frontiers | Frontiers in Marine Science

TYPE Original Research
PUBLISHED 10 July 2024
DOI 10.3389/fmars.2024.1388725

OPEN ACCESS

EDITED BY
Yenny Risjani,
University of Brawijaya, Indonesia

REVIEWED BY
Keisuke Inomura,
University of Rhode Island, United States
Zhibing Jiang,
Ministry of Natural Resources, China

*CORRESPONDENCE
Xin-Wei Wang
wangxinwei@nbu.edu.cn
Hai-Bo Jiang
jianghaibo@nbu.edu.cn

RECEIVED 20 February 2024
ACCEPTED 13 June 2024
PUBLISHED 10 July 2024

CITATION
Li S-Q, Xiao Y, Huang H-L, Luo Z, Sun T-R, Gao H-Y, Wang X-W and Jiang H-B (2024) Experimental evidence that nitrogen-fixing cyanobacterium *Trichodesmium* spp. supplies new nitrogen source to marine phytoplankton.
Front. Mar. Sci. 11:1388725.
doi: 10.3389/fmars.2024.1388725

Experimental evidence that nitrogen-fixing cyanobacterium *Trichodesmium* spp. supplies new nitrogen source to marine phytoplankton

Shuang-Qing Li[1,2], Yan Xiao[3], Hai-Long Huang[1,2], Zhen Luo[1], Tao-Ran Sun[1], Hua-Yang Gao[1], Xin-Wei Wang[1,2]* and Hai-Bo Jiang[1,2]*

[1]School of Marine Sciences, Ningbo University, Ningbo, China, [2]Southern Marine Science and Engineering Guangdong Laboratory, Zhuhai, China, [3]School of Life Sciences, Central China Normal University, Wuhan, China

In oligotrophic oceans, primary productivity is widely limited by nitrogen bioavailability. The broadly distributed and abundant nitrogen-fixing cyanobacterium *Trichodesmium* plays an important role in the oceanic nitrogen and carbon cycles by providing a "new" source of nitrogen to many non-diazotrophic microbes, thereby driving new primary production in the ocean. However, the underlying process and mechanism of nitrogen supply from *Trichodesmium* to other phytoplankton remain unclear. Here, our results demonstrated that the fixed nitrogen released by *Trichodesmium* could sustain the growth of a non-nitrogen-fixing cyanobacterium *Synechococcus* sp. PCC 7002, including a mutant strain (Mut-*ureA*) that cannot use urea. However, the growth rate of Mut-*ureA* was approximately 20% lower than that of the wild strain when *Trichodesmium* filtrate was used for nitrogen supply. This result was consistent with the composition of the *Trichodesmium* exudate, in which urea comprised more than 20% of the total fixed nitrogen that was released. It is evident from the experiments that a fraction of the *Trichodesmium*-derived nitrogen was not available to Mut-*ureA*. Our results suggested that *Trichodesmium* produces dissolved organic nitrogen, especially a certain amount of urea as a "new" nitrogen source, benefiting in particular populations of surrounding phytoplankton species.

KEYWORDS

***Trichodesmium*, fixed nitrogen, *Synechococcus*, nitrogen supply, urea**

Introduction

The diazotrophic marine cyanobacterium *Trichodesmium* is ubiquitous in tropical and subtropical seas, where it fixes both CO_2 and N_2, making it among the most biogeochemically significant microorganisms in the ocean (Moisander et al., 2010; Zehr, 2011; Walworth et al., 2018). It has been estimated that *Trichodesmium* may release approximately 30 to 50% of its newly fixed nitrogen (N), providing an important source of bioavailable N to other non-diazotrophic phytoplankton species in the N-limited subtropical marine ecosystems (Glibert and Bronk, 1994; Walworth et al., 2018). The global N input via N_2 fixation by *Trichodesmium* is estimated around 60–80 Tg N annually (Capone et al., 1997; Mahaffey, 2005; Carpenter and Capone, 2008), which contributes substantially to the annual global marine N_2 fixation estimated to be around 223 ± 30 Tg N (Shao et al., 2023).

Although *Trichodesmium* is not the sole diazotrophic cyanobacterium in the open ocean (Sohm et al., 2011; Gradoville et al., 2020; Hutchins and Capone, 2022), it is the most intensively explored cyanobacterium. Since it has been in culture from the early 1990s (Prufert-Bebout et al., 1993), numerous studies have focused on the eco-physiological responses of *Trichodesmium* to different environmental factors. Despite many studies emphasizing the physiology and biogeographical distribution of *Trichodesmium*, fewer have examined the fate of the N_2 fixed by *Trichodesmium* (diazotroph-derived N, DDN) in the ocean (Capone et al., 1997). Some of these studies have reported that *Trichodesmium* blooms may encourage the growth of other non-N-fixing organisms (Padmakumar et al., 2010; Basu et al., 2011). For instance, the abundance of bacteria and the growth of diatoms and dinoflagellates were found to be significantly increased during *Trichodesmium* blooms in the Eastern Arabian Sea (Padmakumar et al., 2010; Basu et al., 2011). Both field and laboratory data demonstrated that the co-occurrence of *Trichodesmium* spp. and diatoms might be driven by the transfer of N fixed by *Trichodesmium* spp. in the N-limited oligotrophic ocean (Chen et al., 2011). Similarly, a previous study showed that a unicellular dinoflagellate, *Karenia brevis*, could grow well in the culture medium supplemented with *Trichodesmium* exudates as the sole source of nitrogen (Mulholland, 2007).

In general, *Trichodesmium* helps sustain marine life via both the active release of key nutrients such as carbon and nitrogen, and passive release processes like cell death, virus-induced lysis, and grazing by heterotrophs. Hence, it plays a crucial role in the biogeochemical cycling of bioactive elements in contemporary oceans (Capone and Carpenter, 1982; Capone et al., 1997). However, it is unknown how the specific forms of nitrogen are transferred by *Trichodesmium* to non-N-fixing organisms. Moreover, there is a lack of quantitative information regarding the concentrations, composition, and fate of DDN. With the development of techniques such as nanometer scale secondary ion mass spectrometry (nanoSIMS) coupled with $^{15}N_2$ isotopic labelling and flow cytometry cell sorting, researchers have observed the transfer of DDN to the dissolved N pool and to non-diazotrophic plankton such as diatoms, dinoflagellates, and bacteria (Mulholland et al., 2004; Dore et al., 2008; Lenes and Heil, 2010; Chen et al., 2011). Previous reports have shown that the DDN transfer efficiency of *Trichodesmium* is higher compared to other diazotrophic cyanobacteria, indicating that *Trichodesmium* blooms are more efficient in promoting non-diazotrophic production in N-depleted areas (Konno et al., 2010; Benavides et al., 2013; Berthelot et al., 2016; Bonnet et al., 2016). However, this method based on isotopic conversion does not allow for accurate discrimination of nitrogen sources, as well as the assessment of the nitrogen utilization efficiency of non-nitrogen-fixing marine phytoplankton.

In addition to nitrogen-fixing cyanobacteria, many non-nitrogen-fixing cyanobacteria exist in nitrogen-deficient oceans, such as *Synechococcus* and *Prochlorococcus*, which are among the most abundant phytoplankton in the ocean (Flombaum et al., 2013). However, it is unclear whether a substantial fraction of nitrogen required for phytoplankton growth comes from DDN released by *Trichodesmium*. Previous research has concluded that the transfer of DDN to nearby plankton is mediated through the dissolved N pool, as diazotrophs release a significant fraction of their fixed N (10–50%) in the form of ammonium and dissolved organic nitrogen (DON) (Glibert and Bronk, 1994; Mulholland and Bernhardt, 2005; Konno et al., 2010; Benavides et al., 2013; Berthelot et al., 2015; Klawonn et al., 2020; Benavides et al., 2022). These studies suggest that although more than 50% of recently fixed N_2 is released by diazotrophs as DON, NH_4^+ is the main pathway of DDN transfer from diazotrophs to non-diazotrophs. However, NH_4^+ may not accumulate in the surrounding environment as it could be utilized by the diazotrophs themselves and the surrounding organisms as soon as it is produced (Klawonn et al., 2020). Instead, DON concentrations of 5 to 10 μM are common, making it the most abundant form of dissolved nitrogen (Mulholland et al., 2004; Benavides et al., 2013). This suggests that organic compounds exuded by diazotrophs can fuel primary production, and may have considerable effect on the composition of the plankton community in the oligotrophic ocean. The dominant non-nitrogen-fixing cyanobacteria in these ecosystems, including *Synechococcus* and *Prochlorococcus*, possess pathways to take advantage of DON sources, such as urea or dissolved free amino acids (Ludwig and Bryant, 2012; Veaudor et al., 2019; Muñoz-Marín et al., 2020). However, the underlying potential mechanisms have rarely been explored.

Trichodesmium often forms large macroscopic colonies and acts as relatively nutrient-rich substrate for a diverse microbial community through physical attachment and direct colonization in tropical and subtropical oligotrophic oceans (Eichner et al., 2023). A common feature of these associations is the passive transfer of fixed nitrogen (N), such as ammonia, urea, and other DON, from *Trichodesmium* to epibionts (Mulholland et al., 2004). On the other hand, epibionts assist the host *Trichodesmium* in iron and phosphorus acquisition, vitamin B12 exchange, small carbon compound catabolism, and detoxification of reactive oxygen species (Lee et al., 2017). Thus, it is difficult to maintain *Trichodesmium* in culture and unsuccessful to establish stable axenic cultures, due to its obligate dependency on associated organisms. In this study, *Trichodesmium erythraeum* IMS 101 has been maintained in the laboratory for decades and shown to harbor microbial communities similar to natural microbial populations (Lee et al., 2017). Previous meta-transcriptomics research has detected the community

transcripts involved in the reduction of nitrate, nitrite, and nitrous oxide, and suggested that the associated organisms may play an important role in colony-level nitrogen cycling (Lee et al., 2018). To understand the significance of fixed nitrogen released by *Trichodesmium* to other non-diazotrophic microorganisms, it is prudent to consider the entire *Trichodesmium*-epibiont assemblage for the stable cohabitation of a diverse community within *Trichodesmium* consortia.

Although previous studies have shown that *Trichodesmium* can input nascent nitrogen sources into the habitat, there still lacks direct experimental evidence on whether *Trichodesmium* can drive phytoplankton growth (Glibert and Bronk, 1994; Mulholland et al., 2004). To investigate under what conditions and in what form *Trichodesmium* provides nitrogen sources for other cyanobacteria, we studied the growth and nitrogen-fixing gene expression of *Trichodesmium* under different nitrogen sources as well as nitrogen concentration conditions. The types and amounts of nitrogen sources secreted by the *Trichodesmium* symbiotic system under nitrogen-deficient conditions were analyzed, and marine *Synechococcus* sp. strain PCC 7002 was chosen to study the specific forms of nitrogen sources provided by *Trichodesmium* to other phytoplankton. We also validated this result using a mutant strain that lacked some of the nitrogen source utilization functions. Our results provide experimental evidence that the nitrogen-fixing cyanobacterium *Trichodesmium* can provide a certain amount of urea to the different species of marine phytoplankton around it, a nitrogen source that has been previously overlooked.

Material and methods

Strains, culture conditions

Trichodesmium erythraeum IMS 101 (hereafter *Trichodesmium* IMS 101) was originally isolated from the Atlantic Ocean (Prufert-Bebout et al., 1993; Chen et al., 1998). *Trichodesmium* IMS 101 was cultured semi-continuously in YBC-II medium at 28°C, 50 µmol photons·m^{-2}·s^{-1}, and 12:12 L:D photoperiod. The axenic *Synechococcus* sp. strain PCC 7002 (hereafter *Synechococcus* 7002) originated from Jindong Zhao's laboratory (Peking University, China) and the *Synechococcus* 7002 *ureA* mutant strain was constructed in our lab. The detailed methods have been described in the previous study of Li et al. (2023). *Synechococcus* 7002 wild-type and Mut-*ureA* were cultured in A^+ medium under continuous light of 100 µmol photons m^{-2} s^{-1}, at 30°C, with a rotatory shaker at 110 rpm. All growth media, buffers and solutions used in the experiments were autoclaved or filter sterilized.

Experimental setup

Five experiments were conducted in this study:

Experiment 1. Co-culture of *Trichodesmium* IMS 101 and *Synechococcus* 7002 were conducted under nitrogen deficient condition. *Synechococcus* 7002 cells at the logarithmic growth stage were collected, centrifuged, washed and resuspended with nitrogen-free A^+ medium to ensure the complete removal of artificially added nitrogen. Then 125 mL of *Synechococcus* 7002 (cell number about 1.6×10^7 cells/mL) and 125 mL of *Trichodesmium* IMS 101 (cell number about 8×10^3 cells/mL) cultured in N-free YBC II medium were added to 1 L conical flasks. *Synechococcus* 7002 of the same cell number mixed with fresh YBC-II medium without *Trichodesmium* IMS 101 served as a control. To determine the nitrogen deficiency status of the cells during long-term co-culture in an incubator with photoperiod (12:12 L:D), the color change of *Synechococcus* 7002 was observed every 2 days.

Experiment 2. Biomass growth and *nifH* gene expression were determined during *Trichodesmium* IMS 101 cultivation in media containing different nitrogen resources in varying concentrations. The stock culture of *Trichodesmium* IMS 101 was prepared by growing the cells in nitrogen-free YBC-II medium for 7 days. The *Trichodesmium* cells were then transferred separately into 500 mL flasks containing 300 mL of medium composed of $NaNO_3$, NH_4Cl, or urea (with concentrations of 0, 20, 40 to 80 µM, respectively). Culture samples (5 mL) were collected every 3 days for 12 days to measure the biomass of *Trichodesmium* IMS 101. 100 mL sample of each culture was collected onto a 10 micron polycarbonate (PC) membrane after 24 hours of incubation, and quickly frozen in liquid nitrogen for further analysis of *nifH* gene expression.

Experiment 3. A complete growth curve of *Trichodesmium* IMS 101 was drawed and the secreted nitrogen sources and concentrations were measured. Cells were cultivated in nitrogen free YBC-II medium, and the *in vivo* fluorescence was measured in every three days for 24 days to measure changes in biomass. The culture filtrate was collected on day 12^{th} (late exponential growth phase) to measure the concentrations of the secreted nitrogen sources.

Experiment 4. A nitrogen-deficient *Synechococcus* 7002 strain was cultured for 10 days, and then its recovery was monitored in the presence of various nitrogen sources. To do this, *Synechococcus* 7002 stock culture cells were collected during exponential phase and centrifuged. Then the cells were resuspended and washed twice with nitrogen-free A^+ medium to ensure the removal of any dissolved nitrogen sources outside of the *Synechococcus* 7002 cells. The cells were then transferred to fresh nitrogen free A^+ medium, and maintained for 10 days to induce the nitrogen-deficient condition. Finally, the nitrogen-deficient cells of *Synechococcus* 7002 were collected by centrifugation. These cells were then transferred and resuspended into A^+ medium containing 150 µM of different types of nitrogen source ($NaNO_3$, NH_4Cl, urea or glutamic acid). The recovery rate of *Synechococcus* 7002 was monitored by determining the biomass of *Synechococcus* 7002 in the culture suspensions in every 2 days through turbidity measurement (OD_{730}).

Experiment 5. *Trichodesmium* IMS 101 culture filtrate was used as a sole nitrogen source for wild-type and Mut-*ureA* *Synechococcus* 7002 strains. The mutant strain could not utilize urea. The filtrate of *Trichodesmium* IMS 101 culture was collected on day 12^{th} using a sterile PC membrane (0.22 µm) to remove the filamentous *Trichodesmium* cells and the associated bacteria. Then a mixture of filtrate and N-free medium (1:1) was used to cultivate wild-type and Mut-*ureA* nitrogen-deficient *Synechococcus* 7002 strains at the same time. The biomass contents of wild-type and Mut-*ureA*

Synechococcus 7002 were measured at 0 th, 24 th, 36 th, 48 th, and 72th hours. At the 72nd hour, 5 mL culture of each sample was collected for *Chl a* measurement.

Growth determination

The growth curve of *Trichodesmium* IMS 101 was estimated by measuring minimum yields of chlorophyll-*a* fluorescence (F_0) of the culture solution using a FIRe (Fluorescence Induction and Relaxation) Chlorophyll Fluorescence Analyzer (Satlantic, Canada). The biomass of *Synechococcus* 7002 wild type and mutant strains were determined both by measuring the turbidity (OD_{730}) of individual culture using an ultraviolet-visible spectrophotometer TU1810 (PERSEE, China) and by measuring the cell counts using a CytoFLEX flow cytometer (Beckman Coulter, America). F_0 for *Trichodesmium* IMS 101 and turbidity (OD_{730}) for *Synechococcus* 7002 were highly correlated with cell counts, of R^2 were 0.9931 and 0.9984, respectively (Supplementary Figures S1A, B).

Measurement of Chl*a* content

To determine chlorophyll *a* (Chl *a*) content, the absorption peaks at 648.6 and 664.1 nm of 95% ethanol extracts were measured using an ultraviolet-visible spectrophotometer TU1810 (PERSEE, China) and calculated according to the formula: Chl $a = 13.36 \times A_{664.1} - 5.19 \times A_{648.6}$, and the final results were normalized based on the absorbance value of the algal solution at 730 nm (OD_{730}), for details of the method refer by Li et al. (2023).

Detection of nitrogen sources secreted by *Trichodesmium* IMS 101

The total nitrogen content was determined using the alkaline potassium persulfate method (Hagedorn and Schleppi, 2000). Briefly, an alkaline potassium persulfate solution was added to the sample and digested at 120–124°C. The mixed solution was decomposed to potassium bisulfate and atomic nitrogen under high temperatures. The atomic nitrogen not only converts the nitrogenous compounds to nitrate under high temperatures but also oxidizes or decomposes other organic matter in the sample, thus eliminating the interference caused by other nutrient sources. Finally, the absorbance of nitrate was measured by UV-Vis spectrophotometer (210 PLUS) at 220 nm and 275 nm, and the corresponding total nitrogen content was calculated using a standard curve (Supplementary Figure S2A).

The ammonia nitrogen content was measured by using salicylate spectrophotometry (Le and Boyd, 2012). In an alkaline environment (pH 11.7), NH_4^+ will react chromogenically with salicylate and hypochlorite to produce a blue compound under sodium nitrosoferricyanide as catalyst. The absorbance of this compound was measured at 697 nm, and the ammonia nitrogen content was calculated using a standard curve (Supplementary Figure S2B).

Diacetylmonoxime spectrophotometric method was used to determine the urea content (Chen et al., 2015). Diacetylmonoxime was hydrolyzed under acidic conditions to form diacetyl, which condensed the urea under acidic conditions to produce a red diazine compound. The concentration of urea in the sample corresponded to the color shade of the solution. The maximum absorbance at 525 nm was measured and the urea content in the sample was calculated by using the standard curve (Supplementary Figure S2C).

RNA extraction and *nifH* gene expression

Trichodesmium IMS 101 cells grown in YBC-II media containing different nitrogen sources for 24 h were collected and quickly frozen in liquid nitrogen. Each sample had three replicates collected from three independent cultures. Total RNA was extracted using the TRIzol reagent kit (Invitrogen, USA). Reverse transcription (RT) was performed using the PrimeScript RT reagent kit (TaKaRa, Japan). RNA extraction and RT-PCR were performed as described previously (Jiang et al., 2012). To detect the gene expression level of *nifH*, the real-time quantitative PCR was conducted. The primers of *nif*H gene were as follows: 5'-CAATACGCTCCAGAAGATAACCAA -3' and 5'- GTAGGAA TAGTTAGTTTTTCGTTGTTGATT-3', which was designed using Primer Premier 5.

Global distribution survey of *Trichodesmium* and the co-occurring phytoplankton

Biogeography of *Trichodesmium* IMS 101 16S gene sequence was explored in a pan-oceanic collection of plankton metagenomes using *Tara ocean*'s Microbiome Reference Gene Catalog metagenomes (https://tara-oceans.mio.osupytheas.fr/ocean-gene-atlas/). The 16S nucleic acid sequence of *Trichodesmium* IMS 101 was obtained from NCBI database (https://www.ncbi.nlm.nih.gov/). The expected threshold was set at $1e^{-100}$. The abundance of *Synechococcus* 7002 in each *Trichodesmium* IMS 101 distribution site was also obtained from *Tara ocean*'s Microbiome Reference Gene Catalog Metagenomes. Specific details have been previously reported (Vernette et al., 2022; Villar et al., 2018).

Construction of *Synechococcus* 7002 urease mutant

Using the DNA of wild-type *Synechococcus* 7002 as a template, PCR amplification was carried out with *ureA*-up-F/*ureA*-up-R and *ureA*-DN-F/*ureA*-DN-R primers, respectively, to obtain upstream homologous fragment (*ureA*-UP) and downstream homologous fragment (*ureA*-DN) of *ureA* gene. The recombinant plasmid NBU-KAN-Mut-*ureA* was obtained by inserting the *ureA*-UP and

ureA-DN fragments into the upstream and downstream segments of kanamycin resistance gene (Kan^R) of a pUC-MCS-KAN-MCS plasmid vector, respectively, maintaining the orientation of gene sequences. Then, the recombinant plasmid NBU-KAN-Mut-*ureA* was transformed into the wild-type *Synechococcus* 7002 to obtain the Mut-*ureA* strain.

Data statistics and analysis

All data were tested for homogeneity of variance and normal distribution, and statistical analyses including Student's t-test, ANOVA, and Duncan's multiple range test were performed using the open-source statistical software SPSS 26.0. Values of $p < 0.05$ were considered statistically significant. Data with significant differences are marked with different lowercase letters or ∗.

Results

Global distribution of *Trichodesmium* spp. and co-existing phytoplankton in *Tara Ocean*

To explore the potential co-existing phytoplankton with *Trichodesmium*, we used the macro-genome database of *Tara Ocean* to investigate the global distribution of *Trichodesmium* in the sea and the composition of co-existing planktonic communities. The results showed that *Trichodesmium* is distributed in low to mid-latitude waters worldwide (Figure 1A), with its highest abundance in several sites of southern Atlantic Ocean (e.g., T_68, T_76, T_78).

Organisms co-existing with *Trichodesmium* belonged to various phyla including Proteobacteria, Bacteroidetes, Firmicutes, Cyanobacteria, Actinobacteria, Thermotogae, Planctomycetes, etc, among Cyanobacteria, *Synechococcus* occupied the dominant co-occurrence with *Trichodesmium* (Figures 1B–D), the abundance of *Synechococcus* and *Trichodesmium* was very strongly correlated at each station ($R^2 = 0.8343$) (Supplementary Figure S3).

The ability of other phytoplankton to directly use the nitrogen source fixed and released by *Trichodesmium* needed to be confirmed through physiological experiments. As shown in Figure 1E, when *Synechococcus* 7002 was monocultured with N-free medium, the cells gradually turned yellow and eventually stopped growing, indicating that *Synechococcus* cells underwent chlorosis. Such distinctive color changes were considered typical of nitrogen deficiency in cyanobacteria (Klotz et al., 2016). However, when *Synechococcus* 7002 was co-cultured with *Trichodesmium* in the N-free medium, it is obvious that the addition of *Trichodesmium* could help sustain the growth of *Synechococcus* 7002 in N-free medium (Figure 1E). Due to the mixed culture of the two cyanobacteria and the adhere of *Synechococcus* to *Trichodesmium* filaments, we were unable to make a specific count of cell numbers for both. But we observed a greater density of *Synechococcus* 7002 cells as well as a greener cell color (Figure 1E). Thus, we hypothesized that *Trichodesmium* might produce some nitrogen sources and support the growth of *Synechococcus* 7002. In this context, we conducted a series of experiments focused on what forms of nitrogen sources in *Trichodesmium* N-free culture system could support the growth of *Synechococcus* 7002.

Utilization of different nitrogen sources by *Trichodesmium* IMS 101

Trichodesmium can not only obtain nitrogen source through biological nitrogen fixation, but also directly uptake and assimilate combined nitrogen. To understand the preference of *Trichodesmium* for nitrogen sources and the influence of different nitrogen sources on nitrogen fixation genes, we carried out the following research. As shown in Figure 2, low concentrations of nitrogen (5 or 10 μM) did not affect the cell growth of *Trichodesmium* IMS 101. However, high concentrations of inorganic nitrogen (40 and 80 μM $NaNO_3$ or NH_4Cl) significantly inhibited the growth of *Trichodesmium* IMS 101 ($p < 0.05$), especially in presence of NH_4Cl in high concentration (Figures 2A, B). In contrast, the addition of urea did not significantly affect the cell growth compared with the control without any nitrogen. The cell growth increased significantly at the high urea concentration of 80 μM ($p < 0.05$) (Figure 2C).

When the nitrogen sources were added into the *Trichodesmium* IMS 101 culture at lower concentrations (< 20 μM), no significant difference in the growth of *Trichodesmium* was observed. However, significant down-regulation of *nifH* gene expression in *Trichodesmium* IMS 101 was observed with increasing nitrogen concentration, indicating the relatively lower nitrogen fixation rate of *Trichodesmium* IMS 101 under high nitrogen supply (Figure 3). Although all the three added nitrogen sources inhibited the expression of *nifH* gene, more inhibitory effects were observed after the supply of inorganic nitrogen ($NaNO_3$ and NH_4Cl), as compared with the organic nitrogen (urea) supply. NH_4Cl exhibited the most significant inhibiting effect on *nifH* gene expression, especially at high concentrations, which was consistent with its inhibitory effect on the growth of *Trichodesmium* IMS 101.

Types and concentrations of different nitrogen forms present in the filtrate of *Trichodesmium* IMS 101 cultured without nitrogen supply

The growth curve of *Trichodesmium* IMS 101 cultured in YBC medium (no N supply) included three growth phases: exponential growth phase, plateau phase, and decline phase (Figure 4A). The exponential growth phase lasted for 12 days with a specific growth rate at ~0.1 d^{-1} (Figure 4A). The plateau phase lasted for 6 days and sharply dropped into the decline phase (Figure 4A). In addition, the results showed that the main form of nitrogen source present in the culture filtrate at day 12^{th} was DON, with urea accounting for a relatively considerable proportion of DON (more than 25%) and total nitrogen (around 20%). Other forms such as amino acids and proteins accounted for the majority of DON. Inorganic nitrogen forms, such as NH_4^+, accounted for only a small proportion of the total nitrogen, which was far less than urea (Figure 4B).

10.3389/fmars.2024.1388725

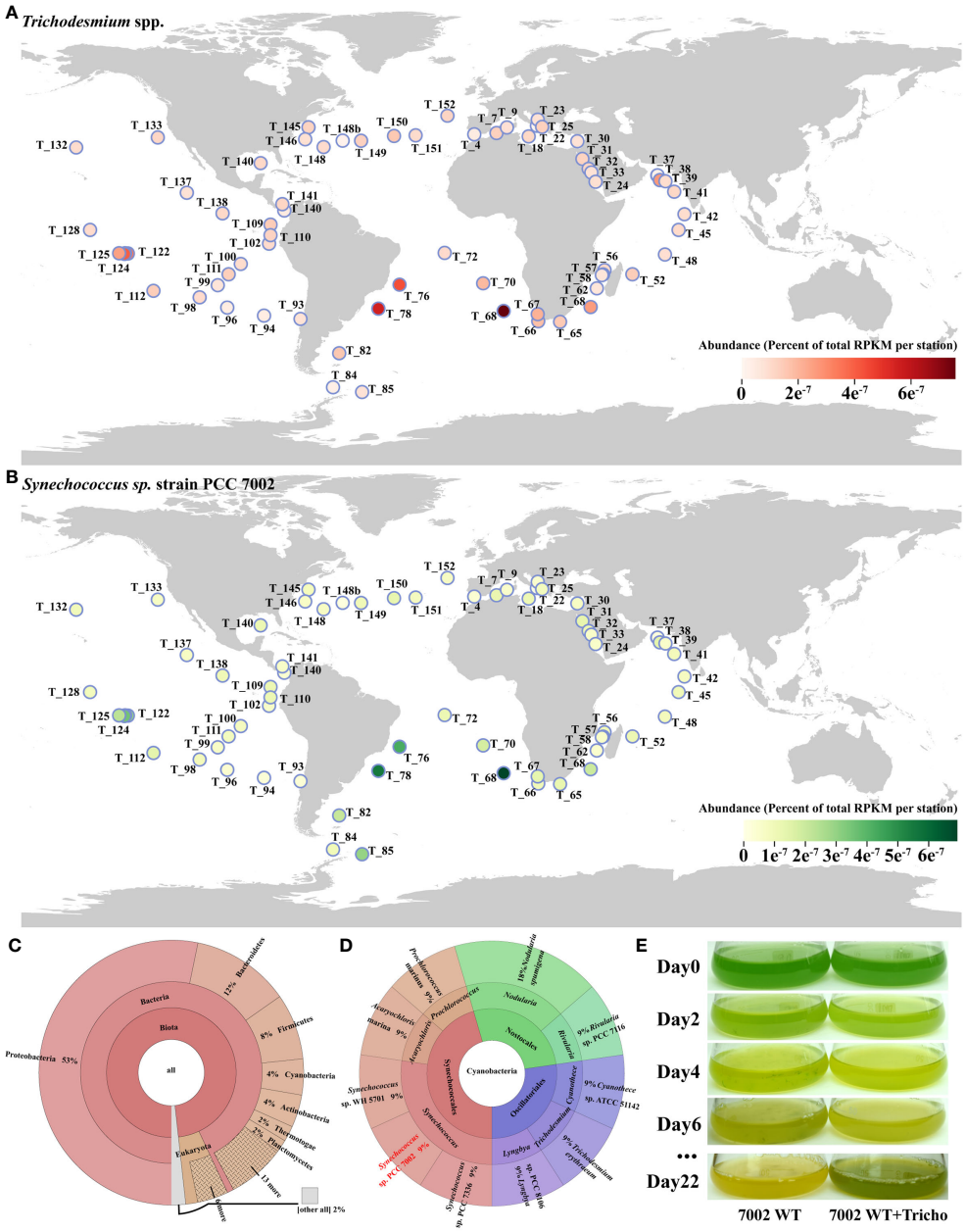

FIGURE 1
(A) Global distribution of *Trichodesmium* spp. from *Tara Ocean* datasets; **(B)** Abundance of *Synechococcus* 7002 at all stations where *Trichodesmium* spp. were present; **(C)** The co-existing phytoplankton at all stations; **(D)** Different species of cyanobacteria co-existing with *Trichodesmium* at all sites; **(E)** The photo of a co-culture of nitrogen-deficient *Synechococcus* 7002 and *Trichodesmium* IMS 101. Tara Ocean sites were analyzed using the partial 16S rRNA gene sequence. T_XXX (e.g., T_128) in the figure represents the station number, and the color of the circles represents the abundance at a specific site.

Tolerance of *Synechococcus* 7002 to chronic nitrogen deficiency and its preference to use different types of nitrogen sources

As shown in Figure 5A, *Synechococcus* 7002 cells cultured without nitrogen showed chlorosis after day 10^{th}. When nitrogen deprivation was further prolonged, the cells of *Synechococcus* 7002 entered a dormant state and stopped growing and dividing. However, upon encountering an external nitrogen source, the cells immediately initiated a recovery program (Figures 5A, B). When nitrogen sources such as NO_3^-, NH_4^+, urea and glutamate were provided to the nitrogen-deficient *Synechococcus* 7002, both inorganic and organic nitrogen sources were absorbed and utilized by *Synechococcus* 7002 for cell division and reproduction (Figure 5C). *Synechococcus* 7002 preferred to use urea and NH_4^+ as the nitrogen source rather than NO_3^- and glutamate (Figure 5B). These results indicate that *Synechococcus* 7002 may serve as an ideal model to study the utilization of nitrogen secreted by *Trichodesmium*.

10.3389/fmars.2024.1388725

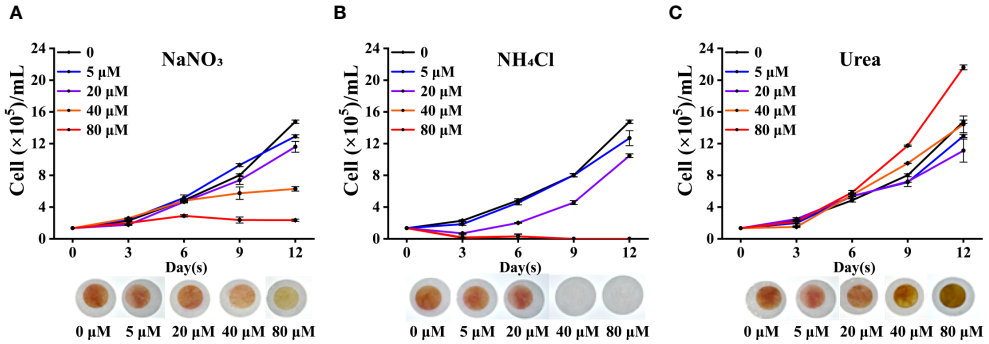

FIGURE 2
The growth curves of *Trichodesmium* IMS 101 in presence of different nitrogen sources (n=3). **(A–C)** are the growth curves of *Trichodesmium* IMS 101 under different concentrations of $NaNO_3$, NH_4Cl, and Urea, respectively. The pictures of culture plates were taken on the 9th day of cultivation. The error bar represents the standard deviation between the three replicates, ($p < 0.05$).

Effect of the nitrogen source secreted by *Trichodesmium* IMS 101 on the growth of wild-type and Mut-*ureA* strain of *Synechococcus* 7002

After the addition of *Trichodesmium* IMS 101 filtrate to the nitrogen-deficient *Synechococcus* 7002 culture, the growth curves and growth rates of *Synechococcus* 7002 showed a significant increase compared to the control treatment with the addition of nitrogen-free YBC-II medium (Figures 6A, C). Moreover, the cellular Chl *a* content of *Synechococcus* 7002 increased significantly ($p < 0.01$) after 72 h of the addition of *Trichodesmium* IMS 101 filtrate, whereas the Chl *a* content of *Synechococcus* 7002 added with nitrogen-free YBC-II medium decreased further, even though the cells could still grow slowly (Figure 6E). These results suggest that the filtrate of *Trichodesmium* IMS 101 can restore the growth of nitrogen-starved *Synechococcus* 7002 in N-free medium.

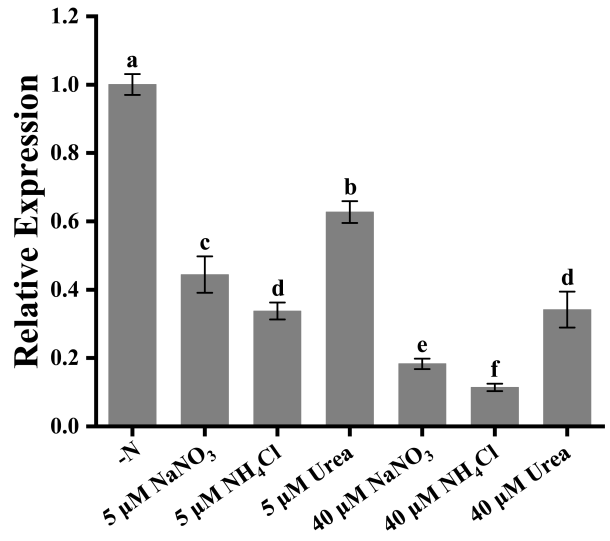

FIGURE 3
nifH gene expression in *Trichodesium* IMS 101 grown for 24 h in YBC-II media supplemented with different nitrogen sources. The error bar represents the standard deviation of the three replicates. Significance analysis was based on Duncan's multiple range test and marked with lowercase letters, with different letters representing significant differences in *nifH* gene expression between different nitrogen source treatments, ($p < 0.05$).

To investigate whether the abundant urea in the *Trichodesmium* filtrate can be utilized by *Synechococcus* 7002, a *Synechococcus* 7002 urease mutant strain (Mut-*ureA*) was obtained using a constructed homologous double-exchange plasmid (Supplementary Figures S4A–C). By using different types of nitrogen sources, it was confirmed that Mut-*ureA* completely lost its urea utilization ability but still had a normal ability to take up and utilize other forms of nitrogen sources (Supplementary Figures S4D–F)

The growth curves and growth rates of chronic nitrogen-deficient *Synechococcus* 7002 Mut-*ureA* strain were also measured after the addition of the same *Trichodesmium* IMS 101 filtrate and the N free YBC-II medium respectively. The result showed that the cells growth and the pigment content of the *Synechococcus* 7002 Mut-*ureA* strain ($p < 0.01$) were also increased significantly with the addition of *Trichodesmium* filtrate. However, its total biomass was lower than that of the wild-type strain (Figures 6B, D). Together, the results indicated that there were multiple nitrogen sources from the *Trichodesmium* filtrate could be used by *Synechococcus* 7002, but urea contributed at least part of the total bioavaible nitrogen. Since the Mut-*ureA* strain lost the urea utilization ability, its growth restoration was slower compared to the wild type strain when they using the N sources released by *Trichodesmium* IMS 101 filtrate. The growth difference of 20% was consistent with the urea content accounting for 20% of the total released nitrogen in *Trichodesmium* IMS 101 filtrate (Figure 4B). Overall, these results indicate that the nitrogen secreted by *Trichodesmium* plays a crucial role in supporting the growth recovery of chronically nitrogen-deficient *Synechococcus* 7002, and urea might contribute a similar fraction of nitrogen source.

Discussion

Trichodesmium may fix dinitrogen (N_2) in the oligotrophic near-surface regions of ocean, alleviating N limitation and encouraging a more diverse plankton community. The nitrogen fixed by

10.3389/fmars.2024.1388725

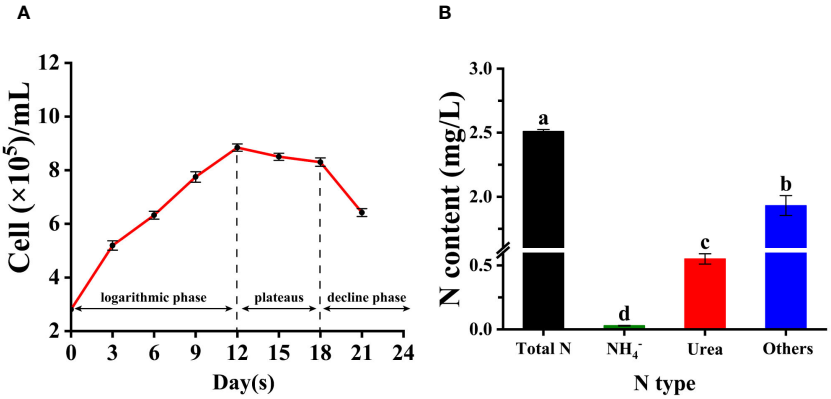

FIGURE 4
The complete growth curve of *Trichodesmium* IMS 101 including exponential phase, plateaus and decline phase **(A)**; and the types and concentrations of different nitrogen forms secreted by *Trichodesmium* IMS 101 during late exponential phase **(B)**. The error bar represents the standard deviation of three replicates. Significance analysis was based on Duncan's multiple range test and marked with lowercase letters, with different letters representing significant differences between the contents of different nitrogen type, ($p < 0.05$).

Trichodesmium has been predicted to provide up to 50% of the new nitrogen-supporting open-ocean food webs in the subtropical North Pacific Gyre (Dore et al., 2002). Many reports have demonstrated that *Trichodesmium* fixes nitrogen under nitrogen deficient conditions and high concentrations of exogenous inorganic nitrogen in the environment may significantly decrease the N_2 fixation rate of *Trichodesmium* (Holl and Montoya, 2005; Sandh et al., 2011; Eichner et al., 2014; Walworth et al., 2018). To date, most diazotrophic nitrogen assimilation studies have focused on the relationship between N_2 fixation and uptake of nitrate, ammonia, and amino acid (Ohki et al., 1991; Mulholland et al., 1999, Mulholland et al., 2001). The absorption and utilization characteristics of *Trichodesmium* for various nitrogen compounds are largely unknown. In this study, the growth and expression of *nifH* gene (encodes nitrogen-fixing enzymes of *Trichodesmium*) were analyzed in the presence of different types of nitrogen sources in varying

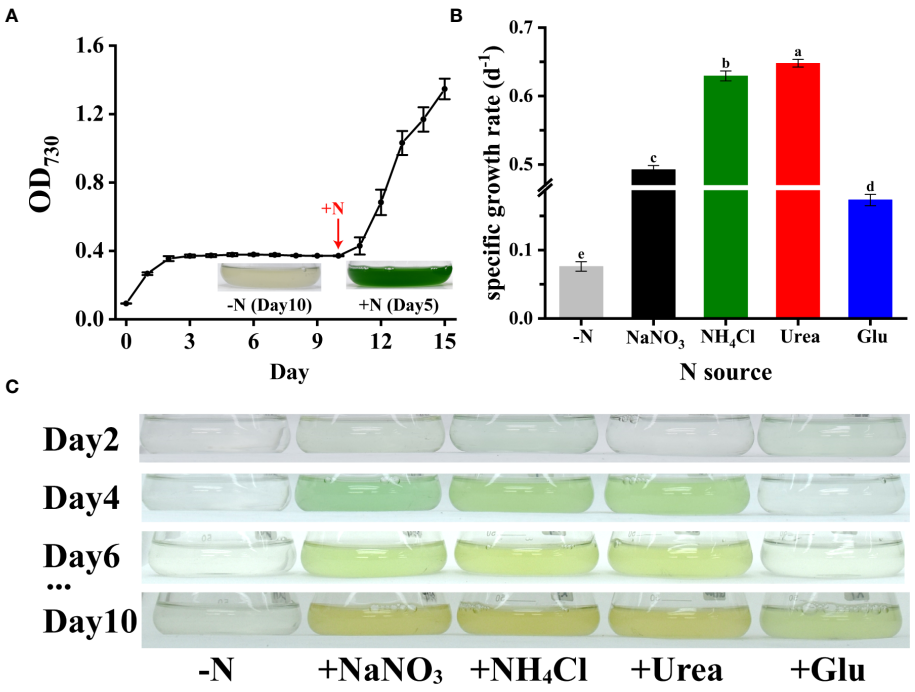

FIGURE 5
Growth rates and photographs of nitrogen-deficient *Synechococcus* 7002 after addition of different nitrogen sources: **(A)** growth curves and photographs of *Synechococcus* 7002 recovered by the addition of nitrogen after 10 days of nitrogen deficiency (added 12 mM $NaNO_3$ on day 10); **(B)** growth rates after supply of different nitrogen sources to nitrogen-deficient *Synechococcus* 7002; **(C)** photos of *Synechococcus* 7002 taken at different days after supply of different nitrogen sources. Error bars represent the standard deviation of three replicates. Significance analysis was based on Duncan's multiple range test and marked with lowercase letters, with different letters representing significant differences between growth rates obtained from different nitrogen sources supplied, ($p < 0.05$).

10.3389/fmars.2024.1388725

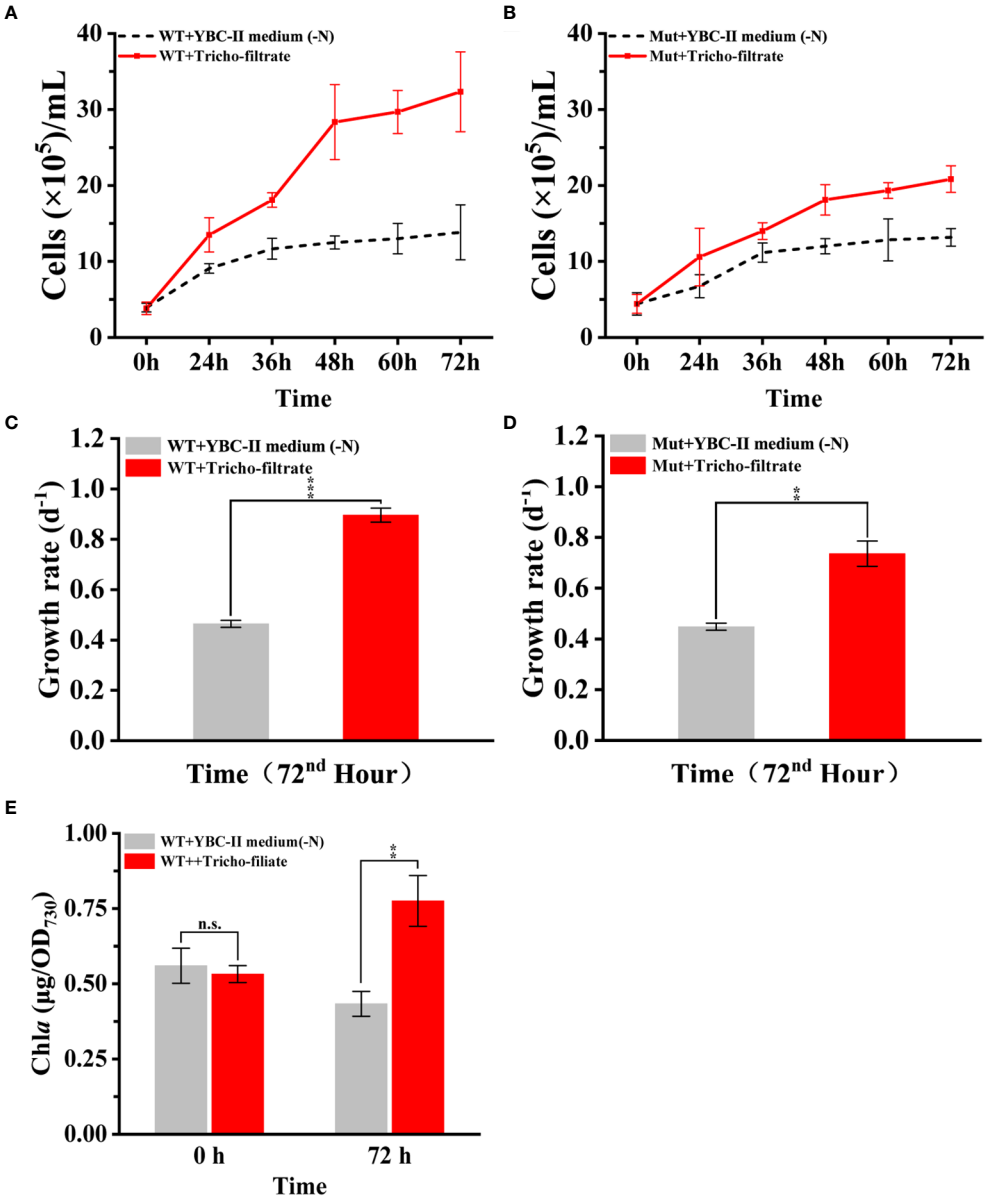

FIGURE 6
(A, C, E) show the effects of *Trichodesmium* IMS 101 filtrate addition on biomass accumulation, growth rate, and Chl *a* content of nitrogen-deficient *Synechococcus* 7002, respectively. **(B, D)** show the effects of *Trichodesmium* IMS 101 filtrate addition on nitrogen-deficient *Synechococcus* 7002 urease mutant strain Mut-*ureA* on biomass accumulation and growth rate, respectively. Error bars represent the standard deviation of three replicates. Significance analysis was based on Duncan's multiple range test and marked with ∗ (∗ $P < 0.05$, ∗∗ $P < 0.01$, ∗∗∗ $P < 0.001$, n.s., not significant).

concentrations. The results demonstrated that urea (common DON in the water) supply at a certain concentration had little effect on *nifH* gene expression in *Trichodesmium*, as compared with the similar concentration of NH_4^+ and NO_3^- (mainly dissolved inorganic nitrogen (DIN) in the water) supply. Moreover, urea supply even could promote the growth of *Trichodesmium*. Similarly, recent research has demonstrated that carbon-, nitrogen- and phosphorus-rich dissolved organic matters (DOMs) enhance N_2 fixation rates and *nifH* gene expression in natural *Trichodesmium* colonies (Stolte et al., 2006; Letscher and Moore, 2015; Benavides et al., 2018). It was speculated that in the presence of exogenous DON, *Trichodesmium* could continue to fix nitrogen and release new nitrogen sources to improve primary productivity in marine ecosystems.

In this study, the results demonstrated that DON accounted for the majority, while NH_4^+ contributed to only a small proportion of *Trichodesmium* derived N left in the filtrate in the late exponential growth phase. This result was consistent with previous studies reporting low DIN and high DON in the culture filtrate of *Trichodesmium* (Mulholland et al., 2001; Glibert et al., 2008). It must be noticed that whether in the lab culture or the field, we should consider the entire *Trichodesmium*-epibiont assemblage as a whole for the stable cohabitation of a diverse community within *Trichodesmium* consortia. Thus, the main form of nitrogen source present in the filtrate was DON after equilibrium between nitrogen fixation by *Trichodesmium* and consumption of nitrogen source by the *Trichodesmium* consortia. However, compared to the released

DON, the remaining NH_4^+ concentration in the surrounding environment of *Trichodesmium* was extremely low (Berthelot et al., 2016). This phenomenon may be explained by the rapid release and uptake of NH_4^+ by the *Trichodesmium* community as a potential major recycling intermediate of *Trichodesmium*-fixed N_2 in the culture system, which facilitates the rapid turnover of the nitrogen pool and leaves little residue in the culture medium (Mulholland et al., 1999, 2001). Whereas DON was the main nitrogen resource accumulated in the medium, contributing a large fraction of the N pool that could be utilized by other microorganisms in the surrounding environment.

Few studies evaluated the bioavailable proportion of *Trichodesmium*-derived DON and the compounds within the uncharacterized DON pool that were utilized by other phytoplankton (Mulholland et al., 2004). DON has been largely understood by extrapolating the data obtained from the studies of dissolved organic carbon flow or through studies of individual compounds, such as urea or dissolved free amino acids (Glibert and Bronk, 1994; Mulholland et al., 2004; Finzi-Hart et al., 2009). In the present study, the nitrogen forms released by *Trichodesmium* were evaluated in the culture medium without exogenous N and the proportion of urea in the total nitrogen was found to be more than 20%. These results indicate that urea may play an important role in marine nitrogen cycle and serve as nitrogen resource for transferring nitrogen to auto- and heterotrophic plankton communities in the surrounding environment.

Many non-nitrogen-fixing cyanobacteria, such as *Synechococcus* and *Prochlorococcus*, co-exist with nitrogen-fixing cyanobacteria in nitrogen-deficient oceans (Flombaum et al., 2013; Masuda et al., 2022). *Synechococcus* 7002, originally isolated from the mud of the Atlantic coast, can be genetically manipulated and grown in axenic cultures. Recent studies have shown that the distribution of *Synechococcus* in coastal regions is far more extensive than previously thought (Lee et al., 2017; Yong et al., 2023). By using the macro-genome database of *Tara Ocean*, the presence of *Synechococcus* 7002 has been detected in the sights where *Trichodesmium* exists. This shows that *Trichodesmium* and *Synechococcus* 7002 (at least strains similar to *Synechococcus* 7002 due to the inaccuracy of bioinformatics prediction) possibly have a close association in the oceans (Figure 1; Supplementary Figure S3). To further reveal the potential interaction between *Trichodesmium* and the "consumer" of its secreted nitrogen, *Synechococcus* 7002 was co-cultured with *Trichodesmium* and its secreted filtrate was used as the sole nitrogen resource. When *Trichodesmium* reached a dynamic equilibrium with *Synechococcus* cells in the culture system, both in terms of biomass and nitrogen turnover, chronically nitrogen-deficient *Synechococcus* 7002 recovered from chlorosis to green cells (Figure 1E). This implied that nitrogen fixation by *Trichodesmium* provided an important source of nitrogen for the recovery of *Synechococcus* 7002. In mono-cultures, both *Synechococcus* 7002 and *Trichodesmium* preferred urea as a nitrogen source than other inorganic nitrogen sources (e.g., ammonium and nitrate). Inhibition of *Trichodesmium* growth was observed at high dose of exogenous inorganic nitrogen (NO_3^-, NH_4^+), but the growth increased at a higher concentration of urea. *Synechococcus* 7002 showed good recovery after a prolonged period of nitrogen deficiency, when exogenous nitrogen sources were supplied. Among the added exogenous nitrogen sources, ammonium and urea were preferred by *Synechococcus* 7002, which was consistent with the previous study reporting ammonia as the preferred microbial nitrogen source for most, if not all, cyanobacteria (Walworth et al., 2018). After the addition of *Trichodesmium* IMS 101 filtrate to *Synechococcus* 7002 culture, the biomass of nitrogen-deficient dormant *Synechococcus* 7002 also increased significantly (Figure 6). These results suggest that the nitrogen source released by *Trichodesmium* can be directly utilized by *Synechococcus* 7002 for its growth.

Some studies investigated the transfer of diazotroph-derived nitrogen to non-diazotrophic planktonic communities (Chen et al., 2011; Sipler et al., 2013). For instance, Chen et al. (2011) conducted ^{15}N tracing experiments and revealed the transfer of newly fixed N from *Trichodesmium* to diatoms. Sipler et al. (2013) used the FT-ICR MS method to detect the changes in DON concentration and the composition of N compound showed that *Trichodesmium* sp. provided a sufficient source of nitrogen to directly or indirectly support *Karenia brevis* blooms. However, the potential mechanism involved in the transfer of *Trichodesmium*-derived DON such as urea and amino acids to non-diazotrophic plankton need to be further studied at physiological and molecular level. In the present study, the biomass of Mut-*ureA* strain was around 20% lower than the biomass of the wild type *Synechococcus*. This result indicated that urea provided a fraction of nitrogen source that *Synechococcus* could obtain from *Trichodesmium* filtrate. The physiological study of genetic mutants provided direct evidence that chronically nitrogen-starved marine *Synechococcus* was able to access urea (produced during nitrogen fixation by *Trichodesmium*) as the nitrogen source, although other forms of nitrogen released by *Trichodesmium* also had positive effects on the growth of *Synechococcus*, which account for ~80% of the biomass accumulation. A previous study examined the transcriptional profile of cyanobacteria co-existing in *Trichodesmium* populations, which was primarily dominated by *Synechococcus* (Lee et al., 2018). The study reported that the cyanobacteria allocated more of their transcriptional pool to ammonium (*amtB*) and urea (*urtA*) transport, with protein products also detected during urea transport, indicating the potential utilization of *Trichodesmium*-derived urea by *Synechococcus* (Lee et al., 2018). In the present study, wild-type and Mut-*ureA* strains of *Synechococcus* 7002 were used in this study to understand the relationship between *Trichodesmium* and non-nitrogen-fixing *Synechococcus* linked by the fixed and released nitrogen of *Trichodesmium*. The results showed that the *Trichodesmium*-derived urea contributed at least part of the nitrogen source for the growth of phytoplankton populations. This suggests that urea exuded by diazotrophs can fuel the primary production and may have a considerable impact on the composition of the plankton community in the oligo-trophic oceans.

Long-term evolution has led to the development of different nitrogen uptake and utilization mechanisms as well as the nitrogen source preferences of various marine phytoplankton. This may challenge the potential of *Trichodesmium* as an important supplier of nitrogen sources to promote marine primary productivity (Moore et al., 2002; Zubkov et al., 2003; Esteves-Ferreira et al., 2018; Li et al., 2023). This study emphasized the crucial role of *Trichodesmium*-derived DON, especially urea, as new nitrogen to support the growth of phytoplankton in the

surrounding environment, represents an important pathway of N and C export to the deeper parts of tropical and subtropical oceans. Further studies are needed to investigate the fate of other dominant *Trichodesmium*-derived N forms and to highlight the complex interactions of diazotrophs with their environment.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

S-QL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. YX: Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. H-LH: Data curation, Investigation, Methodology, Validation, Visualization, Writing – review & editing. ZL: Formal analysis, Investigation, Methodology, Software, Writing – review & editing. T-RS: Formal analysis, Investigation, Software, Writing – review & editing. H-YG: Data curation, Investigation, Methodology, Software, Writing – review & editing. X-WW: Conceptualization, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. H-BJ: Conceptualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was funded by the Science and Technology Innovation 2025 Major Project of Ningbo City (Grant No. 2022Z189), National Natural Science Foundation of China (Grant No. 32170108, No. 42188102, No.91951111 and No. 42206083) and the National Natural Science Foundation of Zhejiang Province (Grant No. LQ21D060004).

Acknowledgments

We are very thankful to Baosheng Qiu (Central China Normal University), David A. Hutchins and Feixue Fu (University of Southern California) for their help and advices for this study.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2024.1388725/full#supplementary-material

References

Basu, S., Matondkar, S. G. P., and Furtado, I. (2011). Enumeration of bacteria from a *Trichodesmium* spp. bloom of the Eastern Arabian Sea: elucidation of their possible role in biogeochemistry. *J. Appl. Phycol* 23, 309–319. doi: 10.1007/s10811-010-9589-4

Benavides, M., Agawin, N., Arístegui, J., Peene, J., and Stal, L. (2013). Dissolved organic nitrogen and carbon release by a marine unicellular diazotrophic cyanobacterium. *Aquat. Microb. Ecol.* 69, 69–80. doi: 10.3354/ame01621

Benavides, M., Bonnet, S., Le Moigne, F. A. C., Armin, G., Inomura, K., Hallstrøm, S., et al. (2022). Sinking *Trichodesmium* fixes nitrogen in the dark ocean. *ISME J.* 16, 2398–2405. doi: 10.1038/s41396-022-01289-6

Benavides, M., Martias, C., Elifantz, H., Berman-Frank, I., Dupouy, C., and Bonnet, S. (2018). Dissolved organic matter influences n_2 fixation in the New Caledonian lagoon (Western Tropical South Pacific). *Front. Mar. Sci.* 5. doi: 10.3389/fmars.2018.00089

Berthelot, H., Bonnet, S., Camps, M., Grosso, O., and Moutin, T. (2015). Assessment of the dinitrogen released as ammonium and dissolved organic nitrogen by unicellular and filamentous marine diazotrophic cyanobacteria grown in culture. *Front. Mar. Sci.* 2. doi: 10.3389/fmars.2015.00080

Berthelot, H., Bonnet, S., Grosso, O., Cornet, V., and Aude, B. (2016). Transfer of diazotroph-derived nitrogen towards non-diazotrophic planktonic communities: a comparative study between *Trichodesmium erythraeum*, Crocosphaera watsonii and *Cyanothece* sp. *Biogeosciences* 13, 4005–4021. doi: 10.5194/bg-13-4005-2016

Bonnet, S., Berthelot, H., Turk-Kubo, K., Cornet-Barthaux, V., Fawcett, S., Berman-Frank, I., et al. (2016). Diazotroph derived nitrogen supports diatom growth in the South West Pacific: A quantitative study using nanoSIMS: Transfer of diazotrophic N into plankton. *Limnol Oceanogr* 61, 1549–1562. doi: 10.1002/lno.10300

Capone, D. G., and Carpenter, E. (1982). Nitrogen fixation in the marine environment. *J. Science* 217, 1140–1142. doi: 10.1126/science.217.4565.1140

Capone, D. G., Zehr, J. P., Paerl, H. W., Bergman, B., and Edward, J. (1997). *Trichodesmium*, a globally significant marine cyanobacterium. *Science* 276, 1221–1229. doi: 10.1126/science.276.5316.1221

Carpenter, E. J., and Capone, D. G. (2008)Chapter 4 - nitrogen fixation in the marine environment in *Nitrogen in the marine environment*, *2nd ed.*Eds. D. G. Capone, D. A. Bronk, M. R. Mulholland and E. J. Carpenter (Academic Press, San Diego), 141–198.

Chen, L., Ma, J., Huang, Y., Dai, M., and Li, X. (2015). Optimization of a colorimetric method to determine trace urea in seawater: Trace urea analysis in seawater. *Limnol Oceanogr-meth* 13, 303–311. doi: 10.1002/lom3.10026

Chen, Y.-B., Dominic, B., Mellon, M. T., and Zehr, J. P. (1998). Circadian rhythm of nitrogenase gene expression in the diazotrophic filamentous nonheterocystous cyanobacterium *Trichodesmium* sp. Strain IMS 101. *J. Bacteriol* 180, 3598–3605. doi: 10.1128/JB.180.14.3598-3605.1998

Chen, Y. L. L., Tuo, S., and Chen, H. (2011). Co-occurrence and transfer of fixed nitrogen from *Trichodesmium* spp. to diatoms in the low-latitude kuroshio current in the NW Pacific. *Mar. Ecol. Prog. Ser.* 421, 25–38. doi: 10.3354/meps08908

Dore, J. E., Brum, J. R., Tupas, L. M., and Karl, D. M. (2002). Seasonal and interannual variability in sources of nitrogen supporting export in the oligotrophic subtropical North Pacific Ocean. *Limnol. Oceanogr.* 47, 1595–1607. doi: 10.4319/lo.2002.47.6.1595

Dore, J. E., Letelier, R. M., Church, M. J., Lukas, R., and Karl, D. M. (2008). Summer phytoplankton blooms in the oligotrophic North Pacific Subtropical Gyre: Historical perspective and recent observations. *Prog. Oceanogr* 76, 2–38. doi: 10.1016/j.pocean.2007.10.002

Eichner, M., Inomura, K., Pierella Karlusich, J. J., and Shaked, Y. (2023). Better together? Lessons on sociality from *Trichodesmium*. *Trends Microbiol.* 31, 1072–1084. doi: 10.1016/j.tim.2023.05.001

Eichner, M., Kranz, S. A., and Rost, B. (2014). Combined effects of different CO_2 levels and N sources on the diazotrophic cyanobacterium *Trichodesmium*. *Physiol. Plantarum* 152, 316–330. doi: 10.1111/ppl.12172

Esteves-Ferreira, A. A., Inaba, M., Fort, A., Araújo, W. L., and Sulpice, R. (2018). Nitrogen metabolism in cyanobacteria: metabolic and molecular control, growth consequences and biotechnological applications. *Crit. Rev. Microbiol.* 44, 541–560. doi: 10.1080/1040841X.2018.1446902

Finzi-Hart, J. A., Pett-Ridge, J., Weber, P. K., Popa, R., Fallon, S. J., Gunderson, T., et al. (2009). Fixation and fate of C and N in the cyanobacterium *Trichodesmium* using nanometer-scale secondary ion mass spectrometry. *Proc. Natl. Acad. Sci. U.S.A.* 106, 6345–6350. doi: 10.1073/pnas.0810547106

Flombaum, P., Gallegos, J. L., Gordillo, R. A., Rincón, J., Zabala, L. L., Jiao, N., et al. (2013). Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proc. Natl. Acad. Sci. U.S.A.* 110, 9824–9829. doi: 10.1073/pnas.1307701110

Glibert, P. M., and Bronk, D. (1994). Release of dissolved organic nitrogen by marine diazotrophic cyanobacteria. *A. Trichodesmium Appl. Environ. Microbiol.* 60, 3996–4000. doi: 10.1128/aem.60.11.3996-4000.1994

Glibert, P. M., Azanza, R., Burford, M., Furuya, K., Abal, E., Al-Azri, A., et al. (2008). Ocean urea fertilization for carbon credits poses high ecological risks. *Mar. pollut. Bull.* 56, 1049–1056. doi: 10.1016/j.marpolbul.2008.03.010

Gradoville, M. R., Farnelid, H., White, A. E., Turk-Kubo, K. A., Stewart, B., Ribalet, F., et al. (2020). Latitudinal constraints on the abundance and activity of the cyanobacterium UCYN-A and other marine diazotrophs in the North Pacific. *Limnol Oceanogr* 65, 1858–1875. doi: 10.1002/lno.11423

Hagedorn, F., and Schleppi, P. (2000). Determination of total dissolved nitrogen by persulfate oxidation. *J. Plant Nutr. Soil Sci.* 163, 81–82. doi: 10.1002/(sici)1522-2624(200002)163:1<81::aid-jpln81>3.0.co;2-1

Holl, C. M., and Montoya, J. P. (2005). Interactions between nitrate uptake and nitrogen fixation in continuous cultures of the marine diazotroph *Trichodesmium* (cyanobacteria). *J. Phycol* 41, 1178–1183. doi: 10.1111/j.1529-8817.2005.00146.x

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

Jiang, H.-B., Lou, W.-J., Du, H.-Y., Price, N. M., and Qiu, B.-S. (2012). Sll1263, a unique cation diffusion facilitator protein that promotes iron uptake in the cyanobacterium *Synechocystis* sp. strain PCC 6803. *Plant Cell Physiol.* 53, 1404–1417. doi: 10.1093/pcp/pcs086

Klawonn, I., Eichner, M. J., Wilson, S. T., Moradi, N., Thamdrup, B., Kümmel, S., et al. (2020). Distinct nitrogen cycling and steep chemical gradients in *Trichodesmium* colonies. *ISME J.* 14, 399–412. doi: 10.1038/s41396-019-0514-9

Klotz, A., Georg, J., Bučinská, L., Watanabe, S., Reimann, V., Januszewski, W., et al. (2016). Awakening of a dormant cyanobacterium from nitrogen chlorosis reveals a genetically determined program. *Curr. Biol.* 26, 2862–2872. doi: 10.1016/j.cub.2016.08.054

Konno, U., Tsunogai, U., Komatsu, D. D., Daita, S., Nakagawa, F., Tsuda, A., et al. (2010). Determination of total N_2 fixation rates in the ocean taking into account both the particulate and filtrate fractions. *Biogeosciences* 7, 2369–2377. doi: 10.5194/bg-7-2369-2010

Le, P. T. T., and Boyd, C. E. (2012). Comparison of phenate and salicylate methods for determination of total ammonia nitrogen in freshwater and saline water. *J. World Aquaculture Soc.* 43, 885–889. doi: 10.1111/j.1749-7345.2012.00616.x

Lee, M. D., Walworth, N. G., McParland, E. L., Fu, F.-X., Mincer, T. J., Levine, N. M., et al. (2017). The *Trichodesmium* consortium: conserved heterotrophic co-occurrence and genomic signatures of potential interactions. *ISME J.* 11, 1813–1824. doi: 10.1038/ismej.2017.49

Lee, M. D., Webb, E. A., Walworth, N. G., Fu, F.-X., Held, N. A., Saito, M. A., et al. (2018). Transcriptional activities of the microbial consortium living with the marine nitrogen-fixing cyanobacterium *Trichodesmium* reveal potential roles in community-level nitrogen cycling. *Appl. Environ. Microb.* 84, e02026–e02017. doi: 10.1128/AEM.02026-17

Lenes, J. M., and Heil, C. A. (2010). A historical analysis of the potential nutrient supply from the N_2 fixing marine cyanobacterium *Trichodesmium* spp. to *Karenia brevis* blooms in the eastern gulf of Mexico. *J. Plankton Res.* 32, 1421–1431. doi: 10.1093/plankt/fbq061

Letscher, R. T., and Moore, J. K. (2015). Preferential remineralization of dissolved organic phosphorus and non-Redfield DOM dynamics in the global ocean: Impacts on marine productivity, nitrogen fixation, and carbon export. *Global Biogeochem Cy* 29, 325–340. doi: 10.1002/2014GB004904

Li, S.-Q., Huang, H.-L., Sun, T.-R., Gao, H.-Y., Wang, X.-W., Fu, F.-X., et al. (2023). Cyanobacteria using urea as a nitrogen source can overcome acid stress. *bioRxiv* 2023, 3.29.534730. doi: 10.1101/2023.03.29.534730

Ludwig, M., and Bryant, D. A. (2012). Acclimation of the global transcriptome of the Cyanobacterium *Synechococcus* sp. Strain PCC 7002 to nutrient limitations and different nitrogen sources. *Front. Microbio.* 3. doi: 10.3389/fmicb.2012.00145

Mahaffey, C. (2005). The conundrum of marine N_2 fixation. *Am. J. Sci.* 305, 546–595. doi: 10.2475/ajs.305.6-8.546

Masuda, T., Inomura, K., Kodama, T., Shiozaki, T., Kitajima, S., Armin, G., et al. (2022). *Crocosphaera* as a major consumer of fixed nitrogen. *Microbiol. Spectr.* 10, e02177–e02121. doi: 10.1128/spectrum.02177-21

Moisander, P. H., Beinart, R. A., Hewson, I., White, A. E., Johnson, K. S., Carlson, C. A., et al. (2010). Unicellular cyanobacterial distributions broaden the oceanic N_2 fixation domain. *Science* 327, 1512–1514. doi: 10.1126/science.1185468

Moore, L. R., Post, A. F., Rocap, G., and Chisholm, S. W. (2002). Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnol. Oceanogr.* 47, 989–996. doi: 10.4319/lo.2002.47.4.0989

Mulholland, M. R. (2007). The fate of nitrogen fixed by diazotrophs in the ocean. *Biogeosciences* 4, 37–51. doi: 10.5194/bg-4-37-2007

Mulholland, M. R., and Bernhardt, P. W. (2005). The effect of growth rate, phosphorus concentration, and temperature on N_2 fixation, carbon fixation, and nitrogen release in continuous cultures of *Trichodesmium* IMS 101. *Limnol Oceanogr* 50, 839–849. doi: 10.4319/lo.2005.50.3.0839

Mulholland, M., Bronk, D., and Capone, D. (2004). Dinitrogen fixation and release of ammonium and dissolved organic nitrogen by *Trichodesmium* IMS 101. *Aquat. Microb. Ecol.* 37, 85–94. doi: 10.3354/ame037085

Mulholland, M. R., Ohki, K., and Capone, D. G. (1999). Nitrogen utilization and metabolism relative to patterns of N_2 fixation in cultures of *Trichodesmium* Nibb1067. *J. Phycology* 35, 977–988. doi: 10.1046/j.1529-8817.1999.3550977.x

Mulholland, M. R., Ohki, K., and Capone, D. G. (2001). Nutrient controls on nitrogen uptake and metabolism by natural populations and cultures of *Trichodesmium* (cyanobacteria). *J. Phycol* 37, 1001–1009. doi: 10.1046/j.1529-8817.2001.00080.x

Muñoz-Marín, M. C., Gómez-Baena, G., López-Lozano, A., Moreno-Cabezuelo, J. A., Díez, J., and García-Fernández, J. M. (2020). Mixotrophy in marine picocyanobacteria: use of organic compounds by *Prochlorococcus* and *Synechococcus*. *ISME J.* 14, 1065–1073. doi: 10.1038/s41396-020-0603-9

Ohki, K., Zehr, J. P., Falkowski, P. G., and Fujita, Y. (1991). Regulation of nitrogen-fixation by different nitrogen sources in the marine non-heterocystous cyanobacterium *Trichodesmium* sp. NIBB1067. *Arch. Microbiol.* 156, 335–337. doi: 10.1007/BF00248706

Padmakumar, K. B., Smitha, B. R., Thomas, L. C., Fanimol, C. L., SreeRenjima, G., Menon, N. R., et al. (2010). Blooms of *Trichodesmium* erythraeum in the south eastern Arabian Sea during the onset of 2009 summer monsoon. *Ocean Sci. J.* 45, 151–157. doi: 10.1007/s12601-010-0013-4

Prufert-Bebout, L., Paerl, H. W., and Lassen, C. (1993). Growth, Nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. *Appl. Environ. Microb.* 59, 1367–1375. doi: 10.1128/aem.59.5.1367-1375.1993

Sandh, G., Ran, L., Xu, L., Sundqvist, G., Bulone, V., and Bergman, B. (2011). Comparative proteomic profiles of the marine cyanobacterium *Trichodesmium erythraeum* IMS 101 under different nitrogen regimes. *Proteomics* 11, 406–419. doi: 10.1002/pmic.201000382

Shao, Z., Xu, Y., Wang, H., Luo, W., Wang, L., Huang, Y., et al. (2023). Global oceanic diazotroph database version 2 and elevated estimate of global oceanic N_2 fixation. *Earth Syst. Sci. Data* 15, 3673–3709. doi: 10.5194/essd-15-3673-2023

Sipler, R. E., Bronk, D. A., Seitzinger, S. P., Lauck, R. J., McGuinness, L. R., Kirkpatrick, G. J., et al. (2013). *Trichodesmium*-derived dissolved organic matter is a source of nitrogen capable of supporting the growth of toxic red tide *Karenia brevis*. *Mar. Ecol. Prog. Ser.* 483, 31–45. doi: 10.3354/meps10258

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

Stolte, W., Balode, M., Carlsson, P., Grzebyk, D., Janson, S., Lips, I., et al. (2006). Stimulation of nitrogen-fixing cyanobacteria in a Baltic Sea plankton community by land-derived organic matter or iron addition. *Mar. Ecol. Prog. Ser.* 327, 71–82. doi: 10.3354/meps327071

Veaudor, T., Cassier-Chauvat, C., and Chauvat, F. (2019). Genomics of urea transport and catabolism in cyanobacteria: biotechnological implications. *Front. Microbiol.* 10. doi: 10.3389/fmicb.2019.02052

Vernette, C., Lecubin, J., Sánchez, P., Coordinators, T. O., Acinas, S. G., Babin, M., et al. (2022). The Ocean Gene Atlas v2.0: online exploration of the biogeography and phylogeny of plankton genes. *Nucleic Acids Res.* 50, W516–W526. doi: 10.1093/nar/gkac420

Villar, E., Vannier, T., Vernette, C., Lescot, M., Cuenca, M., Alexandre, A., et al. (2018). The Ocean Gene Atlas: exploring the biogeography of plankton genes online. *Nucleic Acids Res.* 46, W289–W295. doi: 10.1093/nar/gky376

Walworth, N. G., Fu, F.-X., Lee, M. D., Cai, X., Saito, M. A., Webb, E. A., et al. (2018). Nutrient-colimited *Trichodesmium* as a nitrogen source or sink in a future ocean. *Appl. Environ. Microb.* 84, e02137–e02117. doi: 10.1128/AEM.02137-17

Yong, C.-W., Deng, B., Liu, L.-M., Wang, X.-W., and Jiang, H.-B. (2023). Diversity and evolution of iron uptake pathways in marine cyanobacteria from the perspective of the coastal strain *Synechococcus* sp. strain PCC 7002. *Appl. Environ. Microb.* 89, e01732–e01722. doi: 10.1128/aem.01732-22

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

Zubkov, M. V., Fuchs, B. M., Tarran, G. A., Burkill, P. H., and Amann, R. (2003). High rate of uptake of organic nitrogen compounds by *Prochlorococcus* cyanobacteria as a key to their dominance in oligotrophic oceanic waters. *Appl. Environ. Microbiol.* 69, 1299–1304. doi: 10.1128/AEM.69.2.1299-1304.2003