RESEARCH ARTICLE

Photosynthetic response of *Nodularia spumigena* to UV and photosynthetically active radiation depends on nutrient (N and P) availability

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Abstract

Biomass of *N. spumigena* is distributed within the dynamic photic zone that changes in both light quantity and quality. This study was designed to determine whether nutrient status can mitigate the negative impacts of experimental radiation treatments on the photosynthetic performance of *N. spumigena*. Cyanobacterial suspensions were exposed to radiation consisting of photosynthetically active radiation (PAR = 400–700 nm), PAR+UV-A (= PA, 320–700 nm), and PAR+UV-A+UV-B (= PAB, 280–700 nm) under different nutrient media either replete with external dissolved nitrate (N) and orthophosphate (P; designated as +N/+P), replete with P only (−N/+P), or replete with N only (+N/−P). Under low PAR (75 μmol photons m^{-2} s^{-1}), nutrient status had no significant effect on the photosynthetic performance of *N. spumigena* in terms of $rETR_{max}$, α, and E_k. *Nodularia spumigena* was able to acclimate to high PAR (300 μmol photons m^{-2} s^{-1}), with a corresponding increase in $rETR_{max}$ and E_k. The photosynthetic performance of *N. spumigena* cultured with supplemental nitrogen was more susceptible to experimental PAR irradiance. Under UVR, P-enrichment in the absence of additional external N (−N/+P) induced lower photoinhibition of photosynthesis compared with +N/−P cultures. However, the induction of NPQ may have provided PSII protection under P-deplete and PAR+UVR conditions. Because *N. spumigena* are able to fix nitrogen, access to available P can render them less susceptible to photoinhibition, effectively promoting blooms. Under a P-deficient condition, *N. spumigena* were more susceptible to radiation but were capable of photosynthetic recovery immediately after removal of radiation stress. In the presence of an internal P pool in the Baltic Sea, which may be seasonally available to the diazotrophic cyanobacteria, summer blooms of the resilient *N. spumigena* will persist.

Introduction

Phototrophs require essential macronutrients for photosynthesis and growth. Phytoplankton primary production in freshwater systems is usually controlled by phosphorus (P) availability, whereas in marine environments elemental nitrogen (N) is more commonly the limiting nutrient (McCarthy & Carpenter, 1983). Diazotrophic cyanobacteria, that have the ability to fix atmospheric dinitrogen (N_2) may, ecologically, be at an advantage to other phytoplankton in nitrogen-limited environments.

Freshwater inflow containing high concentrations of inorganic and organic nutrients from land drainage and river discharge contributes to eutrophication of coastal waters. Based on research at various spatial and temporal scales, a scientific consensus has emerged that nitrogen represents the largest pollution problem in coastal waters (NRC, 2000). Excess phosphorus in estuaries and brackish water can, however, interact with the available nitrogen to affect ecological structure and function adversely (Howarth & Marino, 2006). Management efforts in the Baltic Sea have seen the decrease of external phosphorus and nitrogen load

by *c*. 30% in the 1990s (Pitkänen *et al*., 2001). However, due to the poor oxygen condition at the sediment–water interface, there is an increased benthic release of dissolved inorganic phosphorus (DIP) concentration from the internal phosphorus pool (Pitkänen *et al*., 2001).

Cellular synthesis of nucleic acids and membrane phospholipids, and energy transfer through tri- and biphosphorylated nucleotides requires phosphorus. In diazotrophic cyanobacteria, lack of available phosphorus can result in decreased photosynthesis, growth, and cellular phosphorus content, and loss of polyphosphate storage granules. Low phosphorus availability, coupled with low light, can further suppress heterocyst formation with a subsequent reduction in N_2 fixation and cellular nitrogen content, and loss of their prominent gas vacuoles (Thompson *et al*., 1994).

In the Baltic Sea, the annual recurrence of massive cyanobacterial blooms occurs during late spring and summer (Finni *et al*., 2001; Stolte *et al*., 2006) dominated by *Nodularia spumigena*, *Aphanizomenon flos-aquae*, and *Anabaena* spp. These taxa are able to adjust their vertical position in the water column facilitated by their gas vesicles, which can potentially expose them to high solar radiation during certain periods of the day.

Summer bloom-forming cyanobacteria may possess adaptive mechanisms to limit damage by excessive amounts of photosynthetically active radiation (PAR) and UV radiation (UVR). Photoinhibition occurs not only as a photoprotective energy dissipation mechanism but also upon photoinactivation or photodamage to the D1 protein of photosystem II (PSII) reaction centers under conditions of excess PAR. Exposure to UVR could further reduce maximum photosynthetic rates and efficiency. The targets of UVR are numerous, which include: decrease in electron flow from reaction centers to plastoquinone, damage to the oxidizing site and reaction center of PSII, and degradation of parts of the D1/D2 heterodimer, among others (Grzymski *et al*., 2001; Turcsányi & Vass, 2002). On the other hand, UVR protection mechanisms include the production of UV-absorbing compounds such as mycosporine-like amino acids (MAAs). Synthesis of MAAs was reported in several cyanobacteria when exposed under UVR (e.g. Wulff *et al*., 2007; Pattanaik *et al*., 2008).

Initiation of a cyanobacterial bloom is related to a number of abiotic factors including nutrients, temperature, and hydrographic conditions. Abiotically driven mass occurrence of *N. spumigena* is reportedly dependent on phosphorus reserves and an optimum temperature, whereas *A. flos-aquae* occurs in cooler water and is more dependent on wind-induced mixing and upwelling of nutrients (Kononen, 1992; Karjalainen *et al*., 2007). Despite the fact that the diazotrophic hepatotoxic species *N. spumigena* is exposed to high solar radiation during the mid- to late summer bloom in the Baltic Sea, this is, to our knowledge, the first study that has looked into the multifactorial interactive effects of radiation and nutrient condition on the photosynthetic rate and efficiency of *N. spumigena*. Recently, Wulff *et al*. (2007) reported an isolate-specific response of *N. spumigena* under UVR and nutrient-replete conditions.

Most studies of Baltic *N. spumigena* focused on monitoring bloom dynamics and the regulating factors (e.g. nutrient and temperature), hepatoxin production, and other physiological and biochemical parameters, except that very little is known of its photosynthetic performance under different light and nutrient regimes. The seasonal occurrence and morphological characteristics of *N. spumigena* confine the bloom within the dynamic photic zone that changes in both light quantity and quality. In the presence of both, or the absence of one of the essential nutrients, nitrogen and phosphorus, this study aims to better understand the role played by nutrients in affecting the photosynthetic process. Whether the adverse effects of radiation (PAR and PAR+UVR) are eased by supplemental nutrient fertilization is discussed.

Materials and methods

Culture material

Nodularia spumigena Mertens (KAC 71) isolated from the Baltic Sea was obtained from the Kalmar Algal Collection (KAC), Kalmar University, Sweden. The culture, hereafter called *Nodularia* (in reference to this study), was inoculated into seven salinity f/2 medium in sterile 500-mL Nunc bottles and left to grow for 2 weeks at $17 \pm 1\,^{\circ}C$ under a 16 : 8 h light : dark cycle of 75 μmol photons $m^{-2}\,s^{-1}$.

Nutrient treatments

Natural deep seawater (salinity 37) collected at Kosterfjorden, Sweden, was filtered (GF/F) and diluted with MilliQ-water to reduce salinity to seven. The growth medium consisted of three treatments: (1) a medium with a replete external dissolved nitrate (NO_3, 955 μM) and orthophosphate (PO_4^{3-}, 45 μM) designated as +N/+P, corresponding to the f/2 medium; (2) a medium with a replete external dissolved orthophosphate only designated as −N/+P; and (3) a medium with a replete external dissolved nitrate only designated as +N/−P. The nutrients initially present in the seven salinity seawater were 2.8 μM nitrogen and 0.7 μM phosphorus.

Irradiation treatments

PAR (400–700 nm) of 300 μmol photons $m^{-2}\,s^{-1}$ was obtained from 10 white fluorescent tubes (GE Polylux XL,

F36W/860, Great Britain). In another setup, UVR (280–400 nm) was supplemented with the addition of four UVA-340 fluorescent tubes (Q-Panel, Cleveland, OH). To cut off different wavelength ranges from the spectrum emitted by the fluorescent tubes, quartz bottles (25 mL) were covered with one of the following filters: Ultraphan transparent (Digefra GmbH, Germany), Folanorm (Folex GmbH, Germany), or Ultraphan URUV Farblos corresponding to the PAR+UV-A+UV-B (PAB), PAR+UV-A (PA), and PAR treatments, respectively. UVR was measured using a Solar Light PMA 2100 radiometer equipped with the UV-A sensor PMA 2110 and the UV-B Sensor PMA 2106 (Solar Light, Philadelphia). Adjusted UVR below the cut-off filters was 8.90 W m^{-2} UV-A and 0.65 W m^{-2} UV-B. The available PAR was measured using a cosine quantum sensor attached to a LI-COR data logger (LI-1000, LI-COR Biosciences, Lincoln, Nebraska). After high PAR (300 μmol photons m^{-2} s^{-1}) treatment, samples were exposed to low PAR for recovery (1 and 4 h) and during the circadian rhythm experiment (16 : 8 h light : dark photoperiod) under 10 and 60 μmol photons m^{-2} s^{-1}, respectively.

Experimental design

To determine the photosynthetic capacity of *Nodularia* under different nutrient conditions, 100 mL of stock culture was diluted to 200 mL with corresponding media into 12 Nunc bottles corresponding to four replicates per nutrient treatment. The cultures were maintained in semi-continuous growth for 1 week by replacing 50% and 25% of the volume on day 4 and day 7 of the corresponding media, respectively. On the second week, the cultures were grown as batch culture until the end of the experiment. The Nunc bottles were laid prostrate on the exposure table with the lid closed. The bottles were gently shaken many times every day and randomly moved around the exposure table to ensure equal light treatment. The experiment was conducted inside a temperature-controlled room at 17 ± 1 °C. The average temperature under the lamps was 20 ± 2 °C.

To determine the circadian pattern in photosynthetic efficiency under different radiation (PAR, PA, PAB) and nutrient conditions (+N/−P, −N/+P), 24 quartz bottles were prepared. From the above experimental culture, a 120 mL suspension was obtained from the high-light-acclimated +N/−P- and −N/+P-grown cyanobacteria, respectively. Filled into each 25-mL quartz bottle was 10-mL suspension. The 12 quartz bottles corresponding to a specific nutrient treatment were divided into three groups and assigned the radiation treatment to obtain four replicates for each radiation and nutrient treatment combination. Corresponding 10 mL of new media were added to each bottle. The quartz bottles were randomly arranged in the exposure table and covered with corresponding filter foils. The bottles were gently shaken many times every day.

Chlorophyll fluorescence measurements

The photosynthetic efficiency of *Nodularia* was measured as variable fluorescence of PSII using a Water Pulse Amplitude Modulation fluorometer (Water-PAM) consisting of the Emitter-Detector Unit Water-ED and PAM-Control Universal Control Unit connected to a PC operated with WINCONTROL software (Heinz Walz GmbH, Effeltrich, Germany) (Roleda *et al.*, 2006). The Water-PAM is specialized to study a highly diluted cell suspension and applicable to study cyanobacterial photosynthesis using measuring light-exciting chlorophyll fluorescence peaking at 650 nm. Although the photosynthetic physiology and fluorescence pattern of cyanobacteria differ in important respects from those of plants, cyanobacterial $\Delta F/F_m'$ presents a useful integrated measure of PSII activity (Campbell *et al.*, 1998b).

Cell suspension was filled into 5-mL quartz cuvettes to determine effective quantum yield, $\Delta F/F_m'$, calculated as $(F_m'-F)/F_m'$. F is the fluorescence yield of the irradiation-adapted sample and F_m' is the maximum fluorescence yield when a saturating pulse of 600-ms duration (*c.* 2750 μmol photons m^{-2} s^{-1}) was applied inside the Emitter-Detector Unit. F_0 was first measured with a red measuring light pulse (*c.* 0.3 μmol photons m^{-2} s^{-1}, 650 nm).

Rapid photosynthesis (in terms of relative electron transport rate, rETR = PFR × $\Delta F/F_m'$; PFR = photon fluence rate of PAR) vs. irradiance (E) curves (P–E curve) of *Nodularia* under different nutrient conditions was measured in low-light-acclimated samples before the start of the experiment and in high-light-treated samples at day 7 ($n = 3$, chosen at random from the four replicates). Cyanobacteria suspension was exposed to increasing actinic red light intensity, making up to eight points at 21, 30, 47, 69, 98, 137, 227, and 341 μmol photons m^{-2} s^{-1}. Each actinic light treatment was performed for 30 s before application of a saturating pulse to determine rETR. Data points were plotted and curve fits were calculated with the Solver Module of MS-Excel using the least squares method comparing differences between measured and calculated data. The hyperbolic tangent model of Jassby & Platt (1976) was used to estimate P–E curve parameters as:

$$rETR = rETR_{max} \times \tanh\left(\alpha \times E_{PAR} \times rETR_{max}^{-1}\right)$$

where $rETR_{max}$ is the maximum relative electron transport rate, tanh is the hyperbolic tangent function, α is the electron transport efficiency, and E is the PFR of PAR. The saturation irradiance for electron transport (E_k) was calculated as the light intensity at which the initial slope of the curve (α) intercepts the horizontal asymptote ($rETR_{max}$).

To evaluate the photosynthetic performance of the cyanobacteria grown in different media, 2 mL of the suspension was drawn from the Nunc bottles and $\Delta F/F_m'$ of 300 μL of the high-light-acclimated suspension was measured immediately. The rest of the suspension was exposed to low light and $\Delta F/F_m'$ was measured after 1 and 4 h. Photosynthesis was measured on the 1st, 4th, 7th, 9th and 14th day.

The interactive effect of radiation and nutrient conditions on the circadian pattern of photosynthesis of *Nodularia* was measured six times within the day and every 2 days for 1 week. $\Delta F/F_m'$ was measured under low PAR at 1 h after daylight (07 : 00 h), at 1, 4, and 7 h after high PAR and UVR (10 : 00, 13 : 00, and 16 : 00 h), and under low PAR at 1 and 4 h after the end of UV exposure (18 : 00, 21 : 00 h). The non-photochemical-quenching (NPQ) parameter after a 7-h exposure to light stress (high PAR+UVR) was derived according to the equation: $NPQ = (F_m - F_m')/F_m'$. For every measurement, the 300 μL suspension used was discarded.

Nutrient analysis

Samples were regularly taken during the course of the time-series experiment on the day photosynthesis was measured. Nitrate in medium was quantitatively reduced to nitrite by running through a cadmium column coated with metallic copper. The nitrite produced was determined by diazotizing with sulfanilamide and coupling with *N*-(1-naphthyl)-ethylenediamine to form a colored azo dye measured spectrophotometrically at 550 nm. Phosphate in the medium was allowed to react with a composite reagent containing molybdic acid, ascorbic acid and trivalent antimony. The resulting complex was reduced to give a blue solution measured at 885 nm. The standard nutrient analyses were described by Parson *et al.* (1984).

Statistical analysis

Data were tested for homogeneity of variance (Levene Statistics). Corresponding transformations (square root) were performed to heteroskedastic data. Data on time-series measurements were subjected to repeated measure ANOVA (RMANOVA, $P < 0.001$) to determine the main effects of nutrient enrichment and radiation treatment, and their interactive effect on photosynthetic yield. The remaining available nutrient in the medium under different radiation treatments was also tested using appropriate ANOVA ($P < 0.001$). This was followed by Duncan's multiple range test (DMRT, $P = 0.05$). When a significant interaction was observed, significant subgroups were determined by plotting the means of each dependent factor against the level of each independent (main) factor. Groupings were based on a *post hoc* multiple comparison test. Statistical analyses were performed using the SPSS program (SPSS, Chicago, IL).

Results

The rapid P–E curve parameters showed a variable response between low-light- and high-light-acclimated *Nodularia* (Fig. 1). The estimated slope alpha (α), a parameter for the performance of both light-harvesting and photosynthetic conversion efficiency, inclined steeply (α = 0.19–0.26; mean-0.23 ± 0.08) in low-light-acclimated samples compared with high-light-acclimated samples with significantly low-inclined slopes (α = 0.05–0.06; mean = 0.05 ± 0.01). Under low PAR, comparison between different nutrient conditions showed a relatively steeper α in *Nodularia* grown in +N/−P growth media compared with +N/+P- and −N/+P-grown *Nodularia*. One-way ANOVA ($P < 0.001$) showed an insignificant difference in $rETR_{max}$ ($P = 0.060$), α ($P = 0.174$), and E_k ($P = 0.077$) under low PAR. After a 1-week exposure to high PAR, α was also comparable among all nutrient treatments (ANOVA, $P = 0.254$), while a significant difference was observed in $rETR_{max}$ and E_k (ANOVA, $P = 0.001$ and 0.028, respectively). DMRT ($P = 0.05$) showed $rETR_{max}$ of −N/+P > +N/+P > +N/−P while E_k was +N/+P ≥ −N/+P > +N/−P.

In low PAR-grown *Nodularia*, the initial effective quantum yields of the PSII ($\Delta F/F_m'$) were comparable when stock culture was diluted with corresponding experimental growth media at 0.523 ± 0.01, 0.526 ± 0.02 and 0.533 ± 0.01 in +N/+P, −N/+P and +N/−P, respectively. This was reduced by 44–45% after a 1-day exposure to high PAR (Fig. 2a). The $\Delta F/F_m'$ of the semi-continuously grown culture further decreased by 22% on day 4 in +N/+P- and +N/− P-grown cyanobacteria but not in the −N/+P treatment where *Nodularia* was able to maintain their photosynthetic efficiency at a certain 'optimum' level. Photosynthetic performance on day 7 was not significantly different from the day 4 measurement. When the cultures were grown without further dilution and nutrient replenishment (batch culture), $\Delta F/F_m'$ on day 9 consequently decreased further by 19–20% in all nutrient treatments. Subsequently, photosynthesis of *Nodularia* was able to acclimate to the high PAR on the 14th day without further significant reduction in $\Delta F/F_m'$ regardless of the 'available' nutrient in the medium. When transferred to low PAR, *Nodularia* was able to increase photosynthesis (Fig. 2b and c). The increase in $\Delta F/F_m'$ occurs during the first hour by 17–32% across the time-series measurements among the different culture media. An additional 5–14% increase in $\Delta F/F_m'$ was observed after 4 h in low PAR. This was, however, lower compared with the initial $\Delta F/F_m'$. In the course of the time-series measurement, $\Delta F/F_m'$ showed a decreasing trend among the different growth media regardless of whether exposed to high PAR

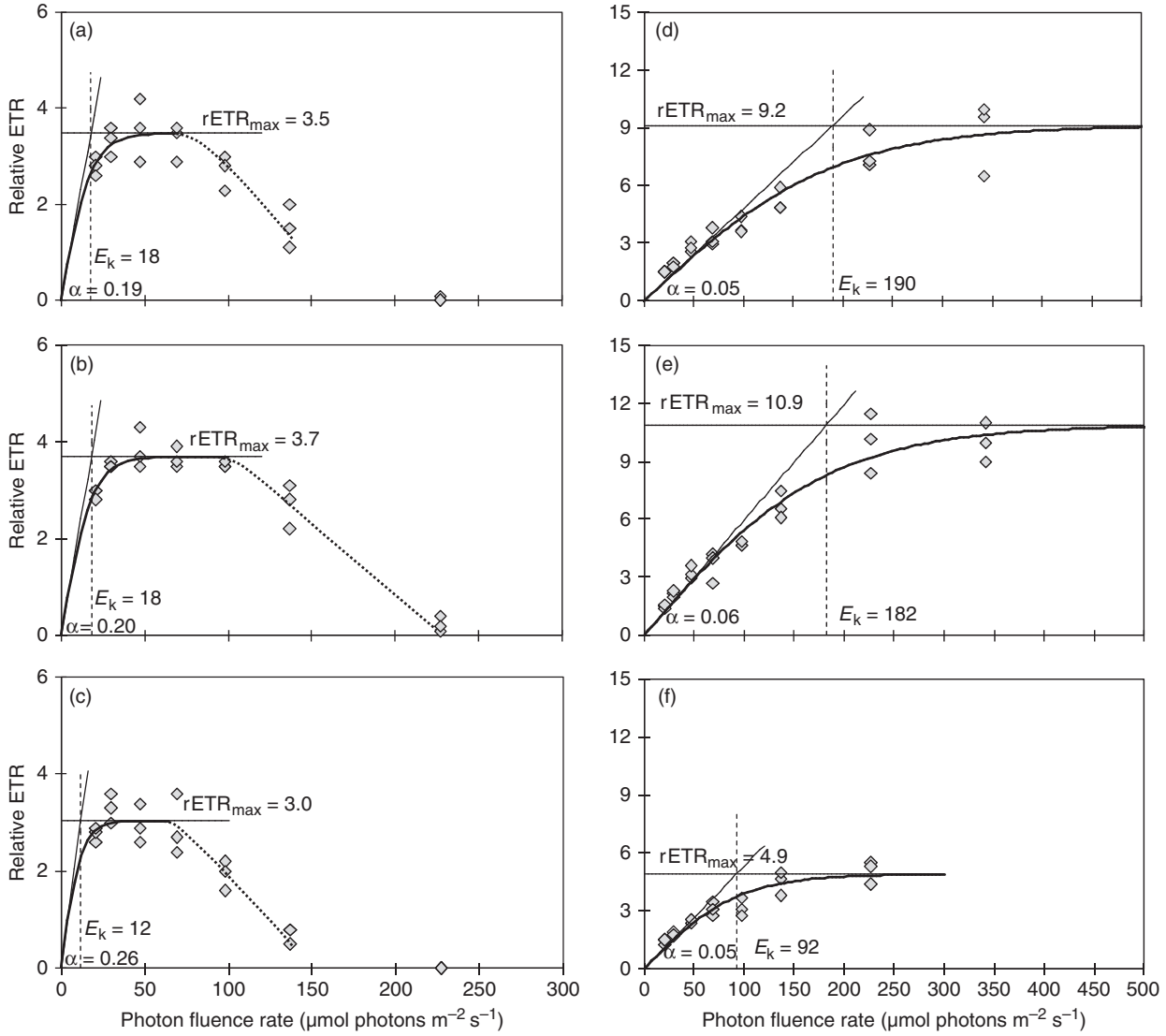

Fig. 1. Rapid photosynthesis–irradiance curve (P–E) of low-light-acclimated (75 μmol photons m^{-2} s^{-1}; left column, a–c) and high-light-acclimated (300 μmol photons m^{-2} s^{-1}; right column, d–f) *Nodularia spumigena* grown under nitrogen- and phosphorus-enriched (+N/+P) medium (a, d), phosphorus-enriched (−N/+P) medium (b, e), and nitrogen-enriched (+N/−P) medium (c, f). PFR is the respective photon fluence rate of actinic light and ETR is the relative electron transport rate. Saturating irradiance (E_k) is estimated as the point at which the initial slope (α) crosses maximum photosynthesis (rETR$_{max}$) using the hyperbolic tangent model of Jassby & Platt (1976).

or allowed to recover in low PAR. Statistical analysis (RMANOVA, $P < 0.001$, Table 1) showed a significant effect of nutrient status in the medium on the photosynthesis of *Nodularia* during exposure to high PAR. The effect of nutrient status was still observed after 1 h incubation under low light, but not after 4 h. DMRT ($P = 0.05$) showed photosynthetic performance of *Nodularia* in different medium was −N/+P > +N/+P > +N/−P under high PAR and −N/+P > +N/+P ≥ +N/−P after 1-h incubation under low PAR.

Available phosphorus in phosphorus-replete (+N/+P and −N/+P) and phosphorus-deficient (+N/−P) growth media was utilized immediately. After 3 days in culture, up to 68% of the external replete orthophosphate and 89% of the natural phosphorus in seawater were consumed by *Nodularia* (Fig. 3a). After replacing 50% and 25% volume (first and second dilution, respectively) of the culture with corresponding fresh media, available phosphorus was replenished but was immediately utilized by *Nodularia*. There was no further reduction in the available phosphorus in different media after cessation of dilution from the 9th day to the last sampling day. The remaining available phosphorus differ significantly between the different media used (Table 1, RMANOVA, $P < 0.001$; DMRT, $P = 0.05$;

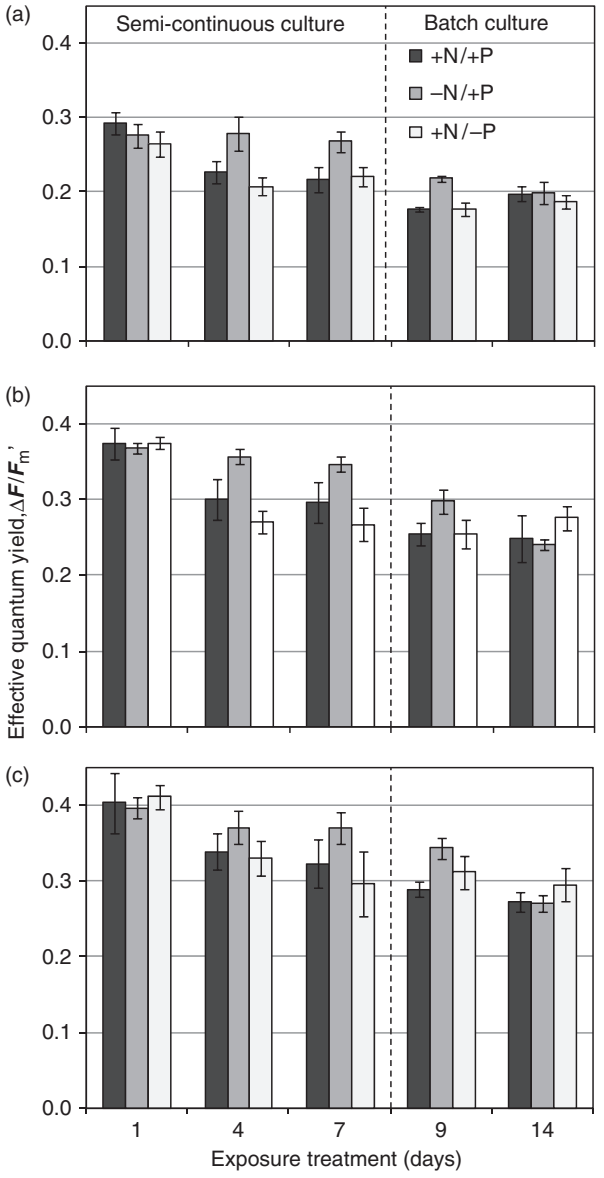

Fig. 2. Mean effective quantum yield ($\Delta F/F_m'$) of *Nodularia spumigena* grown in nitrogen- and phosphorus-enriched (+N/+P), phosphorus-enriched (−N/+P), and nitrogen-enriched (+N/-P) media under 300 μmol photons m^{-2} s^{-1} of PAR (a) and after 1-h (b) and 4-h (c) recovery in low white light (10 μmol photons m^{-2} s^{-1}). The culture was grown under a 16 : 8 h light : dark photoperiod. The growth condition was changed from semi-continuous (week 1) to batch culture (week 2). Error bars are SDs ($n = 5$).

−N/+P > +N/+P > +N/−P). Among phosphorus-replete media, phosphorus absorption was more efficient under a nitrogen-enriched condition.

Total available nitrogen in the medium was not significantly different between the two nitrogen-replete growth media (+N/+P and +N/−P) at anytime during the different water-sampling periods but significantly higher compared with the nitrogen-deficient medium (Table 1, RMANOVA, $P < 0.001$; DMRT, $P = 0.05$, +N/−P ≥ +N/+P > −N/+P). In the nitrogen-deficient medium, the increase in total available nitrogen in the medium after every dilution, while maintaining a semi-continuous culture, was attributed to nitrogen fixation of *Nodularia* (Fig. 3b).

The circadian pattern of photosynthesis in *Nodularia* during the light phase of the day showed a dynamic oscillation in response to PAR fluence and spectral composition of irradiation treatments (Fig. 4). The $\Delta F/F_m'$ generally increased with time (days) regardless of radiation and nutrient treatment except for the slight decrease in $\Delta F/F_m'$ on the 7th day under the +N/−P condition.

The degree of photoinhibition increased significantly with time under a phosphorus-deficient condition. Under a phosphorus-replete condition, the percent photoinhibition of photosynthesis was relatively constant and did not significantly vary between days of repeated exposure to high PAR and UVR (Fig. 5a). Unexpectedly, photoinhibition of photosynthesis under PAB in the phosphorus-deficient medium was observed to level off from day 3 onward and was lower on the 7th day compared with PAR and PA treatment. The average percent photoinhibition over the 7-day period under PAR, PA, and PAB treatment was 43 ± 12%, 50 ± 11%, and 49 ± 6% in +N/−P, respectively, and 31 ± 6%, 43 ± 4%, and 45 ± 4% in −N/+P, respectively. Statistical analysis showed significant effects of the main factors (nutrient and radiation) as well as an interaction between the main factors (Table 1). Multiple *post hoc* comparison tests showed three significantly different subgroups. Arranged in increasing degree of photoinhibition, the subgroups are: −N/+P (PAR) < −N/+P (PA) ≤ +N/−P (PAR) ≤ −N/+P (PAB) < +N/−P (PAB) ≤ +N/−P (PA).

Photosynthetic recovery under low PAR showed a different trend. Under phosphorus-deficient condition, recovery of photoinhibition under PAR, PA, and PAB treatments was on the average maintained across the different sampling periods (Fig. 5b). Under a phosphorus-enriched condition, photosynthetic recovery increased on the 3rd sampling day but was subsequently observed to decrease progressively on the succeeding sampling days until the end of the experiment (Fig. 5b). To compensate loss of PSII function due to photoinhibition, *Nodularia* were able, on average to recover effective quantum yield of photosynthesis over the 7-day period: 36 ± 4%, 50 ± 5%, and 46 ± 4% in +N/−P and 34 ± 7%, 48 ± 6%, and 48 ± 6% in −N/+P under PAR, PA, and PAB treatment, respectively. RMANOVA showed a significant effect of preradiation treatments on the photosynthetic recovery of *Nodularia* (Table 1). Cyanobacteria previously exposed to UVR showed higher photosynthetic recovery after a 4-h incubation under low PAR (DMRT, $P = 0.05$; PA ≥ PAB > PAR).

Table 1. ANOVA (repeated measure, RMANOVA and one-way ANOVA) and significance values for the main effects and interaction of main factors on the photosynthesis of *Nodularia spumigena* and on the nutrient status of culture media

Experiment	Variable	Source of variation	df	*F*-value	*P*-value
Nutrient enrichment	F_v/F_m (under high PAR)	Medium	2	27.72	$< 0.001^*$
	F_v/F_m (after 1 h low PAR)	Medium	2	13.58	0.002^*
	F_v/F_m (after 4 h low PAR)	Medium	2	4.13	0.053^{NS}
	Nutrient				
	Total phosphorus	Medium	2	7924.42	$< 0.001^*$
	Total nitrogen	Medium	2	394.35	$< 0.001^*$
Nutrient × radiation	Photoinhibition	Medium (A)	1	51.88	$< 0.001^*$
		Radiation (B)	2	36.97	$< 0.001^*$
		A × B	2	5.98	0.010^*
	Recovery	Medium (A)	1	1.20	0.288^{NS}
		Radiation (B)	2	112.71	$< 0.001^*$
		A × B	2	2.43	0.116^{NS}
	NPQ	Medium (A)	1	5.37	0.033^*
		Radiation (B)	2	112.71	0.001^*
		A × B	2	2.43	0.711^{NS}
	Nutrient				
	Total phosphorus				
	+N/− P medium	Radiation	2	0.521	0.611^{NS}
	− N/+P medium	Radiation	2	302.04	$< 0.001^*$
	Total nitrogen				
	+N/− P medium	Radiation	2	0.333	0.725^{NS}
	− N/+P medium	Radiation	2	8.841	0.008^*

*Significant.
NS, not significant.

Induction of nonphotochemical quenching (NPQ) was significantly higher under the +N/− P medium than in the − N/+P growth condition (RMANOVA, $P = 0.033$), and under PAR+UVR than in PAR+UV-A and PAR treatment alone (Fig. 6, RMANOVA, $P = 0.007$; DMRT, $P = 0.05$, PAB > PA ≥ PAR). A decline to induce NPQ was observed after prolonged chronic exposure to the irradiation treatments especially under PAR treatment alone.

Exposure to UVR affected nutrient uptake dynamics. Under a phosphorus-replete condition, the remaining phosphorus in the medium were significantly higher when *Nodularia* was exposed to PAR supplemented with either UV-A+UV-B or UV-A alone compared with when exposed to PAR alone (Table 1, ANOVA, $P < 0.001$; DMRT, $P = 0.05$, PAB > PA > PAR). About 75% of the external replete orthophosphate was absorbed by *Nodularia* under PAR treatment compared with 39% and 20% under PA and PAB treatments, respectively (Fig. 7a). Under a phosphorus-deficient condition, no significant difference was observed under different radiation treatments on the absorption of about 40–63% of the initial available phosphorus content in the medium.

Conversely, the initial available total nitrogen in a nitrogen-replete medium was minimally consumed by *Nodularia*, leaving 83–96% of the external nitrogen (and presumably also fixed nitrogen) still available in the medium under different radiation treatments. In the nitrogen-deficient medium, as much as 90% of the initial total available nitrogen in the medium was consumed under PAR treatments, and 75% and 61% under PA and PAB treatment, respectively (Fig. 7b; Table 1, ANOVA, $P < 0.001$; DMRT, $P = 0.05$, PAB ≥ PA > PAR).

Discussion

This study showed no significant nutrient effect on the photosynthetic capacity of *Nodularia* in terms of $rETR_{max}$, α, and E_k under low PAR. Under experimental high PAR, photosynthesis of *Nodularia* was able to acclimate to the increase in PFR with a corresponding increase in $rETR_{max}$ and E_k, but photosynthetic capacity was significantly lower under the phosphorus-deficient nutrient condition. Long-term exposure to a high PFR of PAR showed that supplemental nitrogen with or without simultaneous phosphorus enrichment enhanced photoinhibition of photosynthesis during the semi-continuous growth. The negative 'excess' − N effect was, however, dampened when dilution with new nitrogen-enriched medium was terminated. *Nodularia* were also more susceptible to the negative impact of UVR under nitrogen-replete and phosphorus-deplete nutrient conditions but were capable of dynamic recovery of photosynthesis when UVR was removed.

Changing the growth condition from semi-continuous to batch culture may potentially affect the physiology of

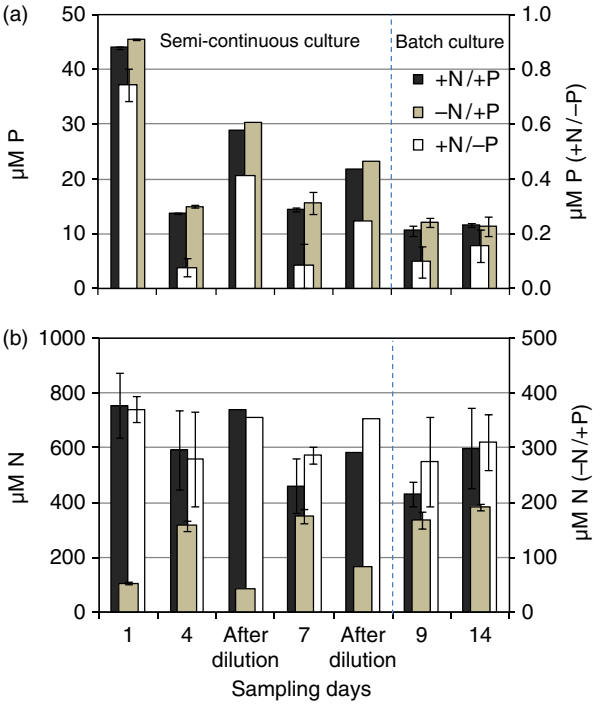

Fig. 3. Time course mean phosphorus (a) and nitrogen (b) concentration in different culture media (+N/+P, −N/+P, +N/−P) of *Nodularia spumigena* grown in a semi-continuous culture (week 1) and in a batch culture (week 2). Values of overlapping bars correspond to the secondary *y*-axis (right-hand side). After each dilution to maintain semi-continuous culture, the nutrient content was estimated (column without error bars). Error bars are SDs ($n = 3$).

Nodularia. The downward trend in $\Delta F/F_m'$ was, however, already observed during semi-continuous culture and stabilized during batch culture. [Correction to previous sentence made on 1 September 2008, after first online publication.] On the other hand, changing growth medium from semi-continuous to batch culture did not seem to affect nitrogen fixation (Fig. 3b).

N_2 fixation by *Nodularia* exceeds their own nitrogen demand by 10–12% and contributes *c*. 50% of the nitrogen demand of the total phytoplankton community (Stal & Walsby, 2000; Stal *et al*., 2003). Besides being able to fix molecular nitrogen, *Nodularia* are known to be able to store phosphorus. In this species, the cellular phosphorus reserve is reported to primarily determine growth and bloom formation rather than the presence of a high nitrogen pool (Karjalainen *et al*., 2007). Model simulations showed that the interannual variation of *Nodularia* bloom in the Gulf of Finland was dependent on phosphorus condition, with the surface layer temperature as the bloom coregulator (Lilover & Laanemets, 2006). *Nodularia* is able to sustain physiological activities relying on cellular phosphorus storage and effective remineralization of organic phosphorus compounds (Vahtera *et al*., 2007), making diazotrophic cyanobacteria probably more iron limited than thought previously (Stal *et al*., 2003) and more competitive under a nitrogen-limited environment.

Our study showed that phosphorus enrichment in the absence of additional external nitrogen (−N/+P) induced lower photoinhibition of photosynthesis under UVR compared with +N/−P cultures. Although no +N/+P-enriched culture was exposed to PAR+UVR, the presence of phosphorus enrichment did not ease the effect of high PAR

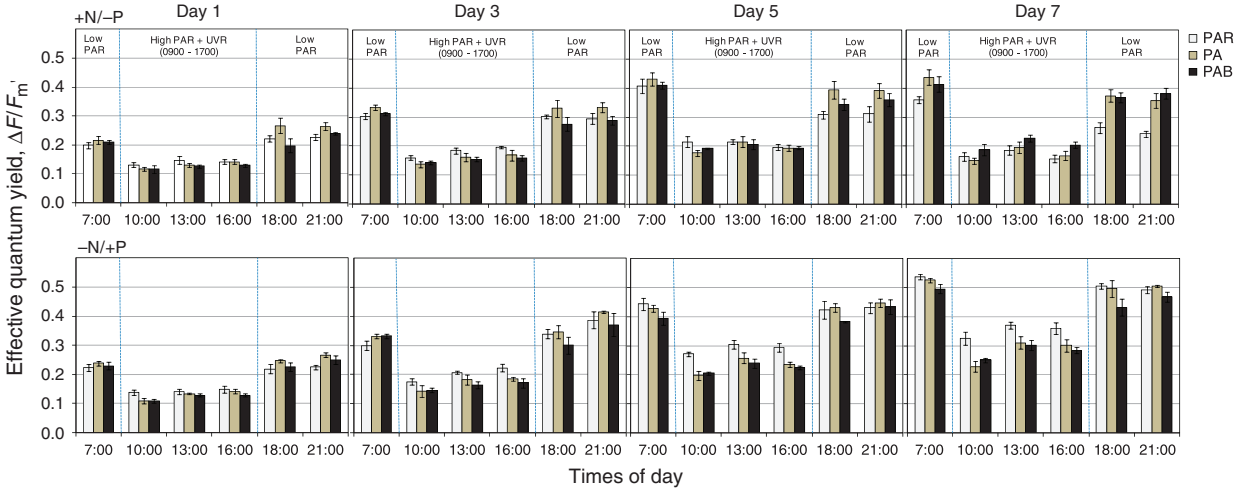

Fig. 4. Circadian pattern of the mean effective quantum yield ($\Delta F/F_m'$) of *Nodularia spumigena* grown in nitrogen-enriched (+N/−P) and phosphorus-enriched (−N/+P) media exposed to different radiation conditions consisting of PAR, PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB) during the light phase of the 16 : 8 h light : dark photoperiod. Low (early morning and late afternoon) and high (middle of the light phase) PFD was used to broadly simulate light intensity variation within the day. Error bars are SDs ($n = 4$).

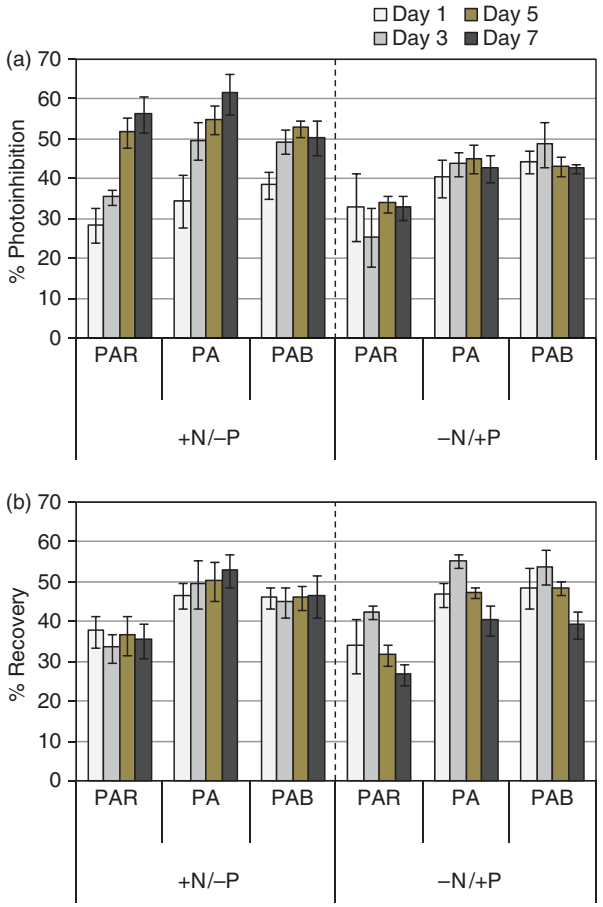

Fig. 5. Time-series photoinhibition of photosynthesis (a) after a 7-h exposure to high PAR and UVR in different radiation and nutrient treatments expressed as percent reduction in $\Delta F/F_m'$ relative to the initial value under low PAR, and photosynthetic recovery (b) expressed as percent increase in $\Delta F/F_m'$ after an 8-h exposure to high PAR and UVR and after 4 h under low PAR. Error bars are SDs ($n = 4$).

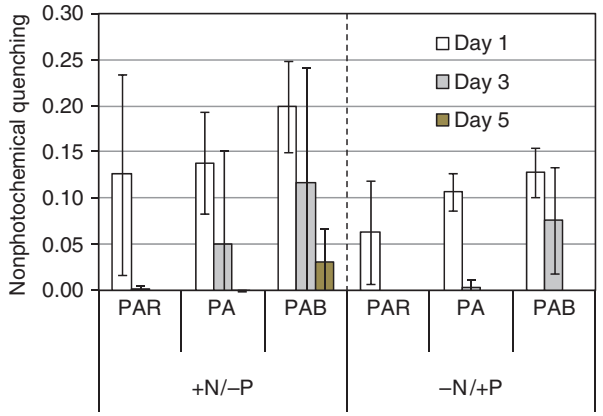

Fig. 6. Nonphotochemical quenching (NPQ) as a function of nutrient and radiation treatment combinations in *Nodularia spumigena*. Values represent NPQ after a 7-h exposure to high PAR and UVR, in the middle of the light phase of the daily 16 : 8 h light : dark photoperiod. No induction of NPQ was observed on Day 7. Error bars are SDs ($n = 4$).

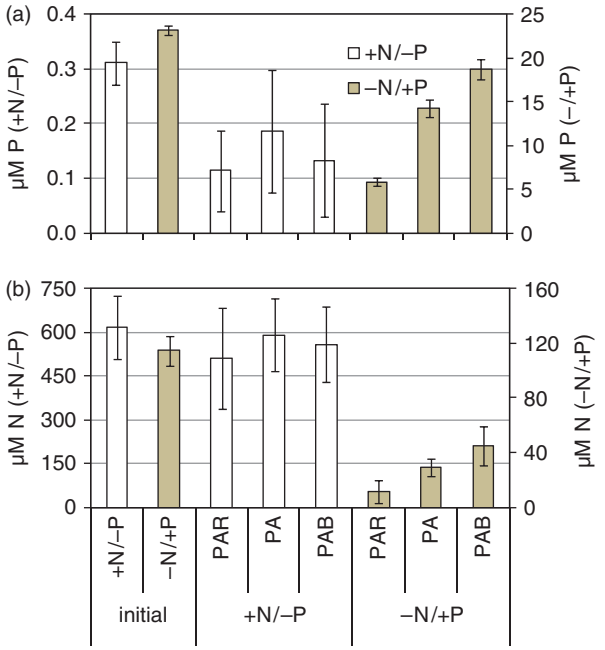

Fig. 7. Mean phosphorus (a) and nitrogen (b) concentrations in *Nodularia spumigena* medium grown under +N/−P (primary y-axis) and −N/+P (secondary *y*-axis) conditions and exposed to different radiation conditions consisting of PAR, PAR+UV-A (PA), and PAR+UV-A+UV-B (PAB). Error bars are SDs ($n = 3$).

in the presence of external nitrogen (first experiment). The photoinhibition of photosynthesis can, therefore, be attributed to the excess nitrogen rather than the absence of phosphorus in the medium. Our materials were previously grown in a +N/+P medium before they were subjected to different combinations of nutrient and radiation treatments. The probability that *Nodularia* cells were able to accumulate enough cellular phosphorus reserved for the duration of the experiment is highly possible. Phosphorus starvation was clearly responsible for the higher photoinhibition of photosynthesis in *Nodularia* during exposure to high PAR and UVR. But adequate cellular phosphorus reserved and sufficient fixed nitrogen may have contributed to their capacity for dynamic recovery of photosynthesis when stress (high PAR and UVR) was removed. This physiological resilience supports previous studies whereby *Nodularia* was reported to be highly tolerant to increased phosphorus starvation (Degerholm *et al.*, 2006) and the eventual collapse of biological functions occurred only after the depletion of the internal phosphorus reserve (Walve & Larsson, 2007).

The results presented here showed that the induction of NPQ provided some protection to PSII as indicated by the inverse relationship between photoinhibition of photosynthesis under +N/−P (Fig. 5a) and the capacity to induce NPQ (Fig. 6). NPQ is associated with the induction of several mechanisms that compete with photochemistry

for the deactivation of Chl *a*-excited states when the rate of captured light exceeds that of the rate of consumption of $NADPH_2$ and ATP by cellular metabolism (Müller *et al.*, 2001; Rodríguez-Román & Iglesias-Prieto, 2005).

Conversely, the progressive reduction in NPQ under −N/+P (Fig. 6) but without a corresponding increase in the photoinhibition of photosynthesis under different radiation treatments (Fig. 5a) was unexpected. In the absence of empirical data to explain this observation, it is tempting to speculate that the modification of the acceptor site of PSII resulting in the development of Q_B nonreducing PSII might be responsible for this phenomenon. The Q_B nonreducing PSII observed in other cyanobacteria is resistant to photoinhibition (Wykoff *et al.*, 1998; Steglich *et al.*, 2001). This may have facilitated the dissipation of excess excitation energy and may have served as an alternate photoprotection mechanism compensating for the failure to induce NPQ.

The negative 'excess' nitrogen effect on the photosynthesis of *Nodularia* is noteworthy and reported for the first time. The biochemical process behind this phenomenon is unknown among cyanobacteria and needs further study. Among kormophytes, reduction in net photosynthesis under high nitrogen treatment was attributed to the depression of carboxylation efficiency, coupled by a decrease in Rubisco concentration and activity (Nakaji *et al.*, 2001; Manter *et al.*, 2005). Other adverse effects of excess nitrogen are reflected in terms of growth inhibition, increased hydrogen peroxide (H_2O_2) accumulation and superoxide radical (O_2^-) production (Yao & Liu, 2006, 2007). Elevated nitrogen does not only negatively influence the physiology and growth of trees (Schulze, 1989) but has also been found to be toxic to certain species of seagrass (Burkholder *et al.*, 1992, 1994).

In diazotrophic cyanobacteria, photosynthesizing vegetative cells differentiate to form N_2-fixing heterocysts upon nitrogen deprivation (Böhme, 1998). In this study, however, the increasing total nitrogen content we found in the medium suggests that regardless of the nitrogen status (e.g. nitrogen enriched), *Nodularia* was still actively fixing N_2 in excess of their metabolic requirement. This conforms to a previous study that showed that cyanobacteria with inactivated genes (*sigB* and *sigC*), expressed under nitrogen deficiency and involved in nitrogen fixation, were still capable of heterocyst differentiation and nitrogen fixation (Brahamsha & Haselkorn, 1992). Assimilation of nitrogen substrates increases energy requirement in the order: ammonium, nitrate and N_2. Regulation of nitrogen fixation is, however, not completely understood. For example, *Nodularia* strains M1 and M2 grown with ammonium at a concentration of 1 mM resulted in the total disappearance of nitrogenase activity and of heterocyst; heterocyst, however, persisted in the presence of 20 mM NO_3^- at a frequency similar to that found in the absence of nitrate. Excess NO_3^- above 25 mM was consequently observed to inhibit growth (Sanz-Alférez & del Campo, 1994). Another *Nodularia spumigena* strain AV1 isolated from the Baltic Sea lost aerobic nitrogen-fixation activity in the presence of ammonium ion and, at the same, time maintained heterocyst frequency along the filaments (Vintila & El-Shehawy, 2007).

Generally, we found transient experimental irradiation effects on the photosynthesis of *Nodularia* regardless of nutrient status. Long-term diel exposure to PAR and PAR+UVR showed dynamic recovery of photosynthesis already 1 h after the exclusion of UVR and under low PAR. Among other phytoplankton species, nitrogen limitation significantly increased the sensitivity of photosynthesis of estuarine dinoflagellates to inhibition by UVR (Litchman *et al.*, 2002), while the marine unicellular chlorophyte *Dunaliella tertiolecta* Butcher also showed the same increased UVR-induced inhibition of photosynthesis but under both nitrogen-limitation and phosphorus-starvation conditions (Shelly *et al.*, 2002, 2005; Heraud *et al.*, 2005).

Most studies on the interactive effects of nutrient and UVR on phytoplankton productivity measured growth as a response parameter. The results showed contrasting responses between phytoplankton species, community assemblage, lotic systems, and altitude and latitudinal gradients. In an alpine lake, the effects of UVR on phytoplankton community depend on temperature and nutrient availability (Doyle *et al.*, 2005). At a low temperature (6 °C), UVR led to a decrease in growth rates of all phytoplankton species regardless of the nutrient condition. At a high temperature (14 °C), no negative UVR effect was observed in the absence of nutrient addition while addition of nitrogen and phosphorus under UVR increased the growth of one diatom species *Fragilaria crotonensis* Kitton and the dinoflagellate *Gymnodinium* sp. (Doyle *et al.*, 2005). In a boreal lake, phytoplankton growth was coregulated by phosphorus-limitation and UVR suppression, with the highest growth rates found in high phosphorus and low UVR treatments (Xenopoulos *et al.*, 2002). Phosphorus input is hypothesized to buffer the harmful UVR effect on algae. However, long-term UVR exposure exerted a significant deleterious effect on the physiology of a natural pelagic algal community in the presence of excess phosphorus (Carrillo *et al.*, 2008). Nutrient limitation was also reported to decrease the sensitivity of the diatom *Chaetoceros brevis* Schütt to photo-induced viability loss relative to nutrient-replete conditions (van de Poll *et al.*, 2005). Conversely, the availability of inorganic nutrients was reported to mitigate the negative UV-B radiation effects on the growth of microphytobenthic communities (Wulff *et al.*, 2000). On the other hand, variable responses of natural phytoplankton population to enhanced UV-B radiation and nitrate enrichment were also reported across a latitudinal gradient (Longhi *et al.*, 2006).

Although our study used only 300 μmol photons m^{-2} s^{-1} of PAR, this was high enough to cause photoinhibition of

photosynthesis. In nature, wind-induced vertical mixing could potentially expose microalgal cells to variable light quantity and quality. Exposure to photoinhibiting PAR ($>1000\,\mu mol\,photons\,m^{-2}\,s^{-1}$) was reported to alter the phycobiliproteins such as the disappearance of the 31.5-kDa linker polypeptide and consequently hinder the energy transfer process within the phycobilisomes in *Spirulina platensis* (Nordstedt) Geitler (Kumar & Murthy, 2007). H_2O_2, as a side product of oxygenic photosynthesis exposed to high irradiance, commonly encountered in an aquatic environment can present a double jeopardy to cyanobacterial photosynthesis. A generated or an external H_2O_2 source does not only inhibit photosynthetic electron transport (Samuilov *et al.*, 2001; Drábková *et al.*, 2007) but also degrades D1 protein (Lupínková & Komenda, 2004) in cells of cyanobacteria.

Despite the artificial laboratory condition with a relatively high UVR : PAR ratio, a probable future scenario under an ozone-depleted stratosphere, UV irradiances comparable with those encountered in the field were observed to have a limited negative impact on the photosynthetic performance of *Nodularia*. Reactive oxygen species (ROS) induced by UV-B affects oxidative damage but may also act as signal molecules and mediate the genetic regulation of photosynthetic genes and the induction of antioxidant enzymes (He & Häder, 2002). Removal of ROS by antioxidants is one of the defense mechanisms providing protection against excessive light absorption in phototrophs (Logan *et al.*, 2006). Moreover, protection of photosynthesis against UVR by carotenoids has been reported in transgenic *Synechococcus* PCC7942 (Götz *et al.*, 1999). The transgenic cyanobacterium further resists UV-B by exchanging PSII reaction-center D1 proteins from the *psbAI*-encoded D1 : 1 to an alternate *psbAII*- and *psbAIII*-encoded D1 : 2 form within 15 min of exposure to moderate UV-B (Campbell *et al.*, 1998a). The fast and reversible recovery of photoinhibited photosynthesis in *Nodularia* can also be attributed to the carotenoid-controlled effective thermal energy dissipation, keeping excessive energy flow in control (Logan *et al.*, 2006).

In conclusion, because *Nodularia* are able to fix nitrogen, access to available phosphorus (internal input or regenerated source) can render them less susceptible to photoinhibition effectively promoting blooms. In the eventual depletion of phosphorus in the system, before the collapse of the bloom, *Nodularia* might be more susceptible to radiation but capable of photosynthetic recovery immediately after removal of radiation stress. Whether individual *Nodularia* filaments are able to actively sink to escape from high solar radiation remains to be studied. Other cyanobacteria like *Anabaena variabilis* and *Oscillatoria tenuis* are reported to migrate from the water surface to lower levels to avoid high solar irradiance (Donkor & Häder, 1995). Otherwise, *in situ* dynamic photosynthetic recovery can occur when filaments are dispersed from the bloom surface to depths under the mat by wind-induced vertical mixing during the day, or eventually during the twilight. Conversely, alternating periods of calm and deep-mixing events can increase the photosynthesis of cyanobacteria when buoyed by gas vacuoles near the water surface (Walsby *et al.*, 1997).

The increasing occurrence and intensity of *Nodularia* bloom in the Baltic Sea can be explained by the resilience of *Nodularia* to radiation and to their capacity for dynamic photoinhibition. With the advent of global climate change, *Nodularia* can potentially acclimate to increasing UVR. Moreover, a high summer temperature will not only increase cyanobacterial growth rate but can also increase water column stability, reducing vertical mixing. Because the phosphacline is located in the upper part of the thermocline (Lilover & Laanemets, 2006), this can give a competitive advantage to buoyant cyanobacteria (Jöhnk *et al.*, 2008) like *Nodularia* to access available phosphorus. Therefore, in the presence of an internal phosphorus pool that can be seasonally available to the diazotrophic cyanobacteria, late spring and summer cyanobacterial blooms in the Baltic Sea will continue to persist.

Synergistic effects might exist between high PAR and UVR. Even if strong PAR is missing, this study presents a piece of the puzzle to explain why extensive summer bloom formation occurs in the Baltic. Future *in situ* photosynthetic studies during the course of bloom initiation and collapse will yield more ecologically relevant data on the basic physiological process controlling bloom formation.

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