LIMNOLOGY and OCEANOGRAPHY

ASLO

Limnol. Oceanogr. 65, 2020, 363–376
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doi: 10.1002/lno.11304

Interactions between ultraviolet radiation exposure and phosphorus limitation in the marine nitrogen-fixing cyanobacteria *Trichodesmium* and *Crocosphaera*

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Abstract

Increased stratification and mixed layer shoaling of the surface ocean resulting from warming can lead to exposure of marine dinitrogen (N_2)-fixing cyanobacteria to higher levels of inhibitory ultraviolet (UV) radiation. These same processes also reduce vertically advected supplies of the potentially limiting nutrient phosphorus (P) to N_2 fixers. It is currently unknown how UV inhibition and P limitation interact to affect the biogeochemical cycles of nitrogen and carbon in these biogeochemically critical microbes. We investigated the responses of the important and widespread marine N_2-fixing cyanobacteria *Crocosphaera* (strain WH0005) and *Trichodesmium* (strains IMS 101 and GBR) to UV-A and UV-B under P-replete and P-limited conditions. Growth, N_2 fixation, and carbon dioxide (CO_2) fixation rates of *Trichodesmium* IMS 101 and *Crocosphaera* were negatively affected by UV exposure. This inhibition was greater for *Trichodesmium* IMS 101 than for *Crocosphaera*, which fixes N_2 only during the night and so avoids direct UV damage. Negative effects of UV on both IMS 101 and *Crocosphaera* were less in P-limited cultures than in P-replete cultures. In contrast, no UV inhibition was observed in GBR, regardless of P availability. UV inhibition was related to different amounts of UV-absorbing compounds produced by these isolates. Responses to UV radiation and P availability interactions were taxon-specific, but our results indicated that in general, UV radiation effects on *Trichodesmium* and *Crocosphaera* range from negative to neutral. UV inhibition and its interactions with P limitation may thus have a substantial influence on the present day and future nitrogen and carbon cycles of the ocean.

An imbalance between N_2 fixation and denitrification leads to much of the surface low-latitude ocean being limited by nitrogen (N) availability (Moore et al. 2013). Biological N_2 fixation is a key source of "new" N in the surface oceans that fuels primary production and influences global carbon cycling (Karl et al. 1997). Cyanobacteria are generally thought to be the major N_2-fixing microorganisms in the open ocean (Zehr 2011). Due to this crucial role that N_2-fixing cyanobacteria play in nitrogen biogeochemistry, numerous studies have been conducted to investigate the responses of N_2-fixing cyanobacteria under different environmental conditions of pCO_2, nutrient availability, and light intensity (e.g., Berman-Frank et al. 2001*a*,*b*; Kranz et al. 2010; Garcia et al. 2013; Hutchins et al. 2013). In particular, limitation by either phosphorus (P) or iron (Fe) (Sañudo-Wilhelmy et al. 2001; Chappell et al. 2012; Moore et al. 2013), or simultaneous colimitation by both nutrients (Mills et al. 2004; Hynes et al. 2009; Walworth et al. 2016), constrains the amount of N_2 fixation that takes place in most of the tropical oceans (Sohm et al. 2011; Hutchins and Boyd 2016; Hutchins and Fu 2017).

Depletion of stratospheric ozone from anthropogenic inputs of chlorinated fluorocarbons (Molina and Rowland 1974) leads to a corresponding increase in solar ultraviolet-B (UV-B, 280–315 nm) radiation reaching the Earth's surface (Kerr and McElroy 1993; Andrady et al. 2017). Because of the success of the Montreal Protocol, global-scale depletion of stratospheric ozone and associated increases in surface UV-B radiation have been avoided (Williamson et al. 2014; Andrady

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et al. 2017), and UV-B irradiance has decreased in recent decades in the middle and high latitudes. However, in the low latitudes where most N_2-fixing cyanobacteria live, UV-B irradiance is still predicted to increase incrementally by the end of the 21st century (Bais et al. 2015; Meul et al. 2016; Andrady et al. 2017).

UV radiation not only damages cellular DNA and protein structure of cyanobacteria directly, but also affects several key physiological and biochemical processes, including growth, survival, photosynthesis, CO_2 uptake, heterocyst formation, cellular morphology, and buoyancy (Falcon et al. 2002; Gao et al. 2007; Gao and Ma 2008; Lesser 2008; Rastogi et al. 2014). The presence of UV-B even alters the morphology of filamentous cyanobacteria (Wu et al. 2005). For some cyanobacteria, exposure to UV-B results in inhibition of N_2 fixation rates (Lesser 2008), inactivating damage to the nitrogenase polypeptide (Kumar et al. 2003), and breakage of cell filaments (Qin et al. 2012). Although studies such as these have focused on freshwater cyanobacteria, little information is available about UV radiation effects on marine diazotrophs.

N_2-fixing cyanobacteria are abundant and widespread in oligotrophic tropical and subtropical oceans, with peak abundances generally found in the upper water column (Zehr et al. 2001; Carpenter et al. 2004; Moisander et al. 2010), where both UV-A (315–400 nm) and UV-B radiation can easily penetrate (Smith et al. 1992; Tedetti and Sempere 2006). Predicted future shoaling of the upper mixed layer and increased stratification of the surface layer induced by global warming are expected to be most pronounced in the subtropical central gyres, where N_2-fixing cyanobacteria are primarily found. Mixed layer shoaling in particular will constrain the vertical distribution of cyanobacteria closer to the sea surface, thus substantially increasing their exposure to higher levels of UV radiation (Gao et al. 2012). Measurements of current UV levels over the oligotrophic gyres are however lacking, and the predicted increases are also difficult to precisely quantify. This difficulty is because predicted higher future UV levels will vary just as current UV does, depending on latitude, season, time of day, local weather, surface reflectivity, and mixed layer conditions (McKenzie et al. 2011; Bais et al. 2015). These same future physical changes in stratification that will increase UV exposure, will also intensify nutrient limitation of N_2-fixing cyanobacteria by limiting nutrients like P that are supplied by vertical advection from deeper water (Hutchins and Fu 2017).

Biological effects of UV radiation have only recently begun to be examined for one of the most abundant and biogeochemically important N_2 fixing cyanobacteria, the filamentous colony-forming *Trichodesmium* spp. UV stress could be a significant problem for this genus, as *Trichodesmium* often forms extensive surface blooms (Zehr et al. 2001; Zehr 2011) and so is regularly exposed to full incident tropical solar radiation. Despite this, a recent study revealed that UV radiation significantly reduced specific growth rates, effective quantum yields of photosystem II, and CO_2 and N_2 fixation rates of *Trichodesmium erythraeum* strain IMS101 (Cai et al. 2017). How UV stress in *Trichodesmium* may interact with commonly limiting nutrients such as P has not been investigated.

Compared to the large, filamentous *Trichodesmium* genus, N_2-fixing cyanobacteria of the genus *Crocosphaera* are unicellular and much smaller. *Trichodesmium* fixes N_2 during the day, using both temporal and spatial segregation of N_2 fixation and photosynthesis within the photoperiod (Chen et al. 1998; Berman-Frank et al. 2001*a*,*b*). In contrast, *Crocosphaera* fixes N_2 at high rates only during the night, using full temporal separation of N_2 fixation and photosynthesis to avoid O_2 inactivation of nitrogenase (Montoya et al. 2004; Needoba et al. 2007). Despite being an important new N provider, the responses of *Crocosphaera* to UV radiation have not yet been examined. The smaller cell size of *Crocosphaera* relative to *Trichodesmium* could make it more vulnerable to UV radiation by increasing penetration of UV radiation.

To improve our understanding of UV effects on marine diazotrophs, we present the results of laboratory culture experiments comparing and contrasting the relationship between UV and diazotrophic growth of *Trichodesmium* and *Crocosphaera*. We also tested for possible intraspecific differences in UV responses using two strains of *Trichodesmium erythraeum* from the North Atlantic (Prufert-Bebout et al. 1993) and from the Great Barrier Reef lagoon, Australia (Fu and Bell 2003). To put our UV results into the context of a more stratified, nutrient-limited future ocean, we additionally examined the interactive effects of P availability with increasing UV radiation. Cultures of *Trichodesmium* and *Crocosphaera* were grown under UV-A, UV-B, and photosynthetically active radiation (PAR) treatments with either sufficient or limiting P concentrations. This study was thus designed to help determine how UV and nutrient limitation may interact with N_2-fixing cyanobacteria in the current ocean, as well as illuminating their possible responses to future conditions of increasing UV radiation and decreasing P availability.

Methods

Cultures and growth conditions

Trichodesmium erythraeum strains used in this study were IMS 101, isolated from the North Atlantic Ocean, and GBR from the Great Barrier Reef lagoon in the South Pacific Ocean (Prufert-Bebout et al. 1993; Fu and Bell 2003). *Crocosphaera watsonii* strain WH0005 was isolated from the North Pacific Ocean (Webb et al. 2009). *Trichodesmium* and *Crocosphaera* cultures were grown without combined N in artificial seawater medium recipes specific to the YBCII (Chen et al. 1996) and Aquil (Sunda et al. 2005) recipes, respectively. Filter-sterilized phosphate, vitamins, and Fe and other trace metals were then added to both media formulations in concentrations equivalent to the Aquil recipe (Sunda et al. 2005). Unialgal stock cultures were grown at 26°C under cool white fluorescent light at an intensity of 32.6 W m^{-2} (equivalent to 150 μmol photons m^{-2} s^{-1}, PAR), with a light–dark cycle of 12:12 h.

Experimental design

Semicontinuous culturing methods were used to maintain cells in the exponential growth phase. Cultures were diluted every 2–3 d with fresh microwave-sterilized medium. Each bottle was diluted individually based on growth rates calculated from in vivo chlorophyll *a* (Chl *a*) fluorescence readings. Volumes of dilutions were adjusted to maintain constant growth rates and allow biomass to reach predilution levels. After three to six consecutive measurements in which specific growth rates were not significantly different (< 15% variability between dilutions), cultures were considered to be in steady-state growth.

To evaluate the combined effect of P and UV radiation on the physiological response of marine N_2-fixing cyanobacteria, *Trichodesmium* (IMS 101 and GBR) and *Crocosphaera* (WH0005) were maintained in P-replete (10 μmol L^{-1} phosphate) and P-limited (0.5 μmol L^{-1} phosphate, which significantly inhibited growth rate of both *Trichodesmium* and *Crocosphaera*) culture media, as described above. The triplicated UV and PAR treatments were initiated when steady-state growth was obtained in both P-replete and P-limited cultures. All PAR/UV treatments were cultured in 120 mL quartz vessels under cool white fluorescence light at an intensity of 32.6 W m^{-2} with a 12:12 h light:dark period. UV treatments used UV-A (315–380 nm, peak at 352 nm) and UV-B (290–315 nm, peak at 306 nm) lamps (Philips UV-A TL and UV-B TL, respectively) placed over the quartz tubes with an exposure time of 4 h d^{-1}, from 11:00 to 15:00 h. Separate incubators were used for UV-A and UV-B treatments, respectively, but all growth conditions (PAR, temperature, etc.) were monitored frequently and kept as uniform as possible.

Under both P conditions, the cultures were exposed to three treatments, including PAR with either no UV radiation exposure, with UV-B, or with UV-A. The three treatments were obtained as follows: (1) *PAR + UV-B treatments*: UV-transparent quartz bottles with PAR (32.6 W m^{-2}, equivalent to 150 μmol photons m^{-2} s^{-1}) and UV-B (0.5 W m^{-2}) attenuated with one layer of gray screen, mimicking the UV-B intensities at a depth of ~ 10 m in the subtropical Atlantic Ocean (Piazena et al. 2002); (2) *PAR + UV-A treatment*: Quartz bottles were exposed to PAR and 4 W m^{-2} UV-A, similar to the UV-A irradiance in oligotrophic seawater at a depth of 60 m (Piazena et al. 2002); and 3) *PAR treatment only*: Quartz bottles were covered with Ultraphan film 395 (UV Opak, Digefra), which passes only PAR irradiance (400–700 nm, Cai et al. 2017). All cultures were also transferred to an additional high UV-B treatment of 1.0 W m^{-2}, equivalent to full tropical sunlight at 0 m depth (Piazena et al. 2002), but none survived more than a few days (data not shown).

All measurements of N_2 fixation and CO_2 fixation were carried out under treatment-appropriate experimental conditions in 27-mL borosilicate bottles, which transmit 77.8% of UV radiation and have been used for this application in previous work (Cai et al. 2017). Transmission spectra of the quartz growth bottles and borosilicate N_2 and CO_2 fixation bottles used in this study were presented in the study of Cai et al. (2017). Incident UV-A and UV-B radiation was measured with a broadband OL 756 Portable High Accuracy UV-Visible Spectroradiometer (Optronic Laboratories, Orlando, FL). After 7–10 generations of steady-state growth, cell cultures were harvested for measurements of N_2 fixation rates, CO_2 fixation rates, and elemental ratios. If the growth rates of cultures decreased significantly after UV exposure, cells were harvested for these assays immediately.

Growth rates and filament lengths

Specific growth rates (μ) were calculated from cell concentrations. The equation $\mu = \ln(C_{t_2}/C_{t_1})/(t_2 - t_1)$ was used to calculate growth rate, where C_{t_2} and C_{t_1} are the average cell concentrations at t_2 and t_1, respectively. One-milliliter cultures from each incubation replicate were fixed with glutaraldehyde (final concentration 0.1%) and preserved at 4°C in the dark. Cell numbers were counted with an Olympus BX51 microscope (Olympus, Tokyo, Japan). Filament lengths of *Trichodesmium* and the cell diameters of *Crocosphaera* were determined with a calibrated micrometer on an Accu-Scope 3032 inverted phase microscope. Filament lengths/cell volumes of 50–60 cells from each treatment were measured. The filament length and cell volume data are presented as mean values with standard deviations.

N_2 fixation rates

N_2 fixation rates were determined using the acetylene reduction method (Capone 1993) with a Shimadzu GC-8a gas chromatograph (Shimadzu Scientific Instruments, Columbia, MD). For each replicate in each treatment, 10 mL of culture was added to a 27-mL serum vial, which was then sealed. After 2 mL of air was extracted from the headspace, 2 mL of acetylene (C_2H_2) was added to each vial and mixed well with the culture. All replicates were incubated under light, UV, and temperature conditions as appropriate for each experimental treatment.

For *Trichodesmium*, incubations were initiated at the beginning of the UV radiation cycle (11:00 h) and terminated at the end of the 4 h UV radiation period. For *Crocosphaera*, the incubation covered the whole dark cycle, from 18:00 to 06:00 h in the next morning. These incubation schedules were chosen to encompass the peak N_2 fixation periods in the diel cycles of each cyanobacterium (Chen et al. 1998; Großkopf and Laroche 2012; Wilson et al. 2017).

At the end of the nitrogen fixation incubation, ethylene production was measured by injecting 200 μL of headspace into the GC-8a gas chromatograph. Sample values were quantified by comparing the reading to that of the same amount of a 100 ppm ethylene standard (GMT10325TC, Matheson Gas Products). A theoretical 3:1 ratio (mol C_2H_2 to mol N_2 reduced) was used to calculate the N_2 fixation based on the rates of ethylene production (Capone 1993). After raw N_2 fixation rates were calculated, the cell counts, particulate organic

carbon (POC), and particulate organic nitrogen (PON) concentrations of each sample were used to normalize N_2 fixation rates.

CO_2 fixation rate

CO_2 fixation rates were measured using the ^{14}C method as described by Fu et al. (2007). Briefly, 0.2 μCi ^{14}C-$NaH^{14}CO_3$ (0.25 kBq mL^{-1}) was added to 15 mL subsamples from each replicate. Samples were incubated over the 4 h UV radiation period under their respective experimental conditions and then filtered onto GF/F filters. To correct for filter adsorption, 15 mL of cultures from each treatment (5 mL from each replicate bottle) was filtered immediately after adding equal amounts of ^{14}C-$NaH^{14}CO_3$. All filters were then placed in 7 mL scintillation vials in the dark overnight after adding 4 mL Ultima Gold™ (Perkin Elmer LLC) scintillation fluid. To determine the total radioactivity added to each incubation, 1 μCi ^{14}C-$NaH^{14}CO_3$ together with 100 μL phenylalanine was placed in identical scintillation vials with the addition of 4 mL scintillation solution. ^{14}C radioactivity was measured using a Wallac System 1400 liquid scintillation counter (Perkin Elmer Life and Analytical Sciences, Wellesley, MA) for total activities, blanks, and samples.

Elemental analysis

Cell samples for POC, PON, and particulate organic phosphorus (POP) were collected onto precombusted (500°C for 5 h) GF/F filters, respectively. POC and PON samples were dried at 60°C and analyzed using a Costech Elemental Combustion System (Costech Analytical Technologies, Valencia, CA), calibrated with methionine and acetanilide as reference materials (Strickland and Parsons 1972; Fu et al. 2007).

The POP sample filters were rinsed with 4 mL 17 mol L^{-1} Na_2SO_4, suspended in a combusted glass scintillation vial with 2 mL 0.017 mol L^{-1} $MgSO_4$, and evaporated to dryness at 95°C. Vials were then baked at 450°C for 2 h. The total of 5 mL 0.2 mol L^{-1} HCl was added to each tightly capped vial after cooling. Vials were heated at 80°C for 30 min to digest POP to orthophosphate. Digested POP samples were analyzed with the standard molybdate colorimetric method (Strickland and Parsons 1972; Fu et al. 2007).

Determination of absorption spectra

Cell samples at the end of the 4-d UV-B treatment were collected by filtering onto GF/F filters. UV-absorbing compounds (UVACs) and Chl *a* were extracted in 4 mL of 100% methanol overnight in darkness at 4°C. The absorption spectra of filtered methanol extracts were obtained between 250 and 800 nm using a scanning spectrophotometer (Beckman Coulter, Fullerton, CA). As cellular Chl *a* did not change after exposure to UV radiation (Supporting Information Fig. S1), the optical density (OD) values were normalized to the Chl *a* peak at OD_{662}. The maximal absorbance at UV region (310–360 nm) was used as an indicator of UVACs (Cai et al. 2017).

Data analysis

UV-induced inhibition of N_2 fixation was calculated according to Cai et al. (2017): UV-B-induced inhibition = $(I_{PAR} - I_{UV\text{-}B})/I_{PAR} \times 100\%$, where I_{PAR} and $I_{UV\text{-}B}$ indicate the values of N_2 fixation under PAR and UV-B treatments, respectively. Data were analyzed using one-way ANOVA and where significant differences existed, multiple comparisons were made using Duncan's test (Duncan 1955). Three replicate bottles for every treatment were sampled for each analysis. Thus, all the data were represented by an average of three replicates (n = 3), with error bars representing standard deviations, with the exception of filament length and cell volume. Filament lengths/cell volumes of 50–60 cells from each treatment were measured. Significance was determined at the 5% level.

Results

Specific growth rates

As defined by the experimental design, growth rates in P-limited cultures were lower than those in P-replete cultures under the PAR treatment ($p < 0.05$, Fig. 1). UV-A treatment only inhibited the growth rate of P-replete IMS 101 ($p < 0.05$, Fig. 1A). None of the three isolates was able to survive more than a few days at UV-B radiation levels of 1.0 W m^{-2}, similar to exposures under unattenuated incident tropical sunlight (Piazena et al. 2002), and therefore data are not shown for this high UV-B treatment. UV-B at 0.5 W m^{-2} (approximately equivalent to 10 m depth in the tropical ocean, Piazena et al. 2002) partially inhibited growth of *Trichodesmium* IMS 101 and *Crocosphaera* in both P-replete and P-limited cultures ($p < 0.05$, Fig. 1B). For both P-replete and P-limited *Trichodesmium* GBR cultures, there was no significant change in growth rates after UV-A exposure, or after UV-B exposure at the 0.5 W m^{-2} level, compared with PAR alone ($p > 0.05$).

N_2 fixation rate

Cells grown in P-limited cultures had lower N-specific N_2 fixation rates than those grown in P-replete cultures under the PAR treatment ($p < 0.05$, Fig. 2). *Trichodesmium* IMS 101 N_2 fixation rates were inhibited by UV-A exposure only in P-replete cultures ($p < 0.05$, Fig. 2A). However, the effect of UV-B was more damaging than that of UV-A. UV-B radiation inhibited N_2 fixation rates of *Trichodesmium* IMS 101 in both P-replete and P-limited cultures, and of *Crocosphaera* in P-replete cultures ($p < 0.05$, Fig. 2B). As with growth rate, though, N_2 fixation rates of *Trichodesmium* GBR were unaffected by either UV-A or UV-B exposure, regardless of P treatment. The treatment-related responses of cell-specific N_2 fixation rates were similar to those of N-specific N_2 fixation rates (Supporting Information Fig. S2).

UV-B exposure inhibited N-specific N_2 fixation by 99.2% and 46.8% in P-replete IMS 101 and *Crocosphaera* cultures, respectively (Table 1). However, P-limitation did not lead to greater inhibition of N_2 fixation in the presence of UV

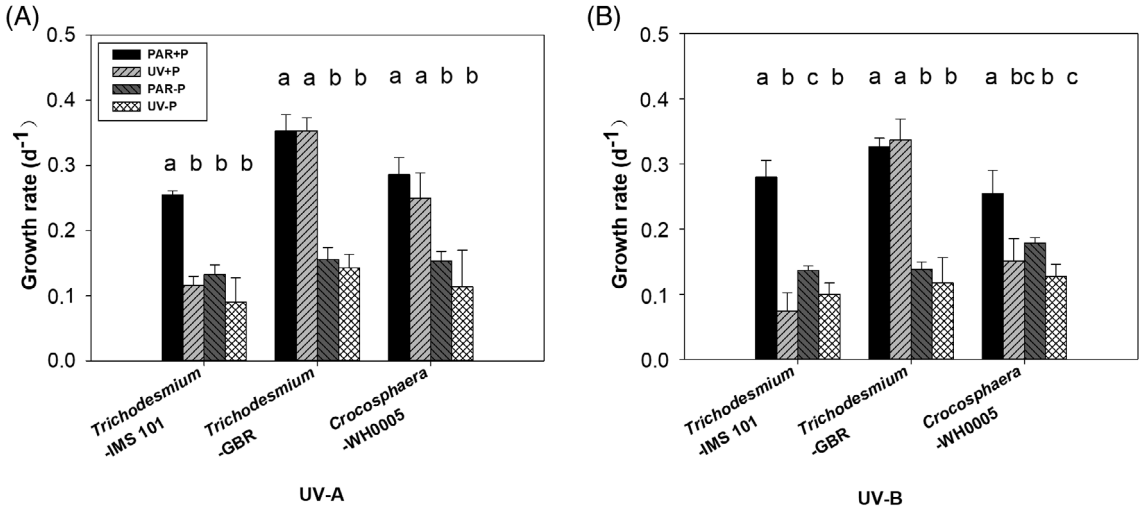

Fig. 1. Effect of UV radiation on the cell-specific growth rate (cells produced per cell per day, units of d^{-1}) of *Trichodesmium* (IMS 101 and GBR) and *Crocosphaera* (WH0005) in P-replete and P-limited cultures. (**A**) UV-A treatment; (**B**) UV-B treatment. Values are means of triplicates ± SD. Mean values denoted by the same letters (a, b, c, or d) for each isolate are not significantly different at $p < 0.05$ (Duncan's test).

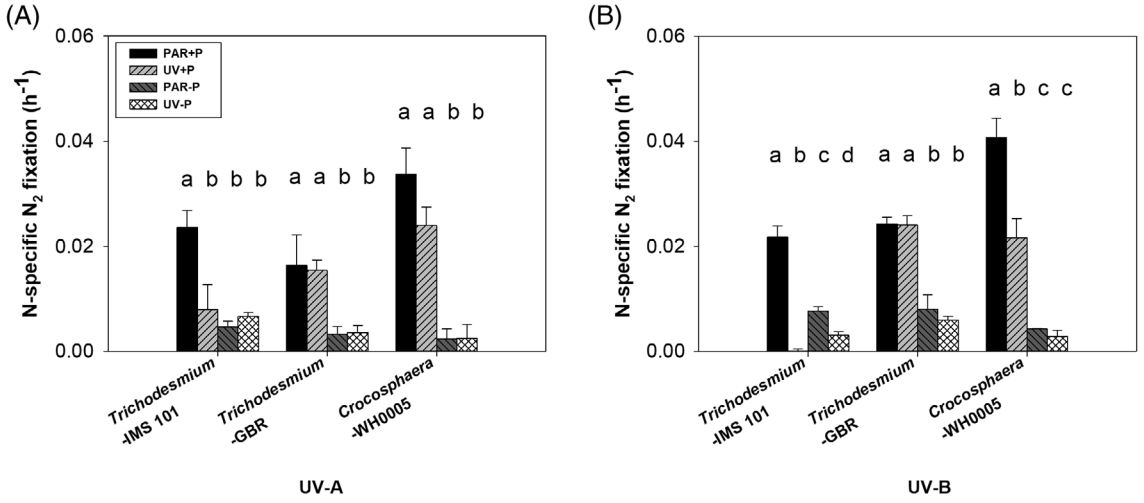

Fig. 2. Effect of UV radiation on N-specific N_2 fixation rate (N fixed per cellular N per hour, units of h^{-1}) of *Trichodesmium* (IMS 101 and GBR) and *Crocosphaera* (WH0005) in P-replete and P-limited cultures. (**A**) UV-A treatment; (**B**) UV-B treatment. Values are means of triplicates ± SD. Different letters (a, b, c, or d) signify significant differences between treatments of each strain at $p < 0.05$ (Duncan's test).

radiation. In fact, UV-induced N_2 fixation inhibition of both *Trichodesmium* IMS 101 and *Crocosphaera* in P-replete cultures was much greater than was observed in P-limited cultures (Table 1). The pattern of UV-B-induced inhibition of cell-specific N_2 fixation was very similar to that seen for UV-B-induced inhibition of N-specific N_2 fixation (Supporting Information Table S1).

CO_2 fixation rate

As expected, the P-limited treatments all had significantly lower C-specific CO_2 fixation rates, compared with the corresponding P-replete treatments ($p < 0.05$, Fig. 3). Exposure to UV-A radiation had no effect on C-specific CO_2 fixation

Table 1. UV-B-induced inhibition of N-specific N_2 fixation and growth rate (% of PAR control) for *Trichodesmium* IMS 101 and GBR, and *Crocosphaera* WH0005 in P-replete and P-limited cultures.

	N_2 fixation inhibition (%)		Growth rate inhibition (%)	
	P-replete	P-limited	P-replete	P-limited
Trichodesmium-IMS101	99.2	59.1	73.4	26.3
Trichodesmium-GBR	n.s.	n.s.	n.s.	n.s.
Crocosphaera	46.8	n.s.	40.6	28.9

n.s., not significant.

rates of all three strains ($p > 0.05$, Fig. 3A). C-specific CO_2 fixation rates of P-replete and P-limited *Trichodesmium* IMS 101 decreased by 50.2% and 32.6%, respectively, under the UV-B treatment ($p < 0.05$, Fig. 3B). C-specific CO_2 fixation rate of *Crocosphaera* decreased by 65.9% and 68.5% in P-replete and P-limited cultures after exposure to UV-B, respectively ($p < 0.05$, Fig. 3B). There was no significant change ($p > 0.05$) in CO_2 fixation rate of *Trichodesmium* GBR after UV-B exposure, compared with PAR.

Elemental ratios

C:N ratio of IMS 101 was not affected by P and UV treatments ($F = 3.2$, $p = 0.067$; $F = 2.627$, $p = 0.103$; Fig. 4A,B). The C:N ratio of GBR increased in P-limited cultures ($p < 0.05$), while the C:N ratio of IMS 101 and *Crocosphaera* remained relatively constant under the P-limited treatment ($p > 0.05$, Fig. 4A,B). Of the three cyanobacteria, only *Crocosphaera* showed increased C:N ratios under either UV-A or UV-B radiation in most treatments. *Crocosphaera* grown in P-replete cultures had 34.7% higher C:N ratios under UV-A treatment than under PAR conditions ($p < 0.05$), although there were no differences in P-limited cultures (Fig. 4A). Exposure to UV-B increased C:N ratios of *Crocosphaera* by 34.0% and 72.7% in P-replete and P-limited cultures, respectively ($p < 0.05$, Fig. 4B). Cellular C:N ratio of *Trichodesmium* IMS 101 and *Trichodesmium* GBR remained unaffected by both UV-A and UV-B radiation, irrespective of P conditions ($p > 0.05$).

As expected, N:P ratios of all strains increased significantly in P-limited cultures ($p < 0.05$, Fig. 4C,D). The N:P ratio was not affected after exposure to UV radiation, except for UV-B-treated P-limited *Trichodesmium* IMS 101, in which N:P ratio was increased by the UV-B treatment ($p < 0.05$, Fig. 4D).

Absorption spectra

Under the PAR treatment, *Trichodesmium* GBR exhibited much higher absorbance in the UV region (300–400 nm) when compared with *Trichodesmium* IMS 101 and *Crocosphaera* (Fig. 5). The ratio of UVACs to Chl *a* was 7.19 in *Trichodesmium* GBR (Fig. 5B), compared to 3.73 in *Trichodesmium* IMS 101 and 4.37 in *Crocosphaera* (Fig. 5A,C). Moreover, the ratio of UVACs to Chl *a* increased by 21.7% and 42.0% in *Trichodesmium* IMS 101 and *Crocosphaera*, respectively, after 4-d UV-B exposure ($p < 0.05$, Fig. 5A,C). However, no significant difference of absorbance (334 nm) between PAR-treated and UV-B-treated *Trichodesmium* GBR was observed ($p > 0.05$, Fig. 5B), suggesting UVACs may be produced constitutively by this isolate.

Cell morphology

Filament length of *Trichodesmium* and cell volume of *Crocosphaera* were represented in a box and whisker plot, as in general these morphological data showed much more variability than the physiological data presented above. Figure 6 illustrates medians for these parameters, 25th and 75th percentiles (box limits) and include whiskers that extend 1.5 times the interquartile range from the 25th and 75th percentiles, as well as outliers represented by dots. Filament length of *Trichodesmium* IMS 101 and *Trichodesmium* GBR showed different responses to P concentration and UV exposure (Fig. 6). There was no significant difference in filament length of *Trichodesmium* IMS 101 between P-replete and P-limited cultures exposed to PAR ($p > 0.05$, Fig. 6A,B). Filament length of P-limited *Trichodesmium* IMS 101 remained unaffected under the UV-A treatment ($p > 0.05$, Fig. 6A), but both UV-A and UV-B reduced IMS 101 filament length in P-replete cultures

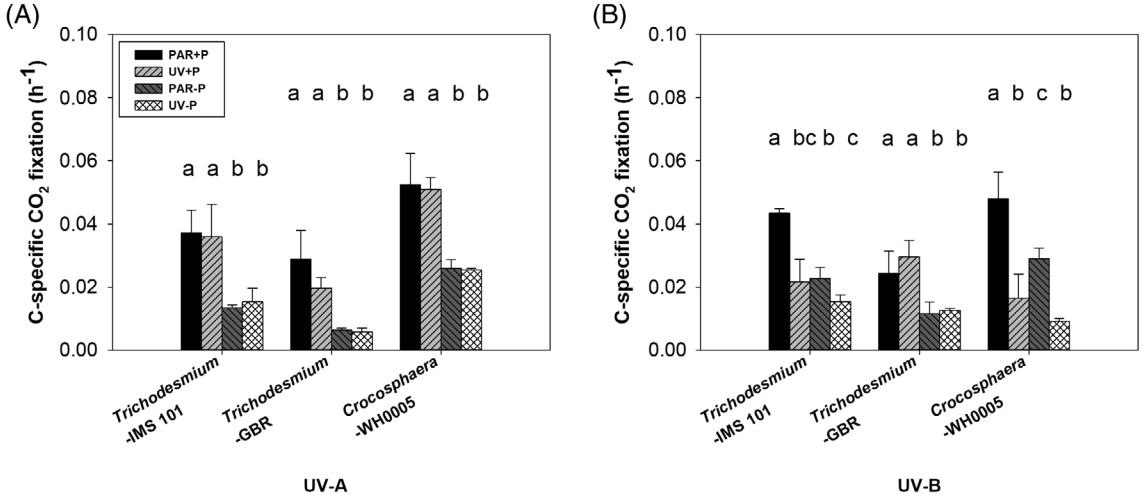

Fig. 3. Effect of UV radiation on C-specific CO_2 fixation rate (C fixed per cellular C per hour, units of h^{-1}) of *Trichodesmium* (IMS 101 and GBR) and *Crocosphaera* (WH0005) in P-replete and P-limited cultures. (**A**) UV-A treatment; (**B**) UV-B treatment. Values are means of triplicates ± SD. Different letters (a, b, c, or d) signify significant differences between treatments of each strain at $p < 0.05$ (Duncan's test).

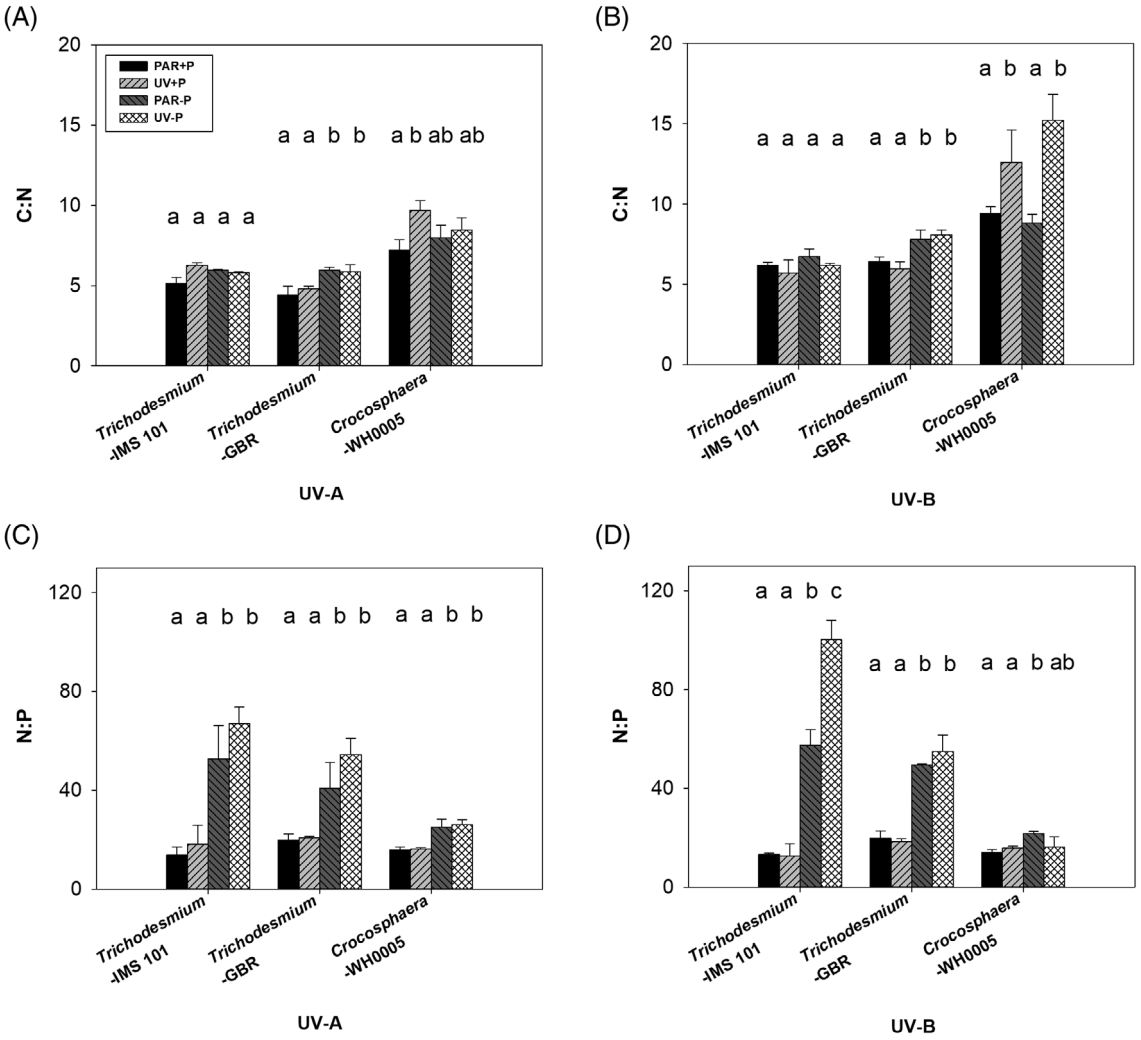

Fig. 4. Effect of UV radiation on C:N and N:P ratios (mol:mol) of *Trichodesmium* (IMS 101 and GBR) and *Crocosphaera* (WH0005) in P-replete and P-limited cultures. (**A**) UV-A treatment, C:N ratio; (**B**) UV-B treatment, C:N ratio; (**C**) UV-A treatment, N:P ratio; and (**D**) UV-B treatment, N:P ratio. Values are means of triplicates ± SD. Different letters (a, b, c, or d) signify significant differences between treatments of each strain at $p < 0.05$ (Duncan's test).

($p < 0.05$, Fig. 6A,B). In contrast, filament length of P-limited IMS 101 was increased by UV-B ($p < 0.05$, Fig. 6B).

P-limitation induced longer filaments in *Trichodesmium* GBR, compared with the P-replete condition ($p < 0.05$, Fig. 6C,D). Filament length of GBR elongated by 28.7% and 30% in P-replete cultures after the UV-A and UV-B treatments, respectively ($p < 0.05$). However, UV radiation had no effect on filament length of GBR in P-limited cultures ($p > 0.05$, Fig. 6C,D).

Filament length of P-replete *Trichodesmium* GBR was not significantly longer than that of P-replete *Trichodesmium* IMS 101 under the PAR treatment ($p > 0.05$, Fig. 6A–D). However, filament length of GBR was 28.7% longer than that of IMS 101 in P-replete cultures after exposed to UV-A ($p < 0.05$, Fig. 6A,C). Following UV-B radiation, the filament length of GBR was 102.7% and 45.4% longer than that of IMS 101 in P-replete and P-limited cultures, respectively ($p < 0.05$, Fig. 6B,D).

No significant difference in cell volumes was found between P-replete and P-limited *Crocosphaera* cells ($p > 0.05$, Fig. 6E,F). UV-A treatment only increased cell volumes of P-replete *Crocosphaera* ($p < 0.05$, Fig. 6E). UV-B radiation significantly increased cellular volumes of *Crocosphaera* in both P-replete and P-limited cultures ($p < 0.05$, Fig. 6F).

Under all the UV and P treatments, both *Trichodesmium* IMS 101 and GBR remained as single filaments (data not shown). However, when exposed to intense UV-B radiation (100% UV-B treatment, 1.0 W m^{-2}) in P-limited or P-replete cultures, fusiform (tuft) aggregates of *Trichodesmium* IMS 101 appeared (Supporting Information Fig. S3A), while *Trichodesmium* GBR remained as single filaments (Supporting Information Fig. S3B).

Discussion

Marine N_2-fixing cyanobacteria provide a critical source of new N and support a large fraction of total biological productivity

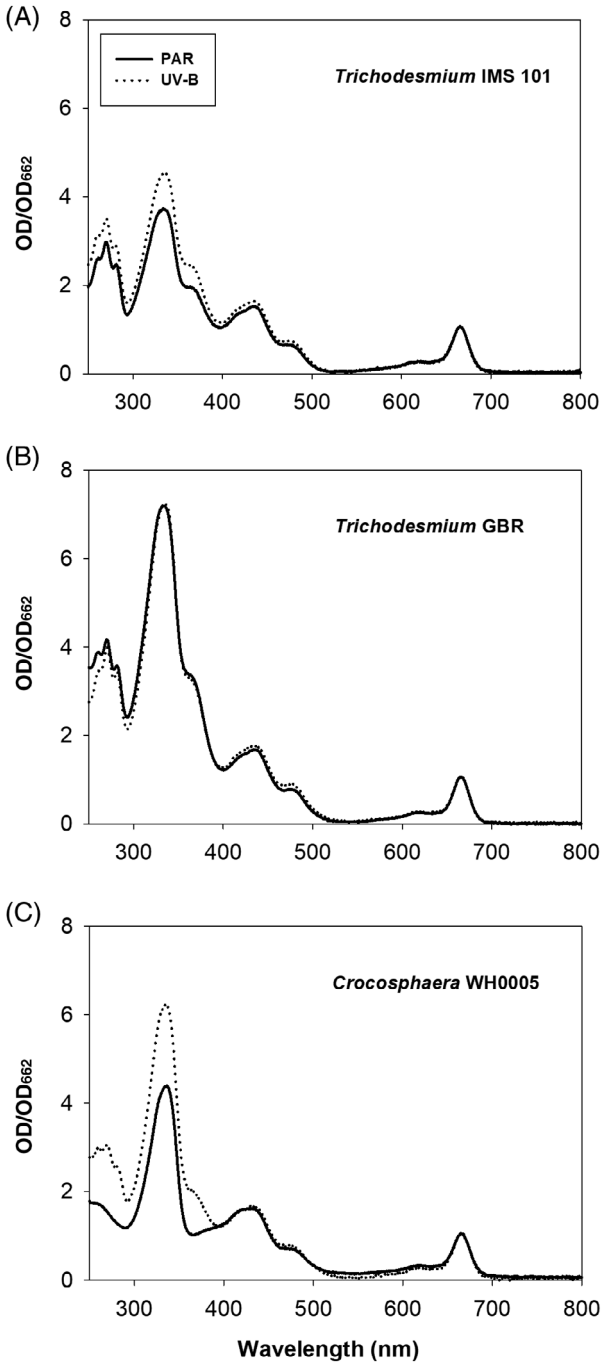

Fig. 5. Absorption spectrum of (**A**) *Trichodesmium* (IMS 101), (**B**) *Trichodesmium* (GBR), and (**C**) *Crocosphaera* (WH0005) grown under PAR (solid lines, ____) and UV-B (dotted lines, ……) conditions for 4 d, with OD values normalized to OD_{662} (Chl *a*).

in the tropical and subtropical ocean (Zehr 2011; Bench et al. 2016). Thus, their responses to global change could have significant consequences for ocean biology and carbon cycling (Hutchins and Fu 2017). Increased exposure of these keystone organisms to UV is a likely consequence of mixed layer shoaling under warmer future climate conditions (Gao et al. 2012).

Trichodesmium's habitat of often forming surface blooms (Capone et al. 1997) likely means that it frequently experiences greater UV stress than *Crocosphaera*, which does not accumulate at the surface and is typically distributed throughout the mixed layer. Many cellular proteins and nucleic acids are subject to damage by excessive UV-B (Atienzar et al. 2000; Pakker et al. 2000; Häder and Gao 2015), including the nitrogenase enzyme itself (Kumar et al. 2003). *Trichodesmium* fixes N_2 during the day, so its nitrogenase enzyme system is directly exposed to UV radiation damage. In contrast, *Crocosphaera* breaks down its nitrogenase each morning and then resynthesizes it just before the dark N_2 fixation period (Saito et al. 2011). Consequently, the nitrogenase of *Crocosphaera* is never exposed to high-intensity UV at midday, and may experience much less direct UV damage than does the nitrogenase of *Trichodesmium*.

The first study of UV radiation effects on *T. erythraeum* IMS 101 showed significant UV inhibition of growth and N_2 fixation, implying a substantial influence of UV on present day and future N and carbon cycling in the ocean (Cai et al. 2017). As with *T. erythraeum* IMS 101 in our study and that of Cai et al. (2017), we found that the unicellular diazotroph *Crocosphaera* WH0005 was also negatively affected by UV exposure (Figs. 1–3). In contrast, however, we observed no inhibition by UV radiation in *T. erythraeum* GBR at the exposure levels used in our experiments. Thus, the results from our culture studies generally suggested that UV radiation can have either negative or neutral effects on both of these ecologically dominant and widespread diazotrophic cyanobacteria groups.

The two isolates of *T. erythraeum* analyzed in this study showed strain-specific responses to UV radiation. The Pacific isolate GBR was much more tolerant of UV radiation than the closely related Atlantic isolate IMS 101, as evidenced by specific growth rates, N_2 fixation rates, CO_2 fixation rates, and C:N and N:P ratios of GBR that did not differ regardless of UV treatment (Figs. 1–4). Different sensitivities to UV radiation could reflect adaptation to their original environments. For instance, solar radiation is typically more intense in the low-latitude tropical regime where GBR was isolated (Heron Island, Great Barrier Reef, Australia, Fu and Bell 2003), than in the more temperate, higher latitude waters where IMS 101 was isolated (North Carolina, U.S. Atlantic coast, Prufert-Bebout et al. 1993). The Australian waters where GBR was isolated are also located much closer to the Southern Hemisphere ozone hole, where ambient UV is likely more intense than in the North Atlantic region where IMS 101 originated. These two strains of *T. erythraeum* also differ in other traits, including phosphate requirements (Fu et al. 2005) and CO_2 responses (Hutchins et al. 2007, 2013). As with *Crocosphaera* subpopulations and *Prochlorococcus* ecotypes (Rocap et al. 2003; Bench et al. 2016), phenotypic divergence of *Trichodesmium* IMS 101 and GBR may reflect genetic differences tied to local selective pressures.

Alternately, the differential UV sensitivity of these two conspecific culture isolates could also reflect changing selective

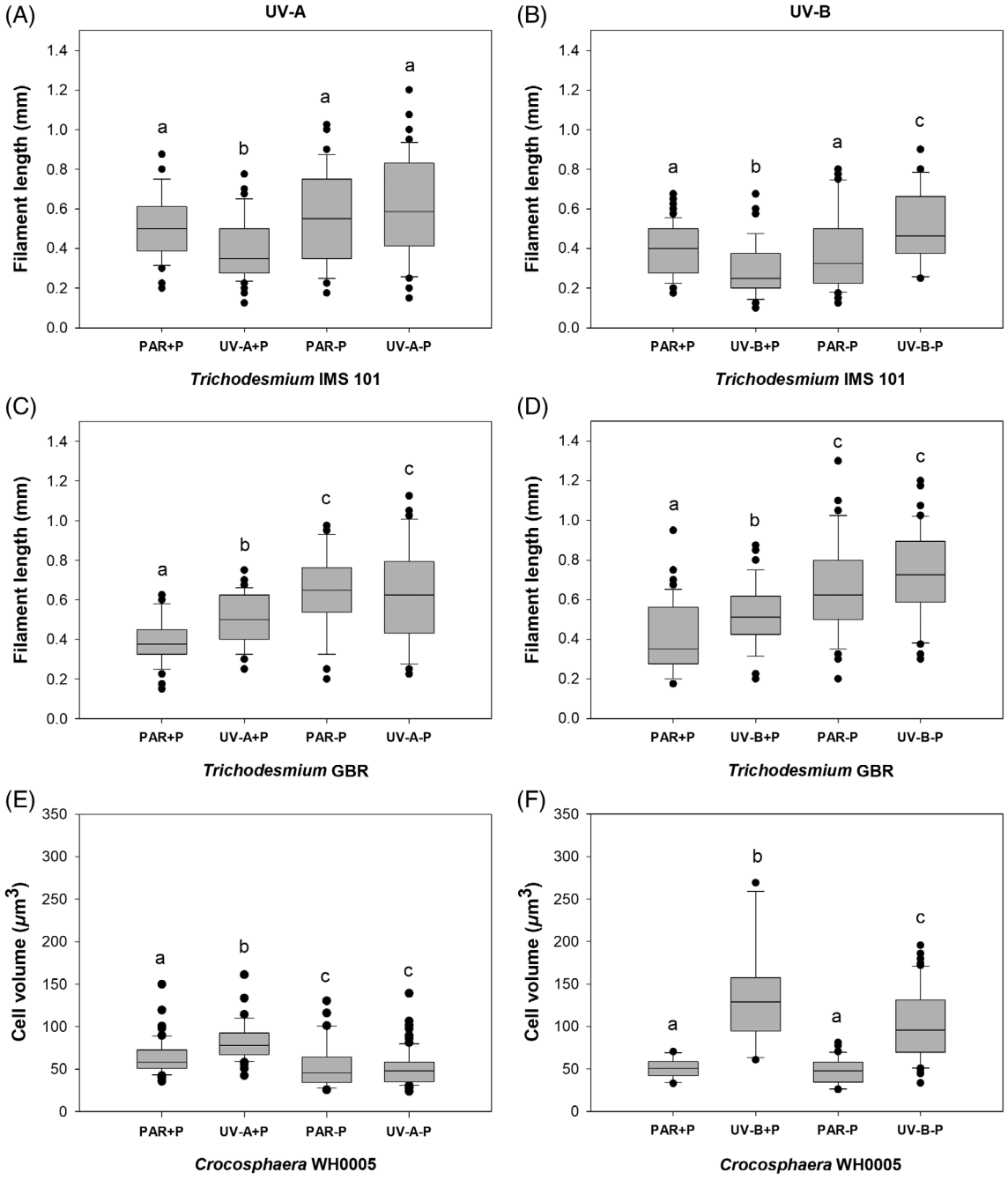

Fig. 6. Effect of UV radiation on filament length of *Trichodesmium* (IMS 101 and GBR) and cell volume of *Crocosphaera* (WH0005) in P-replete and P-limited cultures. (**A**, **C**, **E**) UV-A treatment; (**B**, **D**, **F**) UV-B treatment. Data are shown as box plots; the horizontal lines within the box represent the medians; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; and outliers are represented by dots. Different letters represent values that are significantly different at $p < 0.05$ (Duncan's test).

pressures during extended cultivation. *Trichodesmium* IMS 101 has been in culture about twice as long as *Trichodesmium* GBR (Prufert-Bebout et al. 1993; Fu and Bell 2003). After thousands of generations under artificial lighting relatively deficient in UV wavelengths, it is possible that any UV defense mechanisms *Trichodesmium* IMS 101 originally possessed have been lost due to adaptation (Walworth et al. 2016). A global survey comparing UV responses of wild *T. erythraeum* populations would help to distinguish between intrinsic habitat-related variability and potential long-term cultivation effects.

UV-absorbing mycosporine-like amino acids (MAAs) which have maximum absorption between 310 and 360 nm are effective UV screening compounds that protect cyanobacteria

from high solar UV radiation (Garcia-Pichel and Castenholz 1993). Even under the PAR treatment, *Trichodesmium* GBR had much higher absorption peaks of apparent MAAs (λ_{max} = 334 nm) than *Trichodesmium* IMS 101 and *Crocosphaera* did (Fig. 5), in accordance with the higher UV tolerance of *Trichodesmium* GBR. The UV-B-induced synthesis of protective MAAs has been reported in various cyanobacteria (Singh et al. 2008; Rastogi and Incharoensakdi 2014). However, *Trichodesmium* GBR appeared to constitutively produce elevated levels of MAAs, as no additional induction of MAAs was observed under the UV-B treatment (Fig. 5B). Synthesis of MAAs in *Trichodesmium* IMS 101 and *Crocosphaera*, which were more sensitive to UV radiation, was simulated by UV-B radiation (Fig. 5A,C). Moreover, the short and discontinuous UV-B exposure time used in this study and/or taxon-specific mechanisms might explain the differing responses of MAAs synthesis to UV-B exposure (Rastogi and Incharoensakdi 2014; Cai et al. 2017). Measurements of MAAs after extended UV exposures would allow estimation of their role in long-term UV tolerance of N_2-fixing cyanobacteria.

It is also possible that *Trichodesmium* filament length differences could be related to UV radiation tolerance. Filament length of GBR was much greater than that of IMS 101 under the same UV conditions (Fig. 6). Previous studies have attributed filament/cell elongation in cyanobacteria to adaptation to extreme environmental conditions (Smith and Gilbert 1995; Jezberova and Komarkova 2007; Spungin et al. 2014). Filament diameter of both *Trichodesmium* strains was unaffected by the UV/P treatments (data not shown). However, GBR inherently has a much larger diameter, with an average cellular volume (~ 1740 mm^3) that is nearly double that of IMS101 (~ 950 mm^3) (Fu et al. 2005). Smaller surface area:volume ratios of larger cells might contribute to higher UV tolerance (Karentz et al. 1991).

Besides filament length, we observed another morphological response difference between *Trichodesmium* IMS 101 and GBR in both P-limited and P-replete cultures. Higher UV-B radiation (1.0 W m^{-2}) induced formation of fusiform (tuft) aggregates in IMS 101, while GBR remained as single filaments (Supporting Information Fig. S3A,B). Phytoplankton communities with higher cell densities increase self-shading, and consequently individual cell exposure to high light or UV radiation is decreased (Wu et al. 2005). *Trichodesmium* often grows in a colonial form in nature, with packages of many filaments held together by an extracellular sheath (Capone et al. 1998). Our culture study suggests that *Trichodesmium* IMS 101 may use this protective strategy of adopting a colonial morphotype to help counteract inhibitory UV levels. This is consistent with a recent study by Tzubari et al. (2018), who found that *Trichodesmium* IMS101 grew as single filaments during exponential growth, but formed colonies under oxidative stress or when limited by Fe or P. Although both IMS 101 and GBR died after several days under the highest UV-B radiation treatment (1 W m^{-2}), GBR did not use this potentially protective colonial strategy.

The filamentous *Trichodesmium* and unicellular *Crocosphaera* cultures investigated in this study also showed differing responses to UV radiation. As shown in Table 1, UV-B-induced N_2 fixation inhibition in *Trichodesmium* (IMS 101) was much higher than that in *Crocosphaera*. As noted above, large-celled algal species may sustain less damage per unit of DNA and thus can be less sensitive to UV exposure (Karentz et al. 1991). Nevertheless, despite the fact that *Crocosphaera* is much smaller than *Trichodesmium* IMS 101, it is more tolerant of UV radiation.

The explanation might be the difference in N_2 fixation strategies used by these two very distinct oceanic diazotrophs. As in previous studies (Großkopf and Laroche 2012; Wilson et al. 2017), N_2 fixation in *Crocosphaera* was low to undetectable during the light period, and peaked at the middle of the dark period. The nitrogenase protein NifH is rapidly degraded during the light period, and synthesized de novo in the dark period (Mohr et al. 2010; Saito et al. 2011). Therefore, by fixing N_2 only during the night, *Crocosphaera* avoids direct UV damage to its nitrogenase. However, the CO_2 fixation apparatus in *Crocosphaera* is subject to direct UV exposure during the photoperiod; this vulnerability of the photosystems to UV damage likely explains the lower CO_2 fixation rates of *Crocosphaera* in our UV-B treatments. Nevertheless, N_2 fixation rates of *Crocosphaera* were certainly also inhibited by UV to some extent in our experiments. This may be due to damage by oxidative stress from UV radiation, such as lipid peroxidation and DNA strand breakage (Van Donk and Hessen 1995; Chen et al. 1996), or may be indirectly due to energetic limitation of N_2 fixation caused by prior direct UV inhibition of CO_2 fixation.

As we only examined a single *C. watsonii* isolate, it is unknown whether there are strain-level differences in UV tolerance as we observed in our two *T. erythraeum* isolates. Distinct traits for cell size, temperature optima and range, distribution, and variability in rates of N_2 fixation have been reported between two subpopulations of *Crocosphaera* in the North and South Pacific (Webb et al. 2009; Bench et al. 2016), which raises the possibility of similarly divergent responses to UV stress. In general, our culture study indicates that modeled estimates of the overall influence of UV radiation on oceanic global N_2 fixation will likely be inaccurate if taxon-specific differences in UV inhibition of major diazotroph groups are not taken into consideration.

Limitation by P had a negative impact on growth rate and N_2 fixation of both species, consistent with results previously reported (Hutchins et al. 2007; Spungin et al. 2014). The interactions between P availability and UV radiation effects have been investigated mainly in freshwater algae. Previous work implied that P limitation increases the stress of UV radiation in many algal species and mixed communities, including impacts on growth rates and photosynthetic efficiency (Wulff et al. 2000; Aubriot et al. 2004; Xu and Gao 2009; Yang et al. 2014). This increased stress may be because when P is limiting,

there are insufficient supplies of this key nutrient for repair of UV-induced damage to DNA and to supply energy (via ATP, adenosine triphosphate) for damaged proteins (Murata et al. 2007).

In our study, however, the negative effects of UV on both IMS 101 and *Crocosphaera* were greater in P-replete cultures than in P-limited cultures (Table 1). In contrast to the decrease of *Trichodesmium* filament length in P-replete cultures after UV exposure, filament length was constant or even increased in P-limited cultures (Fig. 6A,B). As noted above, longer *Trichodesmium* filaments might be associated with higher UV tolerance of these P-limited cells. This is consistent with the larger cells found in UV-exposed, P-limited cultures of another filamentous diazotrophic cyanobacterium from the estuarine Baltic Sea, *Nodularia spumigena* Mertens (Mohlin and Wulff 2009). Contrasting responses to UV radiation under varying P availabilities may be evidence for a diversity of cyanobacterial UV tolerance mechanisms.

Previous work has suggested that negative effects of UV radiation may need to be incorporated into biogeochemical models of global N_2 fixation (Cai et al. 2017). Our culture studies suggest that as diazotrophic growth across much of the oligotrophic ocean is often limited by P (Sañudo-Wilhelmy et al. 2001; Mills et al. 2004; Walworth et al. 2016), the negative impacts of UV on global N_2 fixation decline may be ameliorated to a certain extent. A prior study has demonstrated that increased pCO_2 enhanced N_2 and CO_2 fixation and growth rates of *Trichodesmium* even under severely P-limited steady-state growth conditions (Hutchins et al. 2007). Moreover, Fe deficiency actually increases growth and N_2 fixation rates of P-deficient *Trichodesmium* and *Crocosphaera* (Garcia et al. 2015; Walworth et al. 2016).

Similar to these interactions with other growth-controlling factors, it seems that P limitation can also tip the balance between UV damage and repair processes. Therefore, it is important to consider P limitation when exploring the effects of global change (including but not limited to UV increases) on N_2 fixation. Our study indicates that increasing UV radiation has a generally negative effect on *Trichodesmium* and *Crocosphaera*, although some strains such as *Trichodesmium* GBR may exhibit considerable resistance. Based on our culture studies, UV-induced inhibition is likely to lead to lowered new N supplies to the global ocean. Decreased supplies of *Trichodesmium*- and *Crocosphaera*-derived dissolved organic N and C may influence community composition and trophic structure, and hence have large effects on biogeochemical cycles (Smith and Gilbert 1995). Recent work demonstrates the important contribution of noncyanobacterial diazotrophs to oceanic biological N_2 fixation (Bombar et al. 2016; Gradoville et al. 2017; Moisander et al. 2017). Future research should investigate the potential impact of increasing UV exposure on global N_2 fixation, including the currently unknown responses of these noncyanobacterial diazotrophs.

Our observations from the culture studies demonstrate that ocean P availability considerably modifies the effects of UV radiation on diazotrophs. Much of the ocean where diazotrophs live is believed to be P-limited and/or Fe-limited (Sañudo-Wilhelmy et al. 2001; Mills et al. 2004; Fu et al. 2005; Hutchins and Boyd 2016). Furthermore, marine diazotrophs will encounter other challenges caused by global change that may also have unrecognized interactions with each other and with UV radiation, including warming, changes in Fe supplies, elevated CO_2 concentration, and ocean acidification (Hutchins and Fu 2017; Jiang et al. 2018). Future research should consider the interactive effects of all of these multiple environmental stressors for better estimation of the responses of diazotrophs to UV radiation, in order to allow more accurate predictions of the consequences for the marine food webs and biogeochemistry of the present day and future ocean.

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Acknowledgments

We are grateful to BW Hu and MD Chen in ECNU for their assistance with the absorption measurement in this work. Support was provided by U.S. National Science Foundation grants OCE 1538525, OCE 1638804, and OCE 1657757, as well as National Natural Science Foundation of China grant 41720104005.

Conflict of Interest

None declared.

Submitted 27 November 2018
Revised 22 March 2019
Accepted 11 July 2019

Associate editor: Ilana Berman-Frank