




Crocospaera as a Major Consumer of Fixed Nitrogen

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ABSTRACT *Crocospaera watsonii* (hereafter referred to as *Crocospaera*) is a key nitrogen (N) fixer in the ocean, but its ability to consume combined-N sources is still unclear. Using *in situ* microcosm incubations with an ecological model, we show that *Crocospaera* has high competitive capability both under low and moderately high combined-N concentrations. In field incubations, *Crocospaera* accounted for the highest consumption of ammonium and nitrate, followed by picoeukaryotes. The model analysis shows that cells have a high ammonium uptake rate ($\sim 7 \text{ mol N [mol N]}^{-1} \text{ d}^{-1}$ at the maximum), which allows them to compete against picoeukaryotes and nondiazotrophic cyanobacteria when combined N is sufficiently available. Even when combined N is depleted, their capability of nitrogen fixation allows higher growth rates compared to potential competitors. These results suggest the high fitness of *Crocospaera* in combined-N limiting, oligotrophic oceans heightening its potential significance in its ecosystem and in biogeochemical cycling.

IMPORTANCE *Crocospaera watsonii* is as a key nitrogen (N) supplier in marine ecosystems, and it has been estimated to contribute up to half of oceanic N_2 fixation. Conversely, a recent study reported that *Crocospaera* can assimilate combined N and proposed that unicellular diazotrophs can be competitors with non- N_2 fixing phytoplankton for combined N. Despite its importance in nitrogen cycling, the methods by which *Crocospaera* compete are not currently fully understood. Here, we present a new role of *Crocospaera* as a combined-N consumer: a competitor against nondiazotrophic phytoplankton for combined N. In this study, we combined *in situ* microcosm experiments and an ecosystem model to quantitatively evaluate the combined-N consumption by *Crocospaera* and other non- N_2 fixing phytoplankton. Our results suggest the high fitness of *Crocospaera* in combined-N limiting, oligotrophic oceans and, thus, heightens its potential significance in its ecosystem and in biogeochemical cycling.

KEYWORDS *Crocospaera watsonii*, marine N_2 fixer, combined nitrogen, ecological model

Marine phytoplankton contribute about one-half of the global net primary production and play a key role in regulating global biogeochemical cycles (1). Since phytoplankton are biochemically, metabolically, and ecologically diverse (2–4), understanding the contribution of different phytoplankton groups to ecosystem function is central to the precise estimation of the global carbon (C) and nitrogen (N) budget and to predicting the biogeochemical impact of future environmental changes (5).

In the oligotrophic subtropical gyres, combined N (defined as N covalently bonded to one or more elements other than N [6]) limits primary production and controls planktonic community composition (7–10). Therefore, N_2 -fixing microorganisms (diazotrophs) are important as a source of combined N in oligotrophic ecosystems (11, 12). In the subtropic

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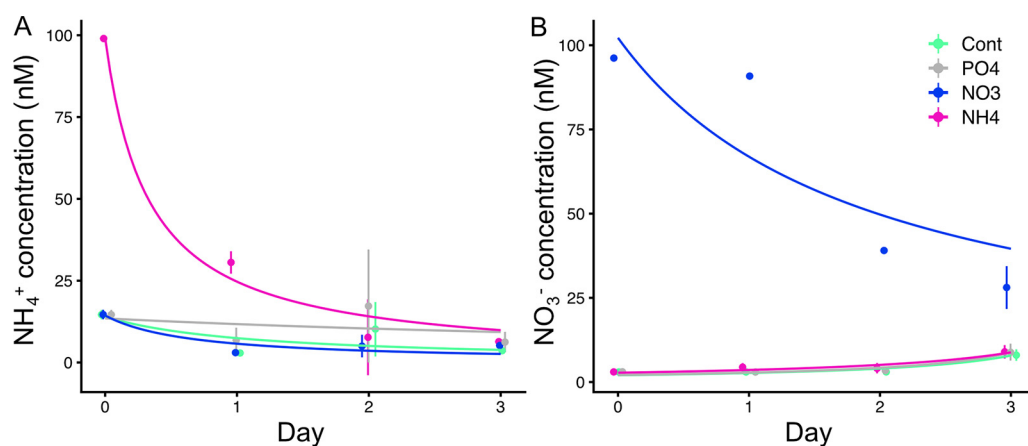


FIG 1 Temporal change in NH₄⁺ and NO₃⁻ concentrations of experiment M3. (A) NH₄⁺ concentration in the NH₄⁺ treatment exponentially decreased during the experiment down to the detection limit of 6 nM on day 3. (B) NO₃⁻ concentrations in the NO₃⁻ treatment exponentially decreased during the experiment, but enriched NO₃⁻ was not always entirely consumed. Error bar shows a standard deviation of triplicate. Temporal change in urea-N concentration is shown in Fig. S2 in the supplemental material.

oligotrophic ocean, the unicellular diazotroph, *Crocospaera watsonii* (2.5 to 6 μ m; hereafter referred to as *Crocospaera*), is widely distributed (10, 13–16) in addition to pico-sized (<3 μ m) cyanobacteria (e.g., *Prochlorococcus* and *Synechococcus*) and picoeukaryotes (17–19). Earlier studies examined the effect of combined N, such as ammonium (NH₄⁺) and nitrate (NO₃⁻), on metabolic activities and reveal the ability of *Crocospaera* to assimilate combined N (20, 21). As reported from *Trichodesmium* (22), increasing concentrations of NH₄⁺ enrichment increases NH₄⁺ uptake activities and inhibits N₂ fixation rates up to ~80% (20, 21), while NO₃⁻ enrichment did not inhibit N₂ fixation rate at any of the tested NO₃⁻ concentrations (up to 10 μ M) (20). When remaining combined-N concentrations in the cultures are at a nanomolar level, *Crocospaera* kept fixing N₂ (20, 21). Model results indicate that using dissolved inorganic nitrogen (DIN) enables *Crocospaera* populations to increase their abundance and expand their niche (23). These studies proposed that unicellular diazotrophs can be competitors with nondiazotrophic phytoplankton for combined N. However, how *Crocospaera* competes for combined N is poorly evaluated. In this study, we combine an *in situ* microcosm experiment with N addition at the nanomolar level and model (24) to evaluate the competitiveness of *Crocospaera* in an N-limiting environment.

RESULTS

Summary of the experiment. We carried out five nitrogen (N) and phosphorus (P) addition bioassays (M1 to M5) every 4 days at a station in the subtropical Northwestern Pacific (12°N, 135°E) from 6 to 25 June 2008 during the MR08-02 cruise on the R/V *MIRAI*. The northward current was dominant until bioassay M3, while a strong southward current occurred on days between bioassays M3 and M4. The initial waters were more oligotrophic during M1 to M3 compared to those during M4 and M5; nutrient concentrations initially were less than 36 nM for ammonium (NH₄⁺), 7 nM for nitrate plus nitrite (NO₃⁻ + NO₂⁻), and 64 nM for phosphorus (PO₄³⁻) (see Table S1 in the supplemental material). The lower initial phytoplankton abundance during M1 to M3 than that during M4 and M5 confirms the oligotrophic characteristics of initial water during M1 to M3 (Table S1). Although we performed prefiltration with a 1- μ m polypropylene cartridge filter (Micropore EU; ORGANO) to eliminate the effect of grazing, water samples contained plankton up to ~5 μ m in size.

Nutrient uptake and fate of enriched DIN. For 3 days of incubation, the phytoplankton community consumed NH₄⁺ entirely at the end, while NO₃⁻ was not always consumed completely (Fig. 1; see also Fig. S1 in the supplemental material). Estimated biomass explains about half of consumed combined-N sources (Fig. 1, 2A).

The greatest portion of estimated C and N in biomass was found in *Crocospaera* (39 to 93% in all N addition incubations) followed by picoeukaryotes (5 to 55% in N addition

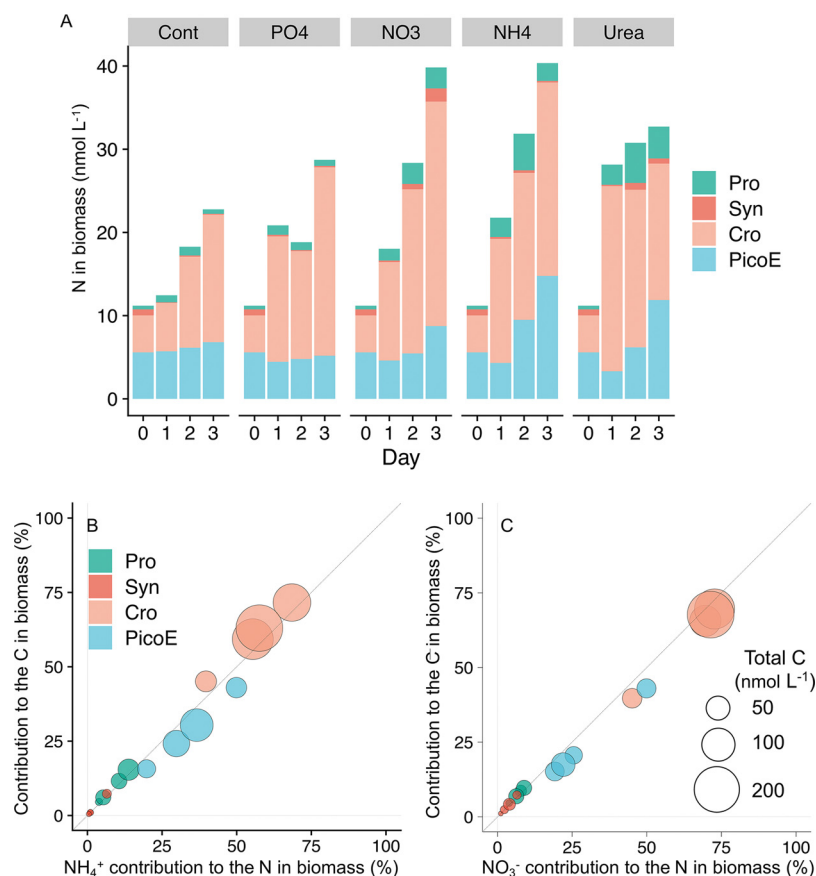


FIG 2 (A) N in biomass in each treatment and its contribution of each phytoplankton group of experiment M3. (B) Contribution to total C in biomass as a function of the contribution of NH₄⁺-N biomass for each phytoplankton group. (C) Contribution to total carbon in biomass as a function of the contribution of NO₃⁻-N biomass for each phytoplankton group. The contributions of NH₄⁺-N or NO₃⁻-N were estimated from either NH₄⁺ or NO₃⁻ enrichment. Each circle shows data from a different day, and the size of the dots represents the total C in biomass (nmol C L⁻¹). Pro, *Prochlorococcus*; Syn, *Synechococcus*; Cro, *Crocospaera*; PicoE, picoeukaryotes.

incubations) (Fig. 2; see also Fig. S3 and S4 in the supplemental material). Although the origin of water mass changed from oligotrophic water to mixed water between experiments M1 to M3 and M4 to M5 (25), with more *Crocospaera* in cell density with higher N₂ fixation in the latter environment (see Tables S1 and S2 in the supplemental material), the dominance of *Crocospaera* as a C and N biomass was observed from all of the experiments. N derived from N₂ fixation was not always sufficient to support the N demand of *Crocospaera*, especially in N amendment (see Fig. S5 in the supplemental material). Estimated N₂ fixation supported 0.5 to 12.7% of N demand of *Crocospaera* in control and 0.5 to 11.6% in NH₄⁺ treatment (Fig. S5), suggesting that *Crocospaera* consumed amended N sources. Assimilation of combined nitrogen (NH₄⁺ and NO₃⁻), together with N₂ fixation by *Crocospaera*, has been reported (20, 21). Although enriched 100 nM NH₄⁺ was completely consumed (<6 nM; detection limit on day 3), increases in N biomass of nondiazotrophs for 3 days were limited to up to 58 nmol L⁻¹, again suggesting *Crocospaera* took up combined nitrogen.

Model analysis of the data. To quantitatively interpret the observed data, we used a simple model of cellular growth, which is based on the uptake of NH₄⁺ and NO₃⁻ (see Materials and Methods). It is natural that such *in situ* incubation experiments display a variation in their results, since the initial conditions vary based on the locations. The ideal conditions to test relaxation from nutrient stress are to use a nutrient-starved phytoplankton community, which spends a long time under low-nutrient conditions (26, 27). Considering the nutrient history of the *in situ* phytoplankton community, we selected M3 as the best example to observe relief from nutrient stress, since the water

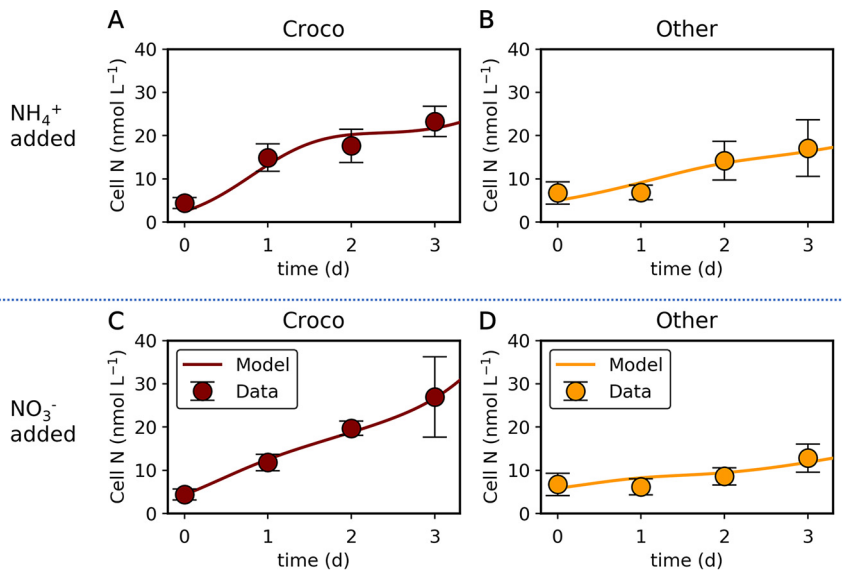


FIG 3 Simulated transition of cellular N with nutrient addition compared with data. (A, B) NH₄⁺-added case. (C, D) NO₃⁻-added case. Croco, *Crocosphaera*; Other, other phytoplankton. Data are from experiment M3.

mass changed from more N-limited to N-rich water between M3 and M4 (see Tables S1 and S3 in the supplemental material). The N-limited nutrient history of phytoplankton was confirmed by low initial nutrient conditions and low biomass of the targeted organisms. Thus, we focus on the data from experiment M3 for modeling analysis.

The model captured the overall trend of the transition of cellular N (Fig. 3) based on the available nutrient (see Fig. S6 in the supplemental material). The parameterization of the model reveals high rates of N uptake by *Crocosphaera*. We used 6.6 mol N (mol N)⁻¹ d⁻¹ for maximum NH₄⁺ uptake under NH₄⁺ limitation to represent the data, which represent high combined-N uptake compared to that of other phytoplankton under the same condition (maximum NH₄⁺ uptake of 1.1 mol N [mol N]⁻¹ d⁻¹) (see Table S4 in the supplemental material). Specifically, such parameterization was needed to reproduce the rapid growth of *Crocosphaera* under NH₄⁺ added conditions between day 0 and day 1. The predicted maximum NO₃⁻ uptake rate for *Crocosphaera* is also higher than for other phytoplankton (Fig. 3), which is supported by the faster growth of *Crocosphaera* with NO₃⁻ addition.

To test the competitiveness of *Crocosphaera*, we simulated a simple ecological situation. Here, we simulated zooplankton with kill the winner (KTW) theory (28). We used this method because it reflects the commonly observed active prey-switching behavior of zooplankton (29–31). The result shows the high competitiveness of *Crocosphaera* under both high and low nutrient concentrations (Fig. 4; see also Fig. S7 in the supplemental material). Under a high nutrient concentration, *Crocosphaera* may dominate other phytoplankton due to the high rate of nutrient uptake (Fig. 4A; see also Fig. S7A). However, under extremely low-nutrient conditions (NH₄⁺ and

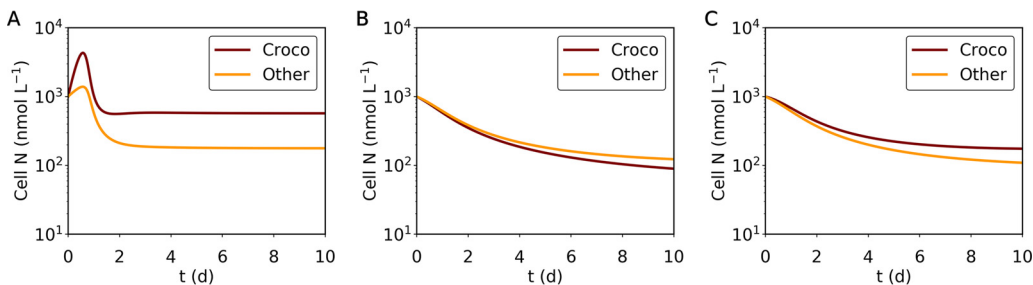


FIG 4 Simulated transition of cellular N in a simple ecosystem model for three different scenarios. (A) The concentrations for NH₄⁺ and NO₃⁻ are both 100 nmol L⁻¹. (B, C) The concentrations for NH₄⁺ and NO₃⁻ are both 1 nmol L⁻¹. In only panel C, *Crocosphaera* may acquire N via N₂ fixation; in panels A and B, the effect of N₂ fixation is neglected. Parameters are based on the NH₄⁺-added case.

NO₃⁻ are both at 1 nmol L⁻¹), *Crocospaera* is slightly outcompeted (Fig. 4B; see also Fig. S7C and D). This is due to the relatively high half-saturation constant for NH₄⁺, which is manifested by the sudden decrease in growth rate with a drop in NH₄⁺ under NH₄⁺ addition (Fig. 3A; see also Fig. S6A). However, this relationship flips if we consider the effect of N₂ fixation, which maintains growth rates at a higher level rather than relying on external N under N depletion (Fig. 4C; see also Fig. S7B). These results suggest that possession of nitrogenase (an enzyme complex involved in N₂ fixation) allows for the survival of *Crocospaera* under low-nutrient environments.

DISCUSSION

Our study shows high uptake of N by *Crocospaera* under relatively high N concentration (Fig. 1 and 2). As described in the method, we estimated cell size of each phytoplankton group based on forward light scatter of flow cytometry, which was calibrated by 1.75- to 10- μ m standard monodisperse polystyrene beads (Polysciences) (see Fig. S8 in the supplemental material). We are aware of the difficulty in estimating the absolute cell size through forward light scatter (FLS) (32) as well as in volume-to-carbon conversion (33, 34). Acknowledging these limitations, we note that the FLS approach has the advantage of taking into account actual cell size variability compared to the application of a constant carbon per cell factor (35). Despite these constraints, our biomass estimation accounted for approximately half of consumed N (Fig. 1, 2A). This gap may be caused by the limitations of an FLS-based approach. Conversely, uncertainties in size estimation will be more pronounced for the smaller cells than for the larger cells, i.e., *Prochlorococcus* in our experiments, since Mie light scattering theory predicts that scattering per cell volume continues to increase with decreasing particle size. The other possibility of the gap may be due to luxury uptake, (27, 36). Although there are uncertainties in estimating absolute size, which leads to uncertainties in cellular N content, these uncertainties do not change our finding of high N uptake by *Crocospaera*, known as a bioavailable nitrogen provider. Our samples, filtrate of 1- μ m polypropylene cartridge filter, consisted of up to 5- μ m cells. This shows the leak of phytoplankton through filters (37, 38). Since *Crocospaera watsonii* (2 to 6 μ m) is larger than other groups (21, 39, 40), higher filtration pressure might lead to underestimation of *Crocospaera* abundance and biomass compared to other groups. The *Crocospaera* abundances observed in our experiment are in the same range with nanocyanobacteria, the temporal name for the *Crocospaera* in reference 25 (see Table 1 in reference 25), which described initial conditions of the same cruise, suggesting that most of the *Crocospaera* cells went through the filter. During our experiment, biomass in any phytoplankton did not increase at experiment M5 (Fig. 2 and 3). Together with the NH₄⁺ concentration remaining at more than 30 nM (see Table S1 in the supplemental material), this suggests the non-N-limited nutrient history of the phytoplankton community in experiment M5 (Fig. 3).

The results counter the general image of *Crocospaera*. It is most known as a diazotroph and is considered to be a provider of N to the environment. Rather, our results support more recent studies, where *Crocospaera* does not increase the productivity of other phytoplankton (41) or even compete with other species over combined N (23). Surprisingly, our study even shows higher maximum uptake rates of NH₄⁺ and NO₃⁻, which allow its dominance just by the uptake of combined N. When the concentration of nitrogen is extremely low, *Crocospaera* could be outcompeted via N uptake; however, its N₂ fixation allows *Crocospaera* to maintain its biomass at a certain level, which can still be higher than that of nondiazotrophic phytoplankton. This high consumption of NO₃⁻ may differ from UCYN-A (15, 42–44), which keeps fixing nitrogen under high NO₃⁻ availability (45, 46), leading to a unique niche acquisition. These results suggest that *Crocospaera* has high competitiveness under conditions with both low and high nutrients.

Despite this, we generally do not observe the oligotrophic ocean completely dominated by *Crocospaera*. This may be due to grazing selection. *Crocospaera* is a unicellular cyanobacterium, a few micrometers to 6 μ m in diameter (47), and its tight coupling with predators has been reported recently (48). The new production of *Crocospaera* is estimated to support up to 400% of C demand of the main grazers, and the grazing rates of the main predator *Protooperidinium* were found to be nearly equivalent to growth rates of *Crocospaera* (48).

Conversely, *Trichodesmium*, another N₂ fixer in the ocean, is reported to produce a toxin (49–51) and create large colonies of ~10⁴ cells (52), potentially protecting themselves from grazing. The other reason might be growth limitation by other nutrients, such as P and Fe. Even though there are reports that *Crocospaera* shows adaptation for low P and low Fe, having high-affinity phosphate transporters (53) as well as availability of several chemical forms of P (54–56) and high affinity to low Fe concentrations (57) and recycling Fe (14), their relative fitness to such low P or low Fe environments compared to other organisms has not been quantified. Since having nitrogenase enzymes requires a high concentration of Fe, nonnitrogen fixers, such as *Prochlorococcus* and *Synechococcus*, may have lower Fe requirements and are more adapted to Fe depletion. Also, *Crocospaera* does not seem to fully utilize sulfolipid, which would save P use when compared with that of other cyanobacteria, such as *Synechococcus* (58, 59), and thus may not compete strongly under P limitation. Neither P limitation nor Fe limitation were observed during our observation ($P < 0.05$; repeated measures analysis of variance [RM-ANOVA], *post hoc* Tukey test) (Fig. 2A; see also Fig. S3 and Tables S1, S5, and S6 in the supplemental material).

At the same time, it is largely possible that *Crocospaera* dominates in some regions in the oligotrophic ocean given its high competitiveness under N limitation, which is the characteristic of the oligotrophic ocean (7, 60). For example, a study of flow cytometry shows a high abundance of *Crocospaera*-like cells in a wide region of the North Pacific (61), where the abundance of *Trichodesmium* seems limited (62). Also, a recent study shows multiple gene copies in *Trichodesmium* (up to ~700 genes copied per cell) (63), which could overestimate their abundance (64). Given these factors and our analysis showing the high fitness of *Crocospaera* to both low and high nitrogen concentrations, it is possible that we are still underestimating the relative abundance and role of *Crocospaera* in global biogeochemical cycling.

MATERIALS AND METHODS

Experimental setup and sample collection. We carried out five macronutrient (N and P)-addition bioassays (M1 to M5) using natural phytoplankton assemblages collected at a station in the subtropical Northwestern Pacific (12°N, 135°E) from 6 to 25 June 2008 during the MR08-02 cruise on the R/V *MIRAI* (see Table S7 in the supplemental material). Water samples were collected from a 10-m depth at 1230 h local time using a Teflon diaphragm pump system. All components of this pump system and associated plastic were washed overnight in a neutral detergent followed by HCl and HNO₃, rinsed with heated Milli-Q water, and flushed with seawater for 30 min immediately prior to sample collection. To reduce grazing pressure, we prefiltered seawater from a 10-m depth through an acid-cleaned 1- μ m in-line cartridge filter (Micropore EU; ORGANO) and distributed it into 4-L polycarbonate bottles, which were rinsed overnight in a neutral detergent, followed by 0.3 N HCl, and rinsed with Milli-Q water. We performed three treatments with 100 nM N addition as NaNO₃, NH₄Cl, or urea. To test P limitation, one treatment with 10 nM NaH₂PO₄ and our control went without nutrient addition. Forty-five bottles, 5 treatments, and 3 incubation periods (1, 2, or 3 days) were incubated in triplicate for each incubation period (1, 2, or 3 days) on deck in flowthrough seawater tanks covered with a neutral density screen to attenuate light intensity to 50% of its corresponding surface value. Each bottle was used for one time period after washing.

To examine Fe limitation, we carried out three Fe addition bioassays (Fe1 to Fe3) at the same station during the same cruise with the macronutrient addition bioassay experiments (see Table S8 in the supplemental material). Prior to the bioassay experiments, the 2-L polycarbonate incubation bottles had been cleaned according to reference 65. Other polyethylene and Teflon lab wares were cleaned according to reference 66. All washing procedures were carried out in an onshore class 1000 clean air room, and plastic gloves were worn during these operations. To reduce grazing pressure, prefiltered seawater was prefiltered through a 10- μ m filter of the same manufacturer. The prefiltered water was then dispensed into the corresponding bioassay incubation bottles. Five duplicate treatments were set up as follows: controls without any nutrient addition, phosphate additions with 10 nM NaH₂PO₄, iron addition with 1 nM FeCl₃, an Fe+P treatment with 1 nM FeCl₃ and 10 nM NaH₂PO₄, and Fe+N treatment with an amendment of 1 nM FeCl₃ and 100 nM NaNO₃. To all treatments containing iron addition, EDTA (1 nM) was added as a buffer. Fe addition treatments were done in an onboard class 100 clean air room. Bottles for the iron addition bioassays were also incubated in on-deck flowthrough seawater tanks covered with neutral density screen to attenuate light intensity to 50% of its corresponding surface value. Iron addition bioassays lasted for 5 days, monitoring total iron (TFe), dissolved iron (DFe), and phytoplankton community composition on days 0, 1, 3, and 5.

Macronutrient and iron concentrations. Concentrations of NO₃⁻ + NO₂⁻ (N+N), NH₄⁺, soluble reactive phosphorus (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). Triplicate samples for the NO₃⁻ + NO₂⁻ (N+N), NH₄⁺, urea, and soluble reactive phosphorus (SRP) (67, 68) analysis were collected in 100 mL of 0.1 N HCl-rinsed polyethylene bottles. All samples were analyzed onboard, with the exception of urea, which was only measured in the urea treatment. Upon collection, all

samples were stored at -20°C until analysis. We analyzed urea concentrations using the diacetyl monoxime method (69). Detection limits of $\text{NO}_3^- + \text{NO}_2^-$, NH_4^+ , and SRP were 3, 6, and 3 nM, respectively.

Iron concentrations of the seawater were measured as total iron (TFe), in the whole-water samples collected directly from the pump system, and as dissolved iron (DFe), in the 125 mL of seawater collected in low-density polyethylene bottles (Nalgen; Nalge Nunc International), cleaned according to the methods of reference 65, and filtered through an acid-cleaned $0.22\text{-}\mu\text{m}$ pore filter (Millipak 100; Millipore). All TFe and DFe samples were acidified with HCl to a pH of <1.5 and stored at room temperature for at least 1 year. Dissolved Fe(III) in seawater samples was determined using catalytic cathodic stripping voltammetry with a detection limit of 6 pM using the approach of reference 70. No contamination during sampling and incubation was detected.

Flow cytometry. Flow cytometry (FCM) identified *Prochlorococcus*, *Synechococcus*, picoeukaryotes, and *Crocospaera* 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 until analysis on land by flow cytometry (PAS-III; Partec, GmbH, Münster, Germany) equipped with a 488-nm argon-ion excitation laser (100 mW). We recorded forward- and side-angle scatter (FSC and SSC), red fluorescence (>630 nm; FL3), and orange fluorescence (570 to 610 nm; FL2). FloMax (Partec, GmbH, Münster, Germany) distinguished *Synechococcus*, *Prochlorococcus*, *Crocospaera*, and picoeukaryotes based on their autofluorescence properties and their size (61). The instrument settings were standardized for fluorescence intensity and size by using 1.75-, 2.0-, 3.0-, 6.0-, and $10\text{-}\mu\text{m}$ standard monodisperse polystyrene beads (Polysciences) (see Fig. S8 in the supplemental material).

Gene analysis. We collected DNA samples from each treatment of the bioassay and collected aliquots of 0.5 to 1.0 L of sample on $0.2\text{-}\mu\text{m}$ SUPOR polyethersulfone membrane filters, which we then placed in sterile tubes containing glass beads, froze in liquid N₂, and stored at -80°C until further analysis. DNA was extracted according to reference 71 to determine the abundance of *Crocospaera watsonii* by quantitative PCR (qPCR) using a 5' nuclease assay as described in reference 72.

Quantitative PCR showed that cell densities of FCM-identified *Crocospaera* were significantly positively correlated with *nifH* gene copies used to quantify the proportion of *Crocospaera*, indicating that *nifH* abundance accounted for 68% of the variation in FCM-identified *Crocospaera* ($r^2 = 0.463$, $n = 48$, $P = 0.001$; Pearson product moment correlation). Therefore, this study treated FCM-identified *Crocospaera* as diazotroph *Crocospaera*. Cell abundance estimated by qPCR was 0.63 ± 0.23 -fold lower than that measured by FCM.

Nitrogen fixation. To measure *in situ* N₂ fixation activity, we used the acetylene reduction assay of references 73, 74. We dispensed a total of 550-ml bioassay samples into 1,200 mL HCl-rinsed glass polyethylene terephthalate modified with glycol (PETG) bottles with 6 replicates and sealed with butyl rubber stoppers. Aliquots of 120 mL of acetylene (99.9999% [vol/vol]; Koatsu Gas Kogyo, Japan) were injected through the stopper by replacing the same volume of headspace. After 24 h in the on-deck flowthrough seawater tanks, we analyzed ethylene concentrations by converting the ethylene to fixed nitrogen with a molar ratio of 4:1 (75).

Cellular C and N estimation. First, we estimated cell size and cell volume based on forward-angle scatter data obtained by flow cytometry following reference 76. Then, we used a conversion factor of $235\text{ fg C } \mu\text{m}^{-3}$ for *Prochlorococcus*, *Synechococcus*, and *Crocospaera* (76) to estimate cellular carbon content. For picoeukaryotes, we represented cell volume by converting it into carbon per cell using a modified Strathmann equation (77) as follows:

$$\log C(\text{pg/cell}) = 0.94 \times \log \text{Vol}(\mu\text{m}^3) - 0.6$$

Then, using an earlier reported C/N ratio (C/N ratio = 9.1 for *Prochlorococcus*, 8.6 for *Synechococcus*, 8.7 for *Crocospaera*, 6.6 for picoeukaryotes), we converted the cellular C content into cellular N (21, 78, 79).

Statistical analysis. Phytoplankton cell densities of each bioassay were first compared between treatments using repeated measures analysis of variance (RM-ANOVA) with nutrient treatments as a between-subjects factor (5 levels) and time (4 levels) as a within-subjects factor. Treatment effects were considered significant if P was <0.05 . Then, the means of five treatments were compared by *post hoc* Tukey test ($n = 3$ replicates per treatment throughout; degrees of freedom = 40). An outlier value in Table S1 in the supplemental material was selected following Smirnov-Grubbs's test ($\alpha = 0.05$).

Quantitative model of microbial growth. To quantitatively analyze the fitness of *Crocospaera* under N-limiting conditions, we ran two simulations. One was to represent the incubation experiment to extract parameters manually, and the other was the simple ecosystem model to simulate their competitiveness under different nutrient concentrations and scenarios. The list of parameters and used values can be found in Tables S4 and S8 in the supplemental material, respectively.

(i) Simulation of the incubation experiment. We used the following equations for the growth of phytoplankton to represent the field incubation experiment:

$$\frac{dN_i}{dt} = \mu_i N_i - m_i N_i \quad (1)$$

where N_i (nmol L⁻¹) is the cellular nitrogen concentration of phytoplankton i ($i = \text{Cro}, \text{Oth}$: *Crocospaera* and other phytoplankton, respectively) per volume water, t (d) is time, μ_i (d⁻¹) is the growth rate of phytoplankton i , and m_i (d⁻¹) is a mortality rate of phytoplankton i .

To represent the growth of *Crocospaera* and other phytoplankton, we used simple growth equations based on the sum of Monod kinetics (80) for each nutrient.

$$\mu_i = V_{Max,i}^{NH_4} \frac{[NH_4^+]}{[NH_4^+] + K_i^{NH_4}} + V_{Max,i}^{NO_3} \frac{[NO_3^-]}{[NO_3^-] + K_i^{NO_3}} \quad (2)$$

$V_{Max,i}^{NH_4}$ and $V_{Max,i}^{NO_3}$ (d⁻¹) are the maximum uptake rate of phytoplankton for NH₄⁺ and NO₃⁻, respectively, $[j]$ (nmol L⁻¹) is the concentration of nutrient j ($j = NO_3^-$, NH₄⁺), and $K_i^{NH_4}$ and $K_i^{NO_3}$ (nmol L⁻¹) are the half-saturation constants of nutrient for phytoplankton i , respectively. We used the data-fitted quadratic curve of nutrient concentrations (see Fig. S6 in the supplemental material).

(ii) Simple ecosystem simulation. To simulate the simple ecosystem situation, we introduced the grazing by zooplankton as follows:

$$\frac{dN_i}{dt} = \mu_i N_i - G_i N_{Zoo} \quad (3)$$

$$\frac{dN_{Zoo}}{dt} = (G_{Cro} + G_{Oth}) N_{Zoo} - m_{Zoo} N_{Zoo}^2 \quad (4)$$

where G_i (d⁻¹) is the grazing rate of phytoplankton i by zooplankton, N_{Zoo} (nmol L⁻¹) is the nitrogen concentration in zooplankton per volume water, and m_{Zoo} (d⁻²) is the quadratic mortality rate of zooplankton. When we allow nitrogen fixation, we used $\mu_{Cro} = 0.31$ (d⁻¹) (a typical growth rate under diazotrophic conditions) (81) if the computation based on equation 2 yielded a value below 0.31 (d⁻¹).

For G_i we have applied the KTW method (28) as follows:

$$G_i = G_{max} \left(\frac{N_i^2}{N_{Cro}^2 + N_{Oth}^2} \right) \left(\frac{(N_{Cro} + N_{Oth})^2}{(N_{Cro} + N_{Oth})^2 + K_G^2} \right) \quad (5)$$

where G_{max} (d⁻¹) is the maximum grazing rate and K_G (nmol L⁻¹) is grazing half saturation. We chose this method since the equation reflects the commonly observed prey-switching behavior of zooplankton (29–31), which stabilizes ecosystems (82, 83). This method allows a diverse phytoplankton to coexist (84) as observed in nature.

Code availability. The model developed in this paper has been uploaded in GitHub/Zenodo and is freely available at <https://zenodo.org/record/5095790>.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 2.3 MB.

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T. Masuda, K. Furuya, and S. Takeda designed the *in situ* microcosm experiments; T. Masuda, T. Kodama, T. Shiozaki, S. Kitajima, and T. Matsui carried out the experiment and analyzed data supervised by K. Furuya, S. Takeda, M. Sato, and K. Suzuki; T. Masuda and K. Inomura shaped the concept of the study with the supervision of O. Prášil; K. Inomura and G. Armin developed and ran the model. T. Masuda and K. Inomura wrote the original draft with substantial input from all of the authors.

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

REFERENCES

- Field C, Behrenfeld M, Randerson J, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* 281:237–240. <https://doi.org/10.1126/science.281.5374.237>.
- Arrigo KR, Robinson DH, Worthen DL, Dunbar RB, DiTullio GR, VanWoert M, Lizotte MP. 1999. Phytoplankton community structure and the drawdown of nutrients and CO₂ in the southern ocean. *Science* 283:365–367. <https://doi.org/10.1126/science.283.5400.365>.
- Richardson TL, Jackson GA. 2007. Small phytoplankton and carbon export from the surface ocean. *Science* 315:838–840. <https://doi.org/10.1126/science.1133471>.

4. Thomas MK, Kremer CT, Klausmeier CA, Litchman E. 2012. A global pattern of thermal adaptation in marine phytoplankton. *Science* 338:1085–1088. <https://doi.org/10.1126/science.1224836>.
5. Le Quéré C, Harrison SP, Prentice IC, Buitenhuis ET, Aumont O, Bopp L, Claustre H, Da Cunha LC, Geider R, Giraud X, Klaas C, Kohfeld KE, Legendre L, Manizza M, Platt T, Rivkin RB, Sathyendranath S, Uitz J, Watson AJ, Wolf-Gladrow D. 2005. Ecosystem dynamics based on plankton functional types for global ocean biogeochemistry models. *Glob Change Biol* 11:2016–2040.
6. Raven JA, Giordano M. 2016. Combined nitrogen, p 143–154. In Borowitzka MA, Beardall J, Raven JA (ed), *The physiology of microalgae*. Springer, New York, NY.
7. Moore CM, Mills MM, Arrigo KR, Berman-Frank I, Bopp L, Boyd PW, Galbraith ED, Geider RJ, Guieu C, Jaccard SL, Jickells TD, La Roche J, Lenton TM, Mahowald NM, Marañón E, Marinov I, Moore JK, Nakatsuka T, Oschlies A, Saito MA, Thingstad TF, Tsuda A, Ulloa O. 2013. Processes and patterns of oceanic nutrient inventories. *Nature Geosci* 6:701–710. <https://doi.org/10.1038/ngeo1765>.
8. Moore JK, Doney SC, Lindsay K. 2004. Upper ocean ecosystem dynamics and iron cycling in a global three-dimensional model. *Global Biogeochem Cycles* 18:GB4028.
9. Monteiro FM, Dutkiewicz S, Follows MJ. 2011. Biogeographical controls on the marine nitrogen fixers. *Global Biogeochem Cycles* 25. <https://doi.org/10.1029/2010GB003902>.
10. Stukel MR, Coles VJ, Brooks MT, Hood RR. 2014. Top-down, bottom-up and physical controls on diatom-diazotroph assemblage growth in the Amazon River plume. *Biogeosciences* 11:3259–3278. <https://doi.org/10.5194/bg-11-3259-2014>.
11. Falkowski PG. 1997. Evolution of the nitrogen cycle and its influence on the biological sequestration of CO₂ in the ocean. *Nature* 387:272–275. <https://doi.org/10.1038/387272a0>.
12. Tyrrell T. 1999. The relative influences of nitrogen and phosphorus on oceanic primary production. *Nature* 400:525–531. <https://doi.org/10.1038/22941>.
13. Monteiro FM, Follows MJ, Dutkiewicz S. 2010. Distribution of diverse nitrogen fixers in the global ocean. *Global Biogeochem Cycles* 24:GB3017.
14. Saito MA, Bertrand EM, Dutkiewicz S, Bulgin VV, Moran DM, Monteiro FM, Follows MJ, Valois FW, Waterbury JB. 2011. Iron conservation by reduction of metalloenzyme inventories in the marine diazotroph *Crocospaera watsonii*. *Proc Natl Acad Sci U S A* 108:2184–2189. <https://doi.org/10.1073/pnas.1006943108>.
15. Zehr JP, Waterbury JB, Turner PJ, Montoya JP, Omoregie E, Steward GF, Hansen A, Karl DM. 2001. Unicellular cyanobacteria fix N₂ in the subtropical North Pacific Ocean. *Nature* 412:635–638. <https://doi.org/10.1038/35088063>.
16. Moisaner PH, Beinart RA, Hewson I, White AE, Johnson KS, Carlson CA, Montoya JP, Zehr JP. 2010. Unicellular cyanobacterial distributions broaden the oceanic N₂ fixation domain. *Science* 327:1512–1514. <https://doi.org/10.1126/science.1185468>.
17. Waterbury JB, Watson SW, Guillard RRL, Brand LE. 1979. Widespread occurrence of a unicellular, marine, planktonic, cyanobacterium. *Nature* 277:293–294. <https://doi.org/10.1038/277293a0>.
18. Platt T, Rao DVS, Irwin B. 1983. Photosynthesis of picoplankton in the oligotrophic ocean. *Nature* 301:702–704. <https://doi.org/10.1038/301702a0>.
19. Buitenhuis ET, Li WKW, Vulot D, Lomas MW, Landry MR, Partensky F, Karl DM, Ulloa O, Campbell L, Jacquet S, Lantoiné F, Chavez F, Macias D, Gosselin M, McManus GB. 2012. Picophytoplankton biomass distribution in the global ocean. *Earth Syst Sci Data* 4:37–46. <https://doi.org/10.5194/essd-4-37-2012>.
20. Dekaezemaeker J, Bonnet S. 2011. Sensitivity of N₂ fixation to combined nitrogen forms (NO₃⁻ and NH₄⁺) in two strains of the marine diazotroph *Crocospaera watsonii* (Cyanobacteria). *Mar Ecol Prog Ser* 438:33–46. <https://doi.org/10.3354/meps09297>.
21. Masuda T, Furuya K, Kodama T, Takeda S, Harrison PJ. 2013. Ammonium uptake and dinitrogen fixation by the unicellular nancyanobacterium *Crocospaera watsoniini* nitrogen-limited continuous cultures. *Limnol Oceanogr* 58:2029–2036. <https://doi.org/10.4319/lo.2013.58.6.2029>.
22. Holl CM, Montoya JP. 2005. Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (Cyanobacteria). *J Phycology* 41:1178–1183. <https://doi.org/10.1111/j.1529-8817.2005.00146.x>.
23. Inomura K, Masuda T, Gauglitz JM. 2019. Active nitrogen fixation by *Crocospaera* expands their niche despite the presence of ammonium - a case study. *Sci Rep* 9:15064. <https://doi.org/10.1038/s41598-019-51378-4>.
24. Inomura K, Deutsch C, Masuda T, Prášil O, Follows MJ. 2020. Quantitative models of nitrogen-fixing organisms. *Comput Struct Biotechnol J* 18:3905–3924. <https://doi.org/10.1016/j.csbj.2020.11.022>.
25. Shiozaki T, Kodama T, Kitajima S, Sato M, Furuya K. 2013. Advective transport of diazotrophs and importance of their nitrogen fixation on new and primary production in the western Pacific warm pool. *Limnol Oceanogr* 58:49–60. <https://doi.org/10.4319/lo.2013.58.1.0049>.
26. Glover HE, Garside C, Trees CC. 2007. Physiological responses of Sargasso Sea picoplankton to nanomolar nitrate perturbations. *J Plankton Res* 29:263–274. <https://doi.org/10.1093/plankt/fbm013>.
27. Droop MR. 1974. The nutrient status of algal cells in continuous culture. *J Mar Biol Ass* 54:825–855. <https://doi.org/10.1017/S002531540005760X>.
28. Vallina SM, Ward BA, Dutkiewicz S, Follows MJ. 2014. Maximal feeding with active prey-switching: a kill-the-winner functional response and its effect on global diversity and biogeography. *Prog Oceanogr* 120:93–109. <https://doi.org/10.1016/j.pocean.2013.08.001>.
29. Murdoch WW. 1969. Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecol Monogr* 39:335–354. <https://doi.org/10.2307/1942352>.
30. Kiørboe T, Saiz E, Viitasalo M. 1996. Prey switching behaviour in the planktonic copepod *Acartia tonsa*. *Mar Ecol Prog Ser* 143:65–75. <https://doi.org/10.3354/meps143065>.
31. Kalinkat G, Rall BC, Vucic-Pestic O, Brose U. 2011. The allometry of prey preferences. *PLoS One* 6:e25937. <https://doi.org/10.1371/journal.pone.0025937>.
32. Ackleson SG, Spinrad RW. 1988. Size and refractive index of individual marine particulates: a flow cytometric approach. *Appl Opt* 27:1270–1277. <https://doi.org/10.1364/AO.27.001270>.
33. Zubkov MV, Sleigh MA, Tarran GA, Burkill PH, Leakey RJG. 1998. Picoplanktonic community structure on an Atlantic transect from 50°N to 50°S. *Deep Sea Res Part I Oceanogr Res Papers* 45:1339–1355. [https://doi.org/10.1016/S0967-0637\(98\)00015-6](https://doi.org/10.1016/S0967-0637(98)00015-6).
34. Campbell L, Nolla HA, Vulot D. 1994. The importance of *Prochlorococcus* to community structure in the central North Pacific Ocean. *Limnol Oceanogr* 39:954–961. <https://doi.org/10.4319/lo.1994.39.4.0954>.
35. Shalapyonok A, Olson RJ, Shalapyonok LS. 1998. Ultradian growth in *Prochlorococcus* spp. *Appl Environ Microbiol* 64:1066–1069. <https://doi.org/10.1128/AEM.64.3.1066-1069.1998>.
36. Thingstad TF, Krom MD, Mantoura RFC, Flaten GAF, Groom S, Herut B, Kress N, Law CS, Pasternak A, Pitta P, Psarra S, Rassoulzadegan F, Tanaka T, Tselepidis A, Wassmann P, Woodward EMS, Riser CW, Zodiatis G, Zohary T. 2005. Nature of phosphorus limitation in the ultraoligotrophic Eastern Mediterranean. *Science* 309:1068–1071. <https://doi.org/10.1126/science.1112632>.
37. Stockner JG, Klut ME, Cochlan WP. 1990. Leaky filters: a warning to aquatic ecologists. *Can J Fish Aquat Sci* 47:16–23. <https://doi.org/10.1139/f90-002>.
38. Bombar D, Paerl RW, Anderson R, Riemann L. 2018. Filtration via conventional glass fiber filters in 15N₂ tracer assays fails to capture all nitrogen-fixing prokaryotes. *Front Mar Sci* 5. <https://doi.org/10.3389/fmars.2018.00006>.
39. Webb EA, Ehrenreich IM, Brown SL, Valois FW, Waterbury JB. 2009. Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocospaera watsonii*, isolated from the open ocean. *Environ Microbiol* 11:338–348. <https://doi.org/10.1111/j.1462-2920.2008.01771.x>.
40. Dron A, Rabouille S, Clauquin P, Chang P, Raimbault V, Talec A, Sciadra A. 2012. Lightdark (12:12 h) quantification of carbohydrate fluxes in *Crocospaera watsonii*. *Aquat Microb Ecol* 68:43–55. <https://doi.org/10.3354/ame01600>.
41. Shiozaki T, Bombar D, Riemann L, Sato M, Hashihama F, Kodama T, Tanita I, Takeda S, Saito H, Hamasaki K, Furuya K. 2018. Linkage between dinitrogen fixation and primary production in the oligotrophic South Pacific Ocean. *Global Biogeochem Cycles* 32:1028–1044. <https://doi.org/10.1029/2017GB005869>.
42. Thompson AW, Foster RA, Krupke A, Carter BJ, Musat N, Vulot D, Kuypers MM, Zehr JP. 2012. Unicellular cyanobacterium symbiotic with a single-celled eukaryotic alga. *Science* 337:1546–1550. <https://doi.org/10.1126/science.1222700>.
43. Hagino K, Onuma R, Kawachi M, Horiguchi T. 2013. Discovery of an endosymbiotic nitrogen-fixing cyanobacterium UCYN-A in *Braarudosphaera bigelowii* (Prymnesiophyceae). *PLoS One* 8:e81749. <https://doi.org/10.1371/journal.pone.0081749>.
44. Zehr JP, Shilova IN, Farnelid HM, Munoz-Marin MD, Turk-Kubo KA. 2016. Unusual marine unicellular symbiosis with the nitrogen-fixing cyanobacterium UCYN-A. *Nat Microbiol* 2:16214. <https://doi.org/10.1038/nmicrobiol.2016.214>.
45. Mills MM, Turk-Kubo KA, van Dijken GL, Henke BA, Harding K, Wilson ST, Arrigo KR, Zehr JP. 2020. Unusual marine cyanobacteria/haptophyte symbiosis relies on N₂ fixation even in N-rich environments. *ISME J* 14:2395–2406. <https://doi.org/10.1038/s41396-020-0691-6>.
46. Shiozaki T, Fujiwara A, Inomura K, Hirose Y, Hashihama F, Harada N. 2020. Biological nitrogen fixation detected under Antarctic sea ice. *Nat Geosci* 13:729–732. <https://doi.org/10.1038/s41561-020-00651-7>.

47. Wilson ST, Aylward FO, Ribalet F, Barone B, Casey JR, Connell PE, Eppley JM, Ferron S, Fitzsimmons JN, Hayes CT, Romano AE, Turk-Kubo KA, Vislova A, Armbrust EV, Caron DA, Church MJ, Zehr JP, Karl DM, DeLong EF. 2017. Coordinated regulation of growth, activity and transcription in natural populations of the unicellular nitrogen-fixing cyanobacterium *Crocospaera*. *Nat Microbiol* 2:17118. <https://doi.org/10.1038/nmicrobiol.2017.118>.
48. Dugenne M, Henderikx Freitas F, Wilson ST, Karl DM, White AE. 2020. Life and death of *Crocospaera* sp. in the Pacific Ocean: fine scale predator-prey dynamics. *Limnol Oceanogr* 65:2603–2617. <https://doi.org/10.1002/lno.11473>.
49. Hawser SP, O'Neil JM, Roman MR, Codd GA. 1992. Toxicity of blooms of the cyanobacterium *Trichodesmium* to zooplankton. *J Appl Phycol* 4: 79–86. <https://doi.org/10.1007/BF00003963>.
50. Proença L, Tamanaha M, Fonseca R. 2009. Screening the toxicity and toxin content of blooms of the cyanobacterium *Trichodesmium erythraeum* (EHRENBERG) in northeast Brazil. *J Venom Anim Toxins Incl Trop Dis* 15: 204–215. <https://doi.org/10.1590/S1678-91992009000200004>.
51. Kerbrat AS, Darius HT, Pauillac S, Chinain M, Laurent D. 2010. Detection of ciguatoxin-like and paralyzing toxins in *Trichodesmium* spp. from New Caledonia lagoon. *Mar Pollut Bull* 61:360–366. <https://doi.org/10.1016/j.marpolbul.2010.06.017>.
52. LaRoche J, Breitbarth E. 2005. Importance of the diazotrophs as a source of new nitrogen in the ocean. *J Sea Res* 53:67–91. <https://doi.org/10.1016/j.seares.2004.05.005>.
53. Pereira N, Shilova IN, Zehr JP. 2019. Use of the high-affinity phosphate transporter gene, *pstS*, as an indicator for phosphorus stress in the marine diazotroph *Crocospaera watsonii* (Chroococcales, Cyanobacteria). *J Phycol* 55:752–761. <https://doi.org/10.1111/jpy.12863>.
54. Rabouille S, Tournier L, Duhamel S, Claquin P, Crispi O, Talec A, Landolfi A, Oschlies A. 2022. Organic phosphorus scavenging supports efficient growth of diazotrophic Cyanobacteria under phosphate depletion. *Front Microbiol* 13:848647. <https://doi.org/10.3389/fmicb.2022.848647>.
55. Yamaguchi T, Sato M, Gonda N, Takahashi K, Furuya K. 2020. Phosphate diester utilization by marine diazotrophs *Trichodesmium erythraeum* and *Crocospaera watsonii*. *Aquat Microb Ecol* 85:211–218. <https://doi.org/10.3354/ame01951>.
56. Dyhrman ST, Haley ST. 2006. Phosphorus scavenging in the unicellular marine diazotroph *Crocospaera watsonii*. *Appl Environ Microbiol* 72: 1452–1458. <https://doi.org/10.1128/AEM.72.2.1452-1458.2006>.
57. Jacq V, Ridame C, L'Helguen S, Kaczmar F, Saliot A. 2014. Response of the unicellular diazotrophic cyanobacterium *Crocospaera watsonii* to iron limitation. *PLoS One* 9:e86749. <https://doi.org/10.1371/journal.pone.0086749>.
58. Van Mooy BA, Fredricks HF, Pedler BE, Dyhrman ST, Karl DM, Kobalzek M, Lomas MW, Mincer TJ, Moore LR, Moutin T, Rappe MS, Webb EA. 2009. Phytoplankton in the ocean use non-phosphorus lipids in response to phosphorus scarcity. *Nature* 458:69–72. <https://doi.org/10.1038/nature07659>.
59. Pereira N, Shilova IN, Zehr JP. 2016. Molecular markers define progressing stages of phosphorus limitation in the nitrogen-fixing cyanobacterium, *Crocospaera*. *J Phycol* 52:274–282. <https://doi.org/10.1111/jpy.12396>.
60. Montoya JP, Holl CMZJP, Hansen A, Villareal TA, Capone DG. 2004. High rates of N₂ fixation by unicellular diazotrophs in the oligotrophic Pacific Ocean. *Nature* 430:1027–1031. <https://doi.org/10.1038/nature02824>.
61. Sato M, Hashihama F, Kitajima S, Takeda S, Furuya K. 2010. Distribution of nano-sized Cyanobacteria in the western and central Pacific Ocean. *Aquat Microb Ecol* 59:273–282. <https://doi.org/10.3354/ame01397>.
62. Sohm JA, Subramaniam A, Gunderson TE, Carpenter EJ, Capone DG. 2011. Nitrogen fixation by *Trichodesmium* spp. and unicellular diazotrophs in the North Pacific subtropical gyre. *J Geophys Res* 116. <https://doi.org/10.1029/2010JG001513>.
63. Sargent EC, Hitchcock A, Johansson SA, Langlois R, Moore CM, LaRoche J, Poulton AJ, Bibby TS. 2016. Evidence for polyploidy in the globally important diazotroph *Trichodesmium*. *FEMS Microbiol Lett* 363:fnw244. <https://doi.org/10.1093/femsle/fnw244>.
64. Pierella Karlusich JJ, Pelletier E, Lombard F, Carsique M, Dvorak E, Colin S, Picheral M, Cornejo-Castillo FM, Acinas SG, Pepperkok R, Karsenti E, de Vargas C, Wincker P, Bowler C, Foster RA. 2021. Global distribution patterns of marine nitrogen-fixers by imaging and molecular methods. *Nat Commun* 12:4160. <https://doi.org/10.1038/s41467-021-24299-y>.
65. Takeda S, Obata H. 1995. Response of equatorial Pacific phytoplankton to subnanomolar Fe enrichment. *Mar Chem* 50:219–227. [https://doi.org/10.1016/0304-4203\(95\)00037-R](https://doi.org/10.1016/0304-4203(95)00037-R).
66. Obata H, Karatani H, Nakayama E. 1993. Automated determination of iron in seawater by chelating resin concentration and chemiluminescence detection. *Anal Chem* 65:1524–1528. <https://doi.org/10.1021/ac00059a007>.
67. Wu J, Sunda W, Boyle EA, Karl DM. 2000. Phosphate depletion in the western North Atlantic Ocean. *Science* 289:759–762. <https://doi.org/10.1126/science.289.5480.759>.
68. Karl DM, Björkman KM, Dore JE, Fujieki L, Hebel DV, Houlihan T, Letelier RM, Tupas LM. 2001. Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. *Deep Sea Res II* 48:1529–1566. [https://doi.org/10.1016/S0967-0645\(00\)00152-1](https://doi.org/10.1016/S0967-0645(00)00152-1).
69. Price NM, Harrison PJ. 1987. Comparison of methods for the analysis of dissolved urea in seawater. *Mar Biol* 94:307–317. <https://doi.org/10.1007/BF00392945>.
70. Laglera LM, Santos-Echeandia J, Caprara S, Monticelli D. 2013. Quantification of iron in seawater at the low picomolar range based on optimization of bromate/ammonia/dihydroxynaphtalene system by catalytic adsorptive cathodic stripping voltammetry. *Anal Chem* 85:2486–2492. <https://doi.org/10.1021/ac303621q>.
71. Short SM, Zehr JP. 2005. Quantitative analysis of *nifH* genes and transcripts from aquatic environments. *Methods Enzymol* 397:380–394. [https://doi.org/10.1016/S0076-6879\(05\)97023-7](https://doi.org/10.1016/S0076-6879(05)97023-7).
72. Church MJ, Jenkins BD, Karl DM, Zehr JP. 2005. Vertical distributions of nitrogen-fixing phylotypes at Stn ALOHA in the oligotrophic North Pacific Ocean. *Aquat Microb Ecol* 38:3–14. <https://doi.org/10.3354/ame038003>.
73. Capone DG, Montoya JP. 2001. Nitrogen fixation and denitrification. *Methods Microbiol* 30:501–515. [https://doi.org/10.1016/S0580-9517\(01\)30060-0](https://doi.org/10.1016/S0580-9517(01)30060-0).
74. Kitajima S, Furuya K, Hashihama F, Takeda S, Kanda J. 2009. Latitudinal distribution of diazotrophs and their nitrogen fixation in the tropical and subtropical western North Pacific. *Limnol Oceanogr* 54:537–547. <https://doi.org/10.4319/lo.2009.54.2.0537>.
75. Montoya JP, Voss M, Kahler P, Capone DG. 1996. A simple, high-precision, high-sensitivity tracer assay for N₂ fixation. *Appl Environ Microbiol* 62: 986–993. <https://doi.org/10.1128/aem.62.3.986-993.1996>.
76. Shalapyonok A, Olson RJ, Shalapyonok LS. 2001. Arabian Sea phytoplankton during Southwest and Northeast Monsoons 1995: composition, size structure and biomass from individual cell properties measured by flow cytometry. *Deep Sea Res II* 48:12231–12261.
77. Eppley RW, Reid FM, Strickland JDH. 1970. Estimates of phytoplankton crop size, growth rate and primary production off La Jolla, CA in the period April through September 1967, p 33–42. *In* Strickland JDH (ed), *Bulletin of the Scripps Institution of Oceanography*. Scripps Institution of Oceanography, San Diego, CA.
78. Heldal M, Scanlan DJ, Norland S, Thingstad F, Mann NH. 2003. Elemental composition of single cells of various strains of marine *Prochlorococcus* and *Synechococcus* using X-ray microanalysis. *Limnol Oceanogr* 48: 1732–1743. <https://doi.org/10.4319/lo.2003.48.5.1732>.
79. Redfield AC. 1958. The biological control of chemical factors in the environment. *Am Sci* 45:205–221.
80. Monod J. 1949. The growth of bacterial cultures. *Annu Rev Microbiol* 3: 371–394. <https://doi.org/10.1146/annurev.mi.03.100149.002103>.
81. Follett CL, Dutkiewicz S, Karl DM, Inomura K, Follows MJ. 2018. Seasonal resource conditions favor a summertime increase in North Pacific diatom-diazotroph associations. *ISME J* 12:1543–1557. <https://doi.org/10.1038/s41396-017-0012-x>.
82. Murdoch WW, Avery S, Smyth MEB. 1975. Switching in predatory fish. *Ecology* 56:1094–1105. <https://doi.org/10.2307/1936149>.
83. Morozov AY. 2010. Emergence of Holling type III zooplankton functional response: bringing together field evidence and mathematical modelling. *J Theor Biol* 265:45–54. <https://doi.org/10.1016/j.jtbi.2010.04.016>.
84. Vallina SM, Follows MJ, Dutkiewicz S, Montoya JM, Cermeno P, Loreau M. 2014. Global relationship between phytoplankton diversity and productivity in the ocean. *Nat Commun* 5:4299. <https://doi.org/10.1038/ncomms5299>.