LIMNOLOGY AND OCEANOGRAPHY
Letters

ASLO

Open Access

Limnology and Oceanography Letters 9, 2024, 296–306

doi: 10.1002/lol2.10380

LETTER

Dissolved organic matter offsets the detrimental effects of climate change in the nitrogen-fixing cyanobacterium *Crocosphaera*

Alba Filella,[1,2] Jacqueline Umbricht,[3] Angelina Klett,[3] Angela Vogts,[3] Thomas Vannier,[2] Olivier Grosso,[1] Maren Voss,[3] Lasse Riemann,[4] Mar Benavides[1,2]*

[1]Aix Marseille Univ, Université de Toulon, CNRS, IRD, MIO UM 110, Marseille, France; [2]Turing Center for Living Systems, Aix-Marseille University, Marseille, France; [3]Biological Oceanography, Leibniz Institute for Baltic Sea Research, Warnemünde, Germany; [4]Marine Biological Section, Department of Biology, University of Copenhagen, Copenhagen, Denmark

Scientific Significance Statement

Diazotrophs fix dinitrogen into bioavailable nitrogen, sustaining ocean primary productivity. Increasing anthropogenic CO_2 emissions decrease seawater pH and induce warming enhancing water column stratification. This will reduce inorganic nutrient availability, forcing microbial communities to obtain resources from dissolved organic matter instead. While the source of nitrogen for diazotrophs is inexhaustible, their ability to use dissolved organic matter (DOM) may influence their performance in the future warm and acidified ocean. Here, we examine the response of *Crocosphaera* to DOM additions under three climate change scenarios. Our findings show that the availability of dissolved organic matter helps diazotrophs facing energetic stress under high temperature and low pH, allowing a more balanced metabolism and a winning strategy to survive in the future warm and acidified ocean.

Abstract

Diazotrophs provide a significant reactive nitrogen source in the ocean. Increased warming and stratification may decrease nutrient availability in the future, forcing microbial communities toward using dissolved organic matter (DOM). Not depending on reactive nitrogen availability, diazotrophs may be "winners" in a nutrient-depleted ocean. However, their ability to exploit DOM may influence this success. We exposed cultures of the widespread *Crocosphaera* to low (26°C, pH 8.1), moderate (28°C, pH 8.0), and extreme (30°C, pH 7.9) climate

---

*Correspondence: mar.benavides@ird.fr

Associate editor: Angelicque White

Author Contribution Statement: AF, JU, MV, LR, and MB designed the experiments. AF acclimated the cultures prior to the experiments. AF, JU, and AK performed the experiments and sampling. OG ran MIMS analyses. JU and AV analyzed nanoSIMS samples. TV did the bioinformatic analyses. AF did the formal data analyses, statistics, and graphs. AF and MB wrote the manuscript with input from all co-authors.

Data Availability Statement: All the data generated by this experiment are available in the Dryad and Figshare repositories at https://doi.org/10.5061/dryad.70rxwdc5j and www.doi.org/10.6084/m9.figshare.22773503, respectively. The RNAseq data are deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE229037 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE229037). Additional Supporting Information can be found in the online version of this article. An accompanying paper evaluates the potential changes in dissolved inorganic nitrogen uptake by *Crocosphaera* under the same treatment conditions (Umbricht et al. in prep.).

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

change scenarios, under control or DOM-amended conditions. Growth was suboptimal in the low and extreme treatments and favored in the moderate treatment. DOM was preferred as a carbon source regardless of the treatment and promoted N_2 fixation in extreme conditions. This was reflected in the increased expression of photosynthesis genes to obtain energy. DOM provides *Crocosphaera* with a key ecological advantage, possibly dictating diazotroph-derived nitrogen inputs in the future ocean.

Increasing anthropogenic CO_2 emissions induce ocean warming and acidification, affecting ecosystems and biogeochemical cycling (Gruber 2011; Cheng et al. 2019; Rossi 2023). Stratification inhibits the vertical supply of nutrients impacting biological productivity and consequently biomass transfer and carbon export (Passow and Carlson 2012). In the future ocean, reduced nutrient availability may force microbial communities to depend on regenerated compounds such as dissolved organic matter (DOM; Thornton 2014; Lønborg et al. 2020). Increased CO_2 also alters the seawater carbonate system, weakening the ocean's buffering ability and reducing pH (Orr et al. 2005; Doney et al. 2009). While low pH has been generally observed to decrease phytoplankton growth (Burkhardt et al. 1999; Hinga 2002), increased CO_2 concentrations can improve the affinity of the RuBisCO enzyme enhancing primary productivity (Riebesell et al. 2007; Wu et al. 2010). In contrast, heterotrophic bacteria are more resistant to low pH and their metabolic rates tend to increase with temperature (Grossart et al. 2006; Kirchman et al. 2009; Morán et al. 2017). Thus, ecosystem functioning in the future ocean will be significantly driven by the sensitivity of different planktonic groups to changes in CO_2, pH, and temperature (Riebesell and Tortell 2011; Dutkiewicz et al. 2015; Sunagawa et al. 2015).

Prokaryotic cells called "diazotrophs" replenish the nitrogen oceanic reservoir by fixing dinitrogen (N_2), sustaining primary productivity (Zehr 2011). As the main suppliers of reactive nitrogen, the response of diazotrophs to future ocean conditions can have profound implications in productivity and the ocean's role as a climate buffer (Hutchins and Capone 2022). Diazotrophic cyanobacteria, including *Trichodesmium* and *Crocosphaera* predominate in (sub)tropical waters where temperatures range between ~ 20°C and 27°C (Breitbarth et al. 2007; Sohm et al. 2011), while the symbiotic UCYN-A and noncyanobacterial diazotrophs (NCDs) can be found across wider latitudinal ranges (polar to tropical waters; 4 to > 25°C; Martínez-Pérez et al. 2016; Harding et al. 2018; Turk-Kubo et al. 2022). Despite the diversity of diazotrophs, most climate change research has focused on *Trichodesmium* and *Crocosphaera* (e.g., Kranz et al. 2009; Garcia et al. 2013*a*; Hutchins et al. 2013). The different temperature optima of *Trichodesmium* and *Crocosphaera*, 24–28°C and 26–31°C, respectively (Webb et al. 2009; Sohm et al. 2011; Fu et al. 2014) will likely influence their biogeography and N_2 fixation activity as the ocean becomes warmer (Breitbarth et al. 2007; Hutchins et al. 2007).

Models predict altered nutrient availability changing interspecies competition, favoring diazotrophs (Dutkiewicz et al. 2021). Under increased CO_2 levels, the energy saved in intracellular carbon concentration allows diazotrophs to invest more energy in fixing N_2 (Levitan et al. 2007; Kranz et al. 2011). Previous work examined the response of *Trichodesmium* and *Crocosphaera* cultures to partial pressure of CO_2 (pCO_2) levels between 150 and 1500 ppm (e.g., Hutchins et al. 2007, 2015; Fu et al. 2008; Kranz et al. 2011). Some studies observed increased N_2 fixation from 380 to 750 ppm pCO_2 (Hutchins et al. 2007; Levitan et al. 2007; Boatman et al. 2017), but others only observed enhanced N_2 fixation when comparing much larger pCO_2 ranges (e.g., 190 vs. > 350 ppm; Kranz et al. 2009; Garcia et al. 2013*a*). These positive responses only seem to hold under high iron or light conditions (Fu et al. 2008; Garcia et al. 2013*b*). However, culture-based studies do not reflect field studies, where diazotroph assemblages show a high resilience to short-term pCO_2 perturbations (Böttjer et al. 2014; Gradoville et al. 2014*a*). In contrast, when temperature and pCO_2 increase are combined, *Trichodesmium* growth decreases if temperature exceeds the strain optimum (Boatman et al. 2017), suggesting that the interlinked climate change stressors will affect diazotrophic performance in the future ocean.

Although CO_2 and photosynthesis provide the main sources of carbon and energy for marine cyanobacteria—including diazotrophs—DOM may supply an alternative source of carbon, energy, and nutrients when facing stress (e.g., Muñoz-Marín et al. 2013). For example, the unicellular cyanobacterium *Cyanothece* can grow on glycerol (Feng et al. 2010), the filamentous *Nodularia* can assimilate leucine (Hietanen et al. 2002), and UCYN-A and *Crocosphaera* may use sugars when photosynthesis is energy-limited (Moisander et al. 2012). *Trichodesmium* and *Crocosphaera* are able to use phosphoesters (Dyhrman et al. 2002; Orchard et al. 2003), but only *Trichodesmium* obtains phosphorus from phosphonates (Dyhrman et al. 2006). *Crocosphaera* can obtain both phosphorus and energy from phosphoanhydrides (Filella et al. 2022). These studies point toward an overlooked role of osmotrophy, the uptake of DOM, for cyanobacteria survival in the future ocean. In this study, we sought to understand the potential of DOM as an alternative carbon and/or energy source for *Crocosphaera* when subjected to future temperature and pH conditions (IPCC 2014; Pachauri et al. 2014). Combining molecular, physiological, and biogeochemical techniques, we find that DOM is an important carbon source for *Crocosphaera*, compensating for the energy and stress imbalances faced under future climate change conditions. These results call for a reassessment of the role of DOM for diazotrophic cyanobacteria, as nitrogen inputs in the future stratified ocean may be closely linked to these interactions.

Methods

To provide a comprehensive assessment of the behavior of *Crocosphaera* under future ocean conditions, we combined temperature and pH levels simulating representative concentration pathways (RCP) 2.6 (26°C and pH 8.1), 4.5 (28°C and pH 8.0), and 6.0 (30°C and pH 7.9) (IPCC 2014), here called "low," "intermediate," and "extreme" treatments (10.6084/m9.figshare.22773503). Cultures of *Crocosphaera* were acclimated to the different treatments for 1 month in 500-mL bottles before the start of the experiment in larger volumes (40 liters; duplicate low and triplicate intermediate and extreme treatments) with a starting concentration of $\sim 1.5 \times 10^4$ cells mL^{-1} (Fig. 1A). The experiment was run for 8 d (here days −2 to 5) under a 12-h light : dark cycle with a light intensity of $\sim 250\ \mu$mol photons s^{-1}. The temperature and pH levels of each treatment were maintained constant along the experiment using an IKS aquastar system (Supporting Information). Temperature and salinity were measured daily with a YSI Pro30 probe, while pH was measured spectrophotometrically (Supporting Information). Cell abundance and nutrient concentrations were measured daily using standard microscopy and colorimetry methods (Supporting Information). On alternate days (days 0, 2 and 4), subvolumes were sampled from each treatment and incubated to measure single-cell N_2 and dissolved inorganic carbon (DIC) fixation using dissolved $^{15}N_2$ and $^{13}C\text{-}HCO_3$, respectively. Similarly, a mix of ^{13}C-labeled organic compounds representative of marine DOM including the amino acids alanine, leucine, and arginine, and the carbohydrates glucose and *N*-acetylglucosamine was used to measure DOM uptake rates and RNA expression responses (*see* Supporting Information). RNA was sequenced using an Illumina NovaSeq 6000 platform generating more than 30 million paired end reads per sample (2 × 50 bp). RNA reads were annotated using Diamond and the databases Kofam UniProt's Swissprot, trEMBL, and NCBI's RefSeq (*see* Supporting Information).

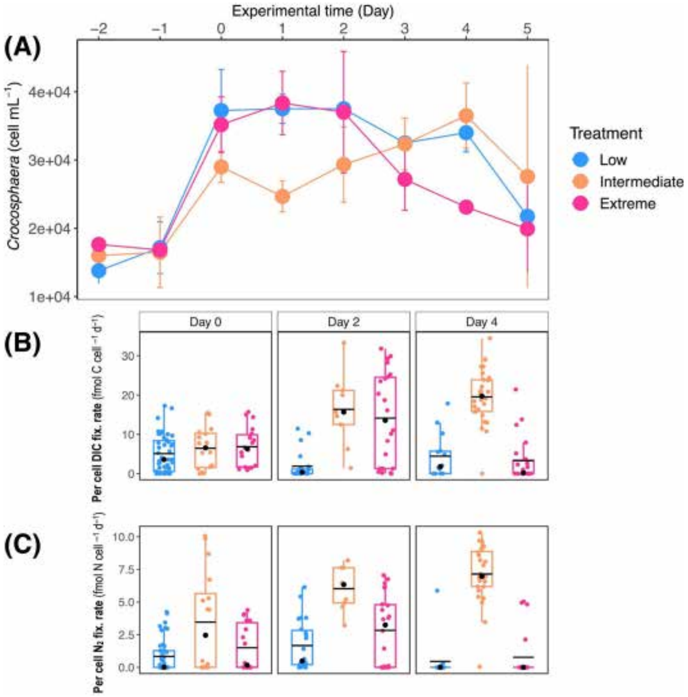

Fig. 1. (**A**) *Crocosphaera* cell abundance in the low, intermediate, and extreme treatments along the experiment, where each dot represents the average, and the error bars the standard deviation of analytical duplicates in each treatment replicate. (**B**) Single-cell DIC and (**C**) N_2 fixation rates in the different treatments on days 0, 2, and 4, where each dot represents the uptake rate for single cells and boxplots show the 25th to 75th percentile, error bars the minimum and maximum percentile and the black dot and crossbar the median and mean values, respectively. Negative day values (days −2 to 0) correspond to the post-acclimation period, when *Crocosphaera* cells are transferred from concentrated cultures to the larger tanks, while positive day values (days 0–5) correspond to the experimental period.

Results and discussion

Responses to combined temperature and pH treatments

Temperature and pH remained close to the target values in all treatments (10.6084/m9.figshare.22773503). Nutrient conditions were similar between treatments and favorable for diazotrophic growth, indicating that any responses in growth, DIC and N_2 fixation can be confidently attributed to the different treatments (Supporting Information). Cells grown in batch incubations without continued nutrient additions, slowly consume limiting elements contained in the culture medium (YBC-II), including vitamins and iron which cause cultures to end collapsing.

Crocosphaera grew exponentially from day −2 until day 0 in all treatments, increasing from ~ 1.5 to $>2.7 \times 10^4$ cell mL^{-1} (Fig. 1A). Growth in the low and extreme treatments reached a plateau between days 0 and 2, then decreased until day 5 (Fig. 1A). Conversely, cells in the intermediate treatment continued growing until day 4 and only started decaying after. The intermediate treatment thus was the most beneficial for *Crocosphaera*, in agreement with previous studies where the thermal range of *Crocosphaera* oscillates between 26°C and 31°C and optimal growth is ~ 28°C (Waterbury and Willey 1988; Falcón et al. 2005; Webb et al. 2009; Fu et al. 2014).

DIC and N_2 fixation rates differed among treatments (ANOVA; $p < 0.001$; Supporting Information Fig. S1), ranging between 0 and 34.4 fmol C $cell^{-1}\ d^{-1}$ and between 0 and 10.3 fmol N $cell^{-1}\ d^{-1}$, respectively (Fig. 1B), in the range of previous field measurements at similar temperatures (i.e., ~ 26–30°C; Foster et al. 2013; Bonnet et al. 2018; Benavides et al. 2022). While neither DIC nor N_2 fixation rates varied significantly with time in the low treatment, rates increased on day 2 in the other two treatments (Fig. 1B,C). However, on day 4 both rates increased in the intermediate treatment where cells were actively growing but decreased in the other two treatments (Fig. 1A,B). Our results agree with previous reports of enhanced DIC and N_2 fixation with increased temperature or pCO_2 during exponential growth (Fu et al. 2014; Gradoville et al. 2014*a*,*b*). The drop in DIC and N_2 fixation rates observed in the extreme treatment on day 4 suggests that cells cannot withstand these harsh conditions for experimental periods of time. Similarly, natural assemblages of picocyanobacteria increase their photosynthetic rates with elevated pCO_2 initially but are unable to maintain elevated rates after 3 d of incubation (Lomas et al. 2012). A closer look at the intrapopulation variability confirms that while extreme conditions may enhance DIC and N_2 fixation momentarily, they became detrimental over time as suggested by the decrease of metabolically active cells between days 2 and 4 (Supporting Information Fig. S4). Similar intra-population variability has been observed in wild and cultured *Crocosphaera* (Foster et al. 2013, Masuda et al. 2020), which questions previous interpretations of increased pCO_2 having a positive effect on *Crocosphaera*.

Comparative transcriptomics captures changes in gene expression identifying regions involved in the regulation of gene expression (Ozsolak and Milos 2011, Wilhelm and Landry 2009). Although gene expression is a complex process regulated at many cellular levels (e.g., Buccitelli and Selbach 2020), it is generally defined as the production of an observable phenotype by a gene, ultimately directing the synthesis of a protein, thus showing how cells attempt to regulate their metabolism (Alberts et al. 2002). Here, we use transcriptomics to investigate differential gene expression of *Crocosphaera* under three future temperature and pH treatments in control or DOM-amended conditions. We screened for the most differentially expressed genes among treatments and experimental time points, covering many important metabolic pathways of this species (Supporting Information Table S1).

Connecting physiological responses to gene expression, we observed that the DIC fixing gene *rbcL*, encoding for part of the RuBisCO enzyme, was least expressed in the extreme treatment and most expressed in the low treatment on day 4 (Supporting Information Fig. S3), as previously observed in *Synechocystis* (Price et al. 2008). This could be due to the thermal damage of RuBisCO, as seen in *Trichodesmium* (Boatman et al. 2017). Increased pCO_2 is thought to alleviate the energy investment that concentrating carbon involves, in turn promoting both DIC and N_2 fixation (Hutchins et al. 2007; Levitan et al. 2007). N_2 fixation (*nif*) genes expression were not mirrored with N_2 fixation rates at any time point of the experiment (Supporting Information Fig. S3; Fig. 1B), which may be due to post-translational modifications and/or differing energy availability for nitrogenase activity (Kranz et al. 2011; Levitan et al. 2010). Alternatively, the time of RNA sampling at sunrise might not reflect the effect of treatments on N_2-fixation genes, as *nif* gene expression in *Crocosphaera* peaks at sunset (Wilson et al. 2017).

Through 1st steps of photosynthesis, light is harvested and transferred to generate energy and reductants that keep most metabolic processes active including DIC and N_2 fixation (Steward 1980; Kranz et al. 2011). The lower expression of the light-harvesting machinery coincided with lower DIC and N_2 fixation rates in the low and extreme treatments (Fig. 1B; Supporting Information Fig. S3), reflecting a close link between the expression of energy-producing genes and *Crocosphaera*'s main metabolic activity. Stress-induced protein genes such as heat shock chaperones were highly expressed in the low and extreme treatments (Supporting Information Fig. S3). Molecular chaperones allow cyanobacteria to maintain protein conformation homeostasis, which is key for temperature stress adaptability (Blondin et al. 1993). *Synechocystis* and *Synechococcus* upregulate about 90 different heat shock proteins after 1 h exposure to heat stress (Slabas et al. 2006) and similar responses are observed when exposed to cold temperatures (Porankiewicz and Clarke 1997). Moreover, osmotic and oxidative stresses promote heat shock expression in cyanobacteria (Apte et al. 1989), suggesting that these genes regulate essential cellular functions (Rajaman et al. 2014). Collectively, our comparative transcriptomics analysis confirms that cells in the low and extreme treatment were more stressed than in the intermediate treatment where the higher expression of light-harvesting genes allowed cells to keep balanced DIC and N_2 fixation activity (Supporting Information Fig. S3). Moreover, the high expression of stress-induced proteins maintained over time only in the extreme treatment suggests a strong effect of low pH and high temperature, negatively affecting all cellular functions despite potential energetic benefits of higher pCO_2.

DOM as a source of carbon when facing environmental stress

Crocosphaera obtained at least twice more carbon from DOM as from DIC, independently of the treatment (ANOVA; $p = 0.12$; Fig. 2). DOM thus provided a preferred source of carbon under all conditions tested here, suggesting that osmotrophy is an important trait of *Crocosphaera*. Given the lability of the amended ^{13}C-DOM, these results should be carefully extrapolated to natural environments where most DOM is generally refractory. Considering that 1% of the background DOM in our cultures was labile, DOM uptake rates decreased by 94% and were two times lower than DIC fixation rates in all treatments on day 0, and remained lower (*t*-test, $p < 0.01$) in the intermediate but similar (*t*-test, $p > 0.1$) in the low and extreme treatments for the rest of the experiment. In nature, DOM uptake rates by *Crocosphaera* may be in the above ranges. Furthermore, despite the competitiveness of *Crocosphaera* against other community components for these labile substrates is unknown, this diazotroph shows

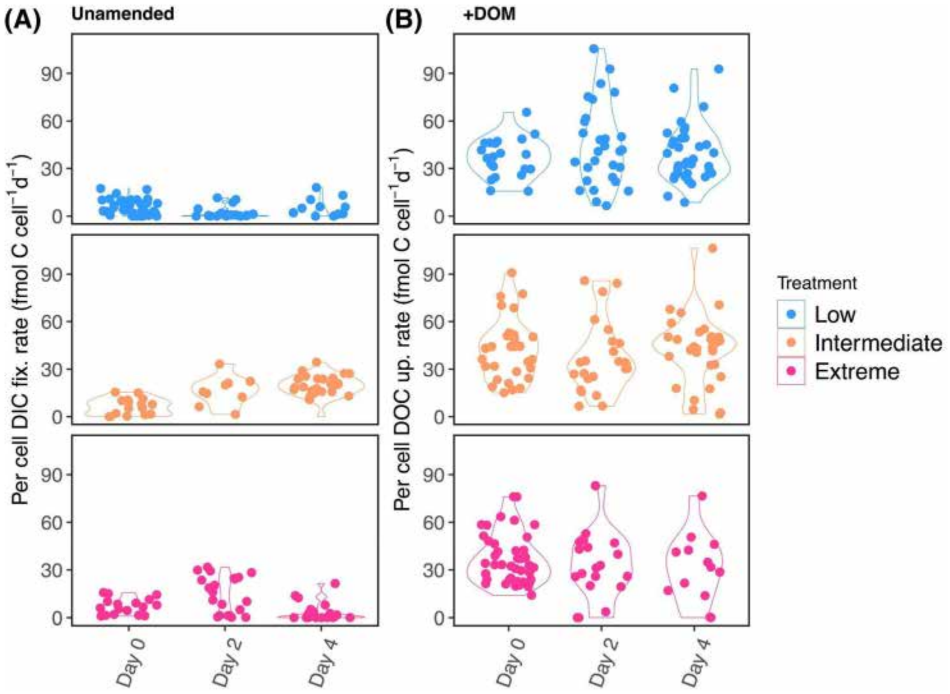

Fig. 2. (**A**) Single-cell DIC fixation and (**B**) DOC uptake rates in unamended and DOM-amended conditions, respectively, for each sampling time point. Each dot represents the uptake rate for single cells.

significant uptake of organic carbon derived from a mixture of representative natural carbohydrates and amino acids (*see* Supporting Information). Diazotrophic cyanobacteria may use DOM as an alternative source of carbon, nutrients and/or energy (Dhyrman et al. 2006; Filella et al. 2022), for example *Trichodesmium* can obtain as much carbon from amino acids as from DIC (Benavides et al. 2017). In contrast, the non-diazotrophic cyanobacterium *Prochlorococcus* obtains more carbon from DIC than from DOM uptake but can assimilate glucose in culture and field conditions (Moisander et al. 2012; Muñoz-Marín et al. 2013; Duhamel et al. 2018). Direct glucose assimilation is more energetically favorable than producing it through the Calvin Cycle (Muñoz-Marín et al. 2013). Moreover, while DIC is tightly coupled to the reactive carbonate system and hence susceptible to pH changes, the chemical composition of DOM does not change significantly with varying pH, providing a more stable carbon source under changing environmental conditions (Zark et al. 2015). The cellular machinery to assimilate DOM may also be more resistant to pH changes than that required to fix DIC, as observed by similar gene expression of the main carbohydrate degradation pathways regardless of pH changes (Supporting Information Fig. S3). In all, DOM seems a more stable source of carbon for cyanobacteria under low pH conditions.

Diazotrophic cyanobacteria are able to obtain carbon and phosphorus from DOM, which can enhance their N_2 fixation activity (Benavides et al. 2017, 2020; Dyhrman et al. 2007). However, organic nitrogen molecules contained in DOM may instead inhibit N_2 fixation (Mulholland and Capone 1999; Walworth et al. 2018). The DOM mix used here contained alanine, arginine and leucine, but average N_2 fixation rates were similar under DOM-amended or unamended conditions with few exceptions (Fig. 3). Again, single-cell analyses showed beneficial responses to DOM additions, masked if treating the population as whole (Fig. 3; Supporting Information Fig. S2). On day 4, N_2 fixation rates were close to zero in the low and extreme treatments (Fig. 3), where only ~ 18% and 21% of the cells were actively fixing N_2, respectively (Supporting Information Fig. S2), while upon DOM amendments ~30% and 54% of the measured cells actively fixed N_2 (Supporting Information Fig. S2; Fig. 3). In contrast, the percentage of cells actively fixing N_2 remained similar over time in the intermediate treatment where cells were healthier (Fig. 1A; Supporting Information Fig. S2). Our results support previous field experiments arguing that DOM aids cyanobacterial diazotrophs fixing N_2 when facing environmental stress (Benavides et al. 2018; Bonnet et al. 2013).

Differential gene expression was observed in response to DOM additions, with changes among growth phases and treatment conditions (Fig. 4). Most genes increased their expression upon DOM additions on day 4, while only a few differences were measured in the intermediate treatment on day 2 (Fig. 4).

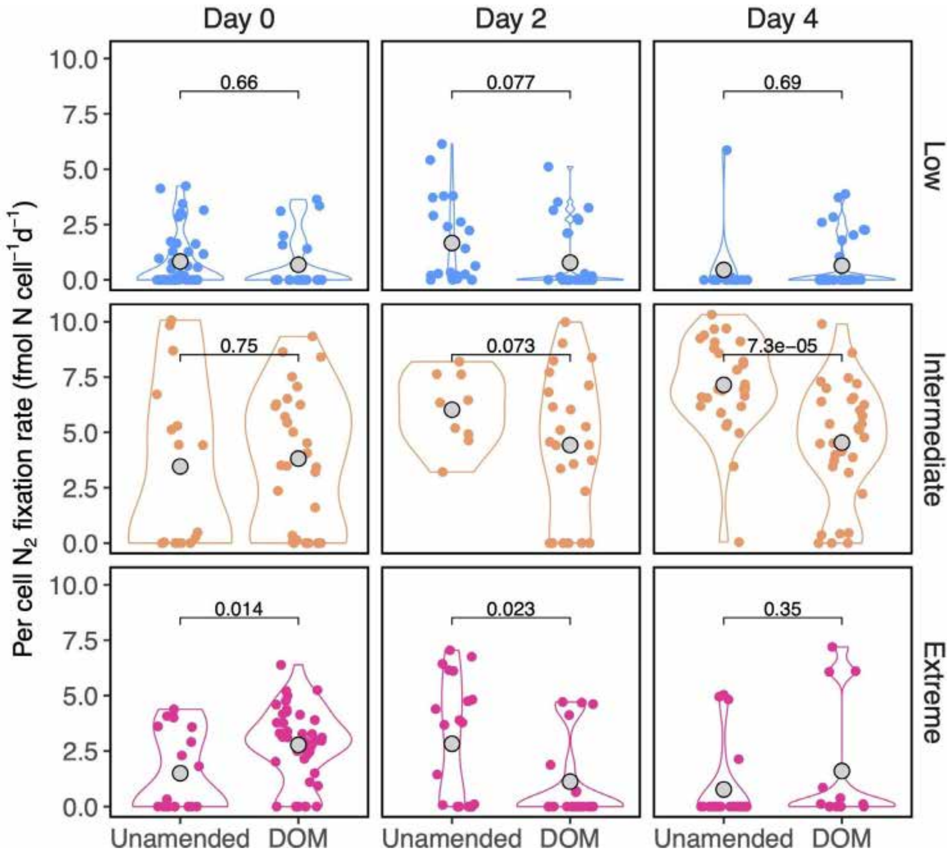

Fig. 3. Single-cell N_2 fixation rates in unamended and DOM-amended conditions, for each experimental treatment and time. Each colored dot represents the uptake rate for single cells and the centered gray dot the average value of these. Differences in the averages between conditions were tested with paired *t*-test (*p*-values are shown for each comparison).

DOM amendments did not influence the expression of DIC fixation genes (Fig. 4). Expression of N_2 fixation genes increased slightly in all treatments on day 4, and in the extreme treatment on day 2 upon DOM additions (Fig. 4), providing inconclusive trends and uncoupled patterns with the measured rates as seen above (Supporting Information Fig. S3). Conversely, DOM additions enhanced the expression of light-harvesting genes in the low treatment and components of the photosystem II in all treatments on day 2 (Fig. 4). In *Crocosphaera*, photosynthesis genes are expressed before sunrise (Wilson et al. 2017) and their expression depends on cellular resources produced on the previous day (Dron et al. 2012). Therefore, cells can easily face energy limitation if metabolic processes are not balanced throughout the diel cycle. For example, *Crocosphaera* shows a temporal delay in cell division of approximately 1 h after sunrise reflecting the need to recover cellular reserves with light-derived energy after night-time activity (Wilson et al. 2017). Hence, DOM could provide an additional and energetically cheaper source of carbon for restoring the light-harvesting components when cells are energetically deprived after the dark period. Overall, DOM enhanced the expression of the machinery required to produce cellular resources to fix DIC and N_2, improving cell performance specially under stress conditions including late growth stage, non-optimal temperature, and low pH.

Crocosphaera in the future ocean

In a future ocean, decreased nutrient supply may limit non-diazotrophic phytoplankton growth, benefitting diazotrophs as long as iron and phosphorus remain available (Weber and Deutsch 2010; Hutchins and Capone 2022). Phosphorus concentrations, unlike N_2 and carbon, will decrease considerably with intensified stratification, while the enhancement of desertification and dust deposition could increase iron and phosphorus inputs regionally, creating diazotroph niches (Jickells et al. 2005; Moulin and Chiapello 2006; Hamilton et al. 2020).

We show that DOM uptake was higher and more constant than DIC fixation regardless of the treatment, providing *Crocosphaera* with an extra and secure carbon source. DOM additions allowed cells in the low and extreme treatments to maintain a greater number of cells actively fixing N_2 than in unamended conditions, which coincided with increased expression of genes involved in the 1st steps of photosynthesis.

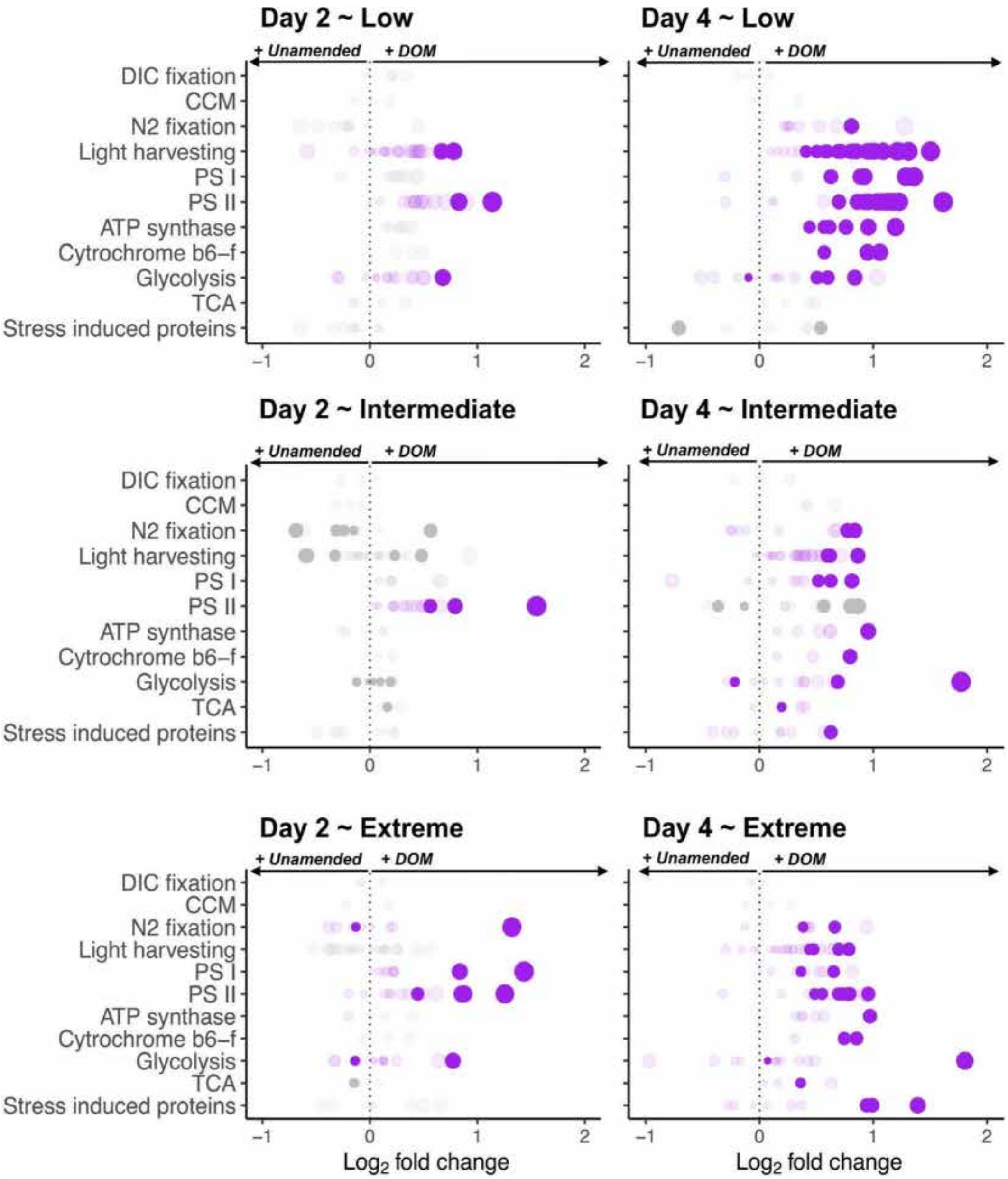

Fig. 4. Differentially expressed genes between DOM-amended and unamended samples ($p < 0.01$) for each treatment on days 2 and 4. Genes are grouped by common pathway or function (*see* Supporting Information Table S1 for gene full names and grouping). Positive fold changes (purple dots) indicate genes that were more expressed in DOM-amended samples than in unamended samples for each treatment. Negative fold changes and genes showing no differences between both conditions are colored in gray. The dot size increases with increasing fold change.

Overall, DOM became especially important in treatments or at growth stages where cells were more limited, presumably lacking stored resources to maintain a balanced metabolism over time.

Osmotrophic *Crocosphaera* may maintain balanced growth in a future stratified, warm, and acidic ocean, contributing to nitrogen inputs where they predominate. Warming may favor *Crocosphaera* relative to other coexisting diazotrophs in (sub) tropical waters like *Trichodesmium* (Fu et al. 2014; Breitbarth et al. 2007; Yang et al. 2021), while increasing $p\mathrm{CO}_2$ may similarly affect both species (Hutchins et al. 2007; Fu et al. 2008). Our results indicate that warming and acidification are more adverse when combined, and that beyond thermal and pH limits, metabolic versatility to exploit DOM is likely to influence the expansion of diazotrophic niches, potentially dictating nitrogen inputs in the future. Therefore, investigating osmotrophy in other prominent diazotrophs such as *Trichodesmium*, UCYN-A and NCDs should be prioritized if we are to understand their role in the future ocean.

References

Alberts, B., and others. 2002. *Molecular biology of the cell*. Garland Press.

Apte, S. K., and A. A. Bhagwat. 1989. Salinity-stress-induced proteins in two nitrogen-fixing *Anabaena* strains differentially tolerant to salt. J. Bacteriol. **171**: 909–915. doi:10.1128/jb.171.2.909-915.1989

Benavides, M., H. Berthelot, S. Duhamel, P. Raimbault, and S. Bonnet. 2017. Dissolved organic matter uptake by *Trichodesmium* in the Southwest Pacific. Sci. Rep. **7**: 41315. doi:10.1038/srep41315

Benavides, M., C. Martias, H. Elifantz, I. Berman-Frank, C. Dupouy, and S. Bonnet. 2018. Dissolved organic matter influences N_2 fixation in the New Caledonian lagoon (Western Tropical South Pacific). Front. Mar. Sci. **5**: 89. doi:10.3389/fmars.2018.00089

Benavides, M., S. Duhamel, F. Van Wambeke, K. M. Shoemaker, P. H. Moisander, E. Salamon, L. Riemann, and S. Bonnet. 2020. Dissolved organic matter stimulates N_2 fixation and nifH gene expression in *Trichodesmium*. FEMS Microbiol. Lett. **367**: fnaa034. doi:10.1093/femsle/fnaa034

Benavides, M., M. Caffin, S. Duhamel, R. A. Foster, O. Grosso, C. Guieu, F. Van Wambeke, and S. Bonnet. 2022. Anomalously high abundance of *Crocosphaera* in the South Pacific Gyre. FEMS Microbiol. Lett. **369**: fnac039. doi:10.1093/femsle/fnac039

Blondin, P. A., R. J. Kirby, and S. R. Barnum. 1993. The heat shock response and acquired thermotolerance in three strains of cyanobacteria. Curr. Microbiol. **26**: 79–84. doi:10.1007/BF01577340

Boatman, T. G., T. Lawson, and R. J. Geider. 2017. A key marine diazotroph in a changing ocean: The interacting effects of temperature, CO_2 and light on the growth of *Trichodesmium erythraeum* IMS101. PLoS One **12**: e0168796. doi:10.1371/journal.pone.0168796

Bonnet, S., J. Dekaezemacker, K. A. Turk-Kubo, T. Moutin, R. M. Hamersley, O. Grosso, J. P. Zehr, and D. G. Capone. 2013. Aphotic N_2 fixation in the eastern tropical South Pacific Ocean. PLoS One **8**: e81265. doi:10.1371/journal.pone.0081265

Bonnet, S., and others. 2018. In-depth characterization of diazotroph activity across the western tropical South Pacific hotspot of N_2 fixation (OUTPACE cruise). Biogeosciences **15**: 4215–4232. doi:10.5194/bg-15-4215-2018

Böttjer, D., D. M. Karl, R. M. Letelier, D. A. Viviani, and M. J. Church. 2014. Experimental assessment of diazotroph responses to elevated seawater pCO_2 in the North Pacific Subtropical Gyre. Global Biogeochem. Cycl. **28**: 601–616. doi:10.1002/2013GB004690

Breitbarth, E., A. Oschlies, and J. LaRoche. 2007. Physiological constraints on the global distribution of *Trichodesmium*—Effect of temperature on diazotrophy. Biogeosciences **4**: 53–61. doi:10.5194/bg-4-53-2007

Buccitelli, C., and M. Selbach. 2020. mRNAs, proteins and the emerging principles of gene expression control. Nature Reviews Genetics. **21**: 630–644. doi:10.1038/s41576-020-0258-4

Burkhardt, S., U. Riebesell, and I. Zondervan. 1999. Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton. Geochim. Cosmochim. Acta **63**: 3729–3741. doi:10.1016/S0016-7037(99)00217-3

Cheng, L., J. Abraham, Z. Hausfather, and K. E. Trenberth. 2019. How fast are the oceans warming? Science **363**: 128–129. doi:10.1126/science.aav7619

Doney, S., W. Balch, V. Fabry, and R. Feely. 2009. Ocean acidification: A critical emerging problem for the ocean sciences. Oceanography **22**: 16–25. doi:10.5670/oceanog.2009.93

Dron, A., S. Rabouille, P. Claquin, P. Chang, V. Raimbault, A. Talec, and A. Sciandra. 2012. Light:dark (12:12 h) quantification of carbohydrate fluxes in *Crocosphaera watsonii*. Aquat. Microb. Ecol. **68**: 43–55. doi:10.3354/ame01600

Duhamel, S., F. Van Wambeke, D. Lefevre, M. Benavides, and S. Bonnet. 2018. Mixotrophic metabolism by natural communities of unicellular cyanobacteria in the western tropical South Pacific Ocean. Environ. Microbiol. **20**: 2743–2756. doi:10.1111/1462-2920.14111

Dutkiewicz, S., J. J. Morris, M. J. Follows, J. Scott, O. Levitan, S. T. Dyhrman, and I. Berman-Frank. 2015. Impact of ocean acidification on the structure of future phytoplankton communities. Nat. Clim. Chang. **5**: 1002–1006. doi:10.1038/nclimate2722

Dutkiewicz, S., P. W. Boyd, and U. Riebesell. 2021. Exploring biogeochemical and ecological redundancy in phytoplankton communities in the global ocean. **27**: 1–18. doi:10.1111/gcb.15493

Dyhrman, S. T., E. A. Webb, D. M. Anderson, J. W. Moffett, and J. B. Waterbury. 2002. Cell-specific detection of phosphorus stress in *Trichodesmium* from the Western North Atlantic. Limnol. Oceanogr. **47**: 1832–1836. doi:10.4319/lo.2002.47.6.1832

Dyhrman, S. T., P. D. Chappell, S. T. Haley, J. W. Moffett, E. D. Orchard, J. B. Waterbury, and E. A. Webb. 2006. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature **439**: 68–71. doi:10.1038/nature04203

Dyhrman, S. T., J. W. Ammerman, and B. A. S. van Mooy. 2007. Microbes and the marine phosphorus cycle. Oceanography **20**: 110–116. doi:10.5670/oceanog.2007.54

Falcón, L. I., S. Pluvinage, and E. J. Carpenter. 2005. Growth kinetics of marine unicellular N_2-fixing cyanobacterial isolates in continuous culture in relation to phosphorus and temperature. Mar. Ecol. Prog. Ser. **285**: 3–9. doi:10.3354/meps285003

Feng, X., A. Bandyopadhyay, B. Berla, L. Page, B. Wu, H. B. Pakrasi, and Y. J. Tang. 2010. Mixotrophic and photoheterotrophic metabolism in *Cyanothece* sp. ATCC 51142 under continuous light. Microbiology **156**: 2566–2574. doi:10.1099/mic.0.038232-0

Filella, A., and others. 2022. Contrasting roles of DOP as a source of phosphorus and energy for marine diazotrophs. Front. Mar. Sci **9**. doi:10.3389/fmars.2022.923765

Foster, R. A., S. Sztejrenszus, and M. M. M. Kuypers. 2013. Measuring carbon and N_2 fixation in field populations of colonial and free-living unicellular cyanobacteria using nanometer-scale secondary ion mass spectrometry. J. Phycol. **49**: 502–516. doi:10.1111/jpy.12057

Fu, F. X., M. R. Mulholland, N. S. Garcia, A. Beck, P. W. Bernhardt, M. E. Warner, S. A. Sañudo-Wilhelmy, and D. A. Hutchins. 2008. Interactions between changing pCO_2, N_2 fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*. Limnol. Oceanogr. **53**: 2472–2484. doi:10.4319/lo.2008.53.6.2472

Fu, F. X., E. Yu, N. S. Garcia, J. Gale, Y. Luo, E. A. Webb, and D. A. Hutchins. 2014. Differing responses of marine N2 fixers to warming and consequences for future diazotroph community structure. Aquat. Microb. Ecol. **72**: 33–46. doi:10.3354/ame01683

Garcia, N. S., F.-X. Fu, C. L. Breene, E. K. Yu, P. W. Bernhardt, M. R. Mulholland, and D. A. Hutchins. 2013*a*. Combined effects of CO_2 and light on large and small isolates of the unicellular N_2-fixing cyanobacterium *Crocosphaera watsonii* from the western tropical Atlantic Ocean. Eur. J. Phycol. **48**: 128–139. doi:10.1080/09670262.2013.773383

Garcia, N. S., F.-X. Fu, and D. A. Hutchins. 2013*b*. Colimitation of the unicellular photosynthetic diazotroph *Crocosphaera watsonii* by phosphorus, light, and carbon dioxide. Limnol. Oceanogr. **58**: 1501–1512. doi:10.4319/lo.2013.58.4.1501

Gradoville, M. R., A. E. White, D. Böttjer, M. J. Church, and R. M. Letelier. 2014*a*. Diversity trumps acidification: Lack of evidence for carbon dioxide enhancement of *Trichodesmium* community nitrogen or carbon fixation at Station ALOHA. Limnol. Oceanogr. **59**: 645–659. doi:10.4319/lo.2014.59.3.0645

Gradoville, M. R., A. E. White, and R. M. Letelier. 2014*b*. Physiological response of *Crocosphaera watsonii* to enhanced and fluctuating carbon dioxide conditions. PLoS One **9**: e110660. doi:10.1371/journal.pone.0110660

Grossart, H. P., G. Czub, and M. Simon. 2006. Algae-bacteria interactions and their effects on aggregation and organic matter flux in the sea. Environ. Microbiol. **8**: 1074–1084. doi:10.1111/j.1462-2920.2006.00999.x

Gruber, N. 2011. Warming up, turning sour, losing breath: Ocean biogeochemistry under global change. Philos. Trans. A Math. Phys. Eng. Sci. **369**: 1980–1996. doi:10.1098/rsta.2011.0003

Hamilton, D. S., and others. 2020. Impact of changes to the atmospheric soluble iron deposition flux on ocean biogeochemical cycles in the anthropocene. Global Biogeochem. Cycles **34**: e2019GB006448. doi:10.1029/2019gb006448

Harding, K., K. A. Turk-kubo, R. E. Sipler, M. M. Mills, and D. A. Bronk. 2018. Symbiotic unicellular cyanobacteria fix nitrogen in the Arctic Ocean. **115**: 115–13375. doi:10.1073/pnas.1813658115

Hietanen, S., J. M. Lehtimäki, L. Tuominen, K. Sivonen, and J. Kuparinen. 2002. *Nodularia* spp. (cyanobacteria) incorporate leucine but not thymidine: Importance for bacterial-production measurements. Aquat. Microb. Ecol. **28**: 99–104. doi:10.3354/ame028099

Hinga, K. R. 2002. Effects of pH on coastal marine phytoplankton. Mar. Ecol. Prog. Ser. **238**: 281–300. doi:10.3354/meps238281

Hutchins, D. A., F.-X. Fu, Y. Zhang, M. E. Warner, Y. Feng, K. Portune, P. W. Bernhardt, and M. R. Mulholland. 2007. CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: Implications for past, present, and future ocean biogeochemistry. Limnol. Oceanogr. **52**: 1293–1304. doi:10.4319/lo.2007.52.4.1293

Hutchins, D. A., F. X. Fu, E. A. Webb, N. Walworth, and A. Tagliabue. 2013. Taxon-specific response of marine nitrogen fixers to elevated carbon dioxide concentrations. Nat. Geosci. **6**: 790–795. doi:10.1038/ngeo1858

Hutchins, D. A., N. G. Walworth, E. A. Webb, M. A. Saito, D. Moran, M. R. Mcilvin, J. Gale, and F. Fu. 2015. *Trichodesmium* experimentally adapted to elevated carbon dioxide. Nat. Commun **6**: 8155. doi:10.1038/ncomms9155

Hutchins, D. A., and D. G. Capone. 2022. The marine nitrogen cycle: New developments and global change. Nat. Rev. Microbiol. **20**: 401–414. doi:10.1038/s41579-022-00687-z

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 151. *In* Core Writing Team, R. K. Pachauri and L. A. Meyer [eds.]. IPCC, Geneva, Switzerland.

Jickells, T. D., and others. 2005. Global iron connections between desert dust, ocean biogeochemistry, and climate. Science **308**: 67–71. doi:10.1126/science.1105959

Kirchman, D. L., X. A. G. Morán, and H. Ducklow. 2009. Microbial growth in the polar oceans—Role of temperature and potential impact of climate change. Nat. Rev. Microbiol. **7**: 451–459. doi:10.1038/nrmicro2115

Kranz, S. A., S. Dieter, K. U. Richter, and B. Rost. 2009. Carbon acquisition by Trichodesmium: The effect of pCO_2 and diurnal changes. Limnology and Oceanography **54**: 548–559. doi:10.4319/lo.2009.54.2.0548

Kranz, S. A., M. Eichner, and B. Rost. 2011. Interactions between CCM and N_2 fixation in *Trichodesmium*. Photosynth. Res. **109**: 73–84. doi:10.1007/s11120-010-9611-3

Levitan, O., G. Rosenberg, I. Setlik, E. Setlikova, J. Grigel, J. Klepetar, O. Prasil, and I. Berman-Frank. 2007. Elevated CO_2 enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*. Glob. Chang. Biol. **13**: 531–538. doi:10.1111/j.1365-2486.2006.01314.x

Levitan, O., C. M. Brown, S. Sudhaus, D. Campbell, J. LaRoche, and I. Berman-Frank. 2010. Regulation of nitrogen metabolism in the marine diazotroph *Trichodesmium* IMS101 under varying temperatures and atmospheric CO2 concentrations. Environ. Microbiol. **12**: 1899–1912. doi:10.1111/j.1462-2920.2010.02195.x

Lomas, M. W., and D. Shi. 2012. Effect of ocean acidification on cyanobacteria in the subtropical North Atlantic. **66**: 211–222. doi:10.3354/ame01576

Lønborg, C., C. Carreira, T. Jickells, and X. A. Álvarez-Salgado. 2020. Impacts of global change on ocean dissolved organic carbon (DOC) cycling. Front. Mar. Sci. **7**: 1–24. doi:10.3389/fmars.2020.00466

Martínez-Pérez, C., and others. 2016. The small unicellular diazotrophic symbiont, UCYN-A, is a key player in the marine nitrogen cycle. Nat. Microbiol. **1**: 1–7. doi:10.1038/nmicrobiol.2016.163

Masuda, T., K. Inomura, N. Takahata, T. Shiozaki, Y. Sano, C. Deutsch, O. Prášil, and K. Furuya. 2020. Heterogeneous nitrogen fixation rates confer energetic advantage and expanded ecological niche of unicellular diazotroph populations. Commun. Biol. **3**: 1–12. doi:10.1038/s42003-020-0894-4

Moisander, P. H., R. Zhang, E. A. Boyle, I. Hewson, J. P. Montoya, and J. P. Zehr. 2012. Analogous nutrient limitations in unicellular diazotrophs and *Prochlorococcus* in the South Pacific Ocean. ISME J. **6**: 733–744. doi:10.1038/ismej.2011.152

Morán, X. A. G., J. M. Gasol, M. C. Pernice, J. Mangot, R. Massana, E. Lara, D. Vaqué, and C. M. Duarte. 2017. Temperature regulation of marine heterotrophic prokaryotes increases latitudinally as a breach between bottom-up and top-down controls. Glob. Chang. Biol. **23**: 3956–3964. doi:10.1111/gcb.13730

Moulin, C., and I. Chiapello. 2006. Impact of human-induced desertification on the intensification of Sahel dust emission and export over the last decades. Geophys. Res. Lett. **33**. doi:10.1029/2006gl025923

Mulholland, M. R., and D. G. Capone. 1999. Nitrogen fixation, uptake and metabolism in natural and cultured populations of *Trichodesmium* spp. Mar. Ecol. Prog. Ser. **188**: 33–49. doi:10.3354/meps188033

Muñoz-Marín, M. d. C., I. Luque, M. V. Zubkov, P. G. Hill, J. Diez, and J. M. García-Fernández. 2013. *Prochlorococcus* can use the Pro1404 transporter to take up glucose at nanomolar concentrations in the Atlantic Ocean. Proc. Natl. Acad. Sci. USA **110**: 8597–8602. doi:10.1073/pnas.1221775110

Orchard, E., E. Webb, and S. Dyhrman. 2003. Characterization of phosphorus-regulated genes in *Trichodesmium* spp. Biol. Bull. **205**: 230–231. doi:10.2307/1543268

Orr, J. C., and others. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature **437**: 681–686. doi:10.1038/nature04095

Ozsolak, F., and P. M. Milos. 2011. RNA sequencing: Advances, challenges and opportunities. Nat. Rev. Genet. **12**: 87–98. doi:10.1038/nrg2934

Pachauri, R. K., and others. 2014. *Climate change 2014: Synthesis report. Contribution of Working Groups I, II and III to the fifth assessment report of the Intergovernmental Panel on Climate Change*. IPCC, 2014.

Passow, U., and C. A. Carlson. 2012. The biological pump in a high CO_2 world. Mar. Ecol. Prog. Ser. **470**: 249–271. doi:10.3354/meps09985

Porankiewicz, J., and A. K. Clarke. 1997. Induction of the heat shock protein ClpB affects cold acclimation in the cyanobacterium *Synechococcus* sp. strain PCC 7942. J. Bacteriol. **179**: 5111–5117. doi:10.1128/jb.179.16.5111-5117.1997

Price, G. D., M. R. Badger, F. J. Woodger, and B. M. Long. 2008. Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): Functional components, ci transporters, diversity, genetic regulation and prospects for engineering into plants. J. Exp. Bot. **59**: 1441–1461. doi:10.1093/jxb/erm112

Rajaram, H., A. K. Chaurasia, and S. K. Apte. 2014. Cyanobacterial heat-shock response: Role and regulation of molecular chaperones. Microbiology **160**: 647–658. doi:10.1099/mic.0.073478-0

Riebesell, U., and others. 2007. Enhanced biological carbon consumption in a high CO_2 ocean. Nature **450**: 545–548. doi:10.1038/nature06267

Riebesell, U., and P. D. Tortell. 2011. Effects of ocean acidification on pelagic organisms and ecosystems. *In Ocean Acidification*. Oxford University Press.

Rossi, S. 2023. Ocean acidification and sea warming-toward a better comprehension of its consequences, p. 111–205. *In SDG 14: Life Below Water*. Springer International Publishing.

Slabas, A. R., I. Suzuki, N. Murata, W. J. Simon, and J. J. Hall. 2006. Proteomic analysis of the heat shock response in *Synechocystis* PCC6803 and a thermally tolerant knockout strain lacking the histidine kinase 34 gene. Proteomics **6**: 845–864. doi:10.1002/pmic.200500196

Sohm, J. A., E. A. Webb, and D. G. Capone. 2011. Emerging patterns of marine nitrogen fixation. Nat. Rev. Microbiol. **9**: 499–508. doi:10.1038/nrmicro2594

Stewart, W. D. P. 1980. Some aspects of structure and function in n fixing cyanobacteria. Annu. Rev. Microbiol. **34**: 497–536. doi:10.1146/annurev.mi.34.100180.002433

Sunagawa, S., and others. 2015. Structure and function of the global ocean microbiome. Science **348**: 1261359. doi:10.1126/science.1261359

Thornton, D. C. O. 2014. Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. Eur. J. Phycol. **49**: 20–46. doi:10.1080/09670262.2013.875596

Turk-Kubo, K. A., M. R. Gradoville, S. Cheung, F. Cornejo-Castillo, K. J. Harding, M. Morando, M. Mills, and J. P. Zehr. 2022. Non-cyanobacterial diazotrophs: Global diversity, distribution, ecophysiology, and activity in marine waters. FEMS Microbiol. Rev. **47**. doi:10.1093/femsre/fuac046

Umbricht, J., A. Filella, A. Klett, A. Vogts, M. Voss, M. Benavides. in prep. Uptake of dissolved inorganic nitrogen and N2 fixation by Crocosphaera watsonii under climate change scenarios.

Walworth, N. G., F.-X. Fu, M. D. Lee, X. Cai, M. A. Saito, E. A. Webb, and D. A. Hutchins. 2018. Nutrient-colimited *Trichodesmium* as a nitrogen source or sink in a future ocean. Appl. Environ. Microbiol. **84**: e02137-17. doi:10.1128/AEM.02137-17

Waterbury, J. B., and J. M. Willey. 1988. Isolation and growth of marine planktonic cyanobacteria, p. 100–105. *In Methods in Enzymology*. Academic Press.

Webb, E. A., I. M. Ehrenreich, S. L. Brown, F. W. Valois, and J. B. Waterbury. 2009. Phenotypic and genotypic characterization of multiple strains of the diazotrophic cyanobacterium, *Crocosphaera watsonii*, isolated from the open ocean. Environ. Microbiol. **11**: 338–348. doi:10.1111/j.1462-2920.2008.01771.x

Weber, T. S., and C. Deutsch. 2010. Ocean nutrient ratios governed by plankton biogeography. Nature **467**: 550–554. doi:10.1038/nature09403

Wilhelm, B. T., and J. R. Landry. 2009. RNA-Seq-quantitative measurement of expression through massively parallel RNA-sequencing. Methods **48**: 249–257. doi:10.1016/j.ymeth.2009.03.016

Wilson, S. T., and others. 2017. Coordinated regulation of growth, activity and transcription in natural populations of the unicellular nitrogen-fixing cyanobacterium *Crocosphaera*. Nat. Microbiol. **2**: 1–27. doi:10.1038/nmicrobiol.2017.118

Wu, Y., K. Gao, and U. Riebesell. 2010. CO_2-induced seawater acidification affects physiological performance of the marine diatom *Phaeodactylum tricornutum*. Biogeosciences **7**: 2915–2923. doi:10.5194/bg-7-2915-2010

Yang, N., and others. 2021. Warming iron-limited oceans enhance nitrogen fixation and drive biogeographic specialization of the globally important cyanobacterium *Crocosphaera*. Front. Mar. Sci. **8**: 1–11. doi:10.3389/fmars.2021.628363

Zark, M., U. Riebesell, and T. Dittmar. 2015. Effects of ocean acidification on marine dissolved organic matter are not detectable over the succession of phytoplankton blooms. Sci. Adv. **1**: e1500531. doi:10.1126/sciadv.1500531

Zehr, J. P. 2011. Nitrogen fixation by marine cyanobacteria. Trends Microbiol. **19**: 162–173. doi:10.1016/j.tim.2010.12.004

Acknowledgments

This research was supported by the NOTION project by the BNP Paribas Foundation for Climate and Biodiversity (MB). The MIMS equipment used in this study was obtained with European FEDER Funds. The NanoSIMS at the Leibniz-Institute for Baltic Sea research in Warnemuende (IOW) was funded by the German Federal Ministry of Education and Research (BMBF), grant identifier 03F0626A. The bioinformatic platform has been funded from France 2030, the French Government program managed by the French National Research Agency (ANR-16-CONV-0001) and from the Excellence Initiative of Aix-Marseille University—A*MIDEX. The authors would like to thank M. Camps and S. Bonnet for their comments and valuable advice on diazotroph culture work. F. Van Wambeke and S. Duhamel for advice regarding the experimental setup and osmotrophy studies, T. Wagener and F. Gazeau for carbonate system measurements advice, and E. Seris, J. Berdun, C. Ducrocq, and J-C. Cancel for their help during pilot experiments. We are indebted to M. Lafont, D. Malengros, K. Idri, N. Bhairy of the SAM platform of the MIO and to L. Thyssen and the Endoume marine station for facilitation and assistance in setting up the experimental infrastructure and advice in handling large volumes. The captain and crew of R/V *Antedon II* are warmly thanked for the collection of seawater and, D. Lefèvre, O. Pringault and S. Alliouane for training and assistance in light, oxygen respiration and total alkalinity measurements, respectively. The PRECYM Flow Cytometry Platform (https://precym.mio.osupytheas.fr/) and S. Nunige are acknowledged for flow cytometry and nutrient analyses respectively, and the Centre de Calcul Intensif d'Aix-Marseille for granting access to its high-performance computing resources.

Submitted 11 May 2023
Revised 20 December 2023
Accepted 24 January 2024