Coexistence of dominant marine phytoplankton species sustained by nutrient specialization

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Coexistence of dominant marine phytoplankton species sustained by nutrient specialization

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Running title: Nutrient affinities allow plankton coexistence

Abstract

Prochlorococcus and *Synechococcus* are the two dominant picocyanobacteria in the low-nutrient surface waters of the subtropical ocean, but the basis for their coexistence in these biomes is still unclear. Here we combine *in situ* microcosm experiments and an ecological model to show that this coexistence can arise from specialization in the uptake of distinct nitrogen (N) substrates. In field incubations, the response of both *Prochlorococcus* and *Synechococcus* to nanomolar N amendments demonstrates N limitation of growth in both populations, but *Prochlorococcus* showed a higher affinity to ammonium whereas *Synechococcus* was more adapted to nitrate uptake. A simple ecological model demonstrates that the differential nutrient affinity of these species can explain their coexistence. Phylogenetic analysis of the presence of nitrate reductase and nitrite reductase further support the higher nitrate affinity of *Synechococcus* compared to *Prochlorococcus*. Our study suggests that the evolution of differential nutrient affinities is an important mechanism for sustaining coexistence of species under resource competition.

Introduction

Marine phytoplankton are key drivers of the global carbon cycle^{1,2}, photosynthesizing about 50×10^{15} g carbon (C) annually, comparable to the global terrestrial biosphere. Nearly a quarter of this net primary

production can be accounted for by the two marine cyanobacteria, *Prochlorococcus* and *Synechococcus*³. The geographical distributions of the two groups are largely overlapping in the band from ~40°N to ~40°S (ref.⁴): both groups have a peak abundance around the equator and range boundary constrained by light, temperature, and nutrient concentrations³. Throughout most of the subtropical gyres, the largest biomes on Earth, *Prochlorococcus* and *Synechococcus* are the dominant phytoplankton groups.

In the stratified open ocean, nutrient supply is weak and concentrations are commonly drawn down to levels that limit the rates of growth and uptake by phytoplankton, consistent with Monod kinetics⁵. In these biomes, competition among species for scarce resources can determine the community composition. Long-standing theory predicts that the species whose nutrient acquisition saturates at the lowest concentration will drive other competitors to extinction⁶ such that coexistence requires specialization on distinct nutrient resources. The overlapping niche of *Prochlorococcus* and *Synechococcus* thus provides a rare opportunity to test this theoretical prediction, if nutrient affinities of both taxa can be determined at levels relevant to those found in the subtropical gyres^{7,8}.

Fluctuations in forms and availability of nutrients directly affect phytoplankton abundance and community structure^{9,10}. Observing community responses to these fluctuations can thus yield important information about the relative nutrient affinities of distinct plankton taxa. Natural variation in nutrient

concentrations in oligotrophic waters occur often on nanomolar scales, but many previous bioassay studies far exceeded naturally occurring concentrations, subjecting phytoplankton to, for example, nitrogen (N) enrichment of more than 1 micromolar, even in oligotrophic waters¹¹⁻¹⁵. Whether naturally occurring nanomolar changes in nutrient availability affect phytoplankton biomass and community composition in the same way as experimental micromolar additions has not been studied and warrants a systematic experimental assessment.

To understand the causes of niche separation and coexistence of *Prochlorococcus* and *Synechococcus*, we conducted a suite of nutrient enrichments at nanomolar level to simulate realistic perturbations at a fixed station in the North Pacific (12°N, 135°E). The North Pacific subtropical gyre, where *Prochlorococcus* and *Synechococcus* are numerically predominant in the phytoplankton community, is characterized by extremely low surface nutrient concentrations at nanomolar level with temporal and spatial variation¹⁶⁻²⁰. We analyzed the data on growth and nutrient uptake using a simple population model for each species, and applied the model to determine the conditions under which the coexistence of these competing species can be maintained. Then, we explored the evolutionary factors that may have led to these conditions.

Results

***In situ* experiments.** To test the effect of increased nutrients at nano-molar level, we conducted five N and phosphorus (P) addition (M1 to M5) and three Fe-addition (Fe1 to Fe3) experiments at the surface of a station in the subtropical Northwestern Pacific (12°N, 135°E) from 6 to 25 June 2008 during the MR08-02 cruise aboard the R/V *MIRAI*. Grazing was eliminated by filtration (see details in Materials and Methods) so that changes in phytoplankton abundance could be considered as cell growth or cell death. Throughout the observation period, nutrients remained very low (<40 nM for N ~30 nM for P (Table S1) and more than 0.05 nM of DFe, (Table S2).

Neither P nor Fe enrichment increased any of the tested phytoplankton groups including *Prochlorococcus* and *Synechococcus* (Fig. S1A, B, Tables S2, S3), suggesting that these nutrients are not the growth limiting factor. However, both *Prochlorococcus* and *Synechococcus* growth were stimulated by N amendments regardless of its chemical form (RM-ANOVA, $\alpha < 0.05$) showing that N was the limiting factor for both groups (Fig. 1, Fig. S1C, D, Tables S2, S3). The magnitude of growth responses differed between the different chemical forms of N, and these differences were species-specific (Fig. 1, Fig. S1C, D, Tables S2, S3). The growth of *Prochlorococcus* was more stimulated by ammonium (NH_4^+), while *Synechococcus* responded more strongly to nitrate (NO_3^-) (Fig. 1).

Fig. 1 The effects of different chemical forms of N, P and Fe additions on cell abundances of *Prochlorococcus* (A) and *Synechococcus* (B) relative to controls (means $\pm$ SD), with number of samples in parentheses. For N and P addition, data is based on Exp. M1-M3, where the initial concentrations of N were small (<10 nm). It shows that *Prochlorococcus* prefers NH₄⁺ while *Synechococcus* prefers NO₃⁻. Note that added NH₄⁺ was depleted on the third day, leading to decreased populations in both organisms.

99

100 *Prochlorococcus* showed significantly higher peak abundance in the NH₄⁺ additions relative to the NO₃⁻

101 additions in all five N and P bioassays (RM-ANOVA, $p < 0.05$) (Fig. 1, Fig. S1C, Table S3), indicating

102 a higher affinity to NH₄⁺ than NO₃⁻. This affinity difference can be explained by the low electron

103 requirement to assimilate NH₄⁺, which has been previously shown in *Prochlorococcus*^{21,22} both in

104 culture and *in situ*¹⁵. Although *Prochlorococcus* was considered to be incapable of NO₃⁻ assimilation^{22,23},

105 recent studies have shown that especially some clades of the High Light (HL) ecotypes appear able to

106 assimilate NO₃⁻ with lower growth rates compared to other nitrogen sources^{21,24,25}. Since our experiment

was conducted using surface phytoplankton, the increased growth rate with NO_3^- addition may indicate a preponderance of HL *Prochlorococcus* ecotypes, as observed in both the North- and South Pacific Oceans^{15,26}.

Unlike *Prochlorococcus*, *Synechococcus* increased in the NO_3^- amendment significantly more than in any other N treatments (ANOVA, $\alpha < 0.05$) (Fig. 1B, Fig. S1D, Table S3). A preference for NO_3^- over NH_4^+ among *Synechococcus* is at odds with both the energetic advantage of reduced N, and with the direct observations in pure culture, which showed faster growth on NH_4^+ ^{22,27}. Thus, the preferential response of *Synechococcus* to NO_3^- arises only in the context of natural phytoplankton assemblages and we therefore turn to explanations for this observation based on *Synechococcus*' broader ecological and evolutionary context.

A simple ecological model. To discern the mechanisms that sustain the coexistence of *Prochlorococcus* and *Synechococcus*, we used a simple ecological model^{20,28-30}. The model represents distinct physiological traits for growth and nutrient affinity for each population, and allows for their ecological interaction through competition for two different N resources: NH_4^+ and NO_3^- . Despite its simplicity, the model reproduces the observed trends in populations over the course of the field study (Figs. 2, S2). The key parameters of the model were half saturation constants (K) (Table S4). A lower K for NH_4^+

(K_{NH_4}) for *Prochlorococcus* was essential to reproduce the rapid *Prochlorococcus* growth up to day 2 and the slower *Synechococcus* growth with NH_4^+ addition (Fig. 2A); otherwise, the model results deviate from the data (see Fig. S3 for the results with the same K_{NH_4} values for both the organisms). On the other hand, K for NO_3^- (K_{NO_3}) must be lower for *Synechococcus* to reproduce its continued high growth up to day 3 (Fig. 2B, compare with Fig. S4 where the same K_{NO_3} values are used for both the organisms). Other than the K values, we have applied similar values of model parameters between these two different microorganisms. Results suggest that these organisms have different nitrogen uptake affinities for NH_4^+ and NO_3^- , circumventing direct competition for the same N species.

Fig. 2 Model data comparison of the time series of abundance (X) of *Prochlorococcus* and *Synechococcus*. (A) NH_4^+ added. (B) NO_3^- added. Points, Data; Curves, Model. Pro,

Prochlorococcus; Syn, *Synechococcus*. Data are based on Exp. M1-M3, where the initial concentrations of N were small (<10 nM). Sample numbers for each data are same as Fig. 1.

To test the effect of different K values on the coexistence of *Prochlorococcus* and *Synechococcus*, we obtained a steady state solution for cellular nitrogen per volume water for various ratios of $K_{NO_3}^{Pro}/K_{NO_3}^{Syn}$ and $K_{NH_4}^{Pro}/K_{NH_4}^{Syn}$. Valid solutions are obtained only where these organisms coexist. The model shows that the set of K values that are consistent with the observations (Figs. 1,2) allows coexistence with a similar amount of biomass when the resource ratio of NO_3^- and NH_4^+ are 1:3, where *Prochlorococcus* and *Synechococcus* mainly use NH_4^+ and NO_3^- , respectively (See the cyan triangle in Fig. 3A and the domain explanation in Fig. 3B, zone i). If $K_{NO_3}^{Pro}/K_{NO_3}^{Syn}$ were lower, we would expect to see only *Prochlorococcus* (Fig. 3B, zone iii), however, if $K_{NH_4}^{Pro}/K_{NH_4}^{Syn}$ were sufficiently higher, *Synechococcus* would exclude *Prochlorococcus* by outcompeting them for NH_4^+ (Fig. 3B, zone ii). If both $K_{NO_3}^{Pro}/K_{NO_3}^{Syn}$ were lower and $K_{NH_4}^{Pro}/K_{NH_4}^{Syn}$ were higher, an alternative state of coexistence could persist (Fig. 3B, zone i). However, this would require *Synechococcus* to be a better competitor for NH_4^+ , in violation of our incubation results.

Fig. 3 Steady state solution of the model for the coexistence of *Prochlorococcus* and *Synechococcus* based on the ratios of half saturation constants for nitrogen sources. (A) Model results of the ratio of *Prochlorococcus* and *Synechococcus* expressed in terms of the proportion of total cellular nitrogen concentration in the water (N_{Pro}/N_{Syn} : see the color bar for values). The cyan triangle in panel A represents the ratios of the half saturation constants predicted from Fig. 2. The model output is based on the resource ratio of NO_3^- and NH_4^+ is 1:3 (for other ratios, see Fig. 3, S5). Dashed curves indicate borders between coexistence and non-coexistence. (B) Explanation of each domain; coexistence occurs only in domains i and iv.

Preferential grazing by zooplankton (e.g. nanoflagellates³¹) may further ensure the coexistence of these species³²⁻³⁵, and this has been the main explanation for the coexistence for these organisms. However, our study shows that, without reliance on zooplankton behavior, *Prochlorococcus* and *Synechococcus* have inherent nutrient-based mechanisms for coexistence.

Fig. 4 Resource-ratio based shifts of coexistence. (A) The shift in the ratio of *Prochlorococcus* and *Synechococcus* based on the resource ratios of $\text{NO}_3^-:\text{NH}_4^+$ ($S_{\text{NO}_3^-}/S_{\text{NH}_4^+}$) with the predicted half saturation constants. (B) Relationship between the NO_3^- concentrations and cell count ratios of *Prochlorococcus* and *Synechococcus*. (C) The effect of doubled NO_3^- recourse. The plot shows the model results of the *Prochlorococcus* and *Synechococcus* ratio in terms of cellular N ($N_{\text{Pro}}/N_{\text{Syn}}$; see the color bar for values). Here the resource ratios of $\text{NO}_3^-:\text{NH}_4^+$ is 2:3. Dashed curves indicate borders between coexistence and competitive exclusion when the resource ratio of $\text{NO}_3^-:\text{NH}_4^+$ are 1:3 (same dashed curves as in Fig. 3A). The cyan triangle represents the ratios of predicted half saturation constants for Fig. 2.

154

155 The lower K value for NO_3^- in *Synechococcus* than *Prochlorococcus* predicts that their relative

156 abundance will also depend strongly on the $\text{NO}_3^-/\text{NH}_4^+$ ratio of the nutrient supply. We tested this model

157 prediction using field observations from the Hawaii Ocean Timeseries (HOT³⁶) in the subtropical North

158 Pacific (Fig. 4A). We find that the ratio cell counts of *Prochlorococcus* to *Synechococcus* exhibits a

159 strong relationship to ambient NO_3^- concentration (Fig. 4B). This is consistent with a previously

160 observed positive relationship between nanomolar NO_3^- concentrations at the surface and

161 *Synechococcus* abundance, but not for *Prochlorococcus* abundance^{7,37,38}. A *Synechococcus* bloom was

162 also observed after the transient nanomolar increase of surface NO_3^- in the stratified Sargasso Sea³⁷.

Further, the stimulation of *Synechococcus* photosynthetic and growth activity after 20 nM of NO_3^- enrichment was later confirmed by *in situ* bioassay experiment⁷.

Shifts in the resource ratio, $\text{NO}_3^-:\text{NH}_4^+$, can also expand or contract the range of species traits that permit coexistence (Fig. 4C). When the NO_3^- resource increases, the range of coexistence (iv in Fig. 3B; hereafter domain iv) shifts to the right, favoring higher $K_{\text{NO}_3}^{\text{Pro}}/K_{\text{NO}_3}^{\text{Syn}}$ and $K_{\text{NH}_4}^{\text{Pro}}/K_{\text{NH}_4}^{\text{Syn}}$ for coexistence (Fig. 4C). On the other hand, when the NH_4^+ resource increases, the domain i shifts to the left, favoring lower $K_{\text{NO}_3}^{\text{Pro}}/K_{\text{NO}_3}^{\text{Syn}}$ and $K_{\text{NH}_4}^{\text{Pro}}/K_{\text{NH}_4}^{\text{Syn}}$ for coexistence (Fig. S5A). Due to these shifts, at increased NO_3^- resource, we predict an increased ratio of *Synechococcus* at the predicted K values (represented by cyan triangles in Fig. 3A and Fig. 4C) and further increase in resource ratios of $\text{NO}_3^-:\text{NH}_4^+$ eventually leads to a predominance of *Synechococcus* (Fig. 3B, S5B).

Discussion

Climatic, Ecological and Evolutionary Implications. The niche partitioning of Pro/Syn based on differential affinities for reduced and oxidized forms of N has important climatic, ecological and evolutionary implications. The $\text{NO}_3^-/\text{NH}_4^+$ ratio of nutrient supply can be strongly influenced by climate change, through its impact on ocean stratification, which reduces the supply of NO_3^- to the photic zone of the low latitudes. According to our results, such climate changes are likely to shift the community

composition of these major biomes toward a greater preponderance of *Prochlorococcus* relative to *Synechococcus*. While long-term trends are not available to test this prediction, it is consistent with perturbations observed on shorter time scales, in response to transient increases of surface NO_3^- ^{2,39}. Monthly observations at the Bermuda Atlantic Time-series Study (BATS)², as well as in the Gulf of Aqaba, Red Sea³⁹, observed spring blooms of *Synechococcus* when the water column was deeply mixed and the NO_3^- supply from deep was active, and *Prochlorococcus* domination during summer stratification. Increased primary production supported by *Synechococcus* at upwelling cyclonic eddies was reported from BATS⁴⁰ and off California⁴¹. Further, new production by *Synechococcus* accompanied by diatoms based on enhanced NO_3^- uptake has been confirmed after typhoons in the South China Sea⁴². The higher abundance of *Synechococcus* clades - containing a gene responsible for NO_3^- assimilation (*narB*) in *Synechococcus* - in cyclonic eddy or transient waters^{15,41}, suggest that nanomolar NO_3^- perturbation, induced by strong winds, tropical cyclones, eddies or vertical mixing, stimulates *Synechococcus* growth as a genus by changing the clade structure.

Changes in the $\text{NO}_3^-/\text{NH}_4^+$ supply ratio can be altered by ecological in addition to climatic changes. The low N environments dominated by these taxa are also prime habitats for N_2 fixing diazotrophs, which inject newly fixed NH_4^+ into surface waters. The presence of nitrogen fixing organisms that provide NH_4^+ may favor *Prochlorococcus*, due to its lower *K* value for NH_4^+ , and as a result

197 *Synechococcus* may be outcompeted. This dynamic may underlie the correlations between
198 *Prochlorococcus* and the filamentous diazotroph *Trichodesmium* reported from the oligotrophic South
199 Pacific Ocean⁴³. Similarly, a positive relationship between *Prochlorococcus* and the unicellular
200 diazotroph *Crocospaera*²⁶ has been suggested by showing analogous nutrient limitation in both
201 *Crocospaera* and *Prochlorococcus*²⁶, suggesting the coexistence of these organisms. Also,
202 distributions of both *Prochlorococcus* and *Crocospaera* show a positive correlation with temperature
203 in oligotrophic oceans^{3,44}. On the other hand, a negative relationship between the abundance of
204 *Synechococcus* and a nano-sized cyanobacteria (most likely *Crocospaera*)⁴⁵ has been reported in the
205 Pacific Ocean. Rising surface temperatures, atmospheric CO₂ concentrations and strengthened water-
206 column stratification⁴⁶ may favor diazotrophs^{44,47-49} in the future, supporting the predictions of
207 *Prochlorococcus* domination³ (Fig. 4A).

208 Marine *Synechococcus* and *Prochlorococcus* are estimated to have sequentially diverged from a
209 common ancestor during the Devonian, at about 413 million years ago (Mya), and Carboniferous, at
210 around 360 Mya and 341 Mya, respectively⁵⁰. During these periods, a number of genus-level lineages
211 likely evolved within both *Synechococcus* and *Prochlorococcus*⁵¹⁻⁵³, and our data suggest that many
212 others are still waiting to be described (Fig. S6). These lineages are in many aspects cryptic due to
213 morphological simplification and genome streamlining, driven by convergent selective pressures,

214 especially from nutrient deficiency⁵⁴. Nevertheless, individual ecotypes and even individual cells of
215 picocyanobacteria harbor distinct sets of metabolic genes reflecting their ecological niche
216 partitioning^{55,56}.

217 The ability to assimilate NO_3^- is observed to vary widely among picocyanobacteria. Whereas most
218 genomes of marine *Synechococcus* harbor both nitrate reductase and nitrite reductase genes, these genes
219 exhibit a patchy distribution throughout both low-light and high-light adapted *Prochlorococcus*
220 lineages^{21,25} (Fig. S6). According to previous studies, gene loss as well as homologous recombination
221 among closely related cell populations are frequent in the genomic regions responsible for NO_3^-
222 acquisition in *Prochlorococcus*²⁵. NO_3^- assimilation is energetically demanding and thus more likely to
223 occur at high-light conditions near the water surface, but the concentration of NO_3^- tends to increase
224 with depth in oligotrophic oceans. This trade-off likely shapes the composition of *Prochlorococcus*
225 populations by creating an equilibrium of NO_3^- assimilating and non-assimilating sub-populations that
226 coexist at a given depth and light intensity to maximize the effectivity of N uptake²⁵.

227 Phylogenetic analysis implies that mixed populations of NO_3^- assimilating or non-assimilating
228 *Prochlorococcus* cells may further influence the relative affinity of *Prochlorococcus* to different N
229 substrates. This could be another mechanism underlying the coexistence of *Prochlorococcus* and
230 *Synechococcus*, and their various phylogenetic sub-lineages, at different concentrations of available N

forms. The relative contribution of genomic population diversity versus physiological affinity to specific forms of N to the coexistence of both picocyanobacteria warrants further study.

Methods

A series of bioassay experiments were carried out using natural phytoplankton assemblages collected at a station in the subtropical Northwestern Pacific (12°N, 135°E) from 6-25 June 2008 during the MR08-02 cruise on the R/V *MIRAI*. Five nutrient (N and P)-addition bioassays (M1 to M5), and three Fe-addition bioassays (Fe1 to Fe3) were conducted to elucidate the availability and preference of natural phytoplankton communities for N, P and Fe sources (Table S1).

Seawater collection. Water samples were collected from 10 m depth at 12:30 h local time using Teflon diaphragm pump system consisting of Teflon tubing and associated plastic ware. All components of this pump system and associated plastic ware washed overnight in neutral detergent, followed by HCl, HNO₃, and rinsed with heated Milli-Q water, and flushed with seawater for 30 min immediately prior to sample collection. For the nutrient-addition bioassays, 4-liter polycarbonate incubation bottles, and other plastic instruments were rinsed overnight in neutral detergent, followed by 0.3 N HCl, and rinsed with Milli-Q water. For Fe addition experiments, the 2-liter polycarbonate incubation bottles had been

248 cleaned according to ref.⁵⁷. Other polyethylene and Teflon lab wares were cleaned according to ref.⁵⁸.

249 All washing procedures were carried out in an onshore class-1000 clean air room and plastic gloves
250 were worn during these operations.

251 To reduce grazing pressure, seawater samples were pre-filtrated prior to the bioassay setup. For
252 the bioassay treatments with N and P addition, water was filtered through an acid-cleaned 1 μm in-line
253 cartridge filter (Micropore EU, ORGANO). For Fe additions, seawater was pre-filtered through a 10
254 μm filter of the same manufacturer. The pre-filtered water was then dispensed into the corresponding
255 bioassay incubation bottles.

256 Iron concentrations of the seawater were measured as total iron (TFe), on the whole water samples
257 collected directly from the pump system, and as dissolved iron (DFe), on the 125 mL of seawater
258 collected in low-density polyethylene bottles (Nalgen; Nalge Nunc International) and filtered through
259 an acid-cleaned 0.22 μm pore filter (Millipack-100; Millipore). All TFe and DFe samples were acidified
260 with HCl to pH <1.5 and stored at room temperature for at least one year.

261 Triplicate samples for the $\text{NO}_3^- + \text{NO}_2^-$ (N + N), NH_4^+ , urea and PO_4^{3-} analysis were collected in
262 100 mL of 0.1 N HCl rinsed polyethylene bottles. All samples were analyzed onboard, with the
263 exception of urea, which was only measured in the urea treatment. Upon collection, all samples were
264 stored at -20 °C until analysis.

265

266 **Nutrient addition bioassay set up and incubation.** A total of eight bioassays were carried out, five of
267 which were macro-nutrient bioassays with additions of different forms of nitrogen, or phosphate (M1
268 to M5) and three Fe addition bioassays (Fe1 to Fe3). For macro-nutrient bioassays, pre-filtered seawater
269 was dispensed into 4-liter polycarbonate bottles. Five different triplicate treatments were set up: one
270 control without nutrient addition, three treatment with 100 nM addition of N, wither as NaNO_3 , NH_4Cl ,
271 or urea and one treatment with 10 nM of NaH_2PO_4 (P). Bottles were incubated on-deck in flow-through
272 seawater tanks covered with neutral density screen to attenuate light intensity to 50% of its
273 corresponding surface value. Macro-nutrient bioassays lasted three days with daily sample collections
274 to follow changes in phytoplankton pigments by HPLC and community composition by flow cytometry.
275 For the Fe addition bioassays, filtrate was poured into 2-liter polycarbonate bottles that had been cleaned
276 according to methods of ref.⁵⁷. Five duplicate treatment were set up: controls without any nutrient
277 addition; phosphate additions with 10 nM NaH_2PO_4 ; iron addition with 1nM FeCl_3 ; an Fe+P treatment
278 with 1 nM FeCl_3 and 10 nM NaH_2PO_4 ; and Fe+N treatment with amendment of 1 nM FeCl_3 and 100
279 nM NaNO_3 . To all treatments containing iron addition, EDTA (1 nM) was added as a buffer. Fe addition
280 treatments were done in an onboard class-100 clean air room. Bottles for the iron addition bioassays
281 were also incubated in an on-deck flow-through seawater thanks covered with neutral density screen to

attenuate light intensity to 50% of its corresponding surface value. Iron addition bioassays lasted for five days, monitoring TFe, DFe, N₂ fixation and phytoplankton community composition on days 0, 1, 3, and 5.

Macro-nutrient analysis. Concentrations of NO₃⁻ + NO₂⁻ (N + N), NH₄⁺, SRP and urea were measured using a high-sensitivity colorimetric approach with an AutoAnalyzer II (Technicon) and Liquid Waveguide Capillary Cells (World Precision Instruments, USA) as outlined¹⁷. Urea concentrations were analyzed by diacetyl monoxime method⁵⁹. Detection limits of NO₃⁻ + NO₂⁻, NH₄⁺, and SRP were 3, 6, and 3 nM, respectively.

TFe and DFe concentrations. Dissolved Fe(III) in seawater samples was determined using catalytic cathodic stripping voltammetry with a detection limit of 6 pM using the approach of ref.⁶⁰. No contamination during sampling, and incubation was detected.

Flow cytometry (FCM). *Prochlorococcus* and *Synechococcus* were identified using Flow-cytometry (FCM) based on cell size and chlorophyll- or phycoerythrin-fluorescence. Aliquots of 4.5 mL were preserved in glutaraldehyde (1% final concentration), flash-frozen in liquid N₂, and stored at -80 °C

299 until analysis by flow cytometer (PAS-III, Partec, GmbH, Münster, Germany) equipped with a 488 nm
300 argon-ion excitation laser (100 mW) on land. Forward- and side- angle scatter (FSC and SSC), red
301 fluorescence (>630 nm, FL3) and orange fluorescence (570–610 nm, FL2) were recorded.
302 *Synechococcus* and *Prochlorococcus* were distinguished using FloMax® (Partec, GmbH, Münster,
303 Germany) based on their auto-fluorescence properties and their size.

304

305 **Statistical analysis.** Mean Chl *a* concentration, phytoplankton cell densities, and N₂ fixation rates of
306 each bioassay were first compared between treatments using repeated measurements Analysis Of
307 Variance (RM-ANOVA) with nutrient treatments as between-subjects factor (5 levels) and time (4
308 levels) as the within-subjects factor. Treatment effects were considered significant if $p < 0.05$. Then,
309 means between five treatments were compared by post hoc Turkey test ($n = 3$ replicates per treatment
310 throughout, degrees of freedom = 40).

311

312 **The ecological model of *Prochlorococcus* and *Synechococcus*.** The model follows simple balances of
313 cell densities and nutrient concentrations as used in resource competition theory^{20,28-30}. This theory is
314 suitable in predicting the ecological niches of phytoplankton in aquatic systems such as diatoms²⁸ and

315 nitrogen fixers^{20,29,30} based on different nutrient uptake behavior. We use the following four key
 316 equations:

$$\frac{dX_{Pro}}{dt} = \left(\mu_{maxNO_3}^{Pro} \frac{[NO_3^-]}{[NO_3^-] + K_{NO_3}^{Pro}} + \mu_{maxNH_4}^{Pro} \frac{[NH_4^+]}{[NH_4^+] + K_{NH_4}^{Pro}} - m_{Pro} \right) X_{Pro} \quad [\text{eq. 1}]$$

$$\frac{dX_{Syn}}{dt} = \left(\mu_{maxNO_3}^{Syn} \frac{[NO_3^-]}{[NO_3^-] + K_{NO_3}^{Syn}} + \mu_{maxNH_4}^{Syn} \frac{[NH_4^+]}{[NH_4^+] + K_{NH_4}^{Syn}} - m_{Syn} \right) X_{Syn} \quad [\text{eq. 2}]$$

$$\frac{d[NO_3^-]}{dt} = -\mu_{maxNO_3}^{Pro} \frac{[NO_3^-]}{[NO_3^-] + K_{NO_3}^{Pro}} Q_N^{Pro} X_{Pro} - \mu_{maxNO_3}^{Syn} \frac{[NO_3^-]}{[NO_3^-] + K_{NO_3}^{Syn}} Q_N^{Syn} X_{Syn} + S_{NO_3} \quad [\text{eq. 3}]$$

$$\frac{d[NH_4^+]}{dt} = -\mu_{maxNH_4}^{Pro} \frac{[NH_4^+]}{[NH_4^+] + K_{NH_4}^{Pro}} Q_N^{Pro} X_{Pro} - \mu_{maxNH_4}^{Syn} \frac{[NH_4^+]}{[NH_4^+] + K_{NH_4}^{Syn}} Q_N^{Syn} X_{Syn} + S_{NH_4} \quad [\text{eq. 4}]$$

317 Where,

318 X_i (cell mL⁻¹) = abundance of phytoplankton i ($i=Pro$, Syn ; *Prochlorococcus* and *Synechococcus*

319 respectively)

320 t (d) = time

321 μ_{maxj}^i (d⁻¹) = maximum growth rate of phytoplankton i for nutrient j ($j=NO_3^-$, NH_4^+)

322 $[j]$ (nmol L⁻¹) = Nutrient concentration

323 K_j^i (nmol L⁻¹) = Half saturation constant of nutrient j for phytoplankton i .

324 m_i (d⁻¹) = Mortality rate of phytoplankton i

325 Q_N^i (nmol cell⁻¹) = Cellular nitrogen content of phytoplankton i

326 S_j (nmol L⁻¹ d⁻¹) = Resource term for nutrient j

327 The cell densities are the outcome of the balances between cellular growth and mortality ([eq. 1] and
 328 [eq. 2]). The balances of NO_3^- and NH_4^+ are represented by the uptake by cells and constant source
 329 of each nutrient ([eq. 3] and [eq. 4]). For Q_N^i , we used the average values from Exp. M1-M3, which
 330 were estimated based on the cell size FCM analyzed cell size with the reference carbon per cell volume⁶¹
 331 and carbon to nitrogen ratios⁶². We manually parameterize μ_{maxj}^i , K_j^i , m_i and S_j as well as initial values
 332 for X_i and $[j]$ (hereafter predicted parameters) in order for X_i and $[j]$ to closely represent observation
 333 (Fig. 2, S2) (parameter values and initial values are in Table S4, S5 respectively). After the parameters
 334 are selected, we solve these equations under the steady state to obtain X_i values using Mathematica
 335 11.3⁶³ and plot $Q_N^{Pro} X_{Pro} / Q_N^{Syn} X_{Syn}$ for different resource ratios (Fig. 4A). For Fig. 3A, 4C, S5A,
 336 S5B, we varied the ratios of half saturation constants with $NO_3^-:NH_4^+ = 1:3, 2:3, 1:6$ and $3:1$, respectively.

337

338 **Phylogenetic analysis.** To assess the evolutionary history of picocyanobacteria, a phylogenetic tree
 339 was constructed utilizing *de novo* workflow available in the Genome Taxonomy Database toolkit^{64,65}.
 340 GTDB-Tk v1.3.0, employing the R05-RS95 release of GTDB from July 2020, was used to produce a
 341 standardized Approximately Maximum Likelihood phylogenomic tree (FastTree2)⁶⁶ based on 120
 342 concatenated conserved bacterial markers. The tree spanned all known genome-sequenced
 343 picocyanobacterial clades and was rooted by a closely related outgroup taxon *Synechococcus elongatus*.

To assess the potential of picocyanobacteria to assimilate NO_3^- (and nitrite: NO_2^-), all genomes included in the analysis were screened for the presence of the nitrate reductase (*narB*) and ferredoxin-nitrite reductase (*nirA*) genes. The search for *narB* and *nirA* homologues was performed using BLASTp and tBLASTn with query proteins mined from *S. elongatus* PCC 6301 (genome accession AP008231.1) and default parameters; the resulting hits were verified using conserved domain search available in NCBI⁶⁷. Taxonomic grouping of picocyanobacteria was annotated following recent studies⁵¹⁻⁵³, individual phylogenetic lineages were subsequently assigned to the traditional ecological scheme of low-light vs. high-light adapted ecotypes^{24,25}.

Code availability. The model has been written in Python 3 and freely available in Zenodo at <https://zenodo.org/record/4568561>.

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363

364 **Author contributions**

365 T.Masuda, K.F and S.T designed the *in situ* microcosm experiments, T.Masuda., T.K., T.S and
366 T.Matsui carried out the experiment and analyzed data supervised by K.F., S.T. and K.S.. T.Masuda
367 and K.I shaped the concept of the study with the supervision of O.P. and C.D. K.I. developed the model
368 with the supervision of C.D. J.M. conducted phylogenetic analysis. T.Masuda, K.I. and J.M wrote the
369 original draft with substantial input from all the authors.

370

371 **Competing interests**

372 The authors declare no competing financial interests.

373

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Table S1 The physical and biological parameters of seawater collected for each bioassay experiment. For all data, means are shown with $\pm$ standard deviation for triplicate samples. ND, no data.

Exp.	Date	Chl <i>a</i>	Salinity	Tempe	N+N	NH ₄ ⁺	SRP	TFe	DFe	<i>Prochloro-</i>	<i>Synecho-</i>
	In 2018	(ng L ⁻¹)		rature	(nM)	(nM)	(nM)	(nM)	(nM)	<i>coccus</i>	<i>coccus</i>
				(°C)						(cells mL ⁻¹)	(cells mL ⁻¹)
M1	6 June	24	34.27	29.0	<3	<3	56	0.34	0.11	5275 $\pm$ 1628	70 $\pm$ 5
Fe1	8 June	ND	34.23	29.1	<3	<3	62	0.25	0.15	4722 $\pm$ 1516	428 $\pm$ 500
M2	10 June	25	34.19	29.3	<3	<3	51	0.29	0.17	2235 $\pm$ 226	38 $\pm$ 3
Fe2	12 June	ND	34.16	29.2	<3	10	57	0.38	0.05	4414 $\pm$ 1198	126 $\pm$ 30
M3	14 June	28	34.24	29.1	<3	9	64	ND	ND	3609 $\pm$ 1051	35 $\pm$ 29
Fe3	16 June	ND	34.22	29.3	<3	12	61	0.22	0.39	3004 $\pm$ 1102	346 $\pm$ 125
M4	18 June	61	34.13	29.1	<3	15	31	0.47	0.44	26212 $\pm$ 2227	1280 $\pm$ 131
M5	22 June	98	33.94	28.9	7	36	31	1.01	0.16	26767 $\pm$ 3662	522 $\pm$ 101

Table S2 p-values of the two-way RM-ANOVA for cell density (Fig. 1). Day represents the timing of harvest (i.e., 1, 2, 3); Treatment represents the enriched nutrient (i.e., Cont, PO_4^{3-} , NO_3^- , NH_4^+ , Urea). RM-ANOVA shows that cell densities of both *Prochlorococcus* and *Synechococcus* were different among treatment.

		df	F	p	df	F	p
Exp.		<i>Prochlorococcus</i>			<i>Synechococcus</i>		
M1	Day	2	3.625	>0.05	2	24.332	<0.01
	Treatment	4	8.6115	<0.01	4	6.080	<0.01
	Day × Treatment	8	4.160	0.01<p<0.05	8	2.289	0.01<p<0.05
M2	Day	2	7.762	<0.01	2	19.022	<0.01
	Treatment	4	39.875	<0.01	4	84.280	<0.01
	Day × Treatment	8	3.332	<0.01	8	27.309	<0.01
M3	Day	2	28.584	<0.01	2	36.558	<0.01
	Treatment	4	80.641	<0.01	4	30.091	<0.01
	Day × Treatment	8	8.872	<0.01	8	11.662	<0.01
M4	Day	2	7.040	<0.01	2	12.845	<0.01
	Treatment	4	39.211	<0.01	4	57.375	<0.01
	Day × Treatment	8	5.856	<0.01	8	5.475	<0.01
M5	Day	2	36.767	<0.01	2	135.416	<0.01
	Treatment	4	29.780	<0.01	4	94.340	<0.01
	Day × Treatment	8	3.831	<0.01	8	37.498	<0.01
Fe1	Day	2	38.127	<0.01	2	30.059	<0.01
	Treatment	4	87.178	<0.01	4	34.875	<0.01
	Day × Treatment	8	33.909	<0.01	8	10.743	<0.01
Fe2	Day	2	23.810	<0.01	2	67.473	<0.01
	Treatment	4	6.027	<0.01	4	7.789	<0.01
	Day × Treatment	8	6.027	<0.01	8	7.789	<0.01
Fe3	Day	2	61.100	<0.01	2	34.409	<0.01
	Treatment	4	7.114	<0.01	4	4.460	0.01<p<0.05
	Day × Treatment	8	7.114	<0.01	8	4.460	0.01<p<0.05

Table S3 Summary of the effects of nutrient additions on cell density in each bioassay experiment. Cell density was measured from separate triplicate bottles. The response of the phytoplankton communities to the different treatments was compared by post hoc Tukey test, to compare the means between five treatments (n = 3 replicates per treatment throughout, degrees of freedom = 40). Significant differences ($p < 0.05$) between individual treatments are indicated by <; = indicates no significant differences. Cont, PO4, NO3, NH4, Urea refer to the treatment of control, PO_4^{3+} , NO_3^- , NH_4^+ , respectively. ND refers to no significant difference. Tukey test shows that *Prochlorococcus* prefers recycled N, such as NH_4^+ and urea while *Synechococcus* prefer NO_3^- .

Exp.	Day	<i>Prochlorococcus</i>	<i>Synechococcus</i>
M1	1	Cont = PO4 = NO3 = NH4 = Urea	Cont = PO4 = NO3 = NH4 = Urea
	2	(Cont = PO4 = NO3) < (NH4 = Urea)	(Cont = PO4 = Urea) < NO3 < NH4
	3	(Cont = PO4) < (NO3 = Urea) < Urea	Cont = PO4 = NO3 = NH4 = Urea
M2	1	(Cont = PO4 = NO3) < NH4 < Urea	Cont = PO4 = NO3 = NH4 = Urea
	2	(Cont = PO4 = NO3) < NH4 < Urea	(Cont = PO4 = NH4) < NO3 = Urea
	3	(Cont = PO4 = NO3 = NH4) < Urea	(Cont = PO4 = NH4 = Urea) < NO3
M3	1	(Cont = PO4) < NO3 < Urea < NH4	Cont < (PO4 = NO3 = NH4 = Urea)
	2	(Cont = PO4) < NO3 < (NH4 = Urea)	(Cont = PO4) = NH4 < NO3 < Urea
	3	(Cont = PO4) < NH4 < NO3 < Urea	(Cont = PO4 = NH4 = Urea) < NO3
M4	1	Cont < PO4 < (NO3 = Urea) < NH4	PO4 < (Cont = NO3 = NH4 = Urea)
	2	(Cont = PO4) < NO3 < Urea < NH4	PO4 < Urea < (Cont = NH4) < NO3
	3	(Cont = PO4) < Urea < NH4 < NO3	PO4 < Urea < (Cont = NH4) < NO3
M5	1	PO4 < (Cont = NO3 = NH4 = Urea)	Cont = PO4 = NO3 = NH4 = Urea
	2	(Cont = PO4) < NO3 < Urea < NH4	Cont = PO4 = NO3 = NH4 = Urea
	3	(Cont = PO4) < (NO3 = NH4) < Urea	Cont = PO4 = NO3 = NH4 = Urea
Fe1	1	Cont = PO4 = NO3 = NH4 = Urea	Cont = PO4 = NO3 = NH4 = Urea
	3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3
	5	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3
Fe2	1	Cont = PO4 = NO3 = NH4 = Urea	Cont = PO4 = NO3 = NH4 = Urea
	3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3
	5	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3
Fe3	1	Cont = PO4 = NO3 = NH4 = Urea	Cont = PO4 = NO3 = NH4 = Urea
	3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3
	5	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3	(Cont = Fe = PO4 = Fe+PO4) < Fe+NO3

Table S4 Parameter values for the model.

Parameter	Value	Unit
μ_{maxNO3}^{Pro}	1.9	d ⁻¹
μ_{maxNH4}^{Pro}	2.7	d ⁻¹
μ_{maxNO3}^{Syn}	2.3	d ⁻¹
μ_{maxNH4}^{Syn}	3.6	d ⁻¹
K_{NO3}^{Pro}	100	nmol L ⁻¹
K_{NH4}^{Pro}	10	nmol L ⁻¹
K_{NO3}^{Syn}	20	nmol L ⁻¹
K_{NH4}^{Syn}	40	nmol L ⁻¹
m_{Pro}	0.85	d ⁻¹
m_{Syn}	0.7	d ⁻¹
Q_N^{Pro}	* 1.20×10^{-7}	nmol cell ⁻¹
Q_N^{Syn}	* 2.88×10^{-7}	nmol cell ⁻¹
NH ₄ ⁺ added case		
S_{NO3}	-1	nmol L ⁻¹ d ⁻¹
S_{NH4}	-50	nmol L ⁻¹ d ⁻¹
NO ₃ ⁻ added case		
S_{NO3}	-26	nmol L ⁻¹ d ⁻¹
S_{NH4}	-4	nmol L ⁻¹ d ⁻¹

* Average values from Exp. M1-M3.

Table S5 Initial values for the simulated time series.

Parameter	Value	Unit
NH ₄ ⁺ added case		
X_{Pro}	4000	cell mL ⁻¹
X_{Syn}	40	cell mL ⁻¹
$[NO_3^-]$	10	nmol L ⁻¹
$[NH_4^+]$	100	nmol L ⁻¹
NO ₃ ⁻ added case		
X_{Pro}	3600	cell mL ⁻¹
X_{Syn}	48	cell mL ⁻¹
$[NO_3^-]$	120	nmol L ⁻¹
$[NH_4^+]$	10	nmol L ⁻¹

Fig. S1 Effect of nutrient amendment on *Prochlorococcus* and *Synechococcus* abundance (A) and (B) The effect of Fe and P additions on cell abundance (means $\pm$ SD, n = 3) for Exp. Fe2. Addition of Fe and P had no effect on the growth of *Prochlorococcus* and *Synechococcus* except when N was also added. (C) and (D) The effect of different chemical forms of N and P additions on cell abundance (means $\pm$ SD, n = 3) for Exp. M3. *Prochlorococcus* prefers NH₄⁺ while *Synechococcus* prefers NO₃⁻.

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Fig. S2 Model-data comparison of the time series of NH_4^+ and NO_3^- concentrations. (A) NH_4^+ added. (B) NO_3^- added. Points, Data; Curves, Model. Data are based on Exp. M1-M3. Sample numbers for each data are same as Fig. 1.

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Fig. S3 Model-data comparison of the time series of abundance (X) of *Prochlorococcus* and *Synechococcus* with the same K_{NO_3} values for these organisms. (A) NH_4^+ added. (B) NO_3^- added. Points, Data; Curves, Model. Pro, *Prochlorococcus*; Syn, *Synechococcus*. Data are based on Exp. M1-M3. Here we used the averaged K_{NH_4} value. Sample numbers for each data are same as Fig. 1.

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Fig. S4 Model-data comparison of the time series of abundance (X) of *Prochlorococcus* and *Synechococcus* with the same K_{NO_3} values for these organisms. (A) NH_4^+ added. (B) NO_3^- added. Points, Data; Curves, Model. Pro, *Prochlorococcus*; Syn, *Synechococcus*. Data are based on Exp. M1-M3. We used the averaged K_{NO_3} value. Sample numbers for each data are same as Fig. 1.

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Fig. S5 The effect of the change in the resource ratio. **(A)** $\text{NO}_3^-:\text{NH}_4^+ = 1:6$ **(B)** $\text{NO}_3^-:\text{NH}_4^+ = 3:1$. The cyan triangle represents the ratios of predicted half saturation constants for Fig. 2. Dashed curve indicate borders between coexistence and competitive exclusion when $\text{NO}_3^-:\text{NH}_4^+ = 3:1$ (same dashed curves as in Fig. 3A).

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Fig. S6 Phylogenomic tree of marine picocyanobacteria. The tree is rooted by the freshwater core cluster of *Synechococcus* (type strain *S. elongatus* PCC 6301). Individual clades are annotated following the most recent taxonomic revisions. a, b, c in superscript in the figure represent ref.⁵¹⁻⁵³, respectively. Phylogenetic lineages are further classified using ecological groups of *Synechococcus* and *Prochlorococcus* (right); LL and HL stands for low-light and high-light adapted sub-clades of *Prochlorococcus*²⁰. The clade traditionally considered as *Prochlorococcus sensu lato* is highlighted in cyan. Presence of genes encoding nitrate reductase (*narB*) and nitrite reductase (*nirA*) was assessed for the individual ecological groups and expressed using the total number of occurrences within group along with a relative abundance scale bar. The tree is based on 120 conserved

bacterial proteins using the Approximately Maximum Likelihood method following the GTDB-Tk *de novo* pipeline⁶⁵. Bootstrap values are shown near the nodes, numbers in brackets indicate the number of genomes within collapsed clades.

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Figures

Figure 1

The effects of different chemical forms of N, P and Fe additions on cell abundances of *Prochlorococcus* (A) and *Synechococcus* (B) relative to controls (means $\pm$ SD), with number of samples in parentheses. For N and P addition, data is based on Exp. M1-M3, where the initial concentrations of N were small (<10 nm). It shows that *Prochlorococcus* prefers NH₄⁺ while *Synechococcus* prefers NO₃⁻. Note that added NH₄⁺ was depleted on the third day, leading to decreased populations in both organisms.

Figure 2

Model data comparison of the time series of abundance (X) of Prochlorococcus and Synechococcus. (A) NH_4^+ added. (B) NO_3^- added. Points, Data; Curves, Model. Pro, Prochlorococcus; Syn, Synechococcus. Data are based on Exp. M1-M3, where the initial concentrations of N were small (<10 nM). Sample numbers for each data are same as Fig. 1.

Figure 3

Steady state solution of the model for the coexistence of *Prochlorococcus* and *Synechococcus* based on the ratios of half saturation constants for nitrogen sources. (A) Model results of the ratio of *Prochlorococcus* and *Synechococcus* expressed in terms of the proportion of total cellular nitrogen concentration in the water (N_{Pro}/N_{Syn} : see the color bar for values). The cyan triangle in panel A represents the ratios of the half saturation constants predicted from Fig. 2. The model output is based on the resource ratio of NO_3^- and NH_4^+ is 1:3 (for other ratios, see Fig. 3, S5). Dashed curves indicate borders between coexistence and non-coexistence. (B) Explanation of each domain; coexistence occurs only in domains i and iv.

Figure 4

Resource-ratio based shifts of coexistence. (A) The shift in the ratio of *Prochlorococcus* and *Synechococcus* based on the resource ratios of NO_3^- : NH_4^+ ($S_{NO_3^-}/S_{NH_4^+}$) with the predicted half saturation constants. (B) Relationship between the NO_3^- concentrations and cell count ratios of *Prochlorococcus* and *Synechococcus*. (C) The effect of doubled NO_3^- recourse. The plot shows the model results of the *Prochlorococcus* and *Synechococcus* ratio in terms of cellular N (N_{Pro}/N_{Syn} : see the color bar for values). Here the resource ratios of NO_3^- : NH_4^+ is 2:3. Dashed curves indicate borders between coexistence and competitive exclusion when the resource ratio of NO_3^- : NH_4^+ are 1:3 (same dashed curves as in Fig. 3A). The cyan triangle represents the ratios of predicted half saturation constants for Fig. 2.