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Balancing photosynthetic electron flow is critical for cyanobacterial acclimation to nitrogen limitation

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ABSTRACT

Nitrogen limitation forces photosynthetic organisms to reallocate available nitrogen to essential functions. At the same time, it increases the probability of photo-damage by limiting the rate of energy-demanding metabolic processes, downstream of the photosynthetic apparatus. Non-diazotrophic cyanobacteria cope with this situation by decreasing the size of their phycobilisome antenna and by modifying their photosynthetic apparatus. These changes can serve two purposes: to provide extra amino-acids and to decrease excitation pressure. We examined the effects of nitrogen limitation on the form and function of the photosynthetic apparatus. Our aim was to study which of the two demands serve as the driving force for the remodeling of the photosynthetic apparatus, under different growth conditions. We found that a drastic reduction in light intensity allowed cells to maintain a more functional photosynthetic apparatus: the phycobilisome antenna was bigger, the activity of both photosystems was higher and the levels of photosystem (PS) proteins were higher. Pre-acclimating cells to Mn limitation, under which the activity of both PSI and PSII is diminished, results in a very similar response. The rate of PSII photoinhibition, in nitrogen limited cells, was found to be directly related to the activity of the photosynthetic apparatus. These data indicate that, under our experimental conditions, photo-damage avoidance was the more prominent determinant during the acclimation process. The combinations of limiting factors tested here is by no means artificial. Similar scenarios can take place under environmental conditions and should be taken into account when estimating nutrient limitations in nature.

1. Introduction

Photosynthetic autotrophs are self-reliant in regard to obtaining reduced carbon sources, needing little more than adequate illumination and carbon dioxide. A different aspect of autotrophy is the need for assimilation of essential nutrients directly from the environment. Nutrients are roughly divided into two groups: macronutrients, specifically, nitrogen, phosphorous, sulfur and potassium; and micronutrients like iron, manganese, copper and molybdenum. The bioavailability of nutrients varies and is dependent on different factors such as concentration and chemical speciation. In the aquatic domain the concentrations of nutrients are often limiting, even before taking their chemical state into account. In different parts of the world's aquatic habitats the bioavailability of nutrients such as N, P and Fe is extremely low. These nutrients are considered as major limiting factors in such areas [1,2]. Nutrient levels in aquatic environments fluctuate considerably over time and depth and are affected by numerous environmental phenomena such as Aeolian dust deposition, river runoff, sediment re-suspension, deep water mixing as well as sporadic local anthropogenic pollution events. In these environments, prokaryotic oxygen evolving photosynthetic organisms (cyanobacteria) account for approximately 30% of primary productivity [3]. Over the course of their evolution cyanobacteria developed acclimation mechanisms for dealing with fluctuating and often limiting nutrient regimes; reacting to nutrient shortages in a way that ensures their survival until the next enrichment event occurs.

Nitrogen is one of the most abundant elements on Earth. However, with the exception of diazotrophic bacteria, it is not bioavailable to microorganisms as N_2. As early as 1910, it was observed that non-diazotrophic cyanobacteria lose their typical blue-green hue and become yellow following transfer to nitrogen limiting conditions [4]. This effect was termed nitrogen bleaching and was found to be caused by the breakdown of phycocyanin (PC), a major antenna pigment in cyanobacteria. Research conducted in the 1960s concluded that when grown under nitrogen limiting conditions, the fresh water cyanobacterium *Anacystis nidulans* (Currently *Synechococcus elongatus*) stops growing and shows a substantial loss of PC, nucleic acids and proteins [4]. A reduction in chlorophyll levels was also observed under these conditions. These phenotypes could be reversed by the addition of nitrogen salts to the growth media [4]. The process of phycobilisome (PBS) breakdown and recovery following nitrogen

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supplementation, its kinetic properties and molecular control mechanisms have been subjected to intensive research conducted with different cyanobacterial strains [5–14]. The common feature reported in these studies was a massive, regulated breakdown of PBS on a time scale of a few hours to 48 h following nitrogen step-down. In these reports, the extent of chlorophyll degradation was variable. In certain cases it is reported that chlorophyll levels drop only slightly [4,7,11]. Some accounts, however, report a significant decrease in cellular chlorophyll levels under nitrogen starvation [6,10]. It was suggested that these discrepancies can be attributed to the different cyanobacteria strains analyzed and to a variation in the experimental setup, especially light intensities, CO_2 levels and even nitrogen step-down method [10].

These observations are supported by transcriptomic studies indicating that many cellular activities are down-regulated in the absence of nitrogen [11,15]. When faced with nitrogen limitation, cyanobacteria lower the rate of metabolic processes and change from an anabolic to a catabolic metabolic strategy, represented by the degradation of proteins and other cellular components [14].

Degradation of PBS was suggested to allow for the reallocation of nitrogen from this highly abundant protein to other critical pathways in the cell [16]. At the same time, nitrogen limitation grinds biosynthetic mechanisms to a halt, resulting in over reduction of photosynthetic electron carrier pools [17]. These conditions expose the photosynthetic apparatus to damage from reactive oxygen species. Decreasing PBS absorption cross-section alleviates this stress by reducing excitation pressure.

In this work we examined the response of photosynthetic apparatus to nitrogen limitation. In particular, we attempted to asses which of the stresses exerted on the photosynthetic apparatus during nitrogen limitations determines the changes in its function.

2. Methods

2.1. Cyanobacterial strains and culture conditions

Wild-type *Synechocystis sp.* Strain PCC 6803 cultures were grown in 120 ml of YBG11 medium [18] in acid washed 500 ml glass Erlenmeyer flasks. Cultures were maintained under constant shaking and illumination at 30 °C. Illumination was set at 60 μmol photons $m^{-2}s^{-1}$ (growth light – GL) except when low light conditions were applied (6 μmol photons $m^{-2}s^{-1}$– LL). In order to remove excess nitrogen, cultures were spun down at 4600 g for 5 min and then resuspended in YBG11 containing no nitrate (YBG11 N-). This was repeated once more and then equal aliquots were transferred to flasks containing either standard YBG11 (N+) or YBG11 (N−). Cell densities were counted with a hemacytometer. Mn limitation was achieved by removing it from the trace metal solution of YBG11. Mn levels in Mn− media were lower than 1.7 nM [19].

For the photo-damage measurements, the cells were transferred to N− conditions and grown for 48 h at GL and LL conditions. At that point the protein synthesis inhibitor chloramphenicol was added (25 μg ml^{-1}) and the cultures were transferred to GL conditions.

2.2. Oxygen evolution measurements

Synechocystis 6803 cells were spun down and re-suspended to a final chlorophyll concentration of 10 μg per ml. Oxygen evolution rates were measured using a Clark-type electrode (Pasco, Roseville, CA). Illumination was provided by a white LED array. The measurements were carried out at 30 °C. Oxygen evolution rates were corrected to the dark respiration rate.

2.3. Spectroscopic analysis

P_{700} photo-oxidation was measured in vivo in cultures brought to the same density i.e. constant cell number in all experiments. Where indicated, DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) or DBMIB (2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoqinone) were added to a final concentration of 10 μM. Measurements were performed using a Joliot-type spectrophotometer (JTS-10, Bio-Logic, Grenoble, France) as described previously [19]. A calibration curve confirmed that P_{700} photo-oxidation values are linearly correlated to chlorophyll concentration (supplemental Fig. 2). Phycobilisome absorption spectra were measured from soluble fractions extracted from broken cells, while chlorophyll concentrations were determined by methanol extractions of the membranal fraction and absorbance measurements at 665 nm (ε = 74.5 ml cm mg^{-1}). The soluble fraction was centrifuged at 24,000*g* for 30 min for sedimentation of small membrane vesicles. Fv/Fm measurements were carried out using an imaging PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The cells were dark adapted for 2 min prior to the measurements.

2.4. Protein analysis

For protein separation using BN-PAGE and SDS-PAGE techniques, *Synechocystis* 6803 cells were broken by rigorous bead beating. The resulting membrane fraction was collected by centrifugation [19]. Membranes were resuspended in a buffer containing 330 mM mannitol, 30 mM HEPES, 2 mM EDTA and 3 mM $MgCl_2$, pH 7.8. Linear 4.5–12% BN-PAGE was performed as described by Heinemeyer and co-workers [20] using the mild detergent *n*-dodecylmaltoside at a ratio of 0.03:1 (w/w) *n*-dodecylmaltoside:chlorophyll. SDS-PAGE was performed as described in [21]. Immunodetection was carried out using antibodies against PsbA, PsaA, PsbC and PsaD.

3. Results

Under nitrogen limiting conditions over-reduction of secondary electron transport carriers can occur. Low availability of terminal electron acceptors might lead to a mostly reduced plastoquinone (PQ) pool. A reduced PQ pool can, in turn, induce acceptor side PSII photoinhibition and the formation of harmful reactive oxygen species [22]. Therefore, the breakdown of PBS and other photosynthetic components may serve not only as a nitrogen reallocation mechanism but also as part of the cellular shift toward catabolic metabolism, providing protection against photoinhibition.

3.1. The light intensity dependence of cell density and pigment content in nitrogen limited cultures

In order to study the importance of light stress avoidance in the nitrogen limitation response we designed an experimental setup in which nitrogen limited *Synechocystis* 6803 were grown under very low light intensities (LL = 6 μmol photons $m^{-2}s^{-1}$). The kinetics of growth, PBS and chlorophyll breakdown, photosynthetic activity and protein content were compared to nitrogen limited cultures grown under growth light intensities (GL = 60 μmol photons $m^{-2}s^{-1}$). These light intensities are one to two orders of magnitude lower than the intensities required for saturation of electron transport rates (Supplemental Fig. 1).

Fig. 1 presents changes in cell density in N− and N+ cultures grown under low and growth light intensities. Nitrogen sufficient cell density was highly dependent on light intensities. Under N− conditions, the concentration of LL cultures was slightly, but not significantly, higher. As would be expected, under N− conditions light intensity and photosynthetic activity do not limit growth.

PBS and chlorophyll (Chl) content of the cells were also assessed in that experiment. Due to the variability of optical scattering which is dependent on cell size and morphology, we present the data in its raw form with no baseline manipulations (Fig. 2A). The peaks at 620 and at 678 nm arise from absorption by phycocyanin (PC) and Chl, respectively. The Chl peak was observable even after 49 h of nitrogen

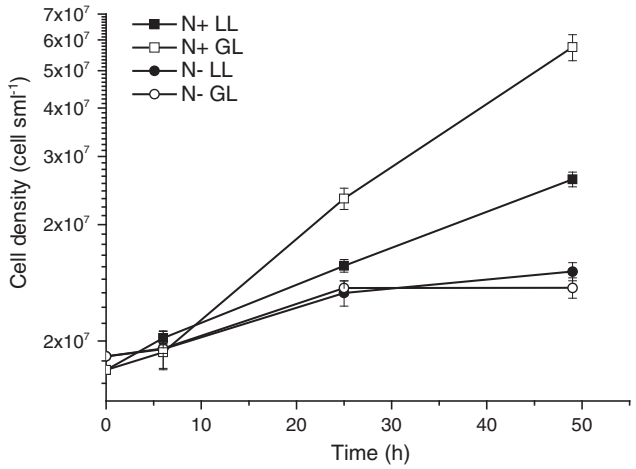

Fig. 1. Growth curves of nitrogen depleted and sufficient cultures under LL and GL. N+ (squares) and N− (circles) cultures were grown at either low light intensity (LL= 6 μmol photons $m^{-2}s^{-1}$, closed symbols) or under growth light intensity (GL= 60 μmol photons $m^{-2}s^{-1}$, open symbols). Error bars represent standard deviation with $n=3$. The experiment was repeated twice with comparable results.

limitation in all samples. Nevertheless, the size of the peak at 678 nm diminished with time in these samples. Nitrogen limited cells grown in GL lost their PC peak faster than corresponding LL cultures, where the peak was still noticeable 49 h after the transfer to N− conditions. The PC peak is not affected in N+ cultures.

To examine the effects of illumination conditions on PBS composition, cell samples were broken and the spectra of the soluble fractions were measured (Fig. 2B). Peaks at 620 and 651 nm result from absorption of PC and allophycocyanin (APC), respectively [23]. APC linker proteins are peripherally attached to the cytoplasmic face of the thylakoid membrane. PC is more abundant than APC and forms rod shaped complexes on top of APC [24]. After 49 h, the ratio of PC/APC in N− LL cultures was 2.46 ± 0.4 ($n=2$) while that of the N− GL cultures was only 1.89 ± 0.03 ($n=2$). Phycobilisome degradation starts at their extremities and progressively advances towards the APC core [25]. The lower PC/APC ratio in N− GL cultures is consistent with the greater loss of phycobilisome absorption in the in vivo spectra, as compared to N− LL cultures.

3.2. Effects of nitrogen deprivation on photosynthetic activity as a function of light intensity

The photosynthetic potential of PSII was measured by chlorophyll PAM fluorometery [26]. Fv/Fm values decreased in N− GL cultures by ~50% over the 2 days of the experiment. N− LL cultures retained their Fv/Fm values throughout this period (Fig. 3). Fluorescence measurements are a common tool for assessing plant photochemical activity [26]. In the case of cyanobacteria, the analysis of this signal is complicated by a number of factors including the overlap of photosynthetic and respiratory electron transport chains [27].

Additional information on the activity of both Photosystem I (PSI) and PSII was gained from measurements of absorption changes at 705 nm in vivo (Fig. 4). Using this method we could quantitatively dissect the contribution of the two photosystems, providing a closer look

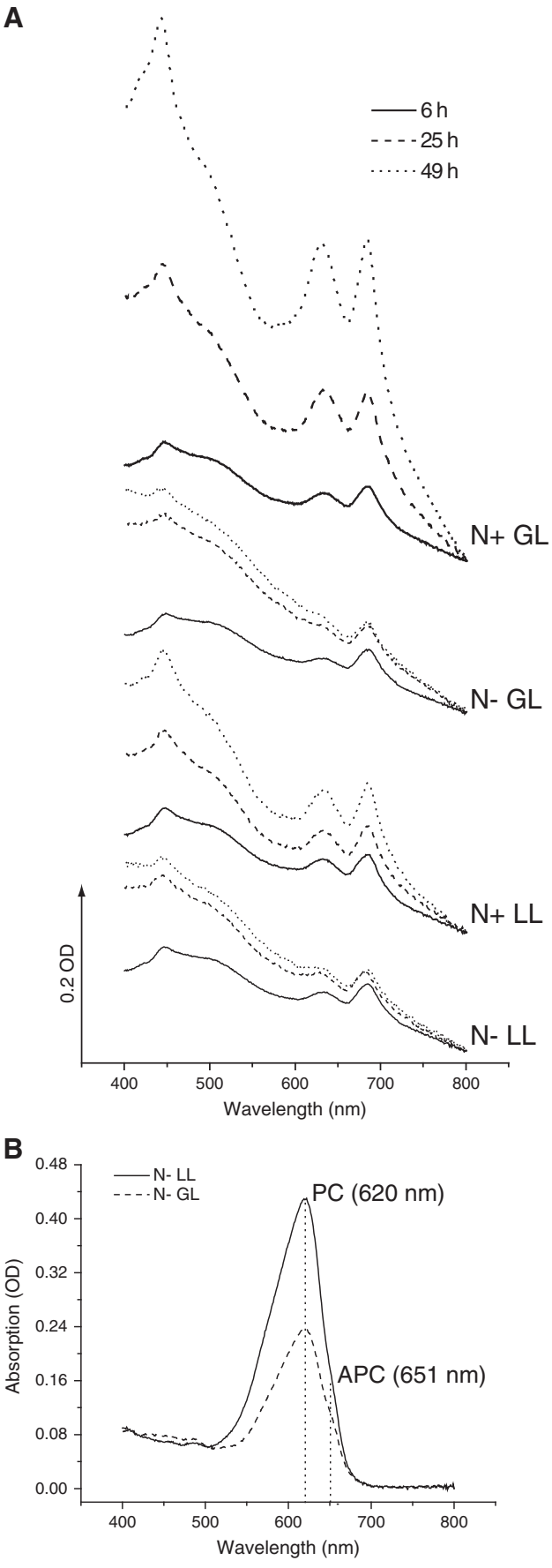

Fig. 2. Absorption spectra of cells and photosynthetic pigments. Visible light absorption spectra of N− and N+ cultures, grown under GL and LL conditions, measured at 6, 25 and 49 h after the nitrogen step-down process (A). The data sets for the different time points are baseline shifted for convenience. Spectra are normalized by subtracting the value at 800 nm. After 49 h the cells were broken and membranal fractions were separated from the soluble fractions by ultra-centrifugation. The absorbance spectra of the soluble fraction of N− samples are presented (B). Chlorophyll was extracted from the membranal fraction and the PC/Chl ratio was calculated to be 43, 34 and 16.3 for the N+ GL, N− LL and the N− GL sample, respectively.

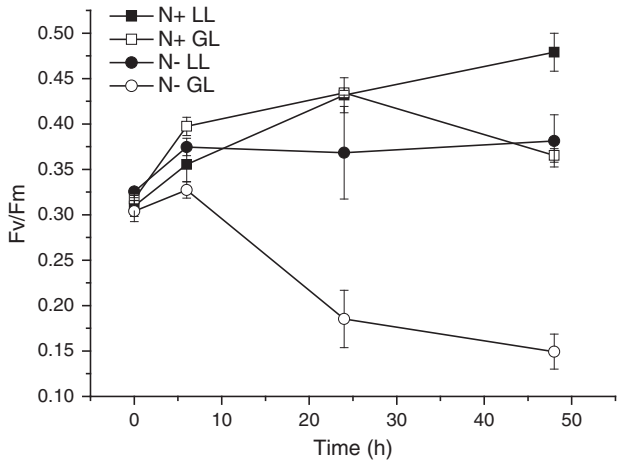

Fig. 3. Changes in the photochemical potential of PSII. The photochemical potential of PSII was measured by chlorophyll fluorescence analysis in vivo. Cultures were grown in N+ or N− conditions under GL or LL, as indicated. Data is presented as the ratio between the variable fluorescence e (Fv) and the maximal fluorescence (Fm), following dark adaptation. Error bars represent standard deviation with $n=3$.

at the photosynthetic apparatus than could be obtained from whole chain oxygen evolution measurements (supplemental Fig. 1) or fluorescence assays (Fig. 3). Three sequential measurements were performed on each sample: (1) control, without inhibitors; (2) a measurement in which DCMU was added, eliminating electron transfer from PSII to the PQ pool; (3) DBMIB was added as well, blocking all electron transport routes from the PQ pool to PSI, including cyclic electron flow. Examples of the experimental results, at the 49 h time point, are presented in Fig. 4A. The maximal absorption change (ΔA_{max}) is proportional to the concentration of photochemically oxidized P_{700} i.e. it is directly related to the maximal photochemical capacity of PSI (supplemental figure 2). Over time, ΔA_{max} decreased in the N− cultures and increased in the N+ cultures (Fig. 4B). The increase in PSI/PSII ratio with growth is well documented [28,29]. The rate of ΔA_{max} loss was faster in GL as compared to LL cultures.

The area between DCMU and control curves represents the contribution of PSII activity to the reduction of PSI. Similarly, the difference between DBMIB & DCMU and DCMU measurements is related to the potential for non-PSII reduction of the electron transport chain. Calculating these values from P_{700} measurements indicated that the contribution of PSII also became progressively lower in N− cultures (Fig. 4C). Here, as in the case of ΔA_{max} values, the effect was stronger in GL as compared to LL. The fraction of non-PSII reduction was small, less than 16% under all experimental conditions (Fig. 4A).

Photosynthetic electron transport is clearly down regulated in nitrogen depleted cells. Our results suggest that this occurs due to a combination of loss of PBS and lower activity of the photosynthetic electron transport chain. The decrease in activity may result either from the loss or from the re-organization of photosynthetic apparatus components.

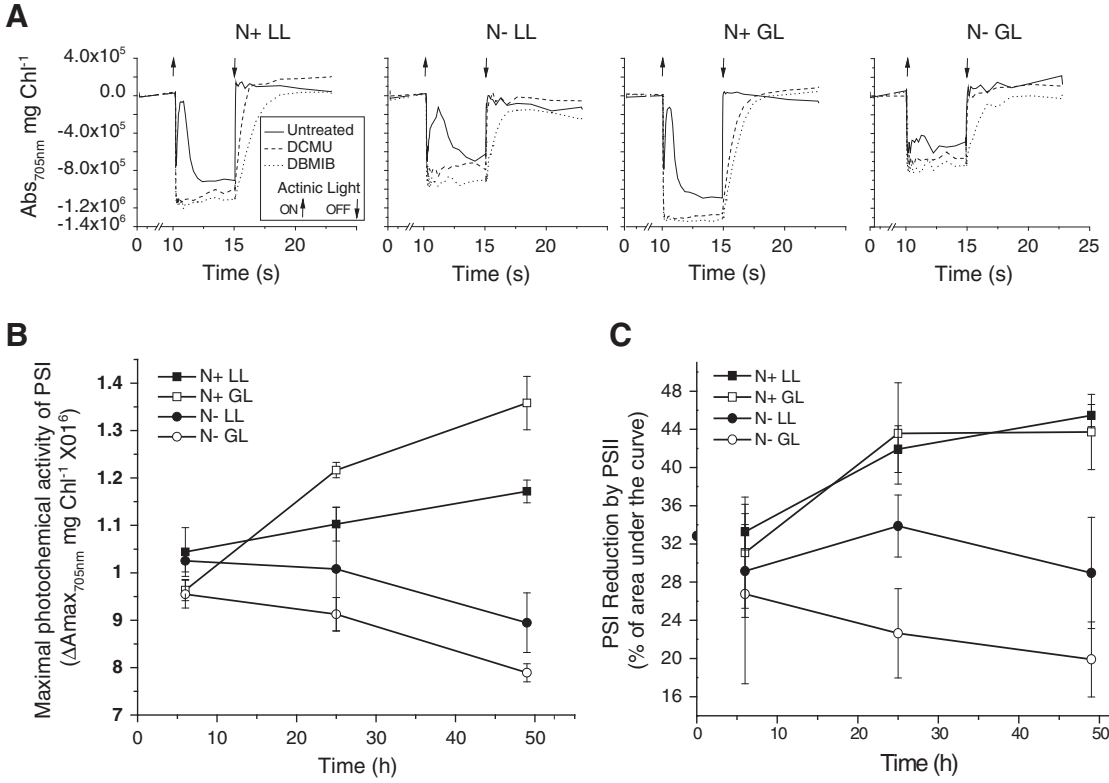

Fig. 4. PSI and PSII photochemical activity. P_{700} photochemical activity was measured in vivo in cultures grown in N+ or N− medium under GL or LL, as indicated. Data are normalized to chlorophyll concentrations. P_{700} oxidation rates were measured as absorbance changes at 705 nm during a 5 s illumination period (590 μmol photons $m^{-2}s^{-1}$), marked by the on and off arrows in panel A. Raw data from measurements conducted at 49 h are presented in panel A. The maximal photochemical activity of PSI is presented as the absolute value of the maximal absorbance change at 705 nm (ΔA_{max}) in panel B. PSII photochemical contribution to the reduction of PSI was calculated as the percent of the area trapped between the control and DCMU measurements, normalized to the DCMU & DBMIB measurement:

$$\frac{\int_{10}^{15}[\text{Control}]-\int_{10}^{15}[\text{DCMU}]}{\int_{10}^{15}[\text{DCMU\&DBMIB}]}\times 100.$$

The kinetics of these values is presented in panel C. Error bars represent standard deviation with $n=3$.

3.3. Effect of nitrogen deprivation on the composition and structure of the photosynthetic apparatus

Western blot analysis of photosystem proteins indicated that their levels are correlated with the decrease in their activity (Fig. 5A). A decrease in the content of the PSII proteins PsbA and PsbC, on an equal chlorophyll basis, was observed. PsbA (D1) levels were lower under GL as compared to LL N− conditions. A similar pattern could be observed for PsbC (CP43), albeit to a smaller extent. This pattern was not observed under N+ conditions (Fig. 5A), suggesting a higher level of PSII light sensitivity under nitrogen limitation. PsaA and PsaD (PSI subunits PSI-A and PSI-D) content were also lower in N− as compared to N+ conditions. The effect of light intensity on PSI proteins was smaller than on PSII proteins.

In cyanobacteria, PSI is found mainly in its trimeric form [30]. It was suggested that PSI conformation can shift between trimeric and monomeric forms in response to changing light conditions and nutrient availability [19,30–33]. Blue native polyacrylamide gel electrophoresis (BN-PAGE) indicated some loss of PSI trimers under N-GL conditions (Fig. 5B), but the extent of that loss is small in comparison to that observed under Mn limitation, for example [19]. This indicates that while the cellular content of PSI and PSII decreased, the overall organization of the photosynthetic electron transfer chain remained relatively unchanged.

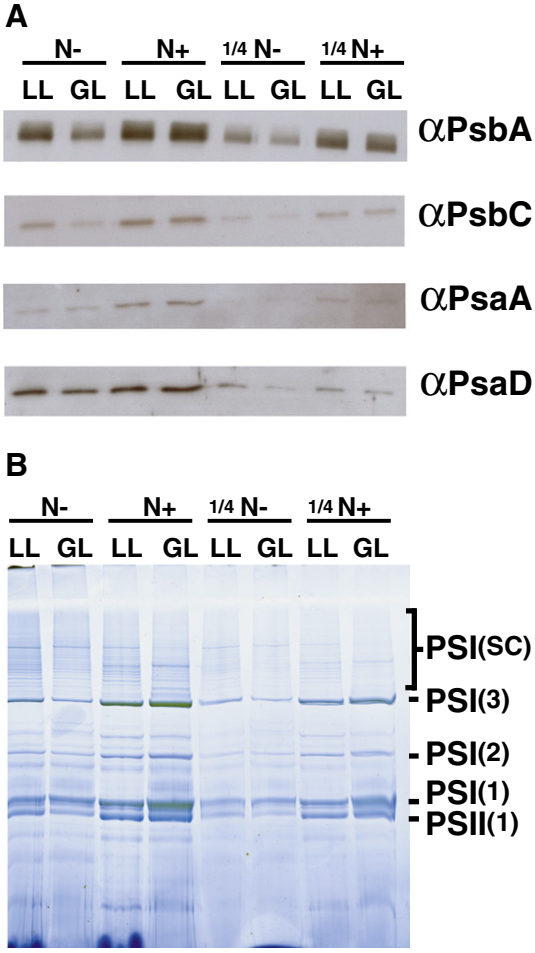

Fig. 5. PSII and PSI protein content and quaternary structure analysis. Panel A: Immunoblot analysis of the PSII proteins PsbA (D1), PsbC (CP43) and the PSI proteins PsaA and PsaD. Proteins were extracted from cells grown on N+ or N− YBG11 for 49 h under GL or LL conditions. Samples were loaded on an equal chlorophyll basis (1 μg per lane). Samples were also loaded at ¼ of the original sample volume, to insure that the saturation of the exposure does not skew the result. Panel B: Blue native gel electrophoresis (BN-PAGE) of the same cell samples presented in panel A. The membranal fraction was solubilized with *n*-dodecylmaltoside and 5 mg chlorophyll per lane was loaded. The locations of PSI supercomplexes (SC), trimers (3), dimers (2) and monomers (1), as well as PSII monomers (1), are indicated (see Salomon and Keren [19], for mass-spectrometric identification of the bands).

3.4. Photoinhibition of nitrogen limited cultures

In N-GL cells the smaller absorption cross-section of the PBS antenna should protect from light induced damage. In order to test this hypothesis a photo-damage experiment was conducted. Cultures grown for 48 h at either N-GL or N-LL conditions were transferred to GL conditions in the presence of the protein synthesis inhibitor chloramphenicol. The results presented in Fig. 6 show that N-LL cultures, retaining a larger cross-section, lost their PSII activity faster than N-GL cultures ($t_{½}$ of 8 h vs. 24 h, respectively). P_{700} activity was not affected. These results demonstrate that even under moderate light levels, the retention of large PBS antenna under nitrogen limitation can accelerate PSII photo-damage considerably.

3.5. Manganese pre-acclimated cells, with lower photosynthetic activity, are less affected by nitrogen starvation

The results presented thus far suggest that, under our growth conditions, a major driving force of PBS breakdown is the damage caused by imbalance between light driven water oxidation and downstream limitation in metabolite reduction rates.

Mn limitation induces the detachment of PBS antenna and reduces the activity of both photosystem II and photosystem I in *Synechocystis* 6803 [19,34]. Under these conditions, where electron flow through the photosynthetic apparatus is low, we expected to see an attenuated response to nitrogen starvation even under growth light conditions.

Synechocystis 6803 cells were transferred YBG11 media with no added Mn (Mn−). Under these conditions Mn levels are in the ~1 nM range [19]. Cultures were allowed to pre-acclimate to Mn limitation for 21 days, diluted with YBG11 Mn− and grown for three more days. Finally, growth media were replaced with YBG11 N−, as described above.

Synechocystis 6803 cultures pre-acclimated to manganese limitation (Mn− N− GL) were less affected by nitrogen deprivation. The rate of phycobilisome degradation was lower in Mn limited cultures as compared to Mn sufficient ones (Fig. 7 and supplemental Fig. 3). The high PC to Chl peak ratio observed in the Mn− spectra (at 620

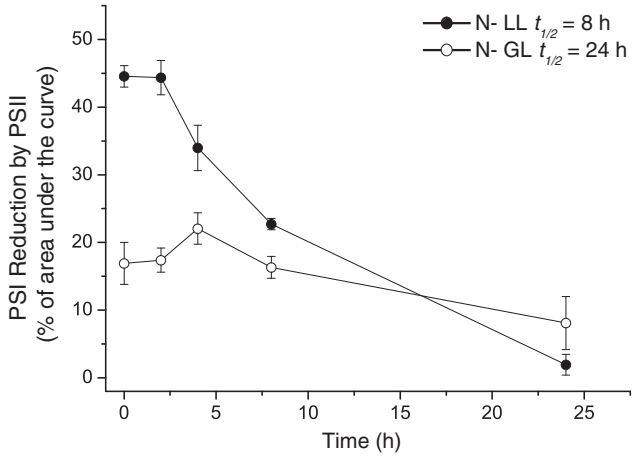

Fig. 6. Light induced inhibition of PSII photochemical activity of N− cultures. N− GL and N− LL *Synechocystis* 6803 cultures were transferred to GL conditions in the presence of the protein synthesis inhibitor chloramphenicol. The photochemical activity of PSII was measured as described in Fig. 4. Error bars represent standard deviation with $n=2$. Under both conditions P_{700} remained constant (99.1%±6.2% and 103.2%±3.1% of the T_0 value after 24 h for the N− LL and N− LL, respectively).

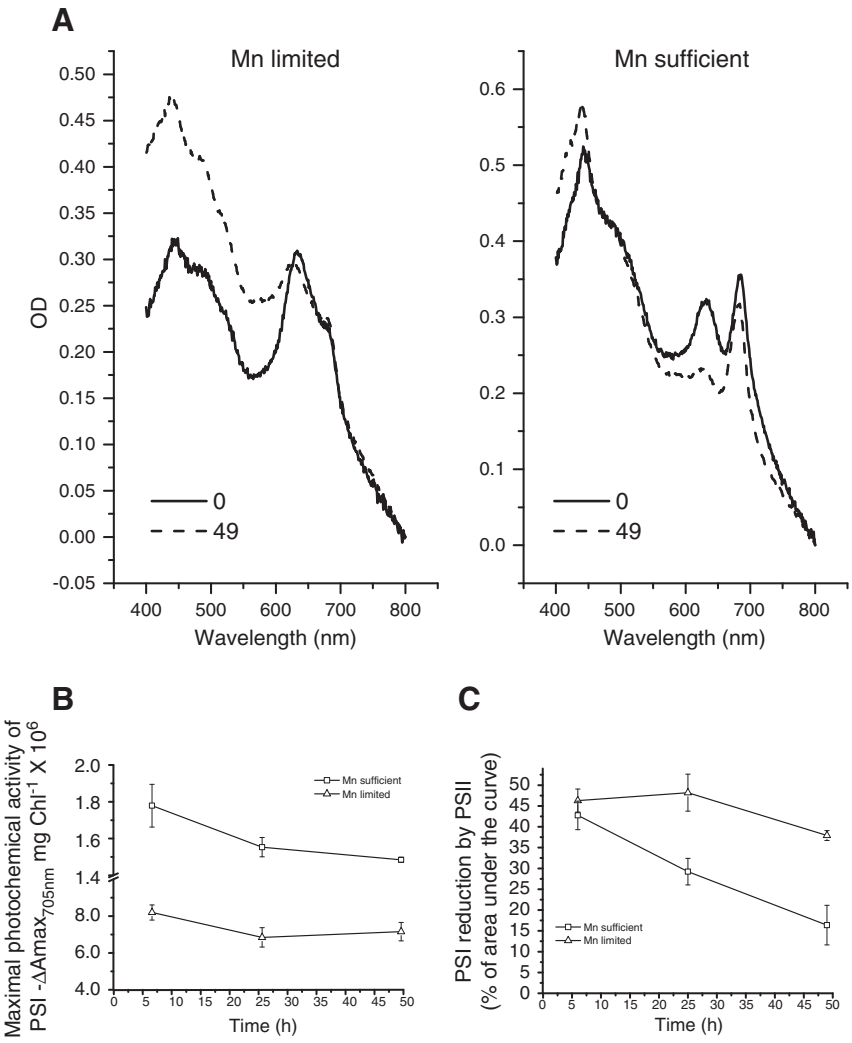

Fig. 7. Effects of Mn limitation on the response to nitrogen deprivation. Panel A: Absorbance spectra of Mn − pre-acclimated cells (left) and Mn sufficient cells (right) at times 0 and 49 h after nitrogen step down. For better visualization, OD values were normalized to the values at 800 nm. Complete spectral data and a picture of the cultures are presented in supplemental figure 3. Panels B and C: PSI photochemical activity and reduction of PSI by PSII, respectively. Values were measured and calculated as described in Fig. 4. Error bars represent standard deviation with $n = 3$.

and 678 nm, respectively) is distinct to Mn limited *Synechocystis* 6803 [19]. In these samples the PC peak was clearly noticeable throughout the duration of the nitrogen limitation experiment (Fig. 7A, left panel). In the Mn sufficient control, the PC peak was still visible 25 h post nitrogen step-down, but was nearly invisible after 49 h of nitrogen stress, as expected (supplemental Fig. 3 and Fig. 7A, right panel).

The protective effects of Mn limitation were extended to the activity of the photosystems. In Mn − N − GL cultures, PSI photochemical activity retained its initial lower amplitude (Fig. 7B). In Mn + N − GL cultures the signal diminished, at a rate comparable to that presented in Fig. 4. The contribution of PSII to the PSI signal diminished at faster rate under Mn + as compared to Mn − conditions (Fig. 7C).

4. Discussion

The results presented in this work demonstrate lower photosynthetic activity, reduced antenna size and lower electron transport rates in nitrogen limited *Synechocystis* 6803 cells. As ample literature suggests, the breakdown of PBS allows for reallocation of amino acids to vital functions. In addition, PBS degradation and downscaling of the photosynthetic apparatus can favor the acclimation process by decreasing excitation pressure during the shift from anabolic to catabolic metabolism. In order to test which of the effects exerts a more prominent effect on the response to nitrogen limitation we devised two strategies. The first was to decrease excitation pressure by drastically decreasing the illumination intensity. The second was to pre-acclimate to Mn limitation where the PBS antenna is detached and electron flow through the photosynthetic apparatus is diminished even under growth light intensities [19].

No difference in growth rates was observed in nitrogen limited cultures grown at either GL or LL intensities (Fig. 1), yet the loss of PBS antenna was slower under N − LL condition as compared to N − GL (Fig. 2). Similarly, the extent of PBS degradation was much lower in Mn − pre-acclimated cells (Fig. 7A). Fv/Fm values, as well as the activity of both PSII and PSI, declined slower in N − LL cultures as compared to N − GL cultures (Figs. 3 and 4). In those parameters, again, the response of Mn − pre-acclimated cells resembled that of N − LL cultures (Fig. 7B and C). The reduction in photosynthetic activity was correlated with a decrease in photosystem protein levels (Fig. 5). These observations provide support to the idea that controlling energy flow in the photosynthetic machinery is a key factor in avoiding photo-damage resulting from over-reduction of downstream electron acceptors. Direct measurements of PSII photoinhibition indicated that this is indeed the case. Under GL conditions, N-LL cells (large PBS cross-section) exhibited much faster PSII inactivation than N-GL cells (small PBS cross-section) (Fig. 6).

The results presented here seem inconsistent with reports demonstrating that the major effect of N limitation is a loss of PBS pigments, with only a minor loss of chlorophyll content [6,11]. Furthermore, in certain cases no decrease of photosynthetic electron transport was reported [11]. A major difference between the experiments in this contribution and prior publications is in the carbon dioxide concentrations used. High CO_2 concentrations (1–3%, [6,11]) can provide extra acceptors and relax the constriction in utilizing photosynthetic energy, thus avoiding photo-damage and maintaining higher functionality of the photosynthetic apparatus. CO_2 concentrations were demonstrated to have a significant effect on the rate of growth, loss of chlorophyll and PBS during the transition into nitrogen limitation [10].

In the absence of downstream acceptors (Nitrogen and CO_2, for example), the photosynthetic apparatus can suffer from light induced damage due to harmful interactions of reduced electron carriers with oxygen [22,35]. One way for decreasing photosynthetic electron transport is by downsizing the effective cross-section of the antenna.

The data presented here suggests that photo-damage avoidance is the more prominent pressure, under our growth conditions. *Synechocystis* 6803 mutants lacking an active nblA gene showed extensive D1 protein degradation under nitrogen limitation [36], further emphasizing the importance of PBS breakdown as protection against photo-damage. An integration point for balancing electron flow and nitrogen availability can be provided by the PII transcriptional regulator [37]. Two factors regulate PII function: (a) the concentrations of 2-oxoglutarate, which affected by nitrogen availability and the carbon reaction of photosynthesis, and (b) the ATP/ADP ratio, directly related to photosynthetic energy conversion.

Loss of photosynthetic activity occurred under light intensities that were much lower than oxygen evolution $K_{½}$ values (supplemental Fig. 1). While originally light induced damage was regarded as a "high light" phenomenon, it is now clear that it can occur at low light intensities as well [38–40, critically reviewed in 41]. The natural habitats of cyanobacteria are often limiting for macronutrients. A scenario in which over-reduction of the photosynthetic apparatus is induced by nitrogen limitation, under low light intensities, is more probable than over-reduction induced by super-saturating light intensities (conditions often used experimentally for generating photo-damage). The effects on the photosynthetic apparatus observed here were recorded under very low light intensities, approximately 2–0.2% of the maximal solar irradiation flux at sea level. These effects should be taken into account when trying to understand the evolutionary forces that molded the dynamic response of the photosynthetic apparatus to stress.

Furthermore, our results suggest that low manganese levels can have beneficial effects on the photosynthetic activity of cyanobacteria when encountering nitrogen shortage. It is important to note that the level of Mn "contamination" in our Mn− media is closer to the concentrations observed in water bodies [42,43] than the μM concentrations used in standard growth media. Mn pre-acclimation induced attenuation of electron transport reduces the chance for over-reduction thus minimizing photo-damage. These results suggest that nutrient co-limitation scenarios can have important and unexpected effects on photosynthetic activity.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2012.11.010.

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