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Research Article

Nitrogen Limitation Decreases the Repair Capacity and Enhances Photoinhibition of Photosystem II in a Diatom

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ABSTRACT

Macronutrient limitation and increased solar exposure coincide with ocean warming-enhanced stratification, with consequences for phytoplankton within the upper mixing layer. In this study, we grew a diatom, *Thalassiosira punctigera,* under nitrogen-limited and replete conditions for more than 14 generations and investigated both the biochemical composition of treated cells and their photochemical responses to high light and UV exposure. The photosynthetic pigment and the particulate organic nitrogen (PON) content significantly decreased in the low nitrate grown cells, with drastic decline of the absorption of UV absorbing compounds. Under an acute exposure to high light or UV radiation, we observed a significant decline in the photochemical yield along with an increase of nonphotosynthetic quenching (NPQ), with the former lowered and the latter raised in the low-nitrogen grown cells. The results reveal a decreased repair rate and enhanced photoinhibition of the diatom under nitrogen limitation when exposed to increased levels of light and UV radiation, suggesting a higher vulnerability of the diatom phytoplankton under influences of oceanic global change.

INTRODUCTION

Human-induced marine environmental changes, such as warming, ocean acidification, and eutrophication, are commonly identified as global problems affecting marine ecosystems (1). However, although nutrient limitation frequently occurs in many regions of the open ocean, this phenomenon has been frequently ignored in laboratory experiments on global change (2). In the future ocean, the warming of surface seawater will intensify the stratification of the water column, which would hinder vertical transport of cold water, with higher nutrient levels, to the upper mixing layer in ocean gyres and upwelling regions where phytoplankton would experience increased light exposure and lower nutrient supply (3-5). These changes are likely to have significant impacts on the primary production and biogeochemical cycling mediated by phytoplankton in the marine ecosystem and their influence on feedback to the global climate (6). Therefore, it is necessary to investigate how phytoplankton perform when encountering high light stress under nutrient-limited conditions.

Diatoms are an important group of primary producers (7), which comprise a very diverse range of species and are widely distributed in the open ocean, coastal regions, and estuaries (8). Due to their generally rapid cell division rate and synthesis of silicic frustules, diatoms also play an important role in the biogeochemical cycling processes for carbon, nitrogen, and silicon (9). As with all photoautotrophic organisms, photosynthesis is the ultimate source of energy for diatoms, and this fuels cellular activities such as uptake and metabolism of nutrients, synthesis of materials for cell division, and mechanisms to cope with external stress. The photosynthetic light reactions function to absorb and convert light energy from solar radiation, via light-harvesting complexes, and electron transport chains, into chemical energy (10). However, intense light and ultraviolet radiation (UV radiation) can cause damage to the photosynthetic apparatus that depresses the efficiency of photosynthesis (11).

The continued operation of the photosystems under light stress relies on replacement of malfunctioning components of the photosynthetic apparatus through *de novo* synthesis of protein (12). Photosystem II (PSII) is more prone to photo-induced damage than photosystem I (13). The D1 protein is a major component of PSII and has a rapid turnover rate during the light reactions of photosynthesis (14). During photosynthesis, particularly under stressful light conditions, the core protein of PSII can be damaged and the inactivation of PSII is reflected in a decrease of photochemical yield (15). To maintain the function of PSII, *de novo* synthesis of D1 protein is activated and damaged components replaced, in a process termed the damage-repair cycle of PSII (16,17). As a consequence, the overall response of photochemical performance depends on the balance between the damage and repair processes of the photosystem.

Ultraviolet radiation is an important, though variable with water depth and water quality in different water bodies, component of the solar spectrum and has multiple effects on photoautotrophs such as direct damage to the electron transport chain of PSII and the recycling of D1 protein (18). The formation of pyrimidine dimers in DNA by UV-B may induce misreading of the coding sequences (19), which can irreversibly inhibit the *de*

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novo synthesis of D1 protein (20). Though most organisms have evolved DNA repair mechanisms, the efficiency of such repair is frequently unable to keep pace with the rate of damage. Therefore, cells usually employ multiple strategies to minimize the effects of ultraviolet radiation (UVR), for example, synthesis of certain components such as β-carotene (21) and aromatic amino acids (mycosporine-like amino acids, MAAs) (22,23), to screen out UVR. In addition, the dissipation of excess energy through nonphotochemical quenching (NPQ) is another important photoprotection mechanism in diatoms (24).

Under nitrogen-limited conditions, the physiological traits of diatoms are likely to adjust to cope with the related stress (25). As an "ultimate limiting nutrient" (26), nitrogen is an essential element for the synthesis of protein, nucleotides, and pigments in photosynthetic organisms. Decreases in cellular content of chlorophyll, D1 protein, and the CO_2-fixing enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) have all been reported in diatoms under nitrogen limitation (27). Effects on protein synthesis influence the turnover rate of photosystem proteins and depress the photosynthetic performance of diatoms (28,29). In theory, N-limitation could induce a series of changes at the macromolecular level, for example, limit D1 protein repair (29), decrease the synthetic capacity for UV absorbing compounds (UVACs) (30) and inhibit electron transport (31), that all ultimately impact the photophysiology of diatoms. Thus, it is foreseeable that the combination of nitrogen limitation and intense solar radiation could enhance the inhibition of photosynthetic efficiency of diatoms in the future marine environment.

There have been previous studies of the physiological responses of marine phytoplankton to nutrient limitation and UV radiation (32). Cultures of a N-limited diatom (*Thalassiosira pseudonana*) were more susceptible to UVR than nutrient-replete cells and suggested that the increase in UV-B sensitivity was a consequence of impaired ability to carry out repair under nutrient limitation (33). Experiments on dinoflagellates showed lower rates of repair together with lower levels of MAAs (leading to higher rates of damage) in N-limited cultures (34). Studies on the green microalga *Dunaliella tertiolecta* have shown that both N and P limitation led to increased susceptibility to UV-B (35,36). However, results from other studies on the effects of nitrogen limitation on UV susceptibility have been contradictory, with an Antarctic diatom showing a lower sensitivity to UV in N-limited compared to N-replete cultures (30).

To improve our understanding of the responses of photochemical performance of diatoms to high light intensity and UV radiation under restricted nitrogen availability, particularly in the context of climate change, we carried out experiments to assess photoinactivation during light exposure of a cosmopolitan marine diatom (*Thalassiosira punctigera*) after acclimation to different nitrogen-nutrient states with the aim of providing insights into the mechanisms involved.

MATERIALS AND METHODS

Species and algal culture conditions. The strain of the marine diatom *Thalassiosira punctigera* Hasle (CCAP 1085/19) was obtained from the Culture Collection of Algae and Protozoa (CCAP, Cambridge, UK) and maintained in a growth chamber equipped with cool white fluorescent tubes.

Before the experiment, the diatom was semicontinuously cultured in artificial seawater enriched with Aquil medium, with a nitrate concentration of 100 μmol L^{-1}, and P and Si concentrations of 10 and 100 μmol L^{-1}, respectively (37). The cultures were renewed every two days with fresh medium to maintain cells in the exponential phase. To obtain low nitrate-acclimated cells, the nitrate added into the medium was gradually decreased from 100 to 5 μmol L^{-1}, with gradients of 100, 30, 10, and 5 μmol L^{-1}. Cultures at each nitrate level were acclimated for 6 days (around 6–8 generations), and subsamples were then inoculated to medium with lower nitrate. The growth rate data indicated that nitrate became limited below 10 μmol L^{-1}. The nitrogen-replete (HN, 100 μmol L^{-1}) and nitrogen-limited (LN, 5 μmol L^{-1}) cells were then maintained simultaneously, as semicontinuous cultures, for another two weeks (more than 14 generations) before the experiment. Other conditions were as follows: The culture photon fluence rate was set at 200 μmol photons m^{-1} s^{-1}, provided by cool white fluorescent lamps in an illuminated incubator (HP 3000 G-C type, Ruihua, Wuhan, China), with a 12:12 light to dark photoperiod. The culture temperature was 20°C. Both treatments were applied to triplicate independent cultures in 500 mL Nalgene bottles (2015-0500, Thermo Fisher).

Determination of growth rate, spectral absorbance, and contents of Chl a, POC, and PON. Specific growth rates (μ) were determined by cell counting using a Sedgwick Rafter chamber under the microscope (Nikon, Eclipse, TS100, Japan). Briefly, 0.5 mL subsamples were withdrawn from cultures every two days before dilution at the end of the photoperiod, then the cells were counted, and growth rates calculated through the following equation:

$$\mu = (\ln N_1 - \ln N_0)/(t_1 - t_0),$$

where μ (day $^{-1}$) represents relative growth rate, N_1 and N_0 are the cell number at day t_1 and t_0 respectively.

For chlorophyll *a* determination, cells from 50 mL medium were collected by filtration using GF/F filter (Whatman) and extracted with pure methanol immediately after cell counting. The extraction was carried out under 4°C in the dark overnight before the samples were centrifuged for 10 min at 5000 g. After centrifugation, the supernatants were transferred to cuvettes for a wavelength scan (250–800 nm) in a spectrophotometer (DU 800, Beckman, Fullerton, CA). In order to compare the absorption spectra, we normalized the values to per cell. Chl *a* content per mL was then calculated from the spectral absorbance through the equation from Ritchie (38):

$$[\text{Chl } a] = 13.2654 \times (A_{665} - A_{750}) - 2.6839 \times (A_{632} - A_{750}),$$

where A_{665}, $A_{750,}$ and A_{632} represent the absorbance of the methanol extracts at wavelengths of 665, 750, and 632 nm, respectively, from the scanned spectrum.

Particulate organic carbon (POC) and particulate organic nitrogen (PON) contents were determined by filtering 50 mL subsamples onto precombusted (450°C, 6 h) GF/F filters immediately after cell counting and stored at −20°C freezer before measurement in an elemental analyzer (PerkinElmer Series II CHNS/O Analyzer 2400).

UV exposure experiment. A solar simulator (Sol 1200, Hönle, Germany) equipped with a 1000 W xenon lamp was used as a light source during the UV exposure experiment. The light condition was adjusted by adding different layers of neutral density screen. Furthermore, cut-off filters Ultraphan 395 or 295 (UV Opak, Digefra, Munich, Germany) were used to create PAR and PAR + UVR (PAB) treatments. The photon fluence rate of PAR and UVR irradiance (radiant flux) during the exposure was set as follows: PAR = 500 μmol m^{-2} s^{-1}, UV-A = 35 W m^{-2}, UV-B = 2 W m^{-2} and low light (PAR = 50 μmol m^{-2} s^{-1}, without UVR, which simulates the light environment of deep water).

Both the LN and HN grown cells were harvested during the middle of the light period and dispensed into 30 mL quartz tubes for UV exposure treatments in triplicate, A parallel experiment was conducted with the addition of lincomycin (which blocks protein synthesis and thus repair processes) to determine the damage rate to PSII; the final concentration of lincomycin was 0.5 mg mL^{-1}. During the light exposure, all quartz tubes were placed into a water bath and temperature was controlled at 20 ± 1 by a circulating-cooling system (D1- 0530, Tianheng, Ningbo, China).

Measurements of Chlorophyll fluorescence. Before light exposure, subsamples from each treatment (LN and HN) were dispensed into quartz tubes and wrapped in aluminum foil for 15 min dark-adaptation before the maximal photochemical yields were measured. Subsequently, all tubes were placed into the water bath and the chlorophyll fluorescence

parameters of each sample were measured at 20-min intervals with an XE-PAM fluorometer (Walz, Eichenring, Germany). After 100 min of light exposure, namely 5 rounds of measurements, samples were transferred to the low light condition for a 60 min recovery. During the light exposure and recovery phase, both photochemical yield and nonphotochemical quenching (NPQ) were measured by monitoring the changes in chlorophyll fluorescence. These fluorescence parameters were calculated by the following equations (39):

$$\text{Maximum photochemical yield} = (F_m - F_0)/F_m,$$

$$\text{Effective photochemical yield} = \left(F'_m - F_t\right)/F'_m$$

$$\text{NPQ} = \left(F_m - F'_m\right)/F_m,$$

where F_m is the maximal fluorescence after dark acclimation, F_0 is the minimal fluorescence after dark acclimation, F_m' is the maximal fluorescence under actinic light, and F_t is the steady-state fluorescence under actinic light.

Data analysis. The rate of damage induced by high light or UVR stress to photosystem II (PSII) (k, min^{-1}) and the corresponding repair rate (r, min^{-1}) were calculated through the following equation according to the Kok model (17):

$$\frac{P_t}{P_0} = \frac{r}{k+r} + \frac{k}{k+r} e^{-(k+r)t},$$

where P_0 and P_t represent the initial photochemical yield (the maximum photochemical yield is used here as the initial value at time 0) and the photochemical yield at a certain time during the experiment, respectively, and t is the time from the exposure to the end of the recovery phase in min. Of particular note is that the r is set as 0 for samples treated with lincomycin as the synthesis of photosystem proteins is blocked by this chemical so that there is assumed to be no repair of photosystem II, while the generated k values are used when fitting the data to obtain values for the repair constant r.

The relative inhibition of photochemical yield induced by UVR is calculated according to (40) by the following equation:

$$\text{Relative inhibition } (\%) = (P_{\text{PAR}} - P_{\text{PAB}})/P_{\text{PAR}} \times 100,$$

where P_{PAR} and P_{PAB} represent the photochemical yield under high light exposure with and without UVR, respectively. This parameter was only introduced when there was a significant difference between P_{PAR} and P_{PAB}.

Statistical differences between different treatments were analyzed using two-sample t-test or two-way analysis of variance (two-way ANOVA) followed by Tukey test, accordingly. The significance level was set at $P < 0.05$ ($n = 3$).

RESULTS

The growth rate (μ) of *Thalassiosira punctigera* under the nitrogen-limited treatment (LN) decreased significantly, by over 22%, compared to the nitrogen-replete treatment (HN) (Fig. 1). Consistent with μ, the cellular content of chlorophyll *a* (Chl *a*) and particulate organic nitrogen (PON) were also significantly decreased (Table 1). The content of Chl *a* decreased from 122.5 (pg cell^{-1}) in HN cells to 57.4 (pg cell^{-1}) in LN grown cells. Due to the sharp reduction of PON and also the increase in POC content, the ratio of POC to PON (C:N) of LN grown cells was twice that of HN cells (Table 1). In contrast to growth rate, both the dark-adapted (F_v/F_m) and the light-adapted (F_v'/F_m') quantum yields were slightly higher for LN grown cells compared with those of HN cells (Fig. 1). The F_v/F_m of LN grown cells was around 0.70, while that of HN cells was around 0.65.

The patterns of absorption spectra normalized to a per cell basis were similar for the LN and HN treatments (Fig. 2), except that there was no absorption peak in the wavelength range of UVR (280–400 nm) (Fig. 2A), with a maximum at 325 nm, in the LN treatment. In general, LN grown cells had a relatively

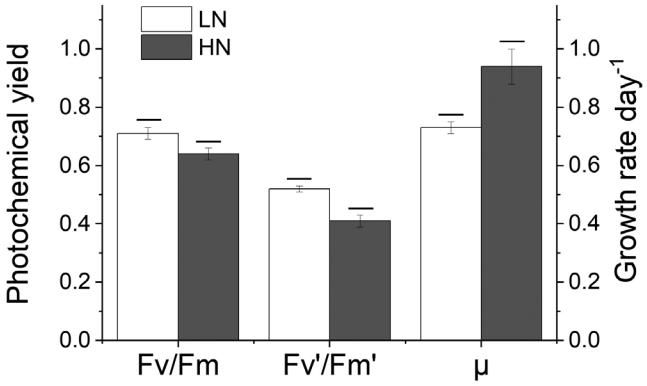

Figure 1. Photochemical yield of *Thalassiosira punctigera* under LN and HN treatments under dark or exposure to growth light. F_v/F_m represents maximum photochemical yield after a dark acclimation, while F_v'/F_m' represent efficient photochemical yield during the light period in the incubator (200 μmol m^{-2} s^{-1}). The horizontal lines represent significant differences between two nutrient treatments under two-sample t-test ($P < 0.05$). Values are the mean ± SD, $n = 3$.

lower optical density than HN grown cells with the overall optical density (OD) of HN grown cells in the range of 400–700 nm being over two times greater than that of LN cells, which reflects the cellular Chl *a* content in both treatments. However, when absorbance of both LN and HN treatments were normalized to the peak of Chl *a* (i.e., 665 nm), the spectra of LN and HN were almost overlapped (Fig. 2B).

During the acute exposure experiment, the photochemical yields of both LN and HN treatments decreased dramatically within the first 40 min of light exposure and showed a slight recovery under PAR treatment in the following exposure period. However, the yields of cells exposed to the PAB treatment remained relatively constant (Fig. 3A). At the end of the light exposure, the photochemical yields of HN-PAR (0.45)-treated cells were over 2 times those in the LN-PAB treatment (<0.2). Compared to the initial value (the maximum photochemical yield at time 0), the photochemical yield in HN-PAR and LN-PAR treatments decreased by 30% and ~ 50%, while those under HN-PAB and LN-PAB decreased by ~ 40% and ~ 30%, respectively (Fig. 3C). After transfer to dim light for 20 min, the HN cells showed higher recovery than the LN cells. The photochemical yield of HN-PAR cells recovered to around 96% of the initial value, and recovery for HN-PAB cells was 76%. For LN grown cells under acute exposure of PAR and PAB conditions, recovery was to relatively lower values, ~75% and ~60% of the initial value, respectively. All the samples treated with lincomycin showed a similar trend in photochemical yield, which decreased gradually and reached the lowest values at the end of the exposure, with a slight recovery during the dim light period (Fig. 3B & D). In general, when exposed to intense PAR or UV radiation the photochemical yield of *Thalassiosira punctigera* was inhibited significantly with the highest inhibition found in the LN-PAB treatment. Photochemical yields of LN grown cells were lower than those of HN cells under both experimental conditions. We also calculated the relative UV inhibition (%) of photochemical yield for both calls cultured under LN and HN medium during the 100 min high light and UV exposure period (Fig. 4). The relative UV inhibition of samples without lincomycin increased from around 20% at time 20 min to 45% at the end of the

Table 1. Cellular component contents and carbon to nitrogen ratio of *Thalassiosira punctigera* cultures under different treatments. Values are the mean ± SD, $n = 3$.

Samples	Chl *a* (pg cell^{-1})	POC (pmol cell^{-1})	PON (pmol cell^{-1})	C : N
LN	57.38 ± 5.44*	689.98 ± 14.54*	57.36 ± 1.17*	12.03 ± 0.39*
HN	122.52 ± 11.14*	501.08 ± 16.65*	85.92 ± 2.71*	5.83 ± 0.06*

*Represents significant differences between different DIN concentration treatments by two-sample t-test ($P < 0.05$).

experiment (Fig. 4A), while this trait was more stable for those cells in the presence of lincomycin, with an average value of around 40% (Fig. 4B). However, there was no statistical difference for relative UV inhibition of photochemical yield between LN and HN grown cells during the exposure.

Under both PAR and PAB treatment, the repair rate (r) of LN grown cells was significantly lower than that for HN ($P < 0.01$, two-way ANOVA) by 55% and 43%, respectively (Table 2). In contrast, the damage rates (k) of LN grown cells showed slight increases in the mean value comparing to that of HN cells though there was no statistically significant difference ($P = 0.057$). The effect of different light treatments was significant for both r and k values ($P < 0.05$), while there was no interaction shown between DIN and light exposure (Table 2). Consequently, the r to k ratio (r/k) of LN grown cells was significantly lower than that of HN cells under all the PAR and PAB conditions, with the highest value found in HN-PAR cells and the lowest found in LN-PAB-treated cells (Fig. 5). This ratio was affected significantly by different light treatments ($P < 0.01$) and the interaction between DIN and light exposure was significant ($P < 0.01$).

The change in the nonphotochemical quenching (NPQ) during the high light exposure period is shown in Fig. 6. For all the treatments, the NPQ values increased from the beginning (time 0) and then became stable. Generally, the NPQ under PAB was higher than that under the PAR condition within each treatment, and the NPQ of LN grown cells was higher than that of HN for the same exposure condition. The highest values were found in the LN-PAB treatment while the lowest ones were found in HN-PAR. With lincomycin, we observed a slightly more rapid increase of NPQ and higher endpoint values (time 100 mins) in all the treatments. For the HN-PAR treatment, the NPQ value after exposure was around 0.5 without lincomycin while that it was a little above 1 with lincomycin.

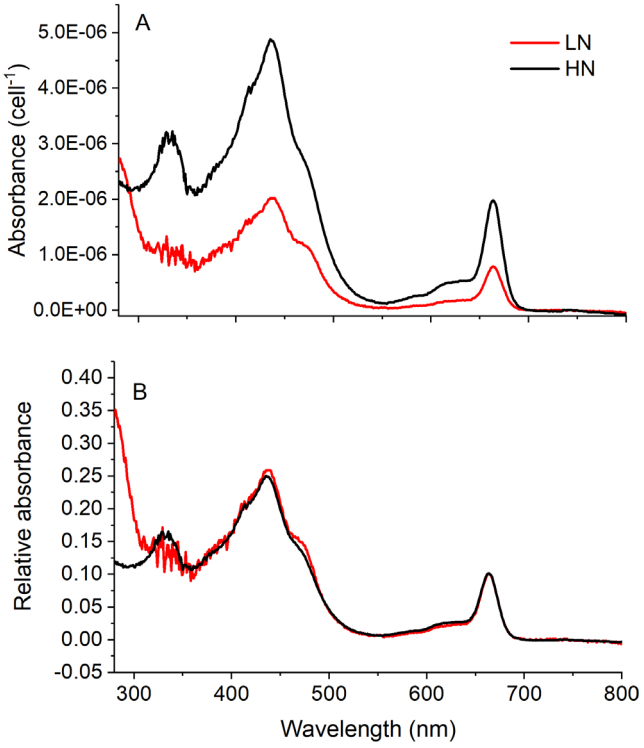

Figure 2. Spectral absorbance of methanol extracts of *Thalassiosira punctigera* under LN and HN conditions. Values are for absorbance of the scanned spectrum (280–800 nm) normalized to per cell (A) or absorbance at 665 nm was normalized to 0.10 (B).

DISCUSSION

Under the impact of climate change, the ocean environment is changing at an unprecedented rate (41) and, as the fundamental basis of the marine ecosystem, phytoplankton are predicted to be exposed to multiple stressors in the future ocean (32). Given the importance of diatoms to global primary production, how these organisms respond to nutrient limitation and UV radiation is of great importance. Previous work on nutrient limitation and UVR interactions under N and P limitation has been carried out on a green alga (35,42) However, the one study showing that nutrient limitation reduced the inhibitory effects of UV and high PAR was on an Antarctic diatom, in which the responses could be complicated by the effect of low temperature which may inhibit repair at low temperature (43,44). Our results revealed that photoinhibition induced by high light and UV radiation was more severe in diatom cells that were grown under limited nitrogen supply (Fig. 3A), which suggested that the nutrient limitation accompanied by excessive radiation would further inhibit the photochemical performance of diatoms in the future ocean. The increased photoinactivation due to UV radiation could partially be attributed to the higher intracellular UV intensity as a result of decreased UVAC, and the limited synthesis of proteins for PSII turnover under nitrogen limitation, which then enhanced damage and suppressed the repair process of PSII. Our results suggest that nitrogen limitation could decrease the N-related resource allocation and increase the sensitivity of the photochemical process to excessive light and UV radiation mainly due to inhibition of the repair process (r).

Diatoms, in common with other algae, have evolved a number of mechanisms to cope with high PAR and UV radiation, such as rapid turnover of proteins within the photosystems to replace damaged apparatus and nonphotosynthetic quenching (NPQ) to dissipate overexcitation (24). Additionally, many phytoplankton species are able to synthesize UV absorbing compounds,

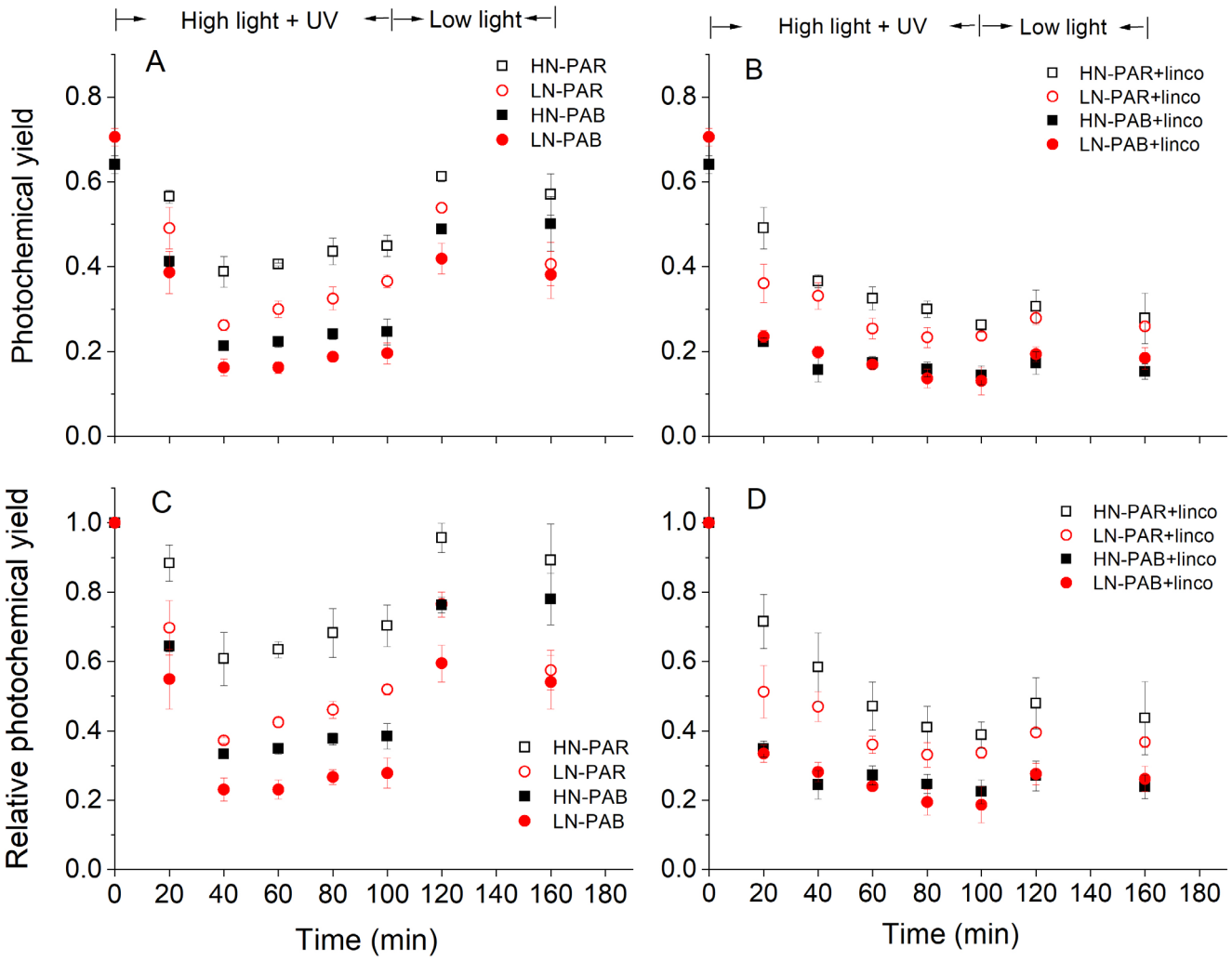

Figure 3. The photochemical yield of *Thalassiosira punctigera* (LN and HN treatment) during light exposure (0–100 min) and recovery under low light without UVR (100–160 min) for two radiation treatments (PAR and PAB) with (B and D) or without (A and C) lincomycin. Panel A and B show the absolute values, while C and D represent the relative values respectively by normalizing photochemical yields to the initial values (set as 1). Different treatments are marked right, the plus sign represents adding with lincomycin. Values are the mean ± SD, $n = 3$.

including mycosporine-like amino acids, to screen out UV radiation (45). Thus, larger phytoplankton cells would be more resistant to radiation, as there will be a longer pathlength for the light to reach the targets inside cells, in addition to self-shading of the light by cellular components (46). In this study, we found a dramatic decrease of chlorophyll *a* (Chl *a*) and organic N content in LN grown cells, which reflects a down-regulated synthesis of pigment and protein under nitrogen limitation. Furthermore, by comparing the absorption spectrum of methanol extracts, we found significantly lower light absorption (per cell) of LN grown cells in both the PAR range (400–700 nm) and the UVR range (300–400 nm) (Fig. 2). Within the UVR range of wavelengths, there was a peak near 330 nm in HN cells while no significant peak was found in LN grown cells, which implies a dramatic reduction in synthesis of UVACs (UV absorbing compounds) under nitrogen limitation (47). Although the results did not show significant increases of damage rate (k) in LN grown cells, further studies are needed to explore whether the reduced cellular components (i.e., pigments and UVACs) under nitrogen limitation would intensify the sensitivity to photoinhibition due to decreased self-screening of UVR. Our results might provide some insights into considering both the direct (reduced nitrogen resource) and indirect (change of physical characteristics as "by-product") effects in physiological studies.

Previous studies showed a high correlation between total nitrogen content with amino acid and protein content in phytoplankton cells when nitrogen supply becomes limited (48,49). A dramatic decrease in total organic nitrogen (Table 1) was observed in the present study, which might reflect a reduction of protein synthesis in the LN treatment. Among all the photosystem proteins, the D1 protein is the most important component for PSII and is found highly dynamic during light exposure (50). The degradation of D1 is light-dependent and highly related to the inactivation and the repair of PSII in diatom (12,51). Therefore, nitrogen limitation might affect the performance of PSII by inhibiting the *de novo* synthesis of D1 protein and influence the dynamic balance of the repair-damage cycle of PSII. This hypothesis is supported by our results as we have shown that the repair rates (r) of LN grown cells are dramatically decreased compared with that in HN cells under both light exposure

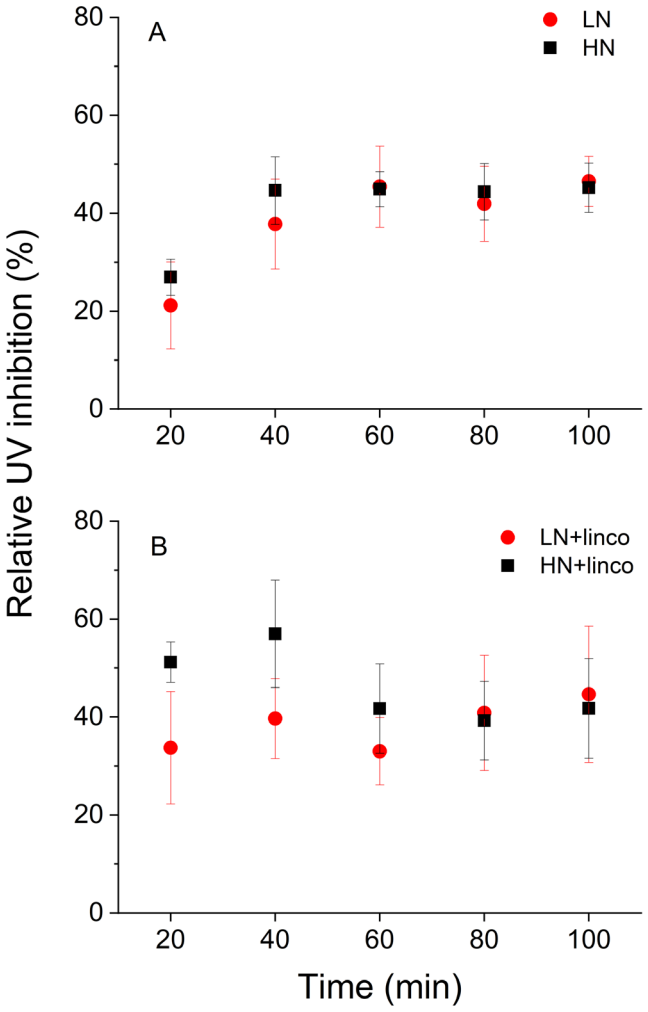

Figure 4. Relative UV inhibition (%) of the photochemical yield of *Thalassiosira punctigera* during the light exposure of 100 min under LN and HN treatments. There is no significant difference between the two DIN treatments under the two-sample t-test ($p > 0.05$). Values are the mean ± SD, $n = 3$.

Table 2. Exponential rate constants (in minute) for repair (r) and damage (k) for different radiation treatments for *Thalassiosira punctigera* during the exposure. Values are the mean, $n = 3$.

Samples	Treatments	r (min^{-1})	SD	k (min^{-1})	SD
LN	PAR	0.009*	0.002	0.017	0.001
	PAB	0.008*	0.000	0.031	0.004
HN	PAR	0.020*	0.003	0.012	0.002
	PAB	0.014*	0.002	0.030	0.003

*Represents significant differences between different DIN treatments ($P < 0.01$). Light treatments have significant effects on both r and k values ($P < 0.05$), while the interaction between DIN and light exposure is not significant. Data from two DIN samples under PAR and PAB treatments were analyzed by two-way ANOVA.

treatments (Table 2), but further studies are needed to reveal details of the underlying mechanisms.

NPQ is an efficient pathway of energy dissipation under overexcitation of PSII, which is assumed to be a mechanism of photoprotection (52). In our study, the NPQ of both treatments

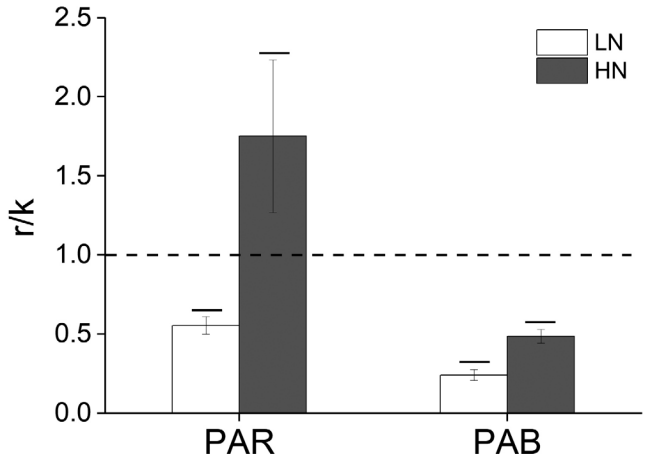

Figure 5. Ratios of repair (r) and damage (k) under LN and HN treatments of *Thalassiosira punctigera* under PAR and PAB conditions. The short horizontal lines above the bars represent significant differences between the two DIN treatments under a two-way ANOVA ($P < 0.05$). Values are the mean ± SD, $n = 3$.

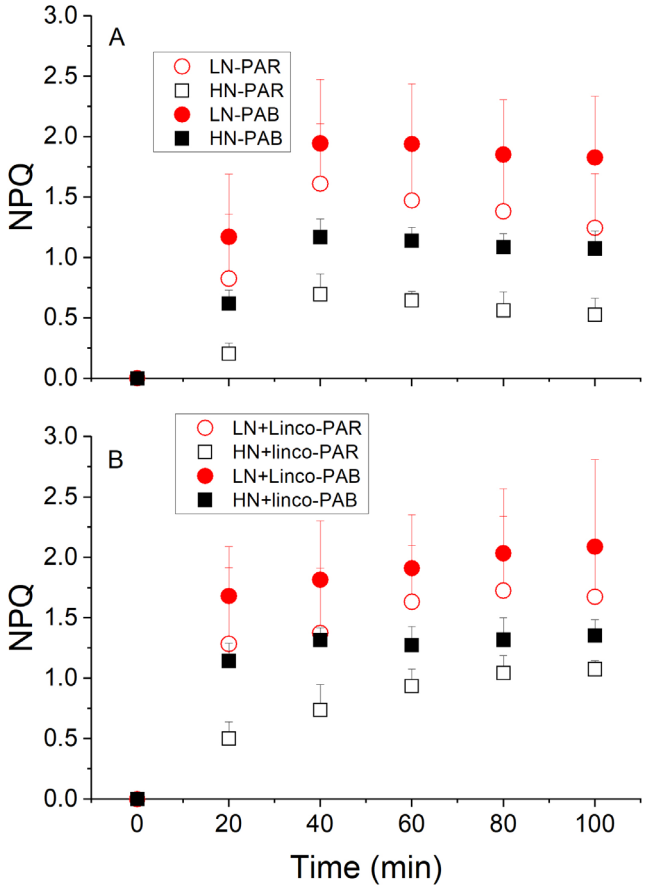

Figure 6. Nonphotochemical quenching (NPQ) of *Thalassiosira punctigera* (LN and HN treatment) during light exposure (0–100 min) and the low light without UVR for two radiation treatments (PAR and PAB) with (A) or without (B) lincomycin. Values are the mean ± SD, $n = 3$.

gradually increased and then remained steady at a high value, which reflected the build-up of photoinhibition during high light or UV exposure (53). The addition of lincomycin significantly

increased NPQ for all light treatments, indicating a trade-off in light energy allocation between the photosystem and NPQ processes. As the stress accumulated and the repair efficiency could not match up with the damage rate, the NPQ increased as a passive response (40). Our results show that under both light conditions LN grown cells have a higher NPQ comparing to HN, which indicated a higher stress level of PSII in diatom under nitrogen limitation as a result of the restricted repair process.

This study explored the photochemical response of a diatom to high light and UVR exposure under nitrogen limitation. The results revealed that nitrogen limitation does not inhibit the photochemical yield of *Thalassiosira punctigera* under growth light conditions. However, the photoinhibition was more severe in nitrogen-limited cells when exposed to both high light and UV radiation as the photochemical yields decreased to a much lower level. That can be explained by lower repair to damage ratios (r/k) mainly due to restricted repair capacity by nitrogen limitation in this study. In the natural environment, the experience of high solar radiation would depress the photosynthetic efficiency of phytoplankton and so affect marine primary production. This phenomenon would become more severe under lower nutrient supply induced by climate change. Thus, in the future ocean, it will be more challenging for phytoplankton to cope with a multi-stressed environment and maintain their ecological function as primary producers and mediators of biogeochemical cycling.

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