.18, and

17.14 mg L^{-1} (the highest level corresponding to the BG-11 reference used in this study). Media were dispensed into culture bottles and sterilized by autoclaving at 121 °C (15 min, 1 atm) prior to inoculation.

Experimental design and cultivation conditions

A completely randomized design (CRD) was employed with five phosphorus treatments and three biological replicates per treatment. Sterile 500 mL glass bottles were filled with 400 mL BG-11 medium and inoculated with 96 mL of *M. braunii* suspension to standardize the initial cell density. Bottles were placed randomly to avoid positional bias, continuously aerated with filtered air via glass capillaries to maintain mixing and CO_2 supply, and incubated at 25 °C under constant illumination (200 µmol photon m^{-2} s^{-1}) for 5 days (Fakhri et al., 2026). The 5-day duration was selected to capture exponential growth and the early metabolic response to phosphorus availability, in line with microalgal kinetic studies under nutrient manipulation.

Growth analysis

Growth was monitored spectrophotometrically at 730 nm (Thermo Scientific GENESYS 20 UV–Vis). Specific growth rate (µ, d^{-1}) was calculated from logarithmic changes in cell concentration:

$$\mu\ (d^{-1}) = \frac{[Ln(Nt) - Ln(N0)]}{\Delta t} \quad (1)$$

where: $N0$ and Nt represent cell concentrations (expressed as OD values) at the start and end of the cultivation period, and Δt is the time interval in days.

Biomass determination

Final biomass concentration was determined gravimetrically (Fakhri et al., 2021). Twenty-five milliliters of culture were filtered through pre-weighed Whatman GF/C filters (1.2 µm pore size), washed twice with distilled water to remove salts, and dried at 105 °C for two hours. After cooling in a desiccator for 30 min, filters were reweighed. Biomass concentration (g L^{-1}) was calculated as the increase in dry weight divided by the sample volume. Biomass productivity (mg L^{-1} d^{-1}) was obtained by dividing the net biomass gain by the cultivation time.

Pigment analysis

Chlorophyll (chl) a+b and carotenoids were analyzed at day 5. Two milliliters of culture were centrifuged (2000×, 15 min), and the pellet was extracted in 2 mL of 90% methanol. Samples were sonicated for 5–10 min, incubated in a water bath at 70 °C for 10 min, and centrifuged again. Absorbance of the supernatant was measured at 665, 652, and 480 nm. Chlorophyll and carotenoid concentrations were calculated using the equations of Ritchie (2006) and Kim et al. (2014).

Protein determination

Protein content was determined using the Lowry assay (Lowry et al., 1951). One milliliter of culture was hydrolyzed with 1 N NaOH, followed by sonication (10 min) and heating at 90–100 °C for 20 min. The sample was cooled, and alkaline copper reagent was added, followed by Folin–Ciocalteu reagent. After 30 min of incubation, absorbance was measured at 750 nm. Protein concentration was expressed relative to a bovine serum albumin standard curve. Values were normalized to biomass dry weight to obtain percentage content.

Lipid extraction and determination

Total lipid content was analyzed following a modified Bligh and Dyer (1959) method. Approximately 30 mg of dried biomass was homogenized in chloroform:methanol (2:1, v/v). To induce phase separation, 0.8 mL of 0.73% NaCl was added, and the mixture was centrifuged at 2.500 rpm for 2 min. The organic phase was collected, and extraction was repeated once. The pooled extracts were evaporated under nitrogen gas and dried at 65–105 °C until constant weight. Lipid content was expressed as percentage of dry weight (dw%).

$$Lipid\ content\ = \\ = \frac{M3-M2}{M1} \times\ 100\%\ (\text{dw}\%) \quad (2)$$

$M1$ denotes the total dry biomass weight, $M2$ indicates the mass of the empty vial, and $M3$ represents the mass of the vial after lipid extraction containing the recovered lipids.

$$Lipid\ productivity\ (\text{mg}\ L^{-1}\ d^{-1}) = \\ = \frac{W2-W1}{\Delta t} \times\ lipid\ content \quad (3)$$

*W*1 refers to the biomass recorded at the beginning of the cultivation period, while *W*2 represents the biomass measured at the end of the cultivation period.

Nutrient uptake

Nitrate and phosphate concentrations were measured only at day 0 and day 5 to evaluate nutrient utilization. Nitrate was determined by the sodium salicylate method (Witthohn et al., 2023), with absorbance at 405 nm, while phosphate was quantified using the vanadate–molybdate method, with absorbance at 470 nm. Uptake efficiency (%) was calculated as the relative difference between initial and final concentrations.

Statistical analysis

Data are presented as mean ± standard deviation of triplicate samples. One-way ANOVA was applied to evaluate the effect of phosphorus treatments. Significant results ($p < 0.05$) were further analyzed with Tukey's multiple range test. Statistical analysis was conducted using IBM SPSS Statistics version 27.

RESULTS AND DISCUSSION

Effect of various phosphate concentrations on growth of *M. braunii*

The growth of *M. braunii* under different phosphate concentrations are presented in Figure 1A. Optical density at 730 nm (OD_{730}) increased progressively across all treatments except in the phosphate-deprived cultures (0.0 mg L^{-1}). In the absence of phosphate, growth stagnated after day 2, and OD values subsequently declined, indicating that phosphorus is an essential element for sustaining cellular division and photosynthetic activity. This finding is consistent with previous reports that phosphorus deprivation rapidly limits algal proliferation due to its indispensable role in nucleic acid synthesis, energy transfer (ATP, NADPH), and membrane phospholipid biosynthesis (Ruangsomboon et al., 2013).

In cultures supplemented with phosphate, growth trajectories varied depending on concentration. The treatments with 2.02 and 4.0 mg L^{-1} phosphate showed moderate increases in OD, with final values of 0.74 and 0.81 at day 5, respectively. Although these levels supported sustained growth, the rate of increase was slower compared to higher phosphate treatments, suggesting that partial limitation constrained the maximal growth potential. By contrast, cultures with 8.18 and 17.14 mg L^{-1} exhibited the highest growth performance, reaching OD_{730} values above 0.85 at day 5. These results demonstrate that higher phosphate availability enables *M. braunii* to maintain exponential growth for longer periods, which is in line with earlier observations in *Chlorella sp.* where phosphate supplementation enhanced both growth rate and biomass accumulation (Chu et al., 2019; Fakhri et al., 2025).

The growth trends also highlight a clear divergence after day 2. While all phosphate-replete treatments continued to increase, the phosphate-deprived culture plateaued and declined, indicating early entry into stationary and death phases. This suggests that *M. braunii* has limited capacity to recycle or substitute phosphorus under deprivation.

Effect of various phosphate concentrations on biomass of *M. braunii*

The effect of phosphate availability on the final biomass yield of *M. braunii* is presented in Figure 1B. Biomass increased markedly with rising phosphate concentrations, from 0.46 g L^{-1} under phosphate deprivation (0 mg L^{-1}) to a maximum of 1.01 g L^{-1} at 17.14 mg L^{-1}. Statistical analysis ($p < 0.05$) identified three distinct groups: the lowest biomass in the absence of phosphate, intermediate yields at 2.02–8.18 mg L^{-1}, and the highest yield under full phosphate supply.

Phosphate-deprived cultures produced less than half the biomass of the control, underscoring the essential role of phosphorus in cell division and biomass formation. As a key component of nucleic acids, phospholipids, ATP, and NADPH, phosphorus supports both structural integrity and energy transfer. Deficiency therefore disrupts genetic processes and metabolism, limiting cell proliferation (Rausch and Bucher, 2002; Ruangsomboon et al., 2013).

At moderate concentrations (2.02–8.18 mg L^{-1}), biomass was significantly higher than in phosphate-free conditions but statistically similar across treatments. This indicates that low phosphate inputs can sustain growth, though not at maximal rates. The plateau across this range suggests saturation of phosphorus uptake and

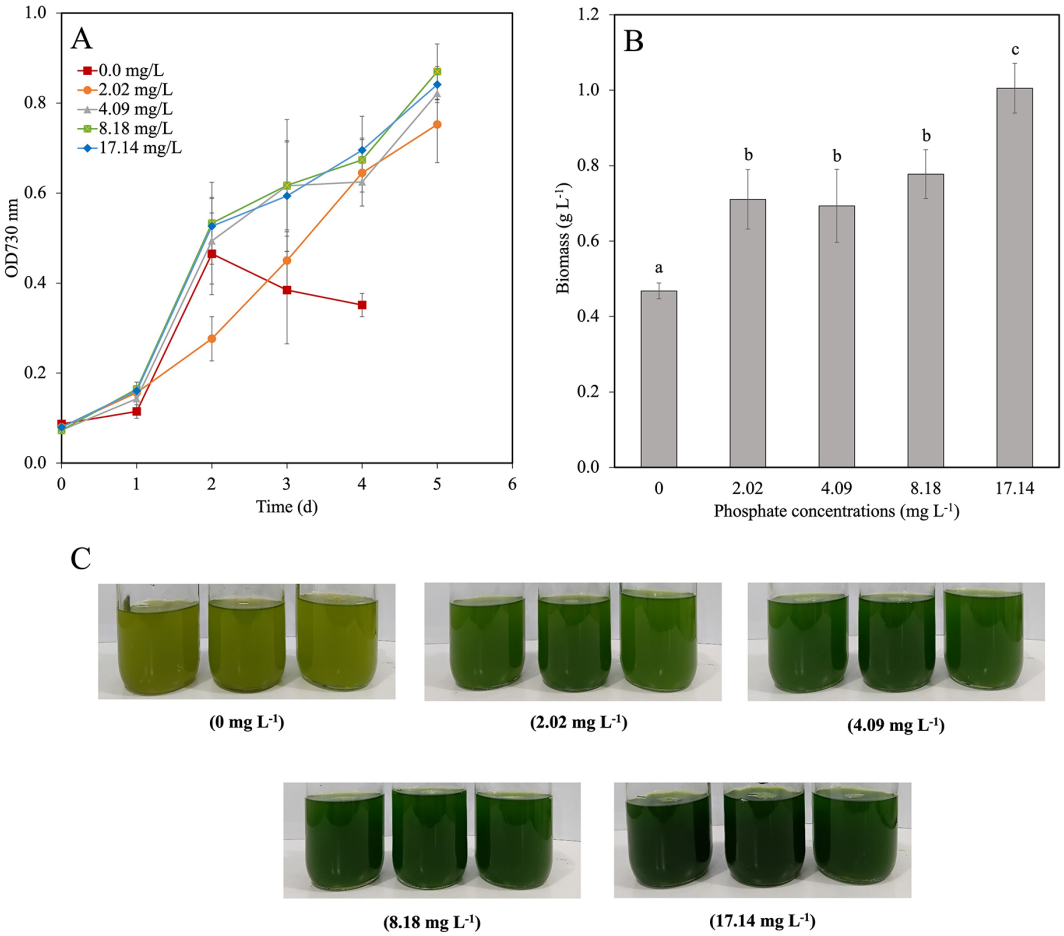

Figure 1. Effects of phosphate concentration on the growth of *Monoraphidium braunii*: (A) growth curves expressed as optical density at 730 nm (OD730) over a 5-day cultivation period under different phosphate levels (0, 2.02, 4.09, 8.18, and 17.14 mg L^{-1}); (B) final biomass concentration (g L^{-1}) after cultivation at the corresponding phosphate concentrations; (C) alterations in microalgal cell coloration observed at the final day of the experiment. Bars represent mean values ± standard deviation. Different lowercase letters above the bars indicate significant differences among treatments ($p < 0.05$)

assimilation pathways, consistent with classical nutrient-quota kinetics (Droop, 1974).

The highest biomass (1.01 g L^{-1}) was achieved at 17.14 mg L^{-1}, consistent with sustained exponential growth under phosphorus sufficiency (Figure 1B). Although growth trajectories at 8.18 and 17.14 mg L^{-1} converged during early cultivation, the fully replete treatment yielded significantly greater final biomass indicating that marginal differences in phosphorus availability compound into substantial yield advantages as internal cellular reserves are progressively depleted over extended culture periods. Visual observation (Figure 1C) further supported these findings, as cultures became progressively darker green with increasing phosphate availability. This result is corroborated by Xin et al. (2010), who reported a twofold enhancement in biomass yield of *Scenedesmus* sp. upon increasing phosphorus concentration from 0.1 to 2 g L^{-1}, reinforcing that phosphorus sufficiency is a principal determinant of cumulative biomass accumulation across green microalgal taxa.

Effect of phosphate concentration on photosynthetic pigments of *Monoraphidium braunii*

Phosphorus availability strongly influenced the photosynthetic pigment composition of *M. braunii*, as reflected by marked variations in chl a+b and carotenoid contents (Figures 2A and 2B). Chl a+b was minimal under complete deprivation (7.8 µg mL^{-1}), rising by 138% at 2.02 mg L^{-1} (18.6 µg mL^{-1}) and peaking at 4.09 mg L^{-1} (37.4 µg mL^{-1}), a 379% increase over the

deprived condition. Further enrichment to 8.18–17.14 mg L^{-1} reduced chlorophyll by approximately 20% to around 30 µg mL^{-1}. Carotenoid content followed an analogous but more muted trajectory. Levels rose modestly from 1.84 µg mL^{-1} under deprivation to 2.4 µg mL^{-1} at 2.02 mg L^{-1} (a 26% increase), before peaking at 7.2 µg mL^{-1} at 4.09 mg L^{-1} (279% above deprived cultures) and subsequently declining to 5.2–5.6 µg mL^{-1} at higher concentrations. The co-occurrence of chlorophyll and carotenoid maxima at 4.09 mg L^{-1} is consistent with observations in *Scenedesmus* sp., where both pigment classes peaked at intermediate phosphorus before declining under excess supply (Raj et al., 2025).

Phosphorus is integral to ATP, NADPH, nucleic acids, and phospholipids, all central to photosynthesis and chloroplast structure (Hu et al., 2025; Kayoumu et al., 2023). Under P deficiency, chloroplast stromal Pi falls, ATP synthase activity declines, ATP production drops, and CO_2 fixation is restricted, leading to reduced chlorophyll biosynthesis and photosynthetic rate (Bossa et al., 2024). With high external P, microalgae often luxury-uptake phosphate and store it as polyphosphate rather than using it proportionally for pigments (Bossa et al., 2024; Raj et al., 2025). At elevated P, several species show stable growth but lower specific chlorophyll and carotenoid content, consistent with pigment dilution by faster biomass accumulation and shifts toward storage metabolites (e.g., lipids in *Chlorella pyrenoidosa* and *Picochlorum* sp.) (Fakhri et al., 2025; Thi et al., 2024).

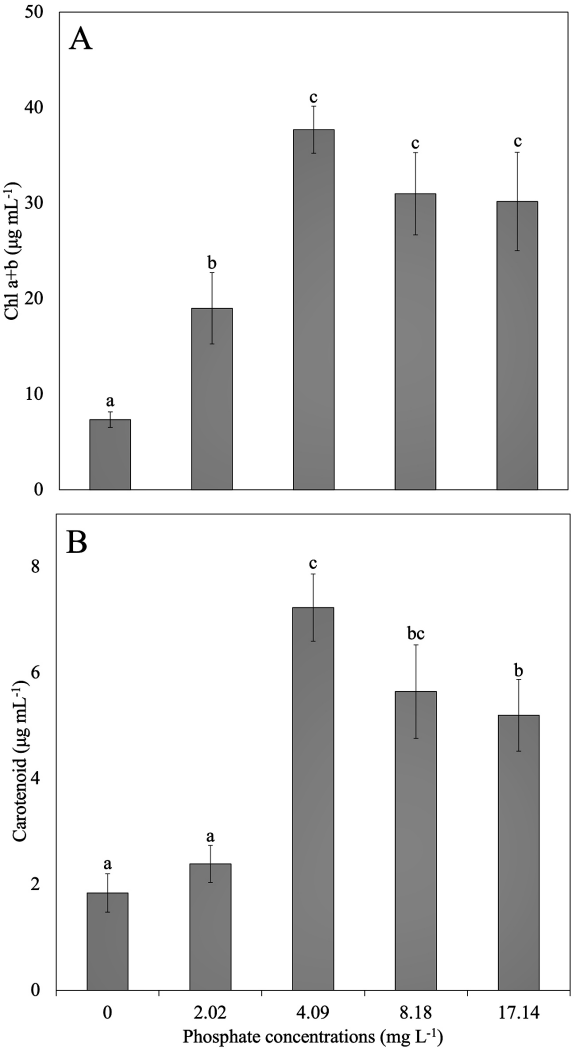

Figure 2. Effects of phosphate concentration on photosynthetic pigment contents of *Monoraphidium braunii*: (A) Chl-a+b content and (B) carotenoid content measured at different phosphate concentrations (0, 2.02, 4.09, 8.18, and 17.14 mg L^{-1}). Values are presented as mean ± standard deviation. Different lowercase letters above the bars indicate significant differences among treatments ($p < 0.05$)

Effect of phosphate concentration on protein content of *Monoraphidium braunii*

Protein content in *M. braunii* increased markedly with phosphate availability, rising from 16.8% DW under complete deprivation to a peak of 37.5 dw% at 8.18 mg L (Figure 3A). Statistical analysis revealed a threshold-dependent rather than graded response: the two lowest treatments (0 and 2.02 mg L^{-1}; ~17–18 dw%) were statistically indistinguishable, while concentrations of 4.09 mg L^{-1} and above produced a significantly elevated group (~32–38% DW), suggesting a critical phosphorus threshold below which translational capacity is fundamentally compromised. In a similar study, Javed et al. (2022) reported that low concentration of phosphorus reduced the protein content in *Chlorella vulgaris* and increased under P sufficient.

This response reflects the obligate dependence of protein biosynthesis on phosphorus availability. Phosphorus is a structural constituent of ribosomal RNA, which directly determines translational capacity, and its deficiency simultaneously suppresses ribosome biogenesis and downregulates nitrogen assimilation enzymes particularly glutamine synthetase, whose activity is tightly coupled to cellular phosphorus status (Raven and Lovelock, 2013).

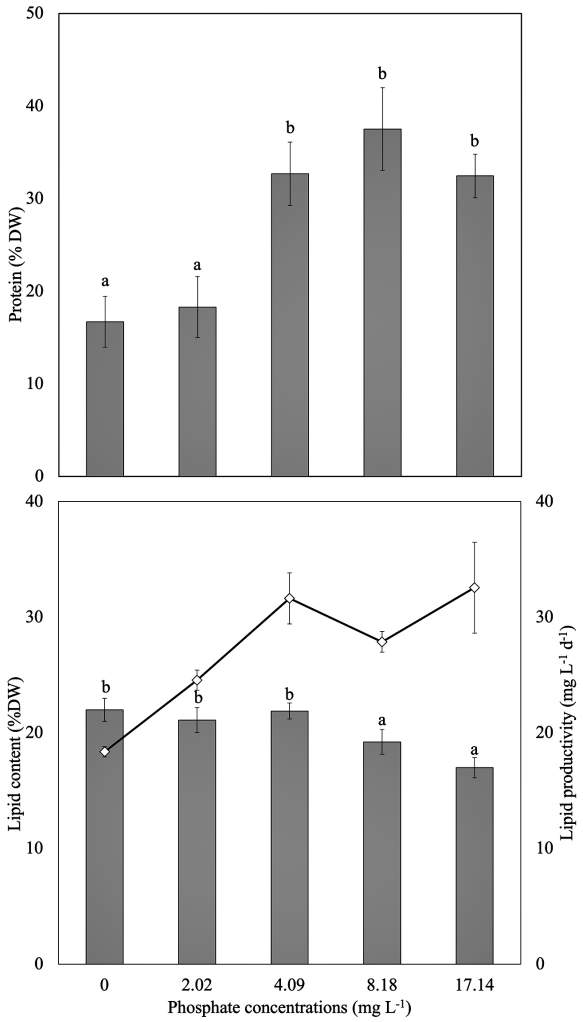

Figure 3. (A) Protein content and (B) lipid content (bars) with lipid productivity (line) of *Monoraphidium braunii* under varying phosphate concentrations (0, 2.02, 4.09, 8.18, and 17.14 mg L^{-1}) after five days of cultivation. Values represent mean ± SD (n = 3). Bars sharing the same lowercase letter are not significantly different ($p < 0.05$)

Phosphate deficiency induced lipid content in *Monoraphidium braunii*

Phosphate concentration exerted a significant influence on lipid accumulation in *M. braunii* (Figure 3B). Interestingly, cultures grown under phosphorus deprivation (0 mg L^{-1}) exhibited relatively high lipid content, averaging 21.9 dw%, comparable to those at 2.02 mg L^{-1} (21.0%) and 4.09 mg L^{-1} (22.1%). Statistical analysis confirmed that these three treatments did not differ significantly ($p > 0.05$), suggesting that moderate phosphorus deficiency did not compromise lipid accumulation. Similiarly, Xin et al. (2010) reported that limited phosphorus concentration increased the accumulation of lipid in *Scenedesmus* sp. This pattern supports the concept that phosphorus limitation can trigger carbon flux redistribution toward lipid synthesis, as reduced phosphorus availability restricts nucleic acid and protein biosynthesis while favoring triacylglycerol (TAG) storage (Hu et al., 2008; Yang et al., 2018). In contrast, cultures maintained at higher phosphorus concentrations (8.18 and 17.14 mg L^{-1}) showed significantly lower lipid content, with values of 17.5% and 18.6%, respectively. This decline indicates that when phosphorus is abundant, cellular metabolism is directed primarily toward biomass and protein accumulation rather than lipid storage. Similar responses have been documented in *Chlorella sp.* and *Scenedesmus sp.*, where nutrient sufficiency supported rapid growth but reduced lipid density per unit biomass (Liang et al., 2013; Xin et al., 2010b).

Lipid productivity followed an inverse trajectory to lipid content, rising 70% from 18.37±0.44 mg L^{-1} d^{-1} under phosphate deprivation to a peak of 31.63±2.21 mg L^{-1} d^{-1} at 4.09 mg L^{-1}, before declining modestly to 27.8 mg L^{-1} d^{-1} at 8.18 mg L^{-1} and recovering to 32.8 mg L^{-1} d^{-1} at 17.14 mg L^{-1} (Figure 3B). The divergence between lipid content and productivity underscores a critical distinction in nutrient stress optimization. As lipid productivity is the product of biomass concentration and lipid content, gains in one variable can be negated by deterioration in the other (Fakhri et al., 2026). Under deprivation, elevated cellular lipid fraction was offset by severely compromised biomass, yielding the lowest volumetric output, confirming that lipid content alone is an insufficient process metric. Conversely, the secondary productivity peak at 17.14 mg L^{-1} demonstrates that biomass yield increasingly dominates the productivity term under full repletion. The optimum at 4.09 mg L^{-1} represents the point at which moderate stress-induced lipid enrichment and sufficient biomass accumulation are most favorably balanced, identifying this concentration as the strategic optimum for biofuel-oriented cultivation of *M. braunii* (Maltsev et al., 2023).

Effect of various phosphate concentrations on nitrate and phosphate assimilation

Nutrient assimilation by *M. braunii* varied systematically with external phosphate availability (Figure 4A and 4B). Nitrate assimilation remained consistently high across treatments

(58–73%), with the greatest uptake observed under phosphate deprivation (0 mg L^{-1}). Although a gradual decline occurred at moderate phosphate concentrations (4.09–8.18 mg L^{-1}), assimilation partially recovered at the highest level (17.14 mg L^{-1}). The persistence of elevated nitrate assimilation under phosphorus limitation indicates that nitrogen was not growth-limiting and suggests continued nitrogen metabolism despite constrained phosphorus availability. Similar responses have been documented in green microalgae such as *Chlorella* and *Scenedesmus*, where nitrate uptake and assimilation remain active during P stress due to intracellular nitrogen recycling and quota-based regulation (Monfet, 2017). According to the Droop cell quota model, nutrient uptake is regulated by internal stores rather than solely by external concentrations, allowing nitrogen assimilation to proceed even when phosphorus becomes limiting (Droop, 1974).

In contrast, phosphate assimilation displayed a saturable response. No uptake occurred in the absence of phosphate, while supplementation stimulated assimilation that peaked at 2.02 mg L^{-1} (44±3.92%) before declining to 25–30% at higher concentrations. This pattern deviates from proportional uptake and instead reflects classical Michaelis–Menten–type kinetics of phosphorus transport systems (Bartosh, 2004). Numerous studies demonstrate that microalgae accumulate excess phosphorus as intracellular polyphosphate granules when available, followed by feedback inhibition of high-affinity transporters once cellular quotas are satisfied (Chu et al., 2019). Such regulatory mechanisms prevent excessive intracellular accumulation and explain the reduced phosphate assimilation at elevated external concentrations.

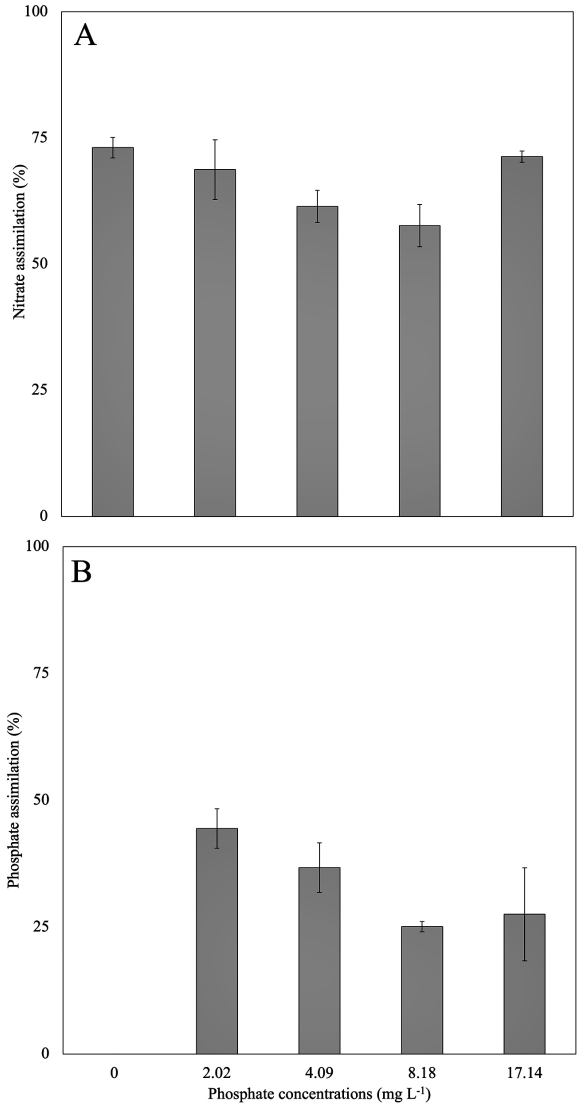

Figure 4. (A) Nitrate assimilation (%) and (B) phosphate assimilation (%) by *Monoraphidium braunii* under varying phosphate concentrations (0, 2.02, 4.09, 8.18, and 17.14 mg L^{-1}) after five days of cultivation. Values represent mean ± SD (n = 3)

CONCLUSIONS

This study demonstrates that phosphorus availability exerts decisive regulatory control over growth, biochemical composition, and carbon partitioning in *Monoraphidium braunii*. Phosphate deprivation severely restricted biomass accumulation and protein synthesis, confirming the indispensable structural and energetic roles of phosphorus in nucleic acid formation, and ATP production. Conversely, full phosphorus repletion (17.14 mg L^{-1}) maximized biomass yield while suppressing cellular lipid fraction, reflecting a metabolic shift toward growth-oriented anabolism. Photosynthetic pigments and lipid productivity converged at a shared optimum of 4.09 mg L^{-1}, where moderate phosphorus supply established a favorable balance between sufficient biomass formation and stress-induced lipid enrichment, resolving the classical trade-off between cellular lipid content and volumetric output that undermines severe deprivation strategies. These findings advance process-level optimization for *M. braunii* cultivation, identifying 4.09 mg L^{-1} as the strategic phosphate concentration for integrated biorefinery applications targeting biofuel,

pigment, and aquaculture feed co-production. Future work should prioritize two-stage cultivation strategies that decouple biomass production from lipid induction, molecular elucidation of phosphorus transport and lipid biosynthesis regulatory networks, and scale-up validation under photobioreactor or outdoor conditions to bridge laboratory optimization with industrial feasibility.

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