

LETTER

Does nutrient scarcity select for *Synechococcus* clade III in the Mediterranean Sea?

Xiaodong Zhang¹, Shunyan Cheung^{2,3}, Hoi Yau Chan¹, Sangwook Scott Lee¹, Ioulia Santi^{4,5}, Paraskevi Pitta⁴, Jiawei Chen^{1*}, Hongbin Liu^{1,6,7*}

¹Department of Ocean Science, The Hong Kong University of Science and Technology, Hong Kong; ²Institute of Marine Biology, National Taiwan Ocean University, Keelung, Taiwan; ³Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan; ⁴Institute of Oceanography (IO), Hellenic Centre for Marine Research (HCMR), Heraklion, Greece; ⁵Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, Greece; ⁶CAS-HKUST Sanya Joint Laboratory of Marine Science Research, Key Laboratory of Tropical Marine Biotechnology of Hainan Province, Sanya Institute of Oceanology, SCSIO, Sanya, China; ⁷State Key Laboratory of Marine Environmental Health, Hong Kong

Scientific Significance Statement

Marine *Synechococcus* are highly diverse, abundant, and globally distributed, playing key roles in global biogeochemical cycles. Among more than 20 clades of *Synechococcus*, clade III are typical warm water and oligotrophic group, which consistently dominate the extremely nutrient-depleted Mediterranean Sea. However, the reasons for their success in such ultraoligotrophic waters have remained unclear. By combining a field mesocosm experiment in the Eastern Mediterranean, dilution approach, amplicon sequencing, genomics, and transcriptomics, we revealed that clade III have a superior capacity to scavenge both nitrogen and phosphorus through multiple high-affinity transporters. These adaptations explain why clade III outcompetes other clades in such nutrient-depleted waters. Understanding this mechanism not only solves a long-standing puzzle in the niche segregation of *Synechococcus* but also helps us predict how ocean carbon export may change as warming expands oligotrophic regions.

Abstract

Synechococcus inhabit both nutrient-rich estuaries and oligotrophic waters, yet exhibit distinct niche segregation across phylogenetic clades. Previous studies indicated clade III predominated in the ultraoligotrophic Mediterranean Sea. However, the mechanisms underlying clade III's dominance over other clades in ultraoligotrophic waters remain unclear. Here, we measured bulk and clade-specific growth rates of *Synechococcus* using a dilution approach during a mesocosm experiment in the Eastern Mediterranean Sea. Clade III exhibited higher growth rates than competing clades and competitive superiority for limited nutrients in mesocosms amended with ammonium and phosphate. Genomic analyses revealed clade III possessed more nitrogen/phosphorus transporters than other clades (except clade VIII and S5.2), which may contribute to their competitive advantage in

*Correspondence: jchenek@connect.ust.hk; liuhb@ust.hk

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ultraoligotrophic conditions. Compared to clade VIII/S5.2, clade III's lower GC content and smaller genome size are consistent with adaptation to oligotrophic systems. The unique adaptive genomic traits of clade III *Synechococcus* may facilitate their ecological success in ultraoligotrophic waters.

Synechococcus are one of the most abundant and globally distributed picocyanobacteria, playing a vital role in global biogeochemical cycles (Flombaum et al. 2013). They are phylogenetically and ecologically diverse, consisting of three subclusters (S5.1, S5.2, and S5.3) and more than 20 physiologically and ecologically distinct lineages (Ahlgren and Rocap 2012; Mazard et al. 2012; Xia et al. 2015, 2019). Within S5.1, clades I–IV are the main clades which are widely distributed and frequently observed, showing heterogeneous biogeographical patterns due to their specific adaptation to different stressors including nutrients, temperature, and light. Clade I and IV co-dominate high-latitude, cold, and nutrient-replete waters, while clade II and III prevail in warm waters (Ahlgren and Rocap 2012; Ferrieux et al. 2022). In contrast to S5.1 which are widely distributed, S5.2 are mainly observed in nutrient-rich estuaries, coastal and shelf waters.

Nitrogen and phosphorus constitute essential elements for microbial growth and metabolism, representing >7% and >1% of cellular biomass, respectively (Geider and La Roche 2002; Moore et al. 2013). Nitrogen is considered as a key limiting nutrient in vast regions of the ocean (Howarth 1988; Ammerman et al. 2003; Paerl 2018). However, increasing evidence showed that phosphorus limitation may also play a key role in biogeochemical cycles in both open waters (Cotner et al. 1997; Karl et al. 2001; Ammerman et al. 2003; Krom et al. 1991, 2004, 2010) and estuaries (Howarth 1988; Harrison et al. 1990; Xu et al. 2008). Nutrient limitation may drive evolutionary adaptations in oligotrophic marine microbes, such as expanded transporter repertoires (Palenik et al. 2003; Tang et al. 2019), phosphonate catabolism (Molina-Pardines et al. 2023), membrane lipid remodeling (Sebastián et al. 2016; Peng et al. 2019), high-affinity alkaline phosphatase (Torcello-Requena et al. 2024), or high-affinity solute-binding proteins (Clifton et al. 2024).

The Mediterranean Sea is among the world's largest oligotrophic areas with extreme phosphorus limitation and low phytoplankton productivity (Bethoux 1989; Krom et al. 1991, 2004, 2005, 2010). Notably, the Mediterranean Sea displays an eastward gradient of nutrient availability (Krom et al. 1991, 2010; Moutin and Raimbault 2002; Moutin et al. 2002), making Eastern Mediterranean Sea a severe ultraoligotrophic region. Many studies have demonstrated that picophytoplankton dominated the phytoplankton community (Magazzu and Decembrini 1995; Ignatiades et al. 2002; Moutin et al. 2002), with clade III the overwhelming dominant clade of *Synechococcus* in the Mediterranean Sea (Zwirgmaier et al. 2008; Mella-Flores et al. 2011; Farrant et al. 2016; Doré et al. 2022). However, the molecular basis for clade III's competitive superiority in the Mediterranean Sea remains unresolved. Given the ultraoligotrophic status,

particularly chronic phosphorus depletion, we hypothesize that differential nutrient acquisition capabilities may constitute the primary selective driver favoring clade III over other clades. To test this hypothesis, we measured the growth rates of bulk *Synechococcus* populations, as well as different clades, using dilution methods and a nutrient-manipulated mesocosm experiment in the EMS. Additionally, we analyzed the genomic information of 87 high-quality genomes covering the main clades, aiming to reveal why clade III *Synechococcus* dominate in the Mediterranean Sea.

Materials and methods

Mesocosm setting

We conducted the mesocosm experiments at the CretaCosmos facility (<https://www.aquacosm.eu/mesocosms/cretacosmos>) of the Hellenic Centre for Marine Research (HCMR; Heraklion) during October 2019. Seawater was collected from 10 m depth at an offshore station near Heraklion in the Cretan Sea on October 5th, 2019 and was transferred to HCMR within 2 h. The seawater was distributed equally among six 3-m³ food-grade polyethylene mesocosm bags. The bags were subsequently submerged in a 350-m³ concrete incubation tank, with water temperature maintained between 21°C and 23°C to reflect in situ conditions. Continuous aeration was provided through an airlift system to maintain homogeneous water column mixing. Each mesocosm was covered with a transparent Plexiglas lid and a neutral-density mesh to exclude atmospheric deposition and mimic ambient light intensity at the original sampling depth, respectively. In Day 0 (October 6, 2019), ammonium and phosphate were added into three mesocosms which were hereby referred to as Mesocosm NP, with the targeted concentration of 3.2 and 0.1 µM, respectively. The N/P ratio of added nutrients was twice the Redfield ratio to maintain the phosphate limitation. Additionally, 5.3 µM carbohydrates (glucose + sucrose) were added to the NP treatment as part of the broader project design to stimulate heterotrophic bacterial activity. Thus, the NP treatment effectively represents a combined N + P + labile dissolved organic carbon (DOC) enrichment, rather than a manipulation of inorganic nutrients alone. No manipulation was set in the other three mesocosms, which were hereby referred to as Mesocosm K. The mesocosm experiment lasted for 12 d.

Measurements of chlorophyll *a*, nutrients concentration, and *Synechococcus* abundance

Water samples were collected daily from each mesocosm for 12 d. For DNA and chlorophyll *a* (Chl *a*) analysis, 500 mL seawater was filtered through 0.22 µm polycarbonate

membranes (GVS) and GF/F filters (Whatman), respectively. Subsamples (1.8 mL) were fixed with paraformaldehyde (1% final concentration) for *Synechococcus* quantification. All these samples were flash-frozen and stored at -80°C . Nutrient concentrations were determined from 100 mL samples filtered through acid-cleaned $0.45\ \mu\text{m}$ cellulose acetate membranes and preserved at -20°C .

DNA was extracted using the DNeasy Plant Kit (Qiagen) and stored at -20°C . For the community, nested polymerase chain reaction of *rpoC1* (primers *rpoC1-C/rpoC1-N5* and barcoded *rpoC1-39F/462R*; Mühling et al. 2006; Xia et al. 2015) was conducted followed by Illumina sequencing (Novogene). Sequences were analyzed with Qiime2 (Bolyen et al. 2019). Sequences processed demultiplexed paired-end sequences via the DADA2 pipeline (Callahan et al. 2016) to generate amplicon sequence variants. Taxonomic classification employed a *rpoC1* reference database (Xia et al. 2019), with a Naïve Bayes classifier trained for precise clade assignment.

Concentrations of nitrate/nitrite, ammonium, and phosphate were measured following Strickland and Parsons (1972), Ivančić and Degobbi (1984), and Rimmelin and Moutin (2005). Chl *a* concentrations were determined fluorometrically (Welschmeyer 1994) on a Turner Designs Trilogy fluorometer (CHL-A NA mode). *Synechococcus* abundance was counted with BD FACSCalibur flow cytometry (488 nm laser; Liu et al. 2014), with $1\text{-}\mu\text{m}$ fluorescent beads (Polysciences) as internal standards.

Synechococcus growth rates were estimated on Days 0, 2, 4, 6, 9, and 11 using a modified two-point dilution method (Landry and Hassett 1982; Chen 2015; Morison and Menden-Deuer 2017). Bulk growth rates were calculated based on abundance measured by flow cytometry, and clade-specific growth rates were derived by combining bulk abundances with relative abundances from *rpoC1* amplicon sequencing. Detailed procedural descriptions are provided in Supporting Information Methods S1.

Strain isolation

Twenty milliliters of seawater were filtered through $1\ \mu\text{m}$ membrane and inoculated in f/2 medium freshly prepared with the autoclaved ambient seawater. Single-cell isolation was achieved through successive serial dilution and multi-round incubation after the culture turned red.

Genome analysis

For DNA extraction, 500 mL *Synechococcus* culture in exponential phase was filtered through $0.22\ \mu\text{m}$ polycarbonate membranes and processed using the DNeasy Plant Kit (Qiagen, Hilden). Qualified DNA samples (A260/A280 = 1.8–2.0) were used for library generation with NEBNext Ultra DNA library prep kits and subsequently sequenced through the NovaSeq 6000 system (Illumina), generating 150 bp paired-end reads.

Clean reads of the two strains were assembled using MEGAHIT v1.2.9 (Li et al. 2016) with parameters $-\text{k-min } 27\ -\text{k-max } 147\ -\text{k-step } 12$. Since our culture is not axenic, *Synechococcus* genomes were obtained through the binning method. Genomic binning was performed using MetaWRAP v1.3 (Uritskiy et al. 2018) with a minimum contig length cutoff of 1.5 kb and refined using the “bin_refinement” module. The completeness and contamination of refined metagenome-assembled genomes were assessed using CheckM2 v1.1.0 (Chklovski et al. 2023). Coding sequences of *Synechococcus* metagenome-assembled genomes were predicted using Prodigal v2.6.3 in meta mode ($-\text{p meta}$) (Hyatt et al. 2010). Functional annotation was performed against multiple databases using DIAMOND v2.1.6 (Buchfink et al. 2015), including KEGG (Kanehisa and Goto 2000), Carbohydrate-active enzymes (Cantarel et al. 2009), and Transporter Classification Database (Saier Jr et al. 2021).

Phylogenetic analysis was constructed based on the genomes of the two isolated strains in this study and 85 reference genomes representing major *Synechococcus* clades (Supporting Information Table S1). A maximum-likelihood tree was inferred using the Genome Taxonomy Database Toolkit v2.3.2 (Chaumeil et al. 2020). The optimal model was then selected using ProtTest v3.4.2 (Abascal et al. 2005), and a phylogenetic tree was constructed using IQ-TREE v1.6.12 (Nguyen et al. 2015) with the ultrafast bootstrap parameter “-bb 1000” (Hoang et al. 2018).

Results

Hydrographic conditions

The ambient seawater at the sampling site exhibited characteristic ultra-oligotrophic conditions with phosphate-depleted status. Dissolved inorganic nitrogen (DIN, nitrate + nitrite + ammonium) concentrations at 2, 10, and 20 m depths were 0.106, 0.174, and 0.189 μM , respectively. In contrast, phosphate concentrations across three depths kept consistently at near or below detection levels. Initial measurements in Mesocosm NP recorded DIN and phosphate concentrations of 6.05 and 65.92 nM on Day 1, respectively (Fig. 1). Measurements 1 h after addition (Day 0) showed DIN and phosphate were close to the 3.2 and 0.1 μM targets (Supporting Information Fig. S1). The subsequent increase of DIN to 6.05 μM by Day 1 coincided with a rise in bacterial abundance (5.19×10^5 cells mL^{-1} , Day 0; 5.95×10^5 cells mL^{-1} , Day 1) and production ($11.59\ \text{ngC L}^{-1}\ \text{h}^{-1}$, Day 0; $35.49\ \text{ngC L}^{-1}\ \text{h}^{-1}$, Day 1), indicating that carbohydrate-stimulated microbial activity may drive ammonium regeneration. Subsequent monitoring revealed progressive DIN depletion from Day 2 onward, contrasted by an abrupt phosphate decrease to 5.22 nM by Day 2 due to the luxury uptake of phosphate, followed by stable concentrations (5.22–5.31 nM) throughout the incubation (Fig. 1). Mesocosm K maintained severe phosphorus limitation throughout the

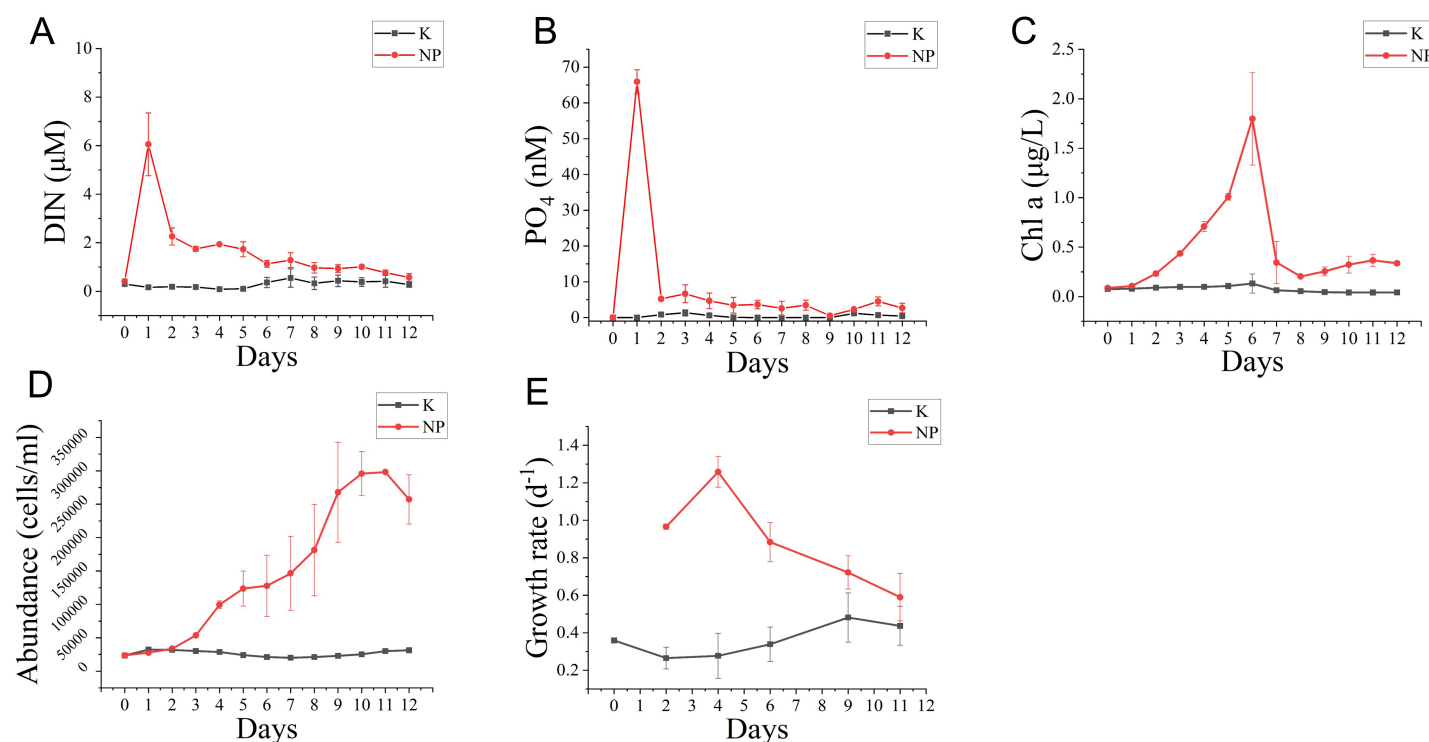


Fig. 1. Nutrients concentration, chlorophyll *a* concentration, and *Synechococcus* abundance and growth rate during the 12-d incubation period in the mesocosm experiment. (A) Dissolved inorganic nitrogen; (B) phosphate; (C) chlorophyll *a*; (D) *Synechococcus* abundance; (E) *Synechococcus* growth rate.

12-d incubation, with phosphate concentrations of 0.33–2.5 nM or below detection. DIN levels in Mesocosm K showed minimal fluctuation, averaging 0.29 μM over the 12-d experimental period (Fig. 1).

In situ Chl *a* concentration at sampling site demonstrated minimal vertical variation (0.11–0.12 $\mu\text{g L}^{-1}$) across the 2–20 m depth profile, with picophytoplankton accounting for 58.8–66.2% of total Chl *a* concentration. Chl *a* concentration in Mesocosm NP reached its peak on Day 6 (1.80 $\mu\text{g L}^{-1}$ on average) and declined rapidly on Day 7 (0.34 $\mu\text{g L}^{-1}$ on average). In contrast, Mesocosm K displayed low Chl *a* concentration without significant fluctuation during the incubation period (Fig. 1).

Synechococcus abundance, community structure, and growth rates

The initial abundance of *Synechococcus* in ambient seawater was 2.3×10^4 cells mL^{-1} . In Mesocosm NP, *Synechococcus* abundance exhibited progressive growth from Day 1 to 11 with the peak of 2.98×10^5 cells mL^{-1} , followed by decline to 1.24×10^5 cells mL^{-1} on Day 12. Mesocosm K maintained stable populations with the mean value of 2.64×10^4 cells mL^{-1} (Fig. 1).

Synechococcus diversity was limited, with only 10 clades detected across all mesocosms. Clade III was the dominant genotypes (>90% relative abundance), whereas clade WPC1 and S5.3 constituted minor components (<5% combined).

Other clades occurred sporadically (<0.5% each; Fig. 2). Throughout the 12-d experimental period, clade III sustained dominance (>90%) in both experimental systems (Fig. 2).

Bulk *Synechococcus* growth rates in ambient seawater averaged 0.30 d^{-1} . Mesocosm K exhibited constrained growth dynamics (0.265–0.481 d^{-1} ; mean 0.34 d^{-1}) (Fig. 1). Mesocosm NP stimulated growth rate to 1.25 d^{-1} by Day 4, followed by progressive attenuation to 0.59 d^{-1} on Day 11 (Fig. 1). Clade-specific growth rates differed between clade III and others. In Mesocosm K, clade III maintained the baseline growth rate of 0.2–0.3 d^{-1} while clade WPC1, S5.3, and other clades possessed undetectable growth. In contrast, clade III displayed significantly higher growth rates than clade WPC1, and others in Day 2, Day 4, and Day 6 in Mesocosm NP (Fig. 2).

Genomic and transcriptomic analysis

Two *Synechococcus* strains (MSI and MSII) were successfully isolated from ambient seawater, and high-quality genomes of them were obtained (MSI completeness: 100%; MSII completeness: 99.84%) (Supporting Information Table S1). The phylogenetic tree showed that MSI and MSII were closely affiliated with clade III and VI, respectively (Fig. 3).

Phosphorus transporter systems (phosphate, phospholipid, phosphonate) were functionally conserved across most strains (Supporting Information Table S2), though limited strains

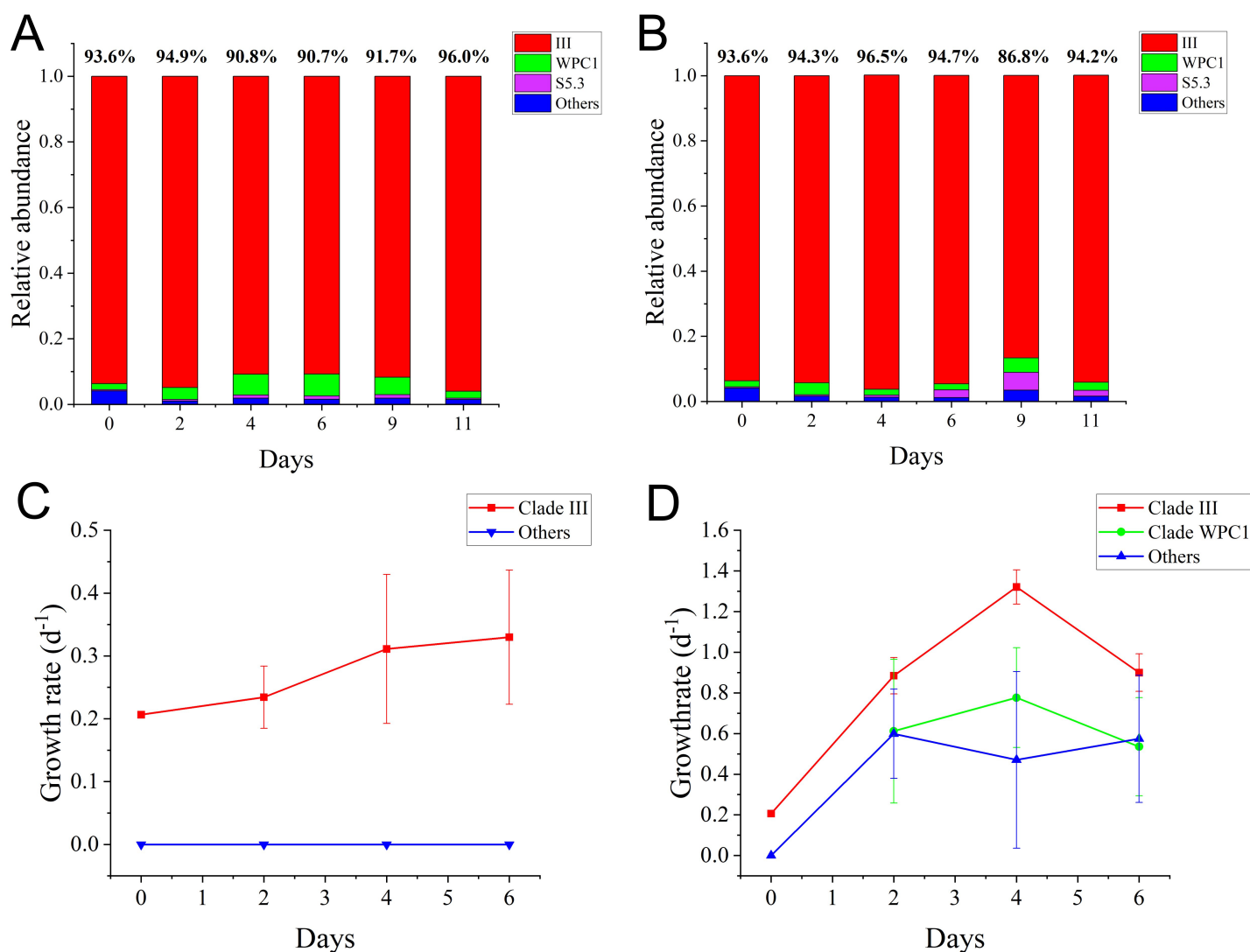


Fig. 2. Community structure and clade-specific growth rates of *Synechococcus* during the incubation period. **(A, B)** Relative abundance of *Synechococcus* clades in Mesocosm K **(A)** and Mesocosm NP **(B)**. Clades with average relative abundance < 5% over the 12-d incubation in both mesocosm were grouped as “Others.” Percentages above each bar indicate the relative abundance of clade III at that time point. **(C, D)** Clade-specific growth rates in Mesocosm K **(C)** and Mesocosm NP **(D)**. Error bars represent standard deviation of triplicate mesocosms.

exhibited reduced transporter complements. Clade III, clade VIII, and subcluster S5.2 possessed significantly elevated phosphorus transporter inventories relative to clades I, II, VI, VII, XVI, CRD1, and S5.3 (Figs. 3, 4). Among these transporters, the high-affinity phosphate-binding protein *pstS* demonstrated the highest gene copy number in clade III (Fig. 3, Supporting Information Table S3).

Nitrogen acquisition systems encompassed transporters for nitrate/nitrite, ammonium, urea, amino acids, and peptides (Fig. 3, Supporting Information Table S2). Notably, the nitrate/nitrite ABC transporter complex (*nrtABCD*) occurred exclusively in clade III and parts of S5.2 strains. The specific S5.2 strains possessing nitrate/nitrite ABC transporter complex (*nrtABCD*) generally also possessed various amino acid

transporters *LivHMGF* and *ABC.PA.S* which were absent in other clades/strains. Clade III and S5.2 maintained a higher number of nitrogen transporters compared with other lineages (Figs. 3, 4). Genomic architecture analysis revealed that clade III strains possessed lower GC content and smaller genomes than clade VIII/S5.2 counterparts (Fig. 4). Intriguingly, while *nrtABCD* occurred universally in clade III, it was undetected in strain MSI. Further examination confirmed *nrtABCD* loci exhibited lower GC content vs. flanking genomic regions (Supporting Information Fig. S2).

Metatranscriptomic analysis of ambient seawater confirmed that both nitrogen and phosphorus transporter genes are actively transcribed, with *pstS* showing the highest expression (Fig. 3), indicating phosphorus acquisition as a priority.

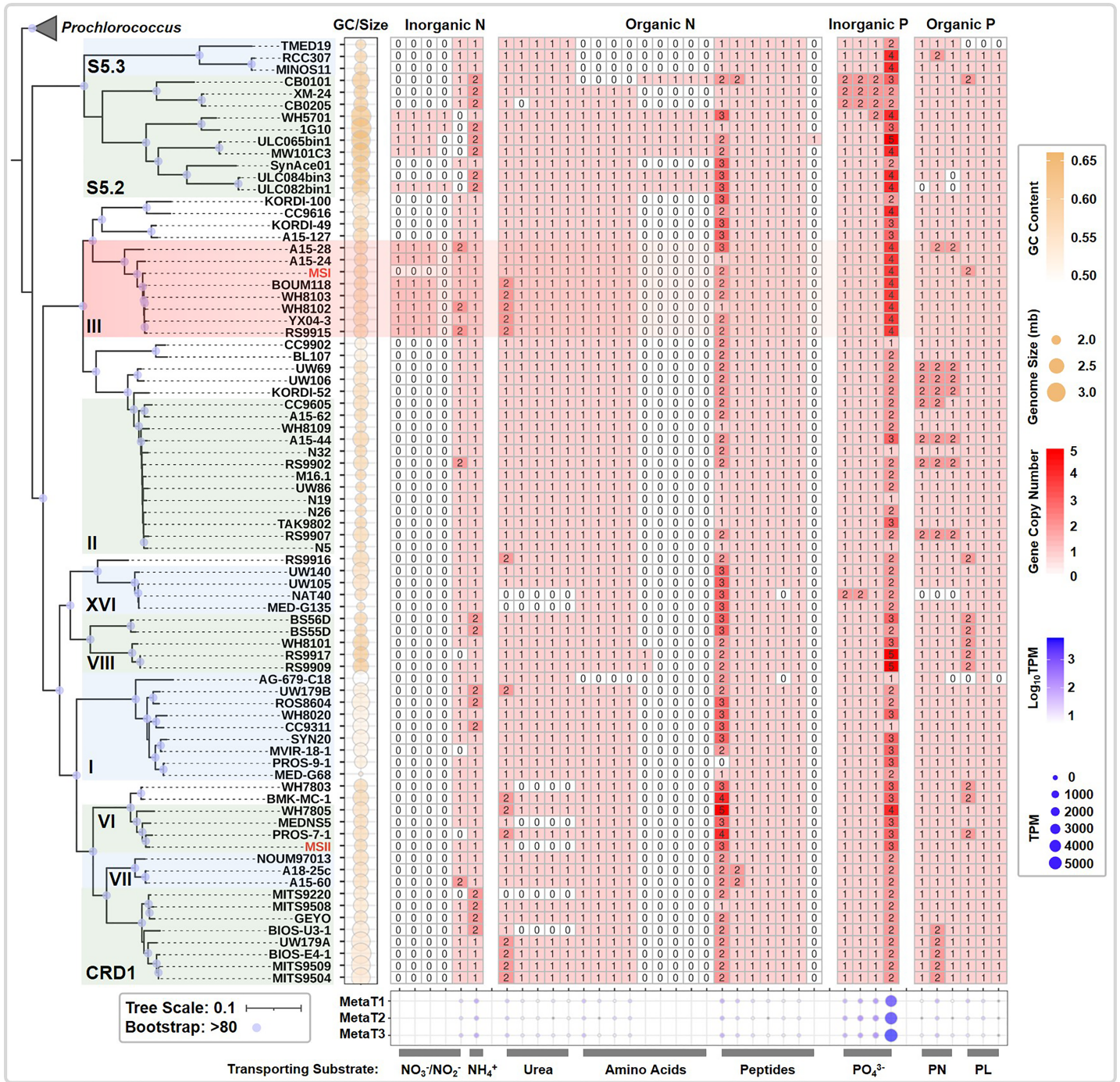


Fig. 3. Comparison of GC content, genome size, gene expression, and gene copy number of nitrogen/phosphorus transporters between different phylogenetic clades/strains of *Synechococcus*. The maximum likelihood tree was constructed using IQ-TREE using the JTT1F1G4 model selected by ProtTest based on a multiple-sequence alignment of single-copy marker genes. Detailed information of each genome and gene annotation is shown in Supporting Information Tables S1 and S2. Transcript abundance (TPM) of the transporter genes shown here is available in Supporting Information Table S3. PL, phospholipid; PN, phosphonate.

Discussion

Methodological constraints

The mismatch between Chl *a* and *Synechococcus* abundance peaks (Day 6 vs. Day 10) in Mesocosm NP reflected the

contribution of picoeukaryotes and microalgae to total Chl *a*, and possible different grazing pressure. The positive growth rates of *Synechococcus* in Mesocosm K ($\sim 0.3 \text{ d}^{-1}$) contrasted with near-zero net Chl *a* change, indicating that eukaryotic

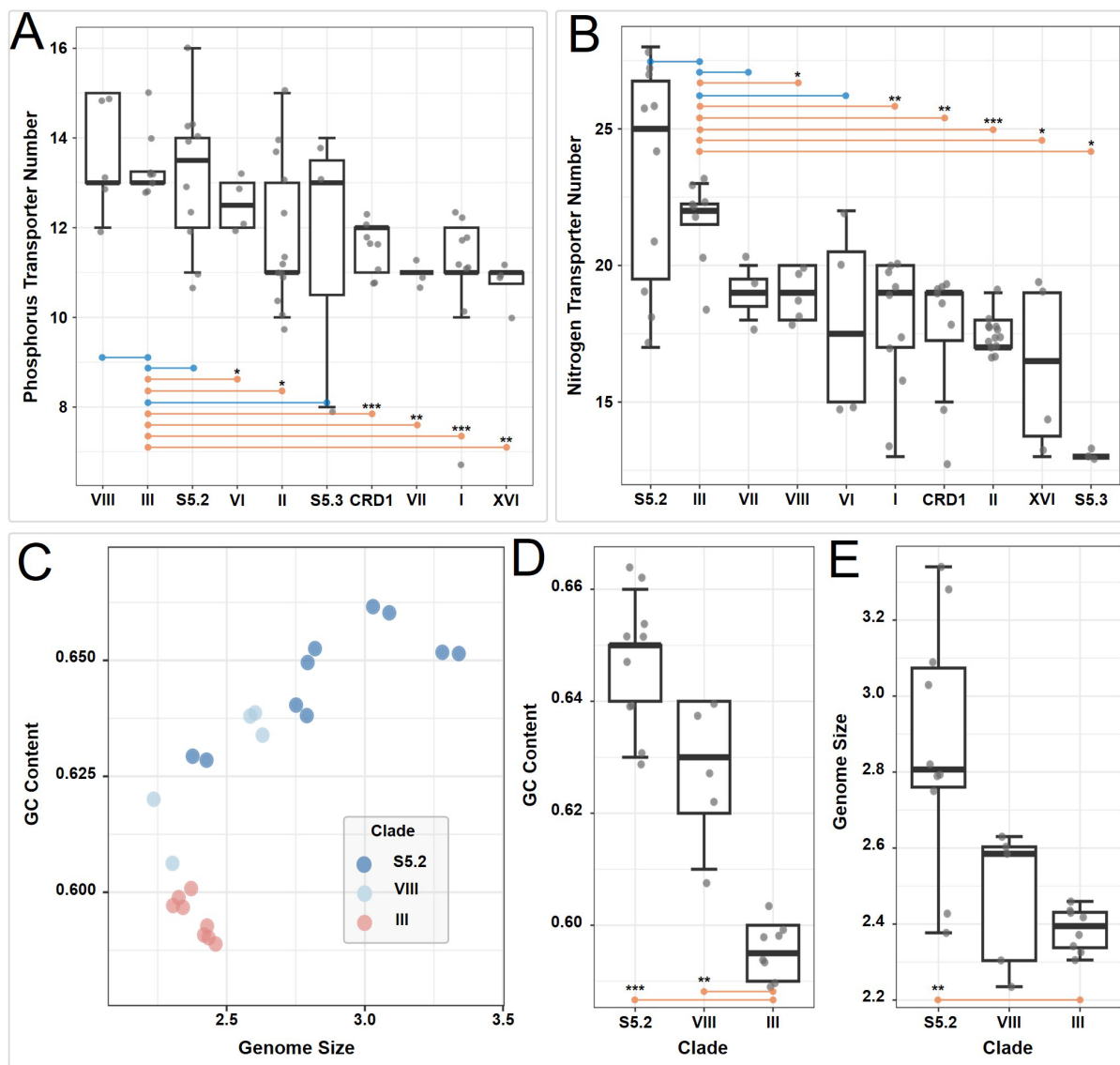


Fig. 4. Gene copy number of nitrogen/phosphorus transporters, GC content, and genome size in different phylogenetic clades/strains of *Synechococcus*. (A) Number of phosphorus transporter gene copy; (B) number of nitrogen transporter gene copy; (C, D) GC content of S5.2, clade VIII, and clade III; (E) Genome sizes of S5.2, clade VIII, and clade III. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$.

phytoplankton were growth-limited or had negative net growth, and growth of *Synechococcus* was masked in the bulk Chl *a* signal. This decoupling underscored the distinct physiological strategies of *Synechococcus* vs. larger phytoplankton under severe nutrient limitation.

The clade-specific growth rates are inferred values rather than direct measurements. Combining bulk abundance with *rpoC1* amplicon data assumes amplicon proportions reflect true compositions, but primer bias, differential amplification, and compositional effects may introduce uncertainty. Thus, these estimated rates are semi-quantitative indicators of

relative competitive performance. Additionally, the two-point dilution method is more sensitive to error and nonlinearity than full series, but previous validations (Chen 2015; Morison and Menden-Deuer 2017) showed that using a highly diluted treatment ($\leq 20\%$) yields accuracy comparable to full-series variability. We therefore emphasize comparisons across treatments and time points rather than absolute values. We also acknowledge that the associations between clade III's nutrient transporter gene abundance/expression and growth advantage are correlative, not causal; direct evidence would require further manipulation.

Clade III dominated the community

Synechococcus clade III dominated in both mesocosms, exhibiting exceptional fitness under ultraoligotrophic conditions especially phosphorus limitation. Their initial > 90% abundance and faster growth (Fig. 2) explained why no community shift occurred. The persistent dominance under both ambient and enriched conditions revealed the dual advantage: high affinity for scarce nutrients and rapid uptake of episodic pulses. The abundant and diverse transporters of nitrogen and phosphorus may help *Synechococcus* clade III compete for the limited nutrients. Notably, clade III encodes four copies of *pstS* (exceeding most other clades; Fig. 2). The highest expression of *pstS* among transporters (Fig. 3) suggested that phosphorus acquisition was a physiological priority, consistent with clade III's exceptional phosphorus recycling efficiency (growth sustained by only 0.1 μ M phosphate addition for 11 days; Figs. 1, 2). Our analysis of phosphorus utilization genes (Supporting Information Figs. S3, S4, Table S4) further showed that clade III had more gene copies than most other clades, reinforcing their advantage in phosphorus-limited waters like the Mediterranean. Notably, the NP treatment included a labile DOC addition, likely stimulating heterotrophic bacterial activity and ammonium regeneration. This bacterial competition for nutrients instead reinforced the robustness of clade III's nutrient acquisition strategy under resource competition.

Although clade III's advantage in nitrogen utilization genes is less pronounced, they still possess higher copy numbers than clade II (Supporting Information Figs. S3, S4). Both clade II and III dominate in warm waters, however, clade III are mainly restricted in ultraoligotrophic waters while clade II display a broader nutrients niche (Zwirgmaier et al. 2008; Farrant et al. 2016; Sohm et al. 2016; Doré et al. 2022). In Mediterranean, clade II and III exhibited mutual exclusivity (Doré et al. 2022) and we detected only limited clade II reads, contrasting with clade III's dominance (> 90% of reads) (Fig. 2). Clade II and III have comparable genome size and GC content (Fig. 3), and no significant differences were found (*t*-test, $p = 0.27$ for genome size, $p = 0.06$ for GC content; $n = 12$ and 8 for each clade). Their niche segregation may result from their distinct adaptive strategies (see Supporting Information Table S5 for a summary of their ecological and genomic features). Proteomic evidence confirmed clade III (e.g., WH8102) prioritized transporter expression for nutrient acquisition (Teoh et al. 2020). This transporter-centric profile aligns with its oligotrophic origin, suggesting evolutionary prioritization of enhanced nutrient scavenging capacity, as evidenced by previous genomic analysis (Tetu and Brahamsha 2009; Mackey et al. 2015) and our study. In contrast, clade II (e.g., CC9605) invested in photosynthetic efficiency and phycobilisome production, optimizing energy yield over nutrient scavenging intensity (Teoh et al. 2020). This energy-centric adaptation likely enables clade II to optimize resource utilization efficiency rather than nutrient

acquisition intensity, partially explaining its wider biogeographic distribution across fluctuating nutrient regimes.

Clade III *Synechococcus* possesses diverse organic nitrogen transporters (urea, amino acids, peptides; Fig. 3), consistent with known organic nitrogen utilization by picocyanobacteria (Wawrik et al. 2009; Muñoz-Marín et al. 2020). In the ultraoligotrophic Eastern Mediterranean, this capacity—together with active expression of these genes (Fig. 3)—likely provides clade III with a competitive advantage by accessing alternative nitrogen pools. We therefore propose that nitrogen mixotrophy is an integral component of clade III's adaptive strategy in ultraoligotrophic waters.

Beyond niche partitioning, clade III's dominance has broader ecological implications. Clade III exhibits intrinsic grazing resistance. Apple et al. (2011) reported that clade III was grazed at significantly lower rates than other clades, potentially diverting less primary production through the microbial loop and enhancing carbon export. Additionally, clade III possesses a unique high-affinity alkaline phosphatase (Psp1) for organic phosphorus sources (Torcello-Requena et al. 2024), which may affect cellular stoichiometry and trophic transfer. Since *Synechococcus* clades II + III contributed up to 2.7% of winter POC flux (Sargasso Sea; De Martini et al. 2018), shifts toward clade III's dominance could greatly influence biogeochemical cycles.

Nitrogen and phosphorus requirements

Although clade III, VIII, and S5.2 all possess numerous nutrient transporters, the underlying drivers may differ. Contrasting with clade III, clade VIII, and S5.2 strains inhabit dynamic, nutrient-rich estuaries (Ahlgren and Rocap 2012; Xia et al. 2015; Xiaomin et al. 2017; Doré et al. 2022). Unlike clade III, which needs competitive advantages for nutrient-limited conditions, clade VIII and S5.2 may require substantially higher nitrogen and phosphorus inputs supported by their abundant nutrient transporters.

Clade VIII and S5.2 exhibited significantly higher GC content and larger genomes than clade III (Fig. 4). Since GC-rich DNA requires more nitrogen (Giovannoni et al. 2005; Grzymalski and Dussaq 2012), and larger genomes demand more phosphorus (Okie et al. 2020) and nitrogen, clade VIII and S5.2 likely have more nutrient requirements than clade III, consistent with their niche partitioning. These observations are consistent with genome streamlining hypotheses (Lynch 2006; Swan et al. 2013), though our data are correlative and do not establish causation. Instead, clade III may rely on numerous transporters to compete under nutrient limitation.

The nutrient strategy of clade III and VIII/S5.2 may also be evidenced by their differences in phycobilisome, which consists of up to 50–60% of soluble protein and is the largest cellular N reservoir (Grossman et al. 1993; Apt et al. 1995). Based on phycobilisome rod pigment composition, *Synechococcus* can be categorized into phycocyanin only and phycoerythrin-

dominant type, while phycocyanin-only type may potentially have higher nitrogen requirements due to the difference in nitrogen atoms between phycocyanin-only (166.4 mol/molecular) and phycoerythrin-dominant (75.4 mol/molecular) (Ruan et al. 2018). Notably, nearly all clade VIII and a large portion of S5.2 strains are phycocyanin-only type, suggesting their elevated nitrogen demands, which are robustly supported by the abundant nitrogen transporters.

Author Contributions

Xiaodong Zhang and Jiawei Chen generated the research idea and analyzed the data. Xiaodong Zhang and Shunyan Cheung performed the mesocosm experiments and sampling. Hoi Yau Chan and Sangwook Scott Lee conducted DNA extraction and polymerase chain reaction. Ioulia Santi and Paraskevi Pitta conceptualized and organized the mesocosm project. Xiaodong Zhang wrote the original manuscript draft, which was reviewed and edited by Jiawei Chen and Hongbin Liu with input from all authors. Hongbin Liu acquired funding. All authors contributed to data compilation and interpretation and approved the final manuscript.

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Conflicts of Interest

None declared.

Data Availability Statement

The *rpoC1* gene and metatranscriptome sequences are deposited in the National Center for Biotechnology Information Sequence Read Archive under accession number: PRJNA1474131. Metadata, reference genomes, and the newly assembled genomes of Mediterranean strains MSI/MSII are accessed at <https://doi.org/10.5281/zenodo.20537489>.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

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