The special and general mechanism of cyanobacterial harmful algal blooms

Wenduo Cheng[†b], Somin Hwang[†b], Qisen Guo[†b], Leyuan Qian[b], Weile Liu[b], Yang Yu[b], Zhenghao Li[b], Tianji Wang[b], Yi Tao*[a], and Huansheng Cao*[b]

[a]Guangdong Provincial Engineering Research Center for Urban Water Recycling and Environmental Safety, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen, 518055, China

[b]Division of Natural and Applied Sciences, Duke Kunshan University, 8 Duke Ave, Kunshan, 215316, China

[†]Equal contribution

[*]Correspondence: Huansheng Cao (hc284@duke.edu) and Yi Tao (tao.yi@sz.tsinghua.edu.cn)

Abstract

Cyanobacterial harmful algal blooms (CyanoHABs) arise as cyanobacteria dominate phytoplankton communities when nutrient levels increase from oligotrophic state. From a wholistic perspective, this longstanding altered phytoplankton structure results from two conditions: one sufficient condition that cyanobacteria can grow maximally with elevated nutrients; one necessary condition that co-living algae cannot grow fast or dominate at the same levels. The sufficient condition, the 'special' mechanism of CyanoHABs at the population level, has been established as the synergistic interaction between superior cyanobacterial ecophysiology and elevated nutrients. But it is unknown how these functions arise or whether they are under directed evolution to water eutrophication. The necessary condition, the 'general' mechanism of CyanoHABs at the community level, is little understood: why co-living algae cannot form blooms as cyanobacteria? Literature and bioinformatics analyses show that the superior ecophysiology undergoes no directed positive evolution to worldwide eutrophication in general or any local eutrophic waters in particular; instead, these functions are under strong purifying selection and likely acquired through early adaptive radiation in nutrient-deficient conditions, as functions enabling extant cyanobacteria to occupy other niches. The general mechanism turns out to be quite straightforward: cyanobacteria are simple life forms and thus have lower per capita nutrient demand for growth, compared to co-existing eukaryotic algae in cell size and structure, genome size, size of genome-scale metabolic networks, cell content, nutrient requirement. Lower nutrient demand is proved by existing field nutrient supplementation. Both the special and general mechanisms of CyanoHABs are tentative frameworks awaiting further theoretic improvement and

empirical assessment.

Keywords

Adaptive radiation, cyanobacterial harmful algal blooms (CyanoHABs), ecophysiology, mechanism, nutrient demand, purifying selection, water eutrophication,

I. Introduction

CyanoHABs are one of the most profound environmental hazards in modern human history in terms of their global geographical scale (Paerl et al. 2020), longstanding duration (over a century) (Francis 1878, Lathrop and Carpenter 1992), and tremendous economic loss (Steffensen 2008). The mere fact that they are still intensifying and expanding under the global climate change (Chapra et al. 2017, Gobler 2020, Paerl et al. 2020) attests the fact CyanoHABs are still not fully understood, such that their ecology remains ‘complicated and confusing’ (Wilhelm et al. 2020). To demystify CyanoHABs, one needs to look at them from a wholistic perspective, considering all the main factors: bloom-forming cyanobacteria, other co-existing algal species, and eutrophic conditions. So far, it has rarely been seen blooms of other groups of algae appear in the same eutrophic waters as cyanobacteria (e.g., Heisler et al. 2008, Steffen et al. 2014, Cook et al. 2020). Considering this upscaled context, the main question now is why cyanobacteria, other than other algae, form blooms in the same eutrophic waters. Apparently, this overarching question can be broken down to two, each of which constitute the sufficient and necessary conditions: (1) how cyanobacteria form blooms in the eutrophic waters, and (2) why co-existing algae do not form blooms in the same eutrophic waters? The second question is equivalent to whether other algae can form blooms under the same eutrophic conditions, whether there are enough supplies of nutrients for them to bloom, or both.

To address these questions, we formulate a perspective primarily based on the comparatively advantage of cyanobacteria in nutrient demand. Simply put, it postulates that cells of cyanobacteria have simple cell structures and small sizes and therefore

require less input of energy and matter to sustain and grow than those of other species, and as a result, can grow fast when nutrient level increases in the eutrophic waters. This simple life structure and operation in turn results from adaptive evolution during nutritiously prohibiting environment of early Earth. This perspective will be established by addressing the sufficient and then the necessary condition, respectively.

II: The special mechanism of CyanoHABs

From an energy and matter perspective, the ubiquitous presence of CyanoHABs attests the fact that bloom-forming cyanobacteria have metabolic machinery able to effectively assimilate external nutrients and convert them to biomass, aided by favorable weather or climate conditions (O'Neil et al. 2012, Zhang et al. 2012). That is to say that CyanoHABs are the outcomes of the synergistic interactions between superior ecophysiology of cyanobacteria and eutrophic conditions. Indeed, both factors have been well established by field work, laboratory simulations, genomics and metatranscriptomics. Specifically, the roles of nutrients, either trace elements or macronutrients, have been recognized as important drivers for CyanoHABs in a consensus and large survey (Heisler et al. 2008, Beaulieu et al. 2013). Meanwhile, the metabolic pathways importing and converting available nutrients have been found to be more complete and have more copies of genes encoded in the genomes of bloom-forming cyanobacteria than non-blooming cyanobacteria (Cao et al. 2020a, Du et al. 2021) (Fig. 1). These metabolic pathways and their genomes are now curated in a webserver CyanoPATH (Du et al. 2021). Most of the functions are peripheral and involved in nutrient uptake and precursor processing to feed central metabolism, and the rest are in the central metabolism converting metabolites for synthesis of

macromolecules in anabolism. Interestingly and expectedly, the gene expression levels of these pathways are correlated with their completeness (Cao et al. 2020a). To sum up, at the population level, the synergistic interactions between superior metabolic capabilities and elevated nutrients suffice as a working mechanism of CyanoHABs.

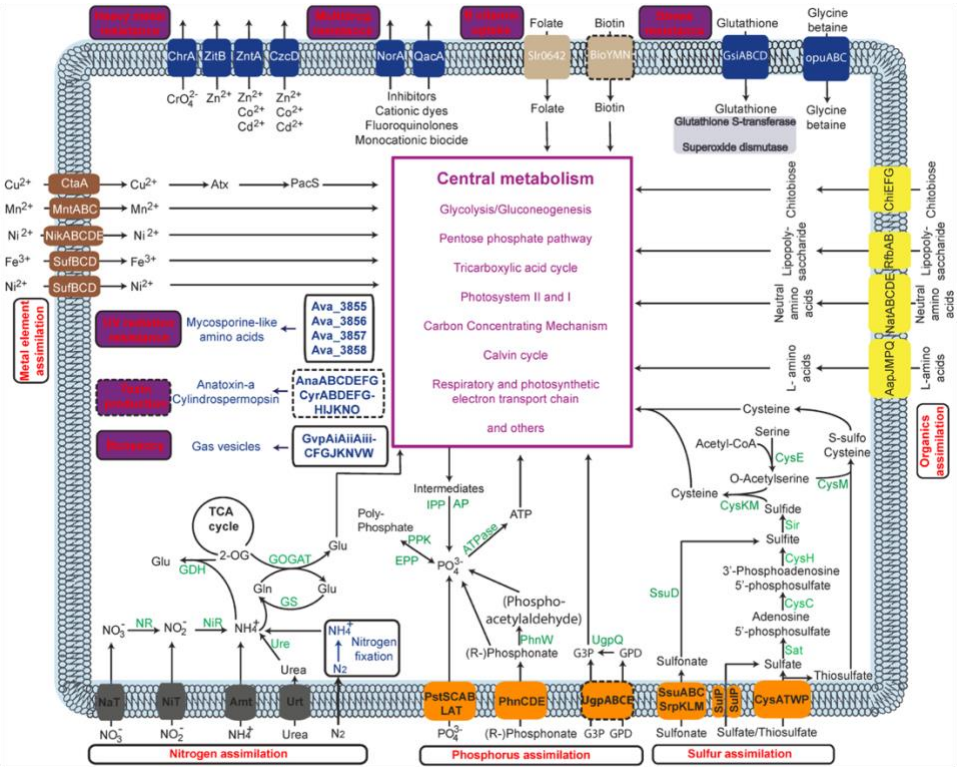


Figure 1. The core and query pathways in *Aphanizomenon flos-aquae* NIES-81. The pathways labeled with dashed borders are not complete (due to the absence of required components) in this strain but may be complete in others.

III: Is there *a priori* directed evolution toward water eutrophication?

Given their global persistence for over a century (Francis 1878, Lathrop and Carpenter

102 1992), one may wonder whether there is prior adaptive evolution in cyanobacteria
103 toward water eutrophication to form bloom, or CyanoHABs are simply ecological
104 consequences of synergistic interactions between superior pre-equipped biological
105 functions and elevated nutrients. We think the latter is more likely than the former. First,
106 short-time whole-lake experiments in fertilization or nutrient reduction (Schindler et al.
107 2016, Wilkinson et al. 2018, Molot et al. 2021) have indisputably shown the causality of
108 eutrophication for CyanoHABs on an ecological time scale, allowing no time for
109 evolution. Second, all evolution of microbes is passive, rather than proactive, response
110 to environmental or genetic changes which are unforeseeable to them, and therefore
111 they cannot proactively prepare themselves through directed evolution.
112 Third, we confirmed there is no directed evolution toward CynaoHABs, by calculating
113 the metric, dN/dS (ratio of non-synonymous to synonymous mutations), for all
114 homologous genes in 50 blooming species of *M. aeruginosa* genomes from different
115 countries. Indeed, almost all homologous genes display dN/dS < 0.8, with a median of
116 0.32 (Fig. 2A), indicating they are all under negative (purifying) selection not positive
117 (divergent) evolution. When grouped into the functional pathways curated in
118 CyanoPAHT (Cao et al. 2020b), the homologous genes associated with CyanoHABs
119 displayed the same levels of purifying selection pressure as those in core metabolism
120 and housekeeping processes (e.g., translation and transcriptional regulation), with a
121 mean of dN/dS at 0.32 (Fig. 2B). This strong purifying selection suggests these gene
122 functions not only are important functions such that cells cannot afford them to be
123 comprised by mutations. Furthermore, the phylogenetic analysis shows that the *M.*
124 *aeruginosa* strains from different countries tend to align in the same clusters (Fig. 2C).

This complex phylogeny clearly shows among other things there is no adaptation to local water conditions. In other words, all bloom-forming cyanobacteria share the set of metabolic capabilities which enable them to grow well and dominate eutrophic waters irrespective of local peculiarity in water conditions.

Last, there are not enough raw materials, mutations, for adaptive evolution to be accumulated during one annual cycle of blooms. The spontaneous mutation rate (μ) in cyanobacteria is estimated at 10^{-7} per genome per generation (García-Villada et al. 2004, López-Rodas et al. 2007, Osburne et al. 2011, Segawa et al. 2018). For a lake of a million m^3 (i.e., with a surface area of 1 million m^2 with a depth of 1 m where cyanobacteria are mostly concentrated), with initial cyanobacteria 20 cells/L growing to 10^8 cells/L after 25 doublings (t) at a growth rate 0.05-1.1 d^{-1} in an annual cycle (bottlenecked in fall and winter), this gives an effective population size (N_e) of 10^8 cells, based on the equation 15 in Wilson et al. (2006). So, the number of beneficial mutations to be fixed can be approximated by, assuming a beneficial rate (s) at 0.02 and a rate of fixation ($2s$) at 0.04, $N_e \mu t 2s$ ($10^8 \times 10^{-7} \times 25 \times 2 \times 0.02 = 10$). Therefore, for a lake with a surface area smaller than 0.1 million m^2, each year there is less than one beneficial mutant will occur, which makes prior adaptive evolution highly unlikely. But large lakes are likely to accumulate beneficial mutations and better-growing mutants.

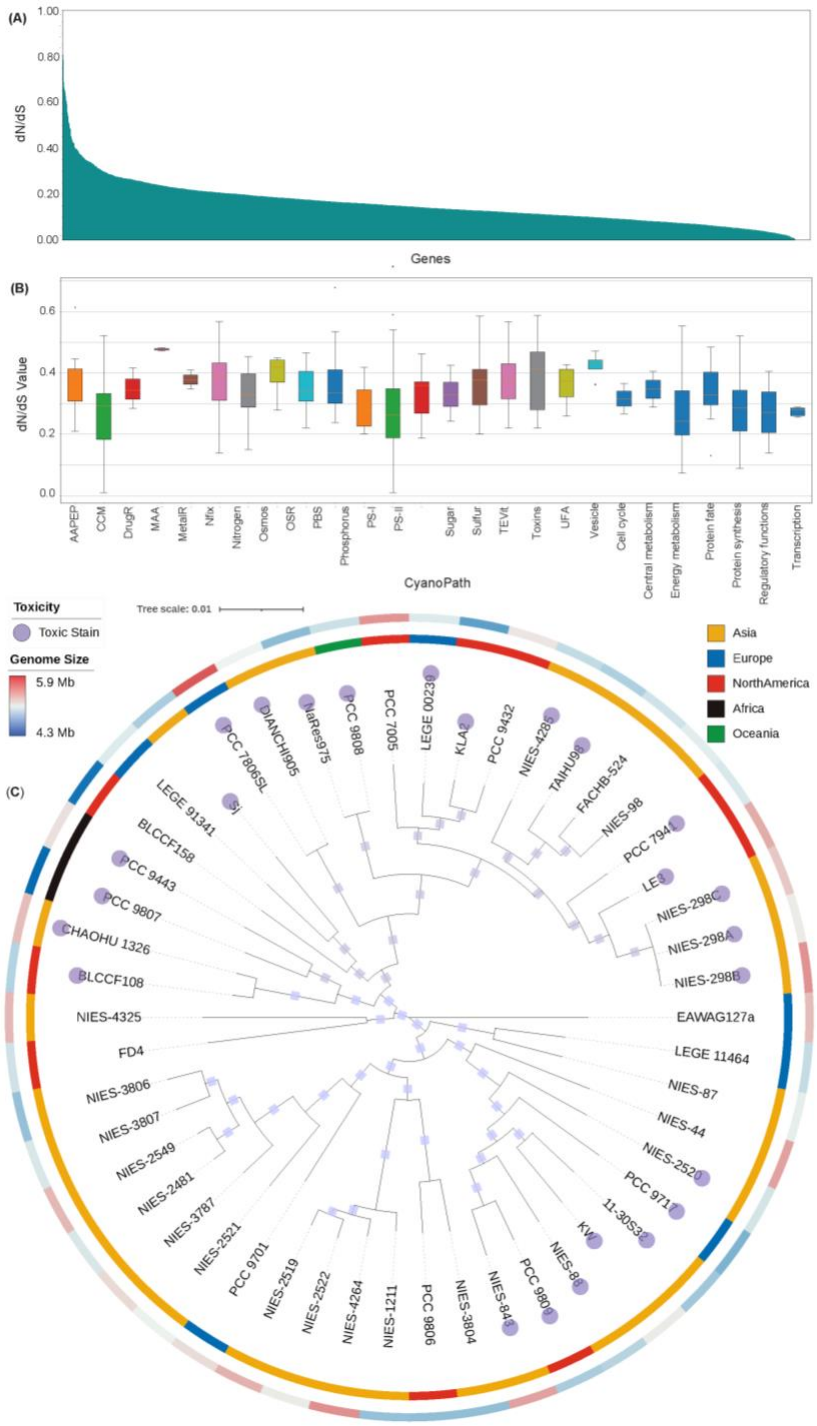


Figure 2. dN/dS for all homologous genes in 50 genomes of *M. aeruginosa*. (A) An overall distribution of dN/dS for all homologous genes; (B) the dN/dS for genes in different groups of functions in CyanoPATH. dN/dS values were calculated by CODEML from the PAML 4.9j package with default settings (Yang 2007). AAPEP: uptake of amino acids and peptides; CCM:

148 CO_2-concentrating mechanism; DrugR: antibiotics resistance; MAA: UV radiation; MetalR:
149 heavy metal resistance; Nfix: nitrogen fixation; Nitrogen: nitrogen utilization; Osmos: osmos
150 homeostasis; OSR: oxidative stress resistance; PBS: phycobilisome; Phosphorus:
151 inorganic/organic utilization; PS-I/PS-II: photosystem I and photosystem II; Sugar: sugar
152 assimilation; TEVit: assimilation of trace metals and vitamins; Toxins: cyanotoxins; UFA:
153 unsaturated fatty acids; Vesicle: gas vesicles. The last seven columns colored dark blue
154 represent essential gene sets (Rubin Benjamin et al. 2015). (C) The phylogeny of the 50 *M.*
155 *aeruginosa* strains collected from different countries.

156 IV. Superior functions are shaped during long adaptive
157 radiation on early Earth

158 The global ubiquity and expansion of CyanoHABs indicate the superiority of metabolic
159 capabilities. The fact these superior functions are under negative selection, instead of
160 assumed positive selection, suggests the metabolic capabilities were well established
161 before the era of water eutrophication. This brings another critical question: how
162 cyanobacteria acquired these functions? Revisiting the origin and evolution of
163 cyanobacteria on early Earth provides important clues: these superior functions are
164 shaped during their course of adaptive radiation.
165
166 Originating in an anoxic biosphere of early Earth, cyanobacteria are likely to evolve in
167 scarcity of macronutrients such as phosphorus and nitrogen (Falkowski 1997, Bjerrum
168 and Canfield 2002, Kipp and Stüeken 2017, Reinhard et al. 2017) and trace metals
169 (Anbar and Knoll 2002, Saito et al. 2003). This humble beginning of life leads to
170 primitive metabolic networks (Goldford and Segrè 2018), which suggests that

171 cyanobacteria could only obtain minimal energy and matter in both variety and amount
172 to support cell survival and division (Schopf 2002). Arguably, their nutrient requirement
173 for life is likely to be quite low. Besides nutrient deficiency, early cyanobacteria also
174 faced various types of stresses which are 'unforeseeable' for them. One type is the
175 rising oxygen level they caused themselves through oxygenic photosynthesis, for which
176 they developed various counterattack strategies in modern cyanobacteria (Raymond
177 and Segrè 2006, Moore et al. 2017). Ultraviolet light is another early stress which has
178 persisted until now, but some cyanobacteria evolved against it by producing sunscreens
179 (Garcia-Pichel et al. 2019). These global stresses were also intertwined with landscape
180 changes through geophysical processes (e.g., volcanism and climatic shifts). Here, we
181 demonstrate the remarkable adaptive ability of cyanobacteria by showcasing their
182 evolution of biological capability and morphology under the rising oxygen levels.
183
184 Since origin, particularly after the Great Oxidation Event (GOE) (Fig. 3), ancestral
185 cyanobacteria quickly acquired versatile antioxidant capabilities against reactive oxygen
186 species (ROSs), derived from O_2 produced as a result of oxygenic photosynthesis.
187 Different classes of superoxide dismutases (SODs) and other types of antioxidant
188 enzymes are evolved to deal with ROSs throughout their entire history (Boden et al.
189 2021). Based on local availability of metal cofactors (another case of adaptation), three
190 types of SODs—NiSOD, CuZnSOD, and MnSOD/FeSOD—have been found in four
191 types of aquatic environments (Fig. 3A). Besides direct enzymatic removal of ROSs,
192 cyanobacteria have also evolved other strategies, e.g., in photosynthetic reaction center
193 (RC) which split into two types as a response to the rising oxygen (Orf et al. 2018).

194 Another adaptation lies in the redundancy (heavy investment) of key functions. For
195 example, in photosystem I, cyanobacteria have two electron transporters that evolved
196 separately: copper-dependent plastocyanin (PC) and iron-dependent cytochrome c_6
197 ($Cytc_6$). Distinct in the primary sequence and tertiary structure of protein/amino acid,
198 their expression is controlled by a BlaI/CopY-family transcription factor (PetR) and a
199 BlaR-membrane protease (PetP), depending on the availability of the metal cofactors
200 (García-Cañas et al. 2021).
201
202 With a continuous rise of oxygen level, morphological diversification was called upon to
203 provide an extra dimension of protection. It has been confirmed that the evolution of
204 multicellular morphotypes and the rate of morphological diversification coincide with the
205 GOE onset (Schirrmeister et al. 2013). Heterocyst became a specialized cell form for
206 nitrogen fixation, in which the oxygen level is reduced to pre-GOE levels (Fig. 3B).
207 Nitrogen fixation is viewed as a leap forward in promoting marine primary production
208 and contributed to increased O_2 levels, coinciding with the rise of animals (Lyons et al.
209 2014). Similarly, cyanobacteria also obtained a specific photosynthetic structure for
210 carbon fixation, carboxysome, in which CO_2 is increased from 10-15μM to 40mM
211 outside and greatly improved the efficiency of enzymatic fixation of carbon dioxide
212 (Mangan and Brenner 2014). After the evolution of geological timescales (Fig. 3C and
213 3D), modern cyanobacteria are among the most diverse prokaryotic phyla, with
214 morphotypes ranging from unicellular to multicellular filamentous forms, including those
215 able to terminally (i.e., irreversibly) differentiate in form and function (Schirrmeister et al.
216 2013, Hammerschmidt et al. 2020).

217

218 Billions of years of adaptive evolution have allowed structure elaboration and functional
219 diversification in coping with nutrient deficiency, environmental extremes and constraints
220 (Paerl 2014). This evolutionary divergence has enabled cyanobacteria to occupy all
221 geographic habitats in modern Earth, including terrestrial and aquatic ecosystems,
222 ranging from deserts to tropical rain forests, soils and limestones, and from open
223 oceans and brackish waters to freshwaters and hydrothermal vents (Fig. 3E). This
224 broad array of biological functions underlying the adaptive radiation in cyanobacteria is
225 recorded in their genomes (Chen et al. 2021). A large proportion of the genes in their
226 genomes are associated with adaption to the specific habitat they occupy, but not to any
227 other habitats. Therefore, the genomes produce a pangenome with most genes being
228 unique and only a small set of 323 genes being common among cyanobacteria (Shi and
229 Falkowski 2008). These core genes are mainly involved in housekeeping functions, as
230 opposed to interacting with environment, e.g., ribosomal proteins, photosynthetic
231 apparatus, ATP synthesis, chlorophyll biosynthesis, and the Calvin cycle. This small
232 stable core and large variable shell of genomes suggest most of the non-overlapping
233 functions are specialized in adaptation to distinct habitats.

234

235 Based on adaptation to rising oxygen level they produced themselves paralleling the
236 evolution of Earth and adaptation to various habitats (see discussion above), one can
237 conclude that most of the functions in CyanoPATH are also obtained through adaptive
238 radiation. For example, one most prominent function is the ABC transport for phosphate
239 PstSCAB, most cyanobacteria have two copies of operons and some top bloomers such

as M. aeruginosa have three operons. Again, CyanoPATH functions are the part of entire function repertoire which sustains cyanobacteria through the harsh environment before the era of water eutrophication.

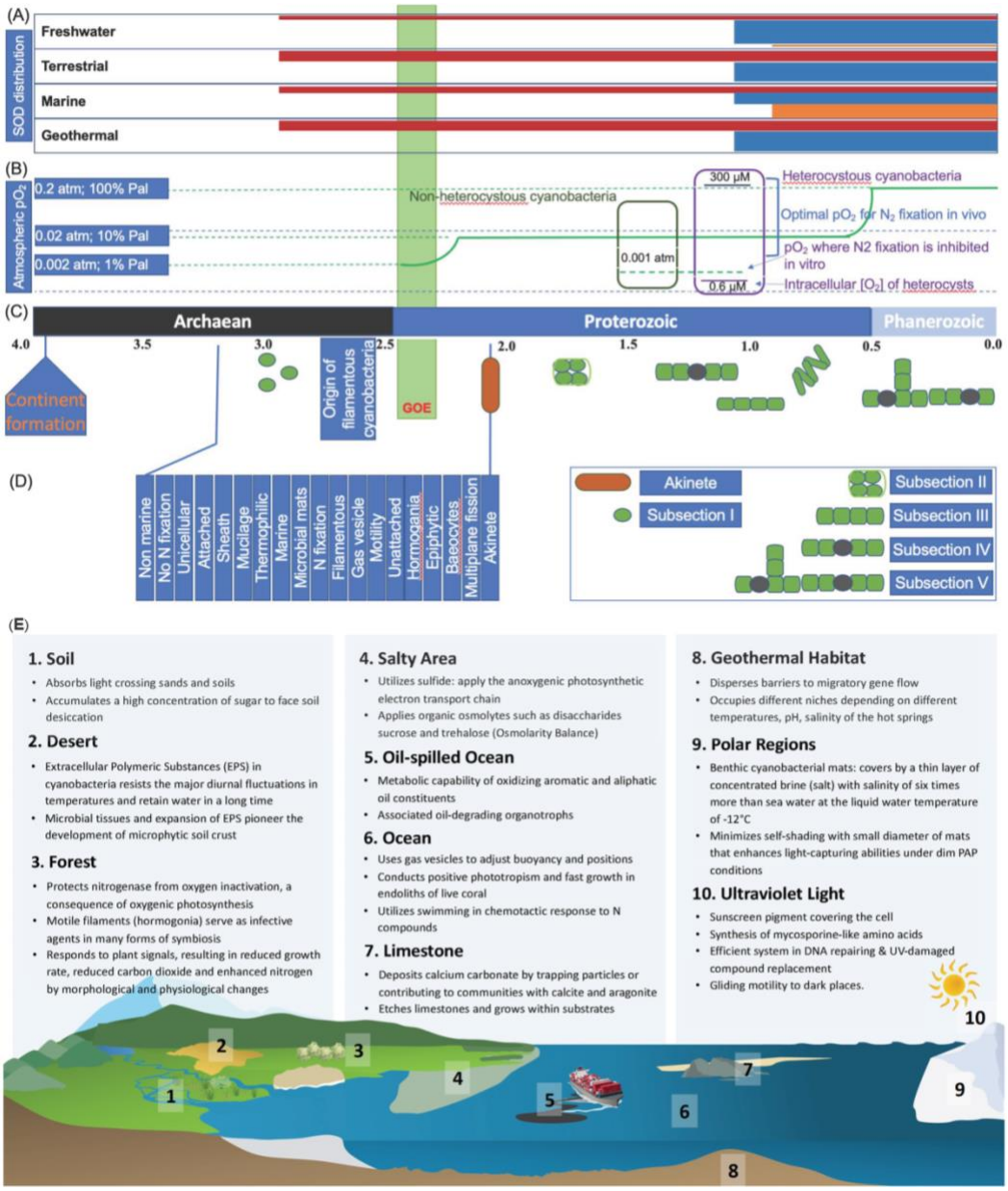


Figure 3. The evolutionary trajectory of cyanobacteria in the presence of oxygen and adaptive radiation. (A) The origin and distribution of SODs in different habitats. The timing of origin and habitat distribution of SOD genes among taxa is created based on Boden et al (Boden et al. 2021). Starting points of horizontal bars represent time of origin and color SOD types: CuZnSOD (blue), NiSOD (green), and Fe- and Mn-

utilizing SODs (yellow). (B) The oxygen level in atmosphere and nitrogen fixation in heterocysts in cyanobacteria in the presence of elevated oxygen levels. The level of oxygen is based on (Tomitani et al. 2006). (C) and (D) The temporal morphological diversification cyanobacteria is based on Schirrmeistera et al (Schirrmeister et al. 2013). (E). Adaptive strategies of cyanobacteria in different habitats. Cyanobacterial adaptation strategies in nine environments, namely soil, desert, forest, salty area, oil-spilled ocean, open ocean, limestone, geothermal water, and polar region. Cyanobacteria also cope with the damage caused by ultraviolet light.

V. The general mechanism of CyanoHABs

In light of the mechanism discussed above that CyanoHABs result from the synergistic interactions between elevated nutrients and the superior functions shaped during evolution, why it is cyanobacteria, not the eukaryotic algae, that form blooms in the same eutrophic waters? In what ways are cyanobacteria better than their co-living counterparts (e.g., Chen et al. 2017, Stockenreiter et al. 2021)? This question is so important such that the population-centric mechanism of CyanoHABs must be amended to accommodate the community-level dominance.

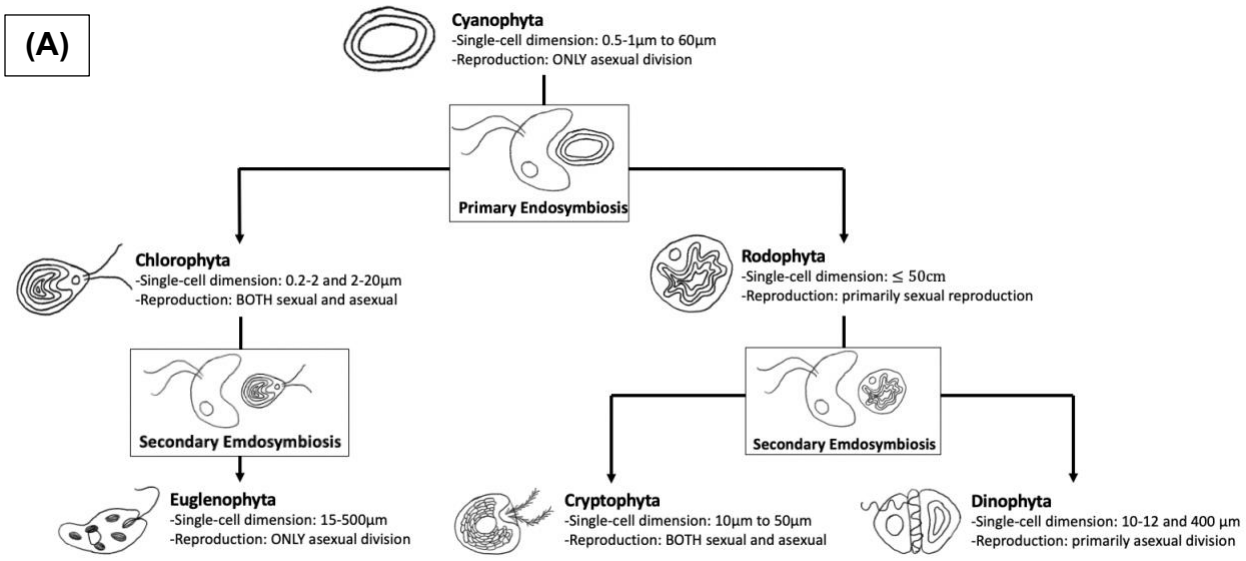
This issue is quite complex, considering high connectedness between populations in a food web. To reduce its complexity to a level amenable to testing without invoking any experiments, here we only consider the issue from an energy perspective. Compared to CyanoHABs as a result of maximum growth, one can inter two reasons why the co-existing algae cannot form blooms. For one, they are not able to obtain enough nutrients for growth; for the other, they have higher demands for nutrients than the current levels can provision. In the discussion below, we focus on the latter and

272 elucidate cyanobacteria are simpler life forms than their co-living eukaryotic algae life

273 forms and thus require less nutrients, through cell structure, size, genome size, per

274 capita cell content, nutrient demand, and field manipulation.

275



276

277 **(B)**

	Cyanophyta	Chlorophyta	Rhodophyta	Euglenophyta	Cryptophyta	Dinoflagellata
Cell Wall	P	P	P	P	P	P
Chloroplast	A[1]	P	P	P	P	P
Nucleus	A	P	P	P	P	P
Golgi Apparatus	A	P	P	P	P[2]	P
Mitochondria	A	P	P	P	P	P
Endoplasmic Reticulum	A	P	P	P	P	P
Starch Granule	P[3]	P	P	P	P	P

278 [1]Cyanophyta contains chlorophylls distributed over cytoplasm but not in an aggregated form, chloroplast.

279 [2]The function of Golgi apparatus in cryptophyte is found to be limited.

280 [3]It does contain starch granule, but it is found much smaller in size compared to other eukaryotic algae
281 lineages.
282 **Figure 4.** Cell structure of cyanobacteria and eukaryotic algae commonly seen in fresh waters
283 and their evolutionary relationship. A: absent; P: present. (A) The major eukaryotic groups have
284 risen from a common prokaryotic ancestor, Cyanophyta, via endosymbiotic events. Through the
285 primary endosymbiosis, Chlorophyta and Rhodophyta acquired primary plastids, membrane-
286 bound organelles usually found in eukaryotic organisms. Chlorophyta was preyed upon by a
287 second eukaryotic cell, resulting in Euglenophyta acquiring their plastids. Similarly, Cryptophyta
288 and Dinophyta have arisen as a result of secondary endosymbiosis on Rhodophyta. (B)
289 Comparison of cellular components in six common major algal phyla. Cyanophyta shows the
290 absence of nucleus, chloroplast, and mitochondria compared to other eukaryotic algae lineages.
291 Cyanophyta, being the common prokaryotic ancestor, do not contain membrane-bound
292 organelles.
293
294 ***Cell Structure and size***
295 Early deep origin and subsequent adaptive radiation (see Section IV) have shaped
296 cyanobacteria as simplest form of life among algae. Additionally, cyanobacteria are the
297 ancestor of eukaryotic algae, Chlorophyta, Rhodophyta, Euglenophyta, Cryptophyta,
298 and Dinophyta commonly seen in fresh waters, e.g., in Lake Taihu (China) (Niu et al.,
299 2011). Therefore, Cyanophyta, being a prokaryote, have the simplest cell structure
300 among modern algae (Fig. 4A): Cyanophyta does not contain a nucleus and membrane-
301 bound organelles such as mitochondria, Golgi apparatus, and endoplasmic reticulum.
302 Throughout the algal evolution, diverse eukaryotic algae have gained structural
303 complexity and acquired plastids via endosymbiotic events on their common prokaryotic
304 ancestor, Cyanophyta (Fig. 4B). In parallel to simple structure, cyanobacteria have the

305 smallest size among all algae gropus (Agusti et al. 1987). One example is provided with
306 lab cultures: cyanobacterium *M. aeruginosa* and chlorophyte *Cholorella vulgaris* are
307 3.30 ± 0.91 μm and 5.32 ± 1.27 μm in diameter, respectively (Hu 2014).
308
309 ***Genome Size***
310 Consistent with the structural simplicity, the genome sizes of cyanobacteria are the
311 smallest compared to those of eukaryotic algae, which are smaller by one or two orders
312 of magnitude (Fig. 5A). Most genomes of the eukaryotic algae are about the same size
313 of around 100 Mb base pairs, except those of Phaeophyceae and Dinophyceae being
314 one more order of magnitude larger. Cyanobacteria usually have around 5000 genes
315 (Kaneko et al. 2007, Frangeul et al. 2008) while eukaryotic genomes have at least twice
316 as many (e.g., Armbrust et al. 2004, Chen et al. 2020). These differences in genome
317 size and gene count reflect the difference in cell structure and functions.
318
319 ***Genome-Scale Metabolic Networks (GSMs)***
320 Cyanobacteria have half the number of the genes encoded in eukaryotic genomes at
321 the most. For some species, GSMs have been reconstructed based on the gene-
322 protein/enzyme-reaction rule and experimental measurement of the macromolecule
323 components (Table 1). In terms of the number of reactions and metabolites, the GSMs
324 of cyanobacteria have, on average, 780 metabolites and 820 reactions, which are only
325 less than half those of the eukaryotic GSMs. As the reactions are involved in
326 catabolism, core metabolism, and anabolism (Li et al. 2018), these smaller GSMs in
327 cyanobacteria make it clear that less nutrient input is needed than their more complex

328 eukaryotic counterparts for life maintenance and growth. Therefore, as nutrient levels
329 increase from the oligotrophic states during the early process of eutrophication,
330 cyanobacteria are the first group that can be saturated for growth.
331 Besides the size of GSMs, the metabolic rate (time^{-1}) is known to often scale with cell
332 size with a volume scaling exponent $b = -0.25$ (López-Urrutia et al. 2006, Finkel et al.
333 2010).
334
335 **Table 1**. Comparison of genome-scale metabolic networks between cyanobacteria and
336 eukaryotic algae.

	Organism	**Genes**	**Model Genes**	**Metabolites**	**Reactions**	**Reference**
Cyanophyta	Arthrospira platensis	6631	620	673	746	(Yoshikawa et al. 2015)
	Cyanothece sp. ATCC 51142	4912	806	651	667	(Vu et al. 2012)
	Spirulina platensis C1	6153	692	837	875	(Klanchui et al. 2012)
	Synechococcus elongatus	2723	785	768	850	(Broddrick et al. 2016)
	Synechococcus sp PCC	3179	821	777	792	(Qian et al. 2017)
	Synechocystis sp. PCC6803	3221	678	795	863	(Nogales et al. 2012)
	Synechocystis sp. PCC6803	3221	669	790	882	(Montagud et al. 2010)
Chlorophyta	Chlamydomonas reinhardtii	16709	866	1862	1725	(Gomes de Oliveira
	Chlamydomonas reinhardtii	16709	1080	1068	2190	(Chang et al. 2011)
	Chlorella variabilis	9879	526	1236	1455	(Juneja et al. 2016)
	Chlorella vulgaris	7100	843	1770	2286	(Zuñiga et al. 2016)
Bacillariophyta	Phaeodactylum tricornutum CCAP 1055/1	12280	1027	2172	4456	(Levering et al. 2016)
	Schizochytrium limacinum SR21	63000	1170	1659	1769	(Ye et al. 2015)

337

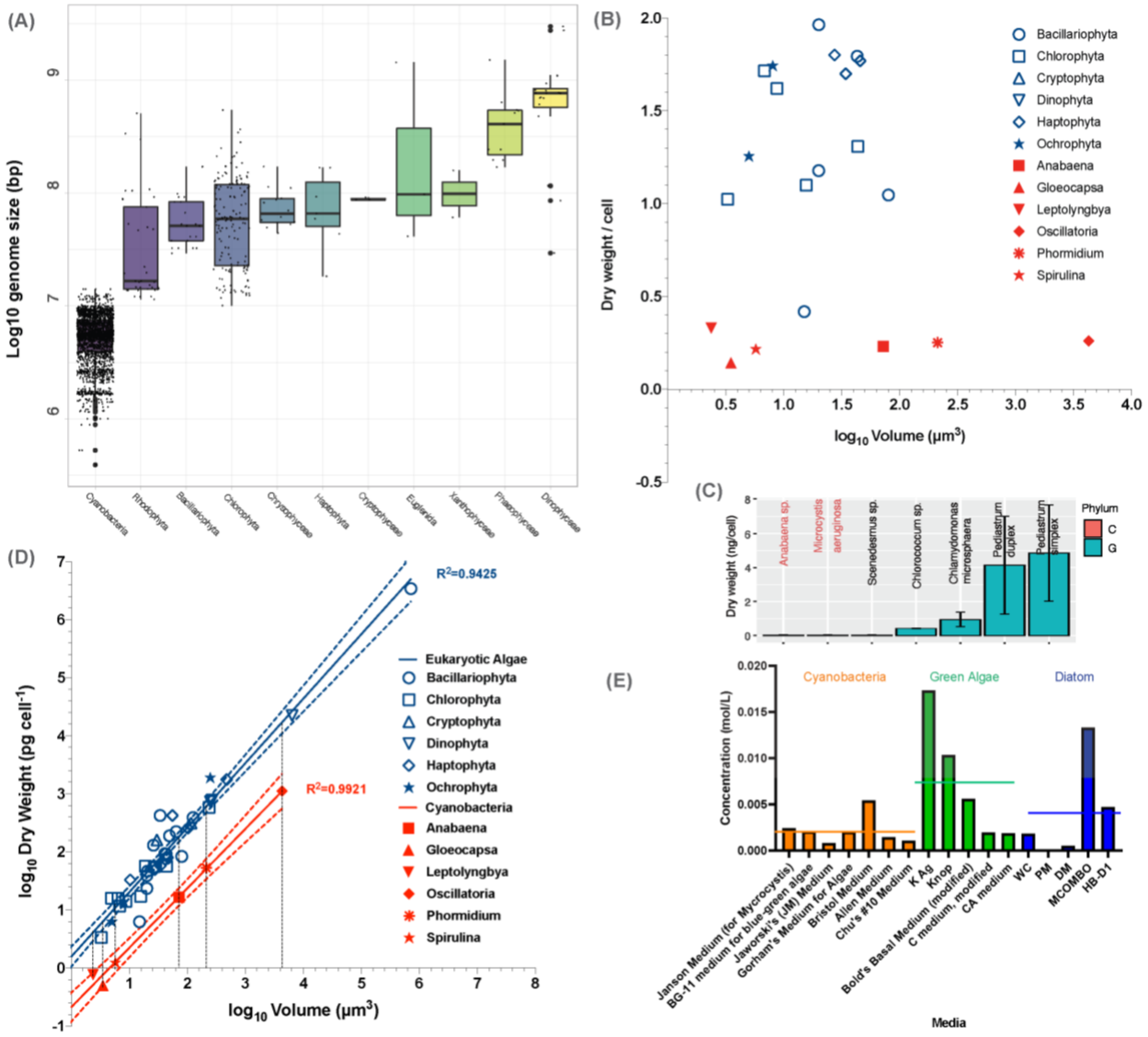


Figure 5. Comparison between cyanobacteria and eukaryotic algae in select key traits. (A): Distributions of log-transformed sequence length in eleven groups of algae. Summary statistics of 2240 genome assemblies were downloaded from NCBI Datasets: 1977 in cyanobacteria, 38 in Rhodophyta, 26 in Bacillariophyta, 156 in Chlorophyta, 14 in Chrysophyceae, 7 in Haptophyta, 3 in Cryptophyceae, 3 in Cryptophyceae, 3 in Euglenida, 2 in Xanthophyceae, 11 in Phaeophyceae and 20 in Dinophyceae, respectively. Each dot in the boxplot represents the log-transformed total sequence length of one assembly. (B): Distributions of dry weight per cell biovolume in Eukaryotic algae and cyanobacteria from literature (Mahlmann et al. 2008, Finkel

et al. 2016). Each dot plot represents the average values of dry weight per unit biovolume and cell dry weights within a class. Blue symbols represent eukaryotic algae cells and red symbols represents cyanobacteria. (C): The per-cell dry weight of two select cyanobacteria and five green algae at mid log phase. C: cyanobacteria; G: green algae. (D): Relationship between cell volume ($\log_{10}V$, μm^3), and dry weight ($\log_{10} DW$, pg $cell^{-1}$). Each dot plot represents the average values of cell volumes and cell dry weights within a class. Linear regressions were applied for eukaryotic algae (blue) and cyanobacteria (red) with 95% confidence intervals. (E): Lower nutrient requirements by cyanobacteria than eukaryotic algae in aquatic environment. Among seven selected media, the recipes of mAC, MJ, VTAC, and CHU-11 (left panel) are used for culturing green algae and the recipes of M11, BG11 and CT (right panel) are used for culturing cyanobacteria.

Per capita cell content

Following the same line of life form complexity, we examined the cell content per cell between cyanobacteria and eukaryotic algae. It is clear that different species of cyanobacteria have lower cell content than those of other algae, using the data from literature (Mahlmann et al. 2008, Finkel et al. 2016) (Wilcoxon Test, $P < 0.01$; Fig. 5B). Besides the public data, we also showed that the same pattern obtained with our own per-cell cell content (as dry weight), we collected with two species of cyanobacteria and five species of green algae (Wilcoxon Test, $P < 0.01$; Fig. 5C). Interestingly, species of cyanobacteria or non-cyanobacteria show a similar trend of within-group variation in per capita cell content (Fig. 5D). Here we provide on specific example of lab cultures. Dry weight of individual *Cholorella vulgaris* cells at exponential phase are $1.70 \pm 0.07 \times 10^{-11}$

370 g/cell, and those of *M. aeruginosa* average 6.38 ± 0.05×10^{-12} g/cell, which is only about
371 of the green alga (Hu 2014).
372
373 ***Concentration of synthetic media***
374 To further support the point that cyanobacteria are simpler life forms and require less
375 energy and mass input, we examined the recipe of the commonly used media optimized
376 for growing different groups of algae. As shown in Fig. 5E, the media for culturing
377 cyanobacteria generally have lower total concentrations of ions than those for green
378 algae or diatom, although the statistic test, Welch's test, produced a high p value (*P*
379 =0.1) for the selected media.
380
381 ***Golden test of nutrient demand: whole-lake nutrient manipulation***
382 To test the hypothesis that cyanobacteria have lower nutrient requirements for
383 maximum growth than co-living eukaryotic algae, we summarized the results, which
384 directly compared the growth of cyanobacteria with eukaryotic algae in the field,
385 laboratory, and cosmos by supplying external nutrients (Table 2). Five case studies we
386 discovered all support our hypothesis: low nutrients (TP, TN, DIN, urea-N, Ca, etc.)
387 leads to dominance of cyanobacteria and high nutrients produce dominance of green
388 algae and even brown algae.
389
390 In a large study of 210 Danish lakes heterocystous cyanobacteria were in dominance
391 when the total phosphorus (TP) at low levels (<0.25 mg P·L^{-1}) and non-heterocystous
392 cyanobacteria were in dominance when TP was at intermediate levels (0.25-0.8 mg

P·L-1), while chlorophytes often dominated when TP was high (>1 mg P·L^{-1}) (Jensen et al. 1994),. In another whole-lake study conducted in Wascana Lake (Canada) (Bogard et al. 2020), different levels (0, 1, 3, 8, and 18 mg N L^{-1}) of urea added into the surface water every seven days (day 0, day 7, and day 14) yielded compositional shifts in the phytoplankton community—after 21 days, colonial cyanobacteria dominated when N level was moderate (≤8 mg N L^{-1}), and chlorophyte dominated when N level was high (>8 mg N L-1). A third support comes as a nutrient-supplement mesocosm in the Archipelago Sea (Table 2). Two ratios of N:P supplement solution, 1N (1.7 μg L^{-1}):1P (1.7 μg L^{-1}) and 7N (12 μg L^{-1}):1P (1.7 μg L^{-1}), were applied to the mesocosm at low initial concentrations of N (mean=2.7 μg L^{-1}) and P (mean=2.8 μg L^{-1}) every day over twenty days. At the onset, heterocystous cyanobacteria made up 70% to 80% of the total phytoplankton community. Compared to 1N:1P treatment, 7N:1P led to the most dramatic decline of the percentage of the heterocystous cyanobacteria and the chlorophyte dominance with the flourishing of *Dictyosphaerium subsolitarium*, *Monoraphidium contortum* and *Oocystis spp*. Besides N and P, the need for calcium is also considered. Lab-based calcium enrichment experiment (Wu & Kow, 2010) showed that both the cell number and the cell column of chlorophytes elevated with the increase of calcium level, in contrast to those of cyanobacteria. More examples of nutrient supplementation to shift dominance from cyanobacteria to other algae are provided in Table 2.

To sum up, cyanobacteria are simpler life forms than co-living eukaryotic algae, in cell size, cell structure, genome size, GSMs, and cell content. Simple structure and small

size provide them with higher metabolic rates leading to higher abundance (Agusti et al. 1987), which have been proved by field nutrient supplementation. Now it is clear that complex eukaryotic algae would form blooms if nutrients were high enough.

Table 2. Case studies of nutrient manipulation on phytoplankton *in situ*, in the mesocosm study, and in laboratory conditions showing lower nutrient requirements of cyanobacteria than eukaryotic algae. “C” represents cyanobacteria; “G” represents green algae. DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorus; TN: total nitrogen; and TP: total phosphorus.

Study	Nutrient Variation	Dominant Species	Dominance
Wascana Lake, Canada Mesocosm Study (Bogard et al. 2020)	1-8 mg Urea-N L^{-1}	Colonial cyanobacteria	C
	>8 mg Urea-N L^{-1}	Chlorophytes	G
210 Danish lakes *In situ* Study (Jensen et al. 1994)	0.25-0.8 mg TP L^{-1}	Non-heterocystous cyanobacteria	C
	>1.0 mg TP L^{-1}	Chlorophytes	G
Lake Tai, China Laboratory and *in situ* study (Zhu et al. 2010)	3 mg/L (TN) and 0.2 mg/L (TP)	*M. aeruginosa*	C
	10, 15, or 20 mg/L (TN), and 1 mg/L (TP)	*Scenedesmus quadricauda*	G
Archipelago Sea Mesocosm Study (Vuorio et al. 2005)	1N:1P 1.7 µg L^{-1}: 1.7 µg L^{-1}	*Aphanizomenon* sp., *Nodularia spumigena*, *Anabaena* spp., *Synechococcus* spp.	C
	7N:1P 12 µg L^{-1}:1.7 µg L^{-1}	*Dictyosphaerium subsolitarium*, *Monoraphidium contortum*, and *Monoraphidium contortum, Oocystis* spp..	G
Taipei Feitsui Reservoir Laboratory and *in situ* study (Wu and Kow 2010)	7-9 mg Ca^{2+} L^{-1}	*Microcystis* spp. and *Aphanocapsa delicatissima*.	C
	9,11,13,15,18 mg Ca^{2+} L^{-1}	*Eutetramorus*, *Coelastrum*, *Coenocystis*, and *Dictyosphaerium*	G
Laboratory Study (Hyenstrand et al. 2000)	32µM (NO_3) after 48h; 32µM NH_4^+ after 48h	*Synechococcus 6301*	C
	32µM NO_3 after 25h; 32µM NH_4 after 18h	*Scenedesmus sp.*	G

Vila Lake, Central Portugal ***In situ study*** **(de Figueiredo et al. 2006)**	0.66-2.42 mg L (DIN); 0-0.24 mg L (SRP)	*Aphanizomenon flos-aquae, Ch roococcus limneticus, M. aeruginosa and Pseudanabaena sp.*	C
	0.7-3.3 mg L (DIN); 0.19-0.93 mg L (SRP)	*Coelastrum reticulatum var. reticulatum, Kirchneriella lunaris, Monoraphidium contortum, Scenedesmus acuminatus var. acuminatus and Pediastrum boryanum var. boryanum*	G

V. Synthesis and outlook

We propose a synthesis of CyanoPATH as a result of the synergistic interactions between cyanobacterial superior functions and elevated nutrients. In this evolutionary ecological perspective, we provided evidence these functions are shaped during the long course of adaptive radiation, but not toward water eutrophication. More importantly, we place CyanoHABs in the community context and show that they are simple life forms than co-living eukaryotic algae in terms of cell size, cell structure, genome size, per capita cell content, metabolic network, nutrient demand. At last, simpler cyanobacteria having low nutrient requirements are proved by various golden tests, field studies of nutrient supplement. This community-centric mechanism of CyanoHABs provides a framework to study them in a wholistic perspective, considering both the differences in energy and matter requirement of various algal cell machineries and the environment dictating their dynamics in relative to each other.

Considering the multiple factors in the mechanism, further empirical tests and theoretic explorations are needed to make a better synthesis. Two types of research should be given priority: (1) comparative studies between cyanobacteria and co-living algae in nutrient requirement and growth in lab and field settings, and their interactions with

varying levels of environmental factors; and (2) the integrated systems biology assessment of the relative roles in promoting growth in cyanobacteria. The latter can be best achieved by genome-scale metabolic networks.

Acknowledgements

This work was supported by National Natural Science Foundation of China (32171565 and 52070117) and Duke Kunshan University Summer Research Scholarships and Signature Work Research Grants (to WC, SH, QG, LQ, WL, and YY).

References

Agusti, S., C. M. Duarte, and J. Kalff. 1987. Algal cell size and the maximum density and biomass of phytoplankton. Limnology and Oceanography **32**:983-986.

Anbar, A. D., and A. H. Knoll. 2002. Proterozoic ocean chemistry and evolution: a bioinorganic bridge? Science **297**:1137-1142.

Armbrust, E. V., A. Berges John, C. Bowler, R. Green Beverley, D. Martinez, H. Putnam Nicholas, S. Zhou, E. Allen Andrew, E. Apt Kirk, M. Bechner, A. Brzezinski Mark, K. Chaal Balbir, A. Chiovitti, K. Davis Aubrey, S. Demarest Mark, J. C. Detter, T. Glavina, D. Goodstein, Z. Hadi Masood, U. Hellsten, M. Hildebrand, D. Jenkins Bethany, J. Jurka, V. Kapitonov Vladimir, N. Kröger, W. Y. Lau Winnie, W. Lane Todd, W. Larimer Frank, J. C. Lippmeier, S. Lucas, M. Medina, A. Montsant, M. Obornik, S. Parker Micaela, B. Palenik, J. Pazour Gregory, M. Richardson Paul, A. Rynearson Tatiana, A. Saito Mak, C. Schwartz David, K. Thamatrakoln, K. Valentin, A. Vardi, P. Wilkerson Frances, and S. Rokhsar Daniel. 2004. The Genome of the Diatom Thalassiosira Pseudonana: Ecology, Evolution, and Metabolism. Science **306**:79-86.

Beaulieu, M., F. Pick, and I. Gregory-Eaves. 2013. Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. Limnology and Oceanography **58**:1736–1746.

Bjerrum, C. J., and D. E. Canfield. 2002. Ocean productivity before about 1.9 Gyr ago limited by phosphorus adsorption onto iron oxides. Nature **417**:159-162.

Boden, J. S., K. O. Konhauser, L. J. Robbins, and P. Sánchez-Baracaldo. 2021. Timing the evolution of antioxidant enzymes in cyanobacteria. Nature Communications **12**.

Bogard, M. J., B. A. Bergamaschi, D. E. Butman, F. Anderson, S. H. Knox, and L. Windham-Myers. 2020. Hydrologic Export Is a Major Component of Coastal Wetland Carbon Budgets. Global Biogeochemical Cycles **34**:e2019GB006430.

Broddrick, J. T., B. E. Rubin, D. G. Welkie, N. Du, N. Mih, S. Diamond, J. J. Lee, S. S. Golden, and B. O. Palsson. 2016. Unique attributes of cyanobacterial metabolism revealed by improved genome-scale metabolic modeling and essential gene analysis. Proceedings of the National Academy of Sciences **113**:E8344.

Cao, H., Y. Shimura, M. Steffen, Z. Yang, J. Lu, A. Joel, L. Jenkins, M. Kawachi, Y. Yin, and F. Garcia-Pichel. 2020a. The trait repertoire enabling cyanobacteria to bloom assessed through comparative genomic complexity and metatranscriptomics. mBio **Accepted**.

Cao, H., Y. Shimura, M. M. Steffen, Z. Yang, J. Lu, A. Joel, L. Jenkins, M. Kawachi, Y. Yin, and

F. Garcia-Pichel. 2020b. The Trait Repertoire Enabling Cyanobacteria to Bloom Assessed through Comparative Genomic Complexity and Metatranscriptomics. mBio **11**:e01155-01120.

Chang, R. L., L. Ghamsari, A. Manichaikul, E. F. Y. Hom, S. Balaji, W. Fu, Y. Shen, T. Hao, B. Ø. Palsson, K. Salehi-Ashtiani, and J. A. Papin. 2011. Metabolic network reconstruction of Chlamydomonas offers insight into light-driven algal metabolism. Molecular Systems Biology **7**:518.

Chapra, S. C., B. Boehlert, C. Fant, V. J. Bierman, J. Henderson, D. Mills, D. M. L. Mas, L. Rennels, L. Jantarasami, J. Martinich, K. M. Strzepek, and H. W. Paerl. 2017. Climate Change Impacts on Harmful Algal Blooms in U.S. Freshwaters: A Screening-Level Assessment. Environmental Science & Technology **51**:8933-8943.

Chen, B.-L., W. Mhuantong, S.-H. Ho, J.-S. Chang, X.-Q. Zhao, and F.-W. Bai. 2020. Genome sequencing, assembly, and annotation of the self-flocculating microalga Scenedesmus obliquus AS-6-11. BMC Genomics **21**:743.

Chen, K., J. Allen, and J. Lu. 2017. Community Structures of Phytoplankton with Emphasis on Toxic Cyanobacteria in an Ohio Inland Lake during Bloom Season. Journal of Water Resource and Protection **9**:1-29.

Chen, M.-Y., W.-K. Teng, L. Zhao, C.-X. Hu, Y.-K. Zhou, B.-P. Han, L.-R. Song, and W.-S. Shu. 2021. Comparative genomics reveals insights into cyanobacterial evolution and habitat adaptation. The ISME Journal **15**:211-227.

Cook, K. V., C. Li, H. Cai, L. R. Krumholz, K. D. Hambright, H. W. Paerl, M. M. Steffen, A. E. Wilson, M. A. Burford, H.-P. Grossart, D. P. Hamilton, H. Jiang, A. Sukenik, D. Latour, E. I. Meyer, J. Padisák, B. Qin, R. M. Zamor, and G. Zhu. 2020. The global Microcystis interactome. Limnology and Oceanography **65**:S194-S207.

de Figueiredo, D. R., A. S. Reboleira, S. C. Antunes, N. Abrantes, U. Azeiteiro, F. Goncalves, and M. J. Pereira. 2006. The effect of environmental parameters and cyanobacterial blooms on phytoplankton dynamics of a Portuguese temperate lake. Hydrobiologia **568**:145-157.

Du, W., G. Li, N. Ho, L. Jenkins, D. Hockaday, J. Tan, and H. Cao. 2021. CyanoPATH: a knowledgebase of genome-scale functional repertoire for toxic cyanobacterial blooms. Briefings in Bioinformatics **22**:bbaa375.

Falkowski, P. G. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO2 in the ocean. Nature **387**:272-275.

Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. A. V. Rees, and J. A. Raven. 2010. Phytoplankton in a changing world: cell size and elemental stoichiometry. Journal of Plankton Research **32**:119-137.

Finkel, Z. V., M. J. Follows, and A. J. Irwin. 2016. Size-scaling of macromolecules and chemical energy content in the eukaryotic microalgae. Journal of Plankton Research **38**:1151-1162.

Francis, G. 1878. Poisonous Australian Lake. Nature **18**:11-12.

Frangeul, L., P. Quillardet, A.-M. Castets, J.-F. Humbert, H. C. Matthijs, D. Cortez, A. Tolonen, C.-C. Zhang, S. Gribaldo, J.-C. Kehr, Y. Zilliges, N. Ziemert, S. Becker, E. Talla, A. Latifi, A. Billault, A. Lepelletier, E. Dittmann, C. Bouchier, and N. Tandeau de Marsac. 2008. Highly plastic genome of Microcystis aeruginosa PCC 7806, a ubiquitous toxic freshwater cyanobacterium. BMC Genomics **9**:274.

García-Cañas, R., J. Giner-Lamia, F. J. Florencio, and L. López-Maury. 2021. A protease-mediated mechanism regulates the cytochrome c₆/plastocyanin switch in Synechocystis sp. PCC 6803. Proceedings of the National Academy of Sciences **118**:e2017898118.

Garcia-Pichel, F., J. Lombard, T. Soule, S. Dunaj, S. H. Wu, M. F. Wojciechowski, and S. J.

534 Giovannoni. 2019. Timing the Evolutionary Advent of Cyanobacteria and the Later Great
535 Oxidation Event Using Gene Phylogenies of a Sunscreen. mBio **10**:e00561-00519.
536 García-Villada, L., M. Rico, M. a. Altamirano, L. Sánchez-Martín, V. López-Rodas, and E.
537 Costas. 2004. Occurrence of copper resistant mutants in the toxic cyanobacteria
538 Microcystis aeruginosa: characterisation and future implications in the use of copper
539 sulphate as algaecide. Water Research **38**:2207-2213.
540 Gobler, C. J. 2020. Climate Change and Harmful Algal Blooms: Insights and perspective.
541 Harmful Algae **91**:101731.
542 Goldford, J. E., and D. Segrè. 2018. Modern views of ancient metabolic networks. Current
543 Opinion in Systems Biology **8**:117-124.
544 Gomes de Oliveira Dal'Molin, C., L.-E. Quek, R. W. Palfreyman, and L. K. Nielsen. 2011.
545 AlgaGEM – a genome-scale metabolic reconstruction of algae based on the
546 Chlamydomonas reinhardtii genome. BMC Genomics **12**:S5.
547 Hammerschmidt, K., G. Landan, F. Domingues Kümmel Tria, J. Alcorta, and T. Dagan. 2020.
548 The Order of Trait Emergence in the Evolution of Cyanobacterial Multicellularity.
549 Genome Biology and Evolution **13**.
550 Heisler, J., P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q.
551 Dortch, C. J. Gobler, C. A. Heil, E. Humphries, A. Lewitus, R. Magnien, H. G. Marshall,
552 K. Sellner, D. A. Stockwell, D. K. Stoecker, and M. Suddleson. 2008. Eutrophication and
553 harmful algal blooms: A scientific consensus. Harmful Algae **8**:3-13.
554 Hu, W. J. 2014. Dry Weight and Cell Density of Individual Algal and Cyanobacterial Cells for
555 Algae Research and Development. Submitted by the University of Missouri--Columbia
556 Graduate School.
557 Hyenstrand, P., U. Burkert, A. Pettersson, and P. Blomqvist. 2000. Competition between the
558 green alga Scenedesmus and the cyanobacterium Synechococcus under different
559 modes of inorganic nitrogen supply. Hydrobiologia **435**:91-98.
560 Jensen, J. P., E. Jeppesen, K. Olrik, and P. Kristensen. 1994. Impact of Nutrients and Physical
561 Factors on the Shift from Cyanobacterial to Chlorophyte Dominance in Shallow Danish
562 Lakes. Canadian Journal of Fisheries and Aquatic Sciences **51**:1692-1699.
563 Juneja, A., F. W. R. Chaplen, and G. S. Murthy. 2016. Genome scale metabolic reconstruction of
564 Chlorella variabilis for exploring its metabolic potential for biofuels. Bioresource
565 Technology **213**:103-110.
566 Kaneko, T., N. Nakajima, S. Okamoto, I. Suzuki, Y. Tanabe, M. Tamaoki, Y. Nakamura, F. Kasai,
567 A. Watanabe, K. Kawashima, Y. Kishida, A. Ono, Y. Shimizu, C. Takahashi, C. Minami, T.
568 Fujishiro, M. Kohara, M. Katoh, N. Nakazaki, S. Nakayama, M. Yamada, S. Tabata, and
569 M. M. Watanabe. 2007. Complete genomic structure of the bloom-forming toxic
570 cyanobacterium *Microcystis aeruginosa* NIES-843. DNA Research **14**:247-256.
571 Kipp, M. A., and E. E. Stüeken. 2017. Biomass recycling and Earth's early phosphorus cycle.
572 Science Advances **3**:eaao4795.
573 Klanchui, A., C. Khannapho, A. Phodee, S. Cheevadhanarak, and A. Meechai. 2012. i AK692: A
574 genome-scale metabolic model of Spirulina platensis C1. BMC Systems Biology **6**:71.
575 Lathrop, R. C., and S. R. Carpenter. 1992. Phytoplankton and Their Relationship to Nutrients.
576 Pages 97-126 *in* J. F. Kitchell, editor. Food Web Management: A Case Study of Lake
577 Mendota. Springer New York, New York, NY.
578 Levering, J., J. Broddrick, C. L. Dupont, G. Peers, K. Beeri, J. Mayers, A. A. Gallina, A. E. Allen,
579 B. O. Palsson, and K. Zengler. 2016. Genome-Scale Model Reveals Metabolic Basis of
580 Biomass Partitioning in a Model Diatom. PloS One **11**:e0155038.
581 Li, G., H. Cao, and Y. Xu. 2018. Structural and functional analyses of microbial metabolic
582 networks reveal novel insights into genome-scale metabolic fluxes. Briefings in
583 Bioinformatics:bby022-bby022.
584 López-Rodas, V., A. Flores-Moya, E. Maneiro, N. Perdigones, F. Marva, M. E. García, and E.

585 Costas. 2007. Resistance to glyphosate in the cyanobacterium Microcystis aeruginosa
586 as result of pre-selective mutations. Evolutionary Ecology **21**:535-547.
587 López-Urrutia, Á., E. San Martin, P. Harris Roger, and X. Irigoien. 2006. Scaling the metabolic
588 balance of the oceans. Proceedings of the National Academy of Sciences **103**:8739-
589 8744.
590 Lyons, T. W., Christopher T. Reinhard, and Noah J. Planavsky. 2014. Evolution: a fixed-nitrogen
591 fix in the early ocean? Current Biology **24**:R276-R278.
592 Mahlmann, D. M., J. Jahnke, and P. Loosen. 2008. Rapid determination of the dry weight of
593 single, living cyanobacterial cells using the Mach-Zehnder double-beam interference
594 microscope. European Journal of Phycology **43**:355-364.
595 Mangan, N. M., and M. P. Brenner. 2014. Systems analysis of the CO2 concentrating
596 mechanism in cyanobacteria. eLife **3**:e02043.
597 Molot, L. A., S. N. Higgins, S. L. Schiff, J. J. Venkiteswaran, M. J. Paterson, and H. M. Baulch.
598 2021. Phosphorus-only fertilization rapidly initiates large nitrogen-fixing cyanobacteria
599 blooms in two oligotrophic lakes. Environmental Research Letters **16**:064078.
600 Montagud, A., E. Navarro, P. Fernández de Córdoba, J. F. Urchueguía, and K. R. Patil. 2010.
601 Reconstruction and analysis of genome-scale metabolic model of a photosynthetic
602 bacterium. BMC Systems Biology **4**:156.
603 Moore, E. K., B. I. Jelen, D. Giovannelli, H. Raanan, and P. G. Falkowski. 2017. Metal
604 availability and the expanding network of microbial metabolisms in the Archaean eon.
605 Nature Geoscience **10**:629-636.
606 Nogales, J., S. Gudmundsson, E. M. Knight, B. O. Palsson, and I. Thiele. 2012. Detailing the
607 optimality of photosynthesis in cyanobacteria through systems biology analysis.
608 Proceedings of the National Academy of Sciences **109**:2678.
609 O'Neil, J. M., T. W. Davis, M. A. Burford, and C. J. Gobler. 2012. The rise of harmful
610 cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful
611 Algae **14**:313-334.
612 Orf, G. S., C. Gisriel, and K. E. Redding. 2018. Evolution of photosynthetic reaction centers:
613 insights from the structure of the heliobacterial reaction center. Photosynthesis Research
614 **138**:11-37.
615 Osburne, M. S., B. M. Holmbeck, A. Coe, and S. W. Chisholm. 2011. The spontaneous mutation
616 frequencies of Prochlorococcus strains are commensurate with those of other bacteria.
617 Environmental Microbiology Reports **3**:744-749.
618 Paerl, H. W. 2014. Mitigating Harmful Cyanobacterial Blooms in a Human- and Climatically-
619 Impacted World. Life **4**:988-1012.
620 Paerl, H. W., K. E. Havens, N. S. Hall, T. G. Otten, M. Zhu, H. Xu, G. Zhu, and B. Qin. 2020.
621 Mitigating a global expansion of toxic cyanobacterial blooms: confounding effects and
622 challenges posed by climate change. Marine and Freshwater Research **71**:579-592.
623 Qian, X., M. K. Kim, G. K. Kumaraswamy, A. Agarwal, D. S. Lun, and G. C. Dismukes. 2017.
624 Flux balance analysis of photoautotrophic metabolism: Uncovering new biological details
625 of subsystems involved in cyanobacterial photosynthesis. Biochimica et Biophysica Acta
626 (BBA) - Bioenergetics **1858**:276-287.
627 Raymond, J., and D. Segrè. 2006. The Effect of Oxygen on Biochemical Networks and the
628 Evolution of Complex Life. Science **311**:1764-1767.
629 Reinhard, C. T., N. J. Planavsky, B. C. Gill, K. Ozaki, L. J. Robbins, T. W. Lyons, W. W. Fischer,
630 C. Wang, D. B. Cole, and K. O. Konhauser. 2017. Evolution of the global phosphorus
631 cycle. Nature **541**:386-389.
632 Rubin Benjamin, E., M. Wetmore Kelly, N. Price Morgan, S. Diamond, K. Shultzaberger Ryan,
633 C. Lowe Laura, G. Curtin, P. Arkin Adam, A. Deutschbauer, and S. Golden Susan. 2015.
634 The essential gene set of a photosynthetic organism. Proceedings of the National
635 Academy of Sciences **112**:E6634-E6643.

Saito, M. A., D. M. Sigman, and F. M. M. Morel. 2003. The bioinorganic chemistry of the ancient ocean: the co-evolution of cyanobacterial metal requirements and biogeochemical cycles at the Archean–Proterozoic boundary? Inorganica Chimica Acta **356**:308-318.

Schindler, D. W., S. R. Carpenter, S. C. Chapra, R. E. Hecky, and D. M. Orihel. 2016. Reducing Phosphorus to Curb Lake Eutrophication is a Success. Environmental Science & Technology **50**:8923-8929.

Schirrmeister, B. E., J. M. de Vos, A. Antonelli, and H. C. Bagheri. 2013. Evolution of multicellularity coincided with increased diversification of cyanobacteria and the Great Oxidation Event. Proceedings of the National Academy of Sciences **110**:1791.

Schopf, J. W. 2002. The Fossil Record: Tracing the Roots of the Cyanobacterial Lineage. Pages 13-35 *in* B. A. Whitton and M. Potts, editors. The Ecology of Cyanobacteria: Their Diversity in Time and Space. Springer Netherlands, Dordrecht.

Segawa, T., N. Takeuchi, K. Fujita, V. B. Aizen, E. Willerslev, and T. Yonezawa. 2018. Demographic analysis of cyanobacteria based on the mutation rates estimated from an ancient ice core. Heredity **120**:562-573.

Shi, T., and P. G. Falkowski. 2008. Genome evolution in cyanobacteria: The stable core and the variable shell. Proceedings of the National Academy of Sciences **105**:2510.

Steffen, M. M., B. S. Belisle, S. B. Watson, G. L. Boyer, and S. W. Wilhelm. 2014. Status, causes and controls of cyanobacterial blooms in Lake Erie. Journal of Great Lakes Research **40**:215-225.

Steffensen, D. A. 2008. Economic cost of cyanobacterial blooms. Pages 855-865 *in* H. K. Hudnell, editor. Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Springer New York, New York, NY.

Stockenreiter, M., J. Isanta Navarro, F. Buchberger, and H. Stibor. 2021. Community shifts from eukaryote to cyanobacteria dominated phytoplankton: The role of mixing depth and light quality. Freshwater Biology **66**:2145-2157.

Tomitani, A., A. H. Knoll, C. M. Cavanaugh, and T. Ohno. 2006. The evolutionary diversification of cyanobacteria: Molecular–phylogenetic and paleontological perspectives. Proceedings of the National Academy of Sciences **103**:5442.

Vu, T. T., S. M. Stolyar, G. E. Pinchuk, E. A. Hill, L. A. Kucek, R. N. Brown, M. S. Lipton, A. Osterman, J. K. Fredrickson, A. E. Konopka, A. S. Beliaev, and J. L. Reed. 2012. Genome-Scale Modeling of Light-Driven Reductant Partitioning and Carbon Fluxes in Diazotrophic Unicellular Cyanobacterium Cyanothece sp. ATCC 51142. PLoS Computational Biology **8**:e1002460.

Vuorio, K., A. Lagus, J. M. Lehtimäki, J. Suomela, and H. Helminen. 2005. Phytoplankton community responses to nutrient and iron enrichment under different nitrogen to phosphorus ratios in the northern Baltic Sea. Journal of Experimental Marine Biology and Ecology **322**:39-52.

Wilhelm, S. W., G. S. Bullerjahn, R. M. L. McKay, and M. A. Moran. 2020. The Complicated and Confusing Ecology of *Microcystis* Blooms. mBio **11**:e00529-00520.

Wilkinson, G. M., S. R. Carpenter, J. J. Cole, M. L. Pace, R. D. Batt, C. D. Buelo, and J. T. Kurtzweil. 2018. Early warning signals precede cyanobacterial blooms in multiple whole-lake experiments. Ecological Monographs **88**:188-203.

Wilson, A. E., W. A. Wilson, and M. E. Hay. 2006. Intraspecific Variation in Growth and Morphology of the Bloom-Forming Cyanobacterium *Microcystis aeruginosa*. Applied and Environmental Microbiology **72**:7386-7389.

Wu, J.-T., and L.-C. Kow. 2010. Alteration of phytoplankton assemblages caused by changes in water hardness in Feitsui Reservoir, Taiwan. Botanical Studies **51**.

Yang, Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Molecular Biology and Evolution **24**:1586-1591.

Ye, C., W. Qiao, X. Yu, X. Ji, H. Huang, J. L. Collier, and L. Liu. 2015. Reconstruction and

687 analysis of the genome-scale metabolic model of schizochytrium limacinum SR21 for
688 docosahexaenoic acid production. BMC Genomics **16**:799.
689 Yoshikawa, K., S. Aikawa, Y. Kojima, Y. Toya, C. Furusawa, A. Kondo, and H. Shimizu. 2015.
690 Construction of a Genome-Scale Metabolic Model of Arthrospira platensis NIES-39 and
691 Metabolic Design for Cyanobacterial Bioproduction. PloS One **10**:e0144430.
692 Zhang, M., H. Duan, X. Shi, Y. Yu, and F. Kong. 2012. Contributions of meteorology to the
693 phenology of cyanobacterial blooms: Implications for future climate change. Water
694 Research **46**:442-452.
695 Zhu, W., L. Wan, and L. Zhao. 2010. Effect of nutrient level on phytoplankton community
696 structure in different water bodies. Journal of Environmental Sciences **22**:32-39.
697 Zuñiga, C., C.-T. Li, T. Huelsman, J. Levering, D. C. Zielinski, B. O. McConnell, C. P. Long, E. P.
698 Knoshaug, M. T. Guarnieri, M. R. Antoniewicz, M. J. Betenbaugh, and K. Zengler. 2016.
699 Genome-Scale Metabolic Model for the Green Alga Chlorella vulgaris UTEX 395
700 Accurately Predicts Phenotypes under Autotrophic, Heterotrophic, and Mixotrophic
701 Growth Conditions. Plant Physiology **172**:589-602.
702
703

704 **Table 1**. Comparison of genome-scale metabolic networks between cyanobacteria and

705 eukaryotic algae.

	Organism	Genes	Model Genes	Metabolites	Reactions	Reference
Cyanophyta	Arthrospira platensis	6631	620	673	746	(Yoshikawa et al. 2015)
	Cyanothece sp. ATCC 51142	4912	806	651	667	(Vu et al. 2012)
	Spirulina platensis C1	6153	692	837	875	(Klanchui et al. 2012)
	Synechococcus elongatus	2723	785	768	850	(Broddrick et al. 2016)
	Synechococcus sp PCC	3179	821	777	792	(Qian et al. 2017)
	Synechocystis sp. PCC6803	3221	678	795	863	(Nogales et al. 2012)
	Synechocystis sp. PCC6803	3221	669	790	882	(Montagud et al. 2010)
Chlorophyta	Chlamydomonas reinhardtii	16709	866	1862	1725	(Gomes de Oliveira
	Chlamydomonas reinhardtii	16709	1080	1068	2190	(Chang et al. 2011)
	Chlorella variabilis	9879	526	1236	1455	(Juneja et al. 2016)
	Chlorella vulgaris	7100	843	1770	2286	(Zuñiga et al. 2016)
Bacillariophyta	Phaeodactylum tricornutum CCAP 1055/1	12280	1027	2172	4456	(Levering et al. 2016)
	Schizochytrium limacinum SR21	63000	1170	1659	1769	(Ye et al. 2015)

706

707

708 Table 2. Case studies of nutrient manipulation on phytoplankton *in situ*, in the mesocosm study,
709 and in laboratory conditions showing lower nutrient requirements of cyanobacteria than
710 eukaryotic algae. "C" represents cyanobacteria; "G" represents green algae. DIN: dissolved
711 inorganic nitrogen; SRP: soluble reactive phosphorus; TN: total nitrogen; and TP: total
712 phosphorus.

Study	Nutrient Variation	Dominant Species	Dominance
Wascana Lake, Canada Mesocosm Study (Bogard et al. 2020)	1-8 mg Urea-N L^{-1}	Colonial cyanobacteria	C
	>8 mg Urea-N L^{-1}	Chlorophytes	G
210 Danish lakes *In situ* Study (Jensen et al. 1994)	0.25-0.8 mg TP L^{-1}	Non-heterocystous cyanobacteria	C
	>1.0 mg TP L^{-1}	Chlorophytes	G
Lake Tai, China Laboratory and *in situ* study (Zhu et al. 2010)	3 mg/L (TN) and 0.2 mg/L (TP)	*M. aeruginosa*	C
	10, 15, or 20 mg/L (TN), and 1 mg/L (TP)	*Scenedesmus quadricauda*	G
Archipelago Sea Mesocosm Study (Vuorio et al. 2005)	1N:1P 1.7 µg L^{-1}: 1.7 µg L^{-1}	*Aphanizomenon* sp., *Nodularia spumigena*, *Anabaena* spp., *Synechococcus* spp.	C
	7N:1P 12 µg L^{-1}:1.7 µg L^{-1}	*Dictyosphaerium subsolitarium*, *Monoraphidium contortum*, and *Monoraphidium contortum, Oocystis* spp..	G
Taipei Feitsui Reservoir Laboratory and *in situ* study (Wu and Kow 2010)	7-9 mg Ca^{2+} L^{-1}	*Microcystis* spp. and *Aphanocapsa delicatissima*.	C
	9,11,13,15,18 mg Ca^{2+} L^{-1}	*Eutetramorus*, *Coelastrum*, *Coenocystis*, and *Dictyosphaerium*	G
Laboratory Study (Hyenstrand et al. 2000)	32µM (NO_3) after 48h; 32µM NH_4^+ after 48h	*Synechococcus 6301*	C
	32µM NO_3 after 25h; 32µM NH_4 after 18h	*Scenedesmus sp.*	G
Vila Lake, Central Portugal *In situ study* (de Figueiredo et al. 2006)	0.66-2.42 mg L (DIN); 0-0.24 mg L (SRP)	*Aphanizomenon flos-aquae, Ch roococcus limneticus, M. aeruginosa and Pseudanabaena sp.*	C
	0.7-3.3 mg L (DIN); 0.19-0.93 mg L (SRP)	*Coelastrum reticulatum var. reticulatum, Kirchneriella lunaris, Monoraphidium contortum, Scenedesmus acuminatus var.*	G

		acuminatus and Pediastrum boryanum var. boryanum	

714 **Figure legends**

715 **Figure 1.** The core and query pathways in *Aphanizomenon flos-aquae* NIES-81. The
716 pathways labeled with dashed borders are not complete (due to the absence of required
717 components) in this strain but may be complete in others.

718 **Figure 2.** dN/dS for all homologous genes in 50 genomes of *M. aeruginosa*. (A) An overall
719 distribution of dN/dS for all homologous genes; (B) the dN/dS for genes in different groups of
720 functions in CyanoPATH. dN/dS values were calculated by CODEML from the PAML 4.9j
721 package with default settings (Yang 2007). AAPEP: uptake of amino acids and peptides; CCM:
722 CO_2-concentrating mechanism; DrugR: antibiotics resistance; MAA: UV radiation; MetalR:
723 heavy metal resistance; Nfix: nitrogen fixation; Nitrogen: nitrogen utilization; Osmos: osmos
724 homeostasis; OSR: oxidative stress resistance; PBS: phycobilisome; Phosphorus:
725 inorganic/organic utilization; PS-I/PS-II: photosystem I and photosystem II; Sugar: sugar
726 assimilation; TEVit: assimilation of trace metals and vitamins; Toxins: cyanotoxins; UFA:
727 unsaturated fatty acids; Vesicle: gas vesicles. The last seven columns colored dark blue
728 represent essential gene sets (Rubin Benjamin et al. 2015). (C) The phylogeny of the 50 *M.*
729 *aeruginosa* strains collected from different countries.

730 **Figure 3.** The evolutionary trajectory of cyanobacteria in the presence of oxygen and adaptive radiation.
731 (A) The origin and distribution of SODs in different habitats. The timing of origin and habitat distribution of
732 SOD genes among taxa is created based on Boden et al (Boden et al. 2021). Starting points of horizontal
733 bars represent time of origin and color SOD types: CuZnSOD (blue), NiSOD (green), and Fe- and Mn-
734 utilizing SODs (yellow). (B) The oxygen level in atmosphere and nitrogen fixation in heterocysts in
735 cyanobacteria in the presence of elevated oxygen levels. The level of oxygen is based on (Tomitani et al.
736 2006). (C) and (D) The temporal morphological diversification cyanobacteria is based on Schirrmeistera
737 et al (Schirrmeister et al. 2013). (E). Adaptive strategies of cyanobacteria in different habitats.
738 Cyanobacterial adaptation strategies in nine environments, namely soil, desert, forest, salty
739 area, oil-spilled ocean, open ocean, limestone, geothermal water, and polar region.

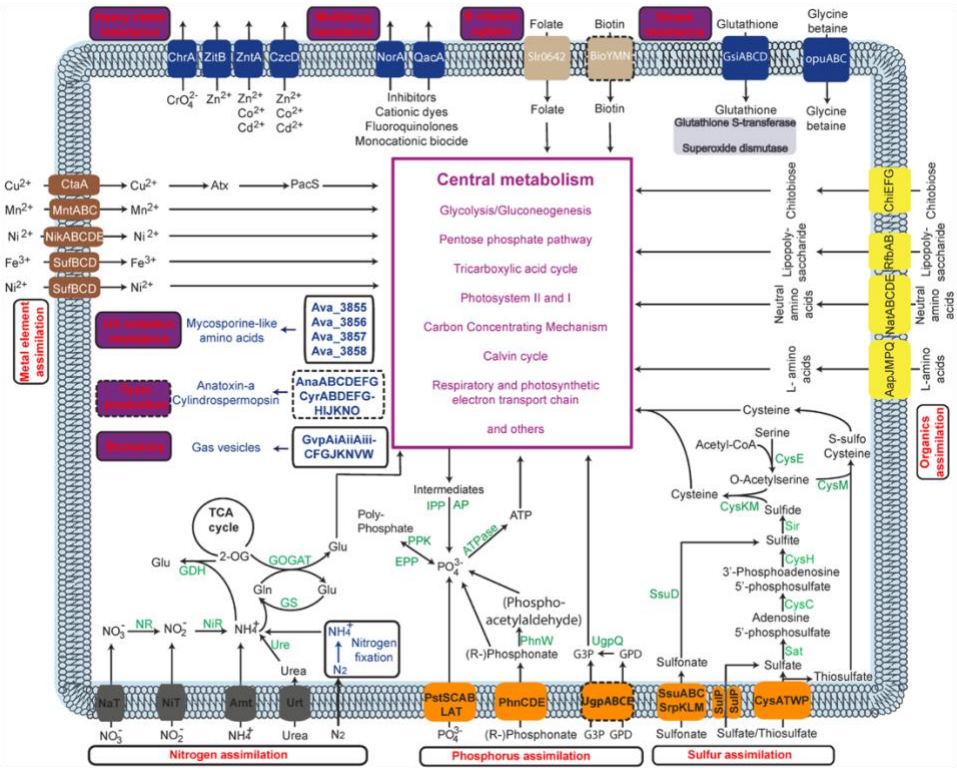
740 Cyanobacteria also cope with the damage caused by ultraviolet light.

741 **Figure 4.** The evolutionary trajectory of cyanobacteria in the presence of oxygen and adaptive radiation.
742 (A) The origin and distribution of SODs in different habitats. The timing of origin and habitat distribution of
743 SOD genes among taxa is created based on Boden et al (Boden et al. 2021). Starting points of horizontal
744 bars represent time of origin and color SOD types: CuZnSOD (blue), NiSOD (green), and Fe- and Mn-
745 utilizing SODs (yellow). (B) The oxygen level in atmosphere and nitrogen fixation in heterocysts in
746 cyanobacteria in the presence of elevated oxygen levels. The level of oxygen is based on (Tomitani et al.
747 2006). (C) and (D) The temporal morphological diversification cyanobacteria is based on Schirrmeistera
748 et al (Schirrmeister et al. 2013). (E). Adaptive strategies of cyanobacteria in different habitats.
749 Cyanobacterial adaptation strategies in nine environments, namely soil, desert, forest, salty
750 area, oil-spilled ocean, open ocean, limestone, geothermal water, and polar region.
751 Cyanobacteria also cope with the damage caused by ultraviolet light.

752 **Figure 5.** Comparison between cyanobacteria and eukaryotic algae in select key traits. (A):
753 Distributions of log-transformed sequence length in eleven groups of algae. Summary statistics
754 of 2240 genome assemblies were downloaded from NCBI Datasets: 1977 in cyanobacteria, 38
755 in Rhodophyta, 26 in Bacillariophyta, 156 in Chlorophyta, 14 in Chrysophyceae, 7 in
756 Haptophyta, 3 in Cryptophyceae, 3 in Cryptophyceae, 3 in Euglenida, 2 in Xanthophyceae, 11 in
757 Phaeophyceae and 20 in Dinophyceae, respectively. Each dot in the boxplot represents the log-
758 transformed total sequence length of one assembly. (B): Distributions of dry weight per cell
759 biovolume in Eukaryotic algae and cyanobacteria from literature (Mahlmann et al. 2008, Finkel
760 et al. 2016). Each dot plot represents the average values of dry weight per unit biovolume and
761 cell dry weights within a class. Blue symbols represent eukaryotic algae cells and red symbols
762 represents cyanobacteria. (C): The per-cell dry weight of two select cyanobacteria and five
763 green algae at mid log phase. C: cyanobacteria; G: green algae. (D): Relationship between cell
764 volume (log10V, μm3), and dry weight (log10 DW, pg cell-1). Each dot plot represents the
765 average values of cell volumes and cell dry weights within a class. Linear regressions were

applied for eukaryotic algae (blue) and cyanobacteria (red) with 95% confidence intervals. (E): Lower nutrient requirements by cyanobacteria than eukaryotic algae in aquatic environment. Among seven selected media, the recipes of mAC, MJ, VTAC, and CHU-11 (left panel) are used for culturing green algae and the recipes of M11, BG11 and CT (right panel) are used for culturing cyanobacteria.

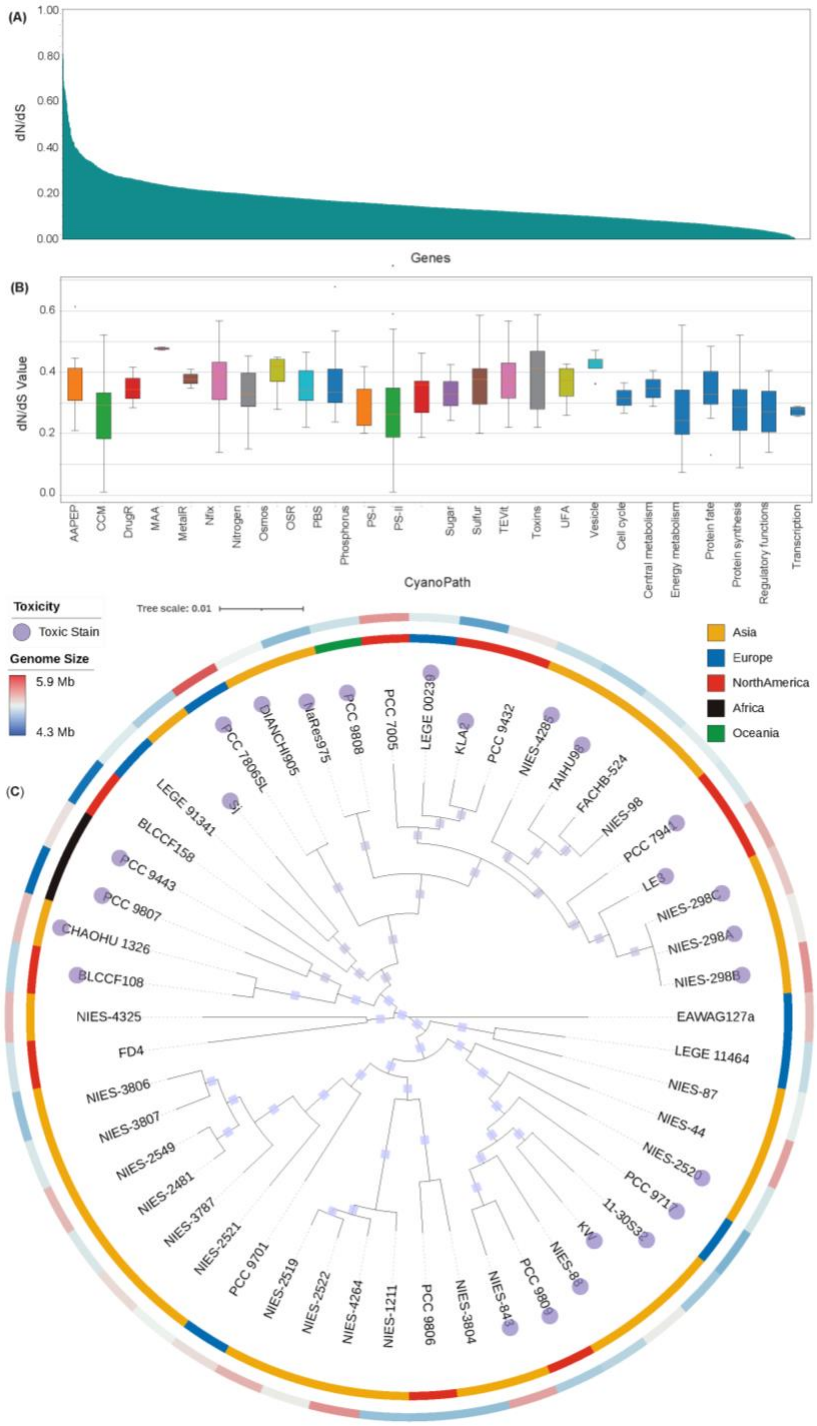
Figure 1



773

774

775 Figure 2



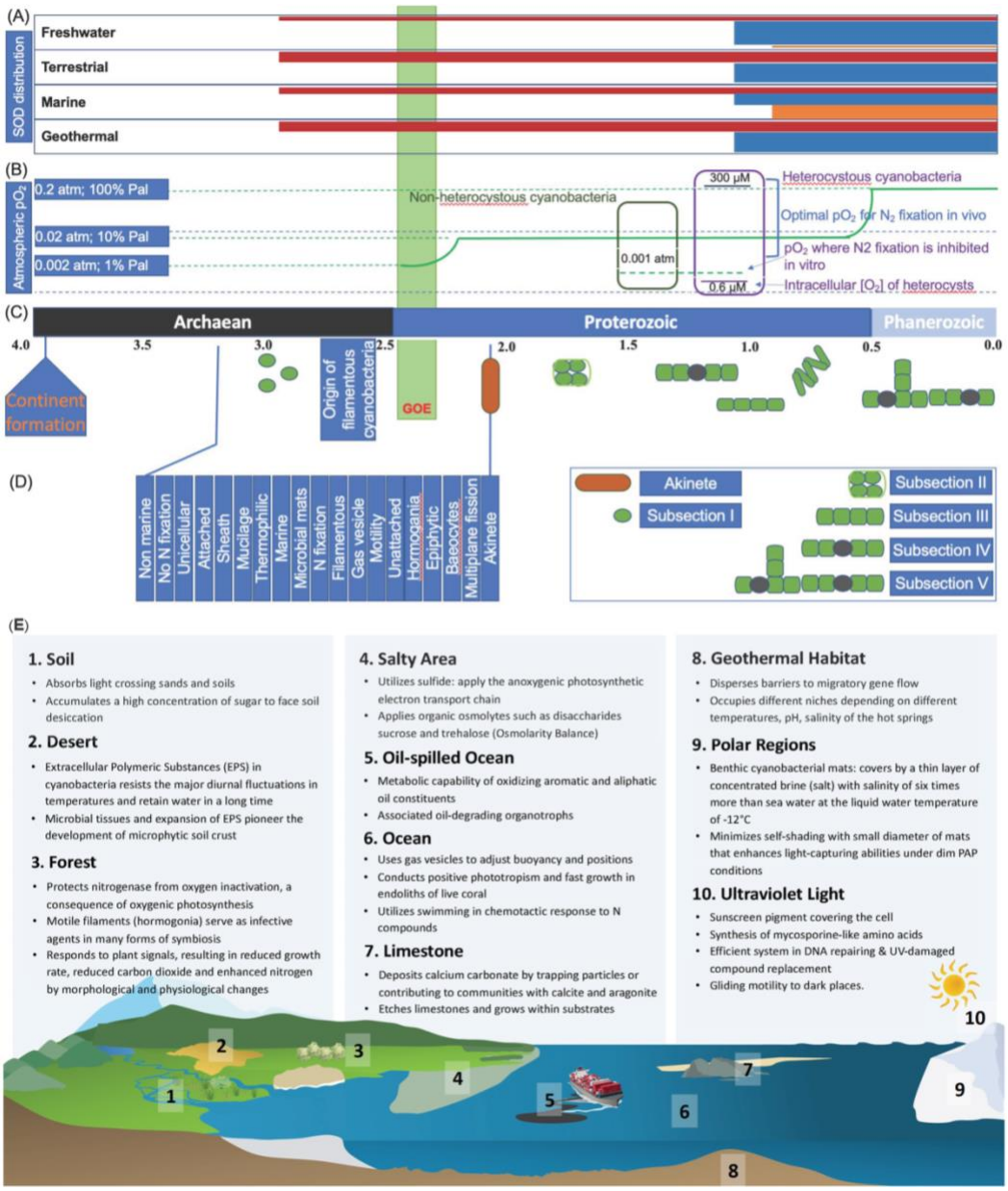
776

777

778

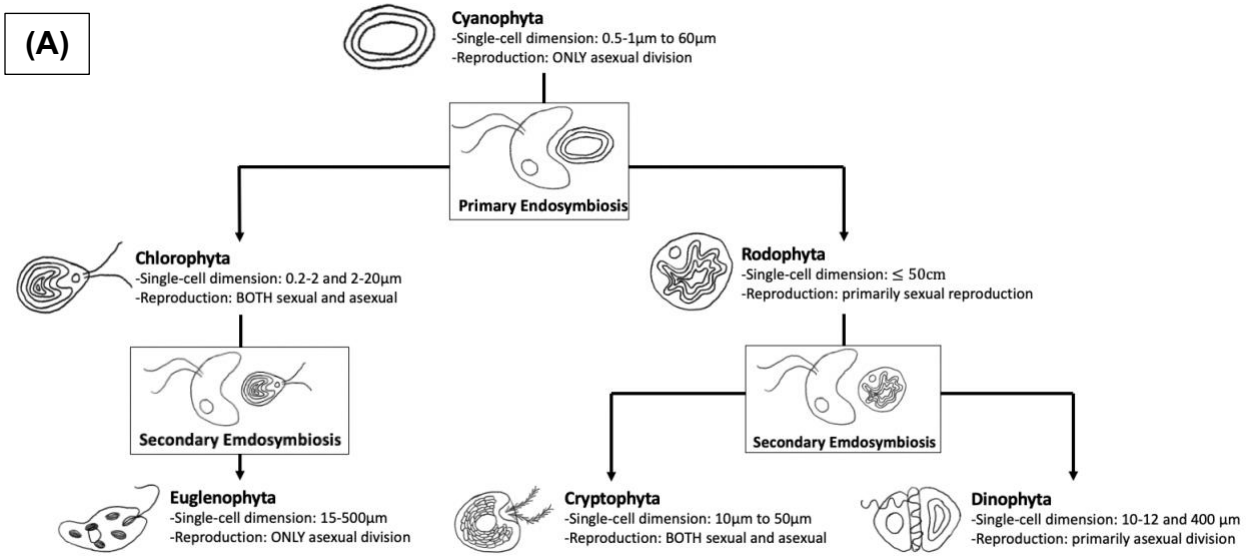
779

Figure 3


(A)
SOD distribution
Freshwater
Terrestrial
Marine
Geothermal
(B)
Atmospheric pO2
0.2 atm; 100% Pal
0.02 atm; 10% Pal
0.002 atm; 1% Pal
Non-heterocystous cyanobacteria
300 μM
0.001 atm
0.6 μM
Heterocystous cyanobacteria
Optimal pO2 for N2 fixation in vivo
pO2 where N2 fixation is inhibited in vitro
Intracellular [O2] of heterocysts
(C)
Archaean
Proterozoic
Phanerozoic
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Continent formation
Origin of filamentous cyanobacteria
GOE
(D)
Non marine
No N fixation
Unicellular
Attached
Sheath
Mucilage
Thermophilic
Marine
Microbial mats
N fixation
Filamentous
Gas vesicle
Motility
Unattached
Hormogonia
Epiphytic
Baeocytes
Multiplane fission
Akinete
Akinete
Subsection I
Subsection II
Subsection III
Subsection IV
Subsection V
(E)
1. Soil
• Absorbs light crossing sands and soils
• Accumulates a high concentration of sugar to face soil desiccation
2. Desert
• Extracellular Polymeric Substances (EPS) in cyanobacteria resists the major diurnal fluctuations in temperatures and retain water in a long time
• Microbial tissues and expansion of EPS pioneer the development of microphytic soil crust
3. Forest
• Protects nitrogenase from oxygen inactivation, a consequence of oxygenic photosynthesis
• Motile filaments (hormogonia) serve as infective agents in many forms of symbiosis
• Responds to plant signals, resulting in reduced growth rate, reduced carbon dioxide and enhanced nitrogen by morphological and physiological changes
4. Salty Area
• Utilizes sulfide: apply the anoxygenic photosynthetic electron transport chain
• Applies organic osmolytes such as disaccharides sucrose and trehalose (Osmolarity Balance)
5. Oil-spilled Ocean
• Metabolic capability of oxidizing aromatic and aliphatic oil constituents
• Associated oil-degrading organotrophs
6. Ocean
• Uses gas vesicles to adjust buoyancy and positions
• Conducts positive phototropism and fast growth in endoliths of live coral
• Utilizes swimming in chemotactic response to N compounds
7. Limestone
• Deposits calcium carbonate by trapping particles or contributing to communities with calcite and aragonite
• Etches limestones and grows within substrates
8. Geothermal Habitat
• Disperses barriers to migratory gene flow
• Occupies different niches depending on different temperatures, pH, salinity of the hot springs
9. Polar Regions
• Benthic cyanobacterial mats: covers by a thin layer of concentrated brine (salt) with salinity of six times more than sea water at the liquid water temperature of -12°C
• Minimizes self-shading with small diameter of mats that enhances light-capturing abilities under dim PAP conditions
10. Ultraviolet Light
• Sunscreen pigment covering the cell
• Synthesis of mycosporine-like amino acids
• Efficient system in DNA repairing & UV-damaged compound replacement
• Gliding motility to dark places.
1
2
3
4
5
6
7
8
9
10

785 **Figure 4.**

786



787

788 **(B)**

	Cyanophyta	Chlorophyta	Rhodophyta	Euglenophyta	Cryptophyta	Dinoflagellata
Cell Wall	P	P	P	P	P	P
Chloroplast	A[1]	P	P	P	P	P
Nucleus	A	P	P	P	P	P
Golgi Apparatus	A	P	P	P	P[2]	P
Mitochondria	A	P	P	P	P	P
Endoplasmic Reticulum	A	P	P	P	P	P
Starch Granule	P[3]	P	P	P	P	P

789 [1]Cyanophyta contains chlorophylls distributed over cytoplasm but not in an aggregated form, chloroplast.

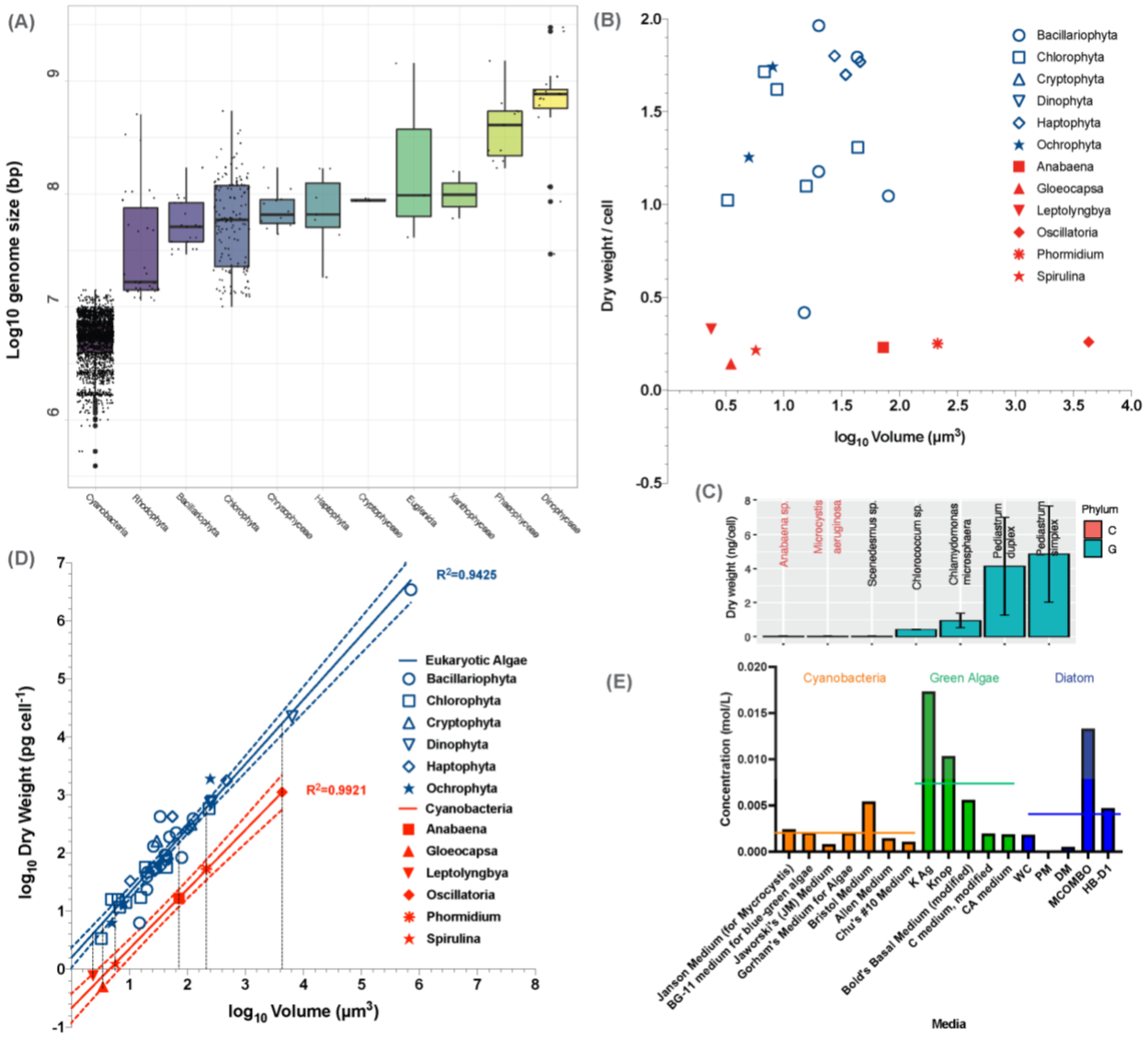
790 [2]The function of Golgi apparatus in cryptophyte is found to be limited.

791 [3]It does contain starch granule, but it is found much smaller in size compared to other eukaryotic algae

792 lineages.

793

Figure 5


(A)
Log10 genome size (bp)
Cyanobacteria
Rhodophyta
Bacillariophyta
Chlorophyta
Chrysophyceae
Haptophyta
Cryptophyceae
Euglenida
Xanthophyceae
Phaeophyceae
Dinophyceae
(B)
Dry weight / cell
log10 Volume (µm3)
Bacillariophyta
Chlorophyta
Cryptophyta
Dinophyta
Haptophyta
Ochrophyta
Anabaena
Gloeocapsa
Leptolyngbya
Oscillatoria
Phormidium
Spirulina
(C)
Dry weight (ng/cell)
Anabaena sp.
Microcystis aeruginosa
Scenedesmus sp.
Chlorococcum sp.
Chlamydomonas microsphaera
Pediastrum duplex
Pediastrum simplex
Phylum
C
G
(D)
log10 Dry Weight (pg cell-1)
log10 Volume (µm3)
R2=0.9425
R2=0.9921
Eukaryotic Algae
Bacillariophyta
Chlorophyta
Cryptophyta
Dinophyta
Haptophyta
Ochrophyta
Cyanobacteria
Anabaena
Gloeocapsa
Leptolyngbya
Oscillatoria
Phormidium
Spirulina
(E)
Concentration (mol/L)
Cyanobacteria
Green Algae
Diatom
Janson Medium (for Mycrocystis)
BG-11 medium for blue-green algae
Jaworski's (JM) Medium
Gorham's Medium for Algae
Bristol Medium
Allen Medium
Chu's #10 Medium
K Aq
Knop
Bold's Basal Medium (modified)
C medium, modified
CA medium
WC
PM
DM
MCOMBO
HB-D1
Media