NITROGEN FIXATION AND RESPIRATORY ELECTRON TRANSPORT IN THE CYANOBACTERIUM *CYANOTHECE* UNDER DIFFERENT LIGHT:DARK CYCLES

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Running Title: Timing of N_2 fixation in *Cyanothece*

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Abstract

Incompatibility of nitrogen fixation and oxygen production compel unicellular diazotrophic cyanobacteria to perform photosynthesis during daytime and restrict nitrogen fixation to nighttime. The marine diazotroph *Cyanothece* BG 043511 was grown in continuous culture under three light:dark regimes (16L:8D, 12L:12D and 8L:16D hours); we monitored nitrogen fixation and potential photosynthetic efficiency simultaneously online to reveal how their temporal separation is affected by different LD regimes. An increase in nitrogen fixation rate at night coincided with a rise in pulse-amplitude modulated fluorescence, indicating that the enhanced respiratory electron transport to fuel diazotrophy affects the oxidation state of the plastoquinone pool. This may offer an alternative approach to assess instantaneous nitrogen fixation activity. Regardless of photoperiod, the maximum rate of nitrogen fixation was conserved at about 20 hours after the onset of the light. Consequently, nitrogen fixation rates peaked at different moments in the dark: relatively early in the 16L:8D cycle, at midnight in 12L:12D, and relatively late in 8L:16D. Under 16L:8D, nitrogen fixation extended into the light, demonstrating the functional plasticity of nitrogen fixation in *Cyanothece*. Highest daily amounts of nitrogen fixed were obtained in 12L:12D, which is consistent with the natural light:dark cycle of subtropical latitudes in which *Cyanothece* thrives.

Keywords: cyanobacteria, light:dark cycle, nitrogen fixation, PAM fluorescence, *Cyanothece*

Abbreviations: PSII, photosystem II; PQ pool, plastoquinone pool

Introduction

Fixation of dinitrogen (N_2) by marine cyanobacteria provides an important source of nitrogen in the tropical and subtropical oceans (Karl *et al.*, 1997). Fixed nitrogen supports 20 to 40 % of the total marine primary production (Lee *et al.*, 2002). Predominant open-ocean N_2 fixers include the filamentous *Trichodesmium* (Carpenter & Romans, 1991; Letelier & Karl, 1996; Capone *et al.*, 1997) and the endosymbiont *Richelia* (Mague *et al.*, 1974; Venrick, 1974). Some unicellular cyanobacteria can also fix nitrogen (Wyatt & Silvey, 1969; Mitsui *et al.*, 1986), although they were, for many years, not recognized as main actors in the open ocean biogeochemistry. The discovery that unicellular diazotrophic cyanobacteria actually thrive in open oceans, and contribute substantially to the oceanic nitrogen budget (Zehr *et al.*, 2001; Moisander *et al.*, 2010), prompted a reconsideration of the role of different N_2 fixers in the oceanic nitrogen cycle (Zehr *et al.*, 2000; Karl *et al.*, 2002; Zehr, 2011).

Autotrophic, unicellular N_2-fixing cyanobacteria are largely confined to the tropics and subtropics (Zehr, 2011). This biogeographical distribution has been attributed to the high temperature requirements of unicellular diazotrophs (e.g., Stal, 2009; Brauer et al., in press). However, it is conceivable that the day-night regime in the (sub)tropics, with an approximately similar duration of day and night, might also play a role. N_2-fixing cyanobacteria handle two processes, mutually exclusive at a first glance: on the one hand, they gather energy and reducing power from oxygenic photosynthesis, while on the other hand the nitrogenase enzyme complex, responsible for N_2 fixation, is inactivated by oxygen (Fay, 1992; Gallon, 1992). To accomplish 'the impossible', temporal separation is the common strategy developed by unicellular cyanobacteria, i.e. restraining N_2 fixation to darkness at night when oxygen evolution of light-dependent photosynthesis is absent (Rippka *et al.*, 1971; Mitsui *et al.*, 1986; Bergman *et al.*, 1997). The principal timing of these processes is via the light on-off rhythm, facilitated by a circadian clock (Sherman *et al.*, 1998;

Johnson & Golden, 1999; Cerveny & Nedbal, 2009; for detail see Cerveny *et al.*, 2013). Such a strategy implies that nitrogenase activity cannot be fuelled by photosynthesis directly, but relies instead on carbon reserves accumulated during the light period (Gallon *et al.*, 1988; Schneegurt *et al.*, 1994; Rabouille *et al.*, 2006; Mohr *et al.*, 2010; Dron *et al.*, 2012; Brauer *et al.*, 2013). We thus expect that the relative duration of the light and dark period will be of ecological significance for the diazotrophic growth of unicellular cyanobacteria.

In this study, we therefore investigate how the diel pattern of N_2 fixation and photosynthesis in the marine, unicellular cyanobacterium *Cyanothece* sp. strain BG 043511 (hereafter *Cyanothece*) responds to different light:dark cycles. We monitored N_2 fixation rates by an online nitrogenase activity assay (Staal *et al.*, 2001) and photosynthetic activity by online fluorescence measurements. Interestingly, the results do not only support the view that the photoperiod is an important determinant of the ecological niche of *Cyanothece*, but also reveal that nighttime fluorescence measurements provide a measure of the respiratory electron transport associated with nitrogen fixation.

Materials and Methods

Cyanothece strain BG 043511, originally isolated as *Synechococcus* BG 043511 near the Bahama Islands in the Atlantic Ocean (León *et al.*, 1986), was kindly provided by the Hawaii Culture Collection (University of Hawaii, Honolulu).

Cyanothece was grown in continuous culture under three different light:dark (LD) regimes. The light regime at tropical latitudes was represented by a 12L:12D cycle, while 16L:8D and 8L:16D cycles reflected summer and winter regimes of the temperate zone. The continuous cultures were specifically designed to study phytoplankton growth (Huisman *et al.*, 2002; Agawin *et al.*, 2007), using a flat culture vessel with an optical path length of 5 cm and an effective working volume of 1.6 L. A water jacket placed between the light source and the culture vessel maintained the temperature of the culture at 26°C. The vessels were heat

sterilized for 1 h at 121°C prior to inoculation. Cultures were grown in modified YBC II medium devoid of any combined nitrogen source (Chen *et al.*, 1996). The mineral medium was pumped into the culture vessel by a peristaltic pump (Watson Marlow 101U/R MkII) at a dilution rate of D = 0.12 d^{-1}. A constant culture volume was maintained by a horizontally placed outflow mounted at the surface level of the culture. Homogeneous mixing and sufficient supply of CO_2 were ensured by aerating the cultures with sterile and moistened air enriched with 2.4 % CO_2, at a flow rate of 70 L h^{-1} regulated by mass flow controllers (Brooks Instruments, Hatfield, PA, USA).

Light was supplied by white fluorescent tubes (Philips PLL 24W/840/4P; Philips Lighting, Eindhoven, The Netherlands) that were placed on the front side of the culture vessel (see Huisman *et al.*, 2002). Light intensities (PAR from 400 to 700 nm) were measured with a Licor LI-189 quantum sensor attached to a LI-250 light meter (LI-COR, Lincoln, Nebraska, USA). The incident light intensity (I_{in}) was set at 50 µmol photons m^{-2} s^{-1} by neutral density filters. Light transmitted through the culture vessel (I_{out}) was measured at the back surface of the culture vessel. To account for spatial variation, I_{in} and I_{out} were measured at 14 regularly spaced positions at the front and back surface of the vessel, respectively.

At start up, two continuous cultures were exposed to a 12L:12D regime, and stabilized at a steady state at which cell growth equals the dilution rate. Once the population densities approached equilibrium values, one of the continuous cultures was shifted to 16L:8D and the second one to a 8L:16D regime.

Cell abundances were measured daily in all cultures, by fixing samples of 2 mL taken in triplicate with 20 µl of a solution of 1% formaldehyde and 10% glutaraldehyde in water. Samples were then frozen with liquid nitrogen and stored at -80°C until cell count analyses with a Coulter Epics Elite flow cytometer (Beckman Coulter Nederland BV, Woerden, The Netherlands). N_2 fixation activity was monitored online using custom designed incubators (Staal *et al.*, 2001). Once or twice per day during four to six consecutive days, samples were

taken from the continuous cultures and filtered on pre-combusted (4h at 400°C) and pre-weighted Watson GF/F glass fiber filters. The filters with cellular material were subsequently placed in the custom designed, gas-tight cell incubator which was placed adjacent to the culture vessel and exposed to the same light regime as the continuous culture. The cell incubator was connected to a Shimadzu Gas Chromatograph GC-14B, controlled by Shimadzu Gas Chromatograph software CLASS-VP (Shimadzu, Kyoto, Japan). Samples were incubated during 12 to 24 hours and nitrogenase activity was monitored online every 10 min using the acetylene reduction assay method (Staal *et al.*, 2001). The N_2 fixation rate was calculated according to Stal (1988), with a conversion factor of 4 to calculate N_2 fixation from acetylene reduction.

Using the same samples, fluorescence emission was measured every 30 min online by pulse amplitude modulated (PAM) fluorescence using a Walz MINI-PAM (Walz GmbH, Effeltrich, Germany) equipped with white excitation light. Records during the light period were taken without pre-adaptation of the cells to dark conditions. Hence, measurements during the light period gave the in-situ fluorescence (F_s), and a saturating light flash gave the maximum fluorescence of light-adapted cells (F'_m). During the dark period, the cells were obviously dark adapted and the same measurements thus gave what is commonly known as the minimum fluorescence (F_0) and maximum fluorescence (F_m). The quantum yield of photosystem II was calculated as: $\Phi = (F'_m - F_s)/ F'_m$ in the light and $(F_m - F_0)/ F_m$ in the dark (Genty *et al.*, 1989).

Similarities in the fluctuations of the nitrogen fixation rate and quantum yield were investigated with cross-correlation analysis, which calculates the Pearson correlation coefficient between the two time series at different time lags. For this purpose, we improved the stationarity of the time series by rescaling both time series between 0 and 1 on a daily basis. Statistical significance of the cross-correlations was assessed by bootstrapping. Each individual bootstrap sample was represented by the cross-correlation between two artificial

time series randomly generated using the same sample size, mean, variance and lag-1 autocorrelation of the residuals as the measured time series of the nitrogen fixation rate and quantum yield. A two-sided 95% confidence interval of the cross-correlation coefficient was estimated based on the 2.5 and 97.5 percentile of the bootstrap distribution obtained from a total of 1,000 bootstrap samples.

Results

Population dynamics

Two continuous cultures (C1 and C2) were inoculated at concentrations of ~8×10^5 cells mL^{-1}, and exposed to a 12L:12D regime. Cell concentrations increased during the transient phase, leading to enhanced self-shading as revealed by a gradual decline in light transmission (I_{out}) (data not shown). At cell densities of $7.33 \pm 1.67\times10^6$ cells per mL^{-1} (n = 9) in C1 and $7.77 \pm 2.61\times10^6$ cells per mL^{-1} (n = 3) in C2, and corresponding stabilized I_{out} values of 2.95 ± 0.12 (n = 6) µmol photons m^{-2} s^{-1} and 2.97 ± 0.23 (n = 5) µmol photons m^{-2} s^{-1}, the light regimes were changed. C1 was shifted to 16L:8D and C2 to 8L:16D. Equilibrium was reached within a few days in both cultures, for which cell abundances were $13.2 \pm 1.07\times10^6$ cells mL^{-1} (n = 13) in C1 (16L:8D) and $9.97 \pm 0.56\times10^6$ cells mL^{-1} (n = 3) in C2 (8L:16D). Corresponding light transmission values were then I_{out} = 1.86 ± 0.10 (n = 6) µmol photons m^{-2} s^{-1} in C1 and I_{out} = 2.97 ± 0.23 (n = 5) µmol photons m^{-2} s^{-1} in C2.

N_2 fixation

At equilibrium, N_2 fixation showed a periodicity of 24 hours under all LD regimes with consistent repeatability. A comparison is made between the 12L:12D regime (control), in which the nitrogenase enzyme was active during the entire dark period, and the 16L:8D and 8L:16D regimes (Fig. 1). Under 12L:12D, cells began to fix N_2 at or very shortly before the

onset of darkness, nitrogenase activity first increased, peaked between 6.5 h and 9 h into the dark period, and then decreased to a negligible activity at the end of the dark period (Fig. 1a). The average maximum N_2 fixation rate for 12L:12D was 0.87 ± 0.2 pmol N_2 $cell^{-1}$ h^{-1} (n = 4) and the total amount of N_2 fixed during an entire day (i.e., 24 hours) averaged at 6.24 ± 1.25 pmol N_2 $cell^{-1}$, from which more than 97 % was acquired in the dark (Table 1). Under 16L:8D, nitrogenase activity was not exclusively restricted to the 8 hours of darkness but started already in the light, shortly before the onset of the dark period, and peaked between 1.5 and 4 h into the dark period (Fig. 1b). Moreover, after this short dark period, nitrogenase activity continued in the light for about 6 hours, albeit at a lower rate (Fig. 1b). Almost 14% of the total daily N_2 fixation was obtained during the light period (Table 1). Nevertheless, both the total amount of N_2 fixed in the dark and the total daily N_2 fixation were substantially lower in the 16L:8D culture than in the 12L:12D culture (Table 1). In the 8L:16D culture, nitrogenase activity initiated after about 8 h in the dark and lasted for about 8 h (Fig. 1c). The total amount of N_2 fixed during the dark period was again substantially lower than in the 12L:12D culture, while we did not observe N_2 fixation activity in the light (Table 1). These results demonstrate that day-night regimes different from 12L:12D reduce the rate and daily amount of N_2 fixation.

Fluorescence

To monitor the potential efficiency of photosystem II during the dark period and its actual efficiency during the light period, pulse-amplitude modulated fluorescence was applied, using the 16L:8D and 8L:16D light regimes. The photosynthetic performance of cell samples was studied in the same chamber as the N_2 fixation assays. Fluorescence showed distinct patterns during the light and dark periods. In the dark, maximum fluorescence F_m first increased and then decreased, in a very similar way as the nitrogenase activity. Both maximum fluorescence and quantum yield peaked during periods of highest N_2 fixation

activity (Fig. 1b,c). Hence, it appears that the temporal dynamics of PSII fluorescence and nitrogen fixation are closely connected. We noticed that minimum fluorescence during the dark period (F_0) showed constant values throughout each night: values fluctuated by only 3% in the 16L:8D regime and by only 5% in the 8L:16D regime (data not shown). In the 16L:8D culture, where the nitrogenase activity started in the late light period shortly before the onset of the dark, both the maximum fluorescence and quantum yield of PSII also began to increase already in the late light period (Fig. 1b). After the nitrogen fixation rate had reached maximum values in the first half of the short night, the maximum fluorescence and quantum yield started to decrease again. Under the 8L:16D regime (Fig. 1c), the maximum fluorescence and quantum yield both decreased during the entire light period, continued decreasing after the onset of the dark period as long as nitrogenase activity remained undetected, and only started to increase later in the dark, together with nitrogenase activity.

Cross-correlation analysis confirmed the similarity in the diel fluctuations of the quantum yield of PSII and the nitrogen fixation rate, both for the 16L:8D regime (Fig. 2a) and the 8L:16D regime (Fig. 2b). For both LD regimes, the cross-correlation was significant. In the 16L:8D regime, the highest value of the cross-correlation coefficient was $r \approx 0.6$ at a time lag of 30 min. In the 8L:16D regime, the cross-correlation coefficient approached $r \approx 0.8$ at time lags of 0 and 30 min. In other words, the temporal variation in quantum yield was closely tracked by the nitrogen fixation rate with a small delay of 0 - 30 min.

Discussion

N_2 fixation dynamics

Our results show that the day-night regime affects the efficiency of N_2 fixation in unicellular cyanobacteria, in agreement with earlier studies (e.g., Ikemoto & Mitsui, 1994; Schneegurt *et al.*, 1994; Sherman *et al.*, 1998; Gallon, 2001; Taniuchi & Ohki, 2007; Toepel *et al.*, 2008; Mohr *et al.*, 2010). In the 12L:12D control, *Cyanothece* showed a typical cyclic

nitrogenase activity in which N_2 fixation was restricted to the dark period and peaked around the mid-dark phase (Fig. 1a). Data of the 16L:8D culture show that light periods longer than 12 hours did not promote an increase in the gross rate and cumulative yield of nitrogen acquisition. Interestingly, the apparent reduction in dark N_2 fixation capacity as a result of a shorter nighttime was partly compensated by continuation of N_2 fixation in the first 4 to 8 h after the onset of the light period (Fig. 1b). Daytime N_2 fixation added a substantial fraction (14%) to the total amount of N_2 fixed over a 24 h time span. Hence, the present experiment confirms the ability of *Cyanothece* to fix nitrogen in the light (see also, e.g., Červený *et al.*, 2013). Despite this continued activity in the light, the total amount of N_2 fixed per cell under 16L:8D was about a third lower than in the 12L:12D culture. In the 8L:16D culture, N_2 was fixed only during the second half of the dark period, leading to a daily amount of N_2 fixation that was only half of the 12L:12D control.

Our results thus indicate that a 12:12 LD regime provided optimal conditions for N_2 fixation by *Cyanothece* BG 043511, as both day lengths longer and shorter than 12 h rendered less product formation by the nitrogenase enzyme. This is most likely caused by an imbalance between the carbon and nitrogen metabolism of the cells. Carbon reserves are needed for the redox and energy requirements of N_2 fixation, but also for covalent bonding of the ammonium ions formed during nitrogen fixation via the GS-GOGAT reaction (Muro-Pastor *et al.*, 2001; von Wobeser *et al.*, 2011; Krasikov *et al.*, 2012). Hence, we hypothesize that a shortage of carbon may limit nitrogen fixation if the light period becomes too short, while there is insufficient time for nitrogen fixation if the nights become too short. N_2 fixation therefore appears to be regulated not only by the prevalence of active nitrogenase enzyme, but also by the accumulation of carbon reserves fueling the nitrogenase activity and providing carbon skeletons for the GS-GOGAT controlled N metabolism.

Irrespective of the length of the light period, nitrogenase activity in our cultures was first detected at 12 to 16 hours and reached maximum values at 18 to 21 hours after the onset

of the light period. Similar results have been reported for the unicellular diazotroph *Gloeothece* sp. 68DGA, where nitrogenase activity consistently peaked 16 hours after the onset of the light period, even when the 12L:12D cycle was modified by the addition or subtraction of a single 6 h light period or dark period (Taniuchi & Ohki, 2007). This indicates that the timing of the onset of N_2 fixation is apparently well conserved and obeys a tight connection to the 24 h biological clock. Energy transducing systems, such as photosynthesis, glycolysis, respiration and nitrogen fixation generate cycles of redox and phosphorylation potentials that both modulate metabolic activities and affect gene transcription, via Transcriptional/Translational Control Loops (Stock *et al.*, 2000; Albrechtova *et al.*, 2006). The assembly of the machinery for nitrogen fixation also responds to this control (Chen *et al.*, 1998) and this may be the reason why, in the 8L:16D culture, N_2 fixation was postponed to the second half of the night. Moreover, the biochemical processes initiated by these control loops will be temperature dependent, which may explain the recent observation that the timing of the onset of N_2 fixation is delayed at low temperature (Brauer *et al.*, 2013).

N_2 fixation and respiratory electron transport

We used pulse-amplitude modulated (PAM) fluorescence to monitor changes in photosynthetic electron transport. During daytime, this technique reports on the quantum yield of photosystem II (Φ), which is commonly used as a measure of the efficiency of photosynthetic electron transport (Schreiber *et al.*, 1986; Genty *et al.*, 1989). Use of the PAM technique during nighttime seems counterintuitive, as there is no photosynthetic electron transport during the night. However, nighttime nitrogen fixation in cyanobacteria makes use of thylakoid membrane bound electron transfer components; in fact, photosynthetic and dark respiratory electron transport both take place in the thylakoid membranes (Hirano *et al.*, 1980; Matthijs *et al.*, 1984a,b; Scherer *et al.*, 1988). These two processes share several components of the electron transport chain including the plastoquinone (PQ) pool and the cytochrome b_6/f

complex (Jones & Myers, 1963; Hirano *et al.*, 1980; Scherer *et al.*, 1988; Vermaas, 2001). Thus, electron transfer during photosynthesis and respiration affects the actual redox state of the PQ pool in the thylakoid membranes, which in turn affects the PAM fluorescence signal.

During the dark period, measurement of the potential quantum yield of photosystem II informs on the PQ redox state. Typically, the quantum yield of photosystem II remains constant during the dark period in phototrophic eukaryotes such as green algae (Flameling & Kromkamp, 1997). In contrast, the quantum yield of *Cyanothece* showed a bell-shaped pattern in close synchrony with nitrogenase activity (Fig 1b,c). Our explanation is that the increased quantum yield during N_2 fixation at night is caused by enhanced respiratory electron transfer through the thylakoid membranes, intended for ATP generation to support the energy requirements for N_2 fixation. Cross-correlation analysis confirmed the close relationship between the fluctuations in quantum yield and nitrogen fixation rate, and pointed at a small time delay of 0 - 30 min (Fig. 2). This minor time delay may roughly indicate the time between the respiratory electron transport in the thylakoid membrane and the actual fixation of N_2 by the nitrogenase enzyme complex.

At the molecular level, these results indicate that Q_A, the first stable acceptor of electrons from PSII, transfers its electrons more efficiently to the plastoquinone pool, due to electron transport associated with the N_2 fixation process (Fig. 3). In particular, enhanced respiration of carbohydrate reserves at night produces NADPH and succinate, which transfer electrons via NADPH dehydrogenase (NDH) and succinate dehydrogenase (SDH; Cooley & Vermaas, 2001) into the PQ pool and onwards to the terminal electron acceptor oxygen via cytochrome c and cytochrome oxidase (Fig. 3). This respiratory electron transport sets up a proton gradient across the thylakoid membrane, which drives ATP production. N_2 fixation in unicellular diazotrophs is probably the most energy-consuming process in the dark and thus constitutes a drain of both ATP and electrons (Fig. 3). High activity of the electron transport chain, to sustain the ATP production required for N_2 fixation, results in relative oxidation of

the PQ pool. An oxidized state of the PQ pool is measured as a high "quantum yield" (Genty *et al.*, 1989), and hence changes in nitrogenase activity are accompanied by changes in the apparent quantum yield of PSII measured by PAM fluorescence. This finding has two important implications: (i) during daytime, changes in PAM fluorescence of N_2-fixing cyanobacteria do not necessarily reflect changes in their photosynthetic activity, because N_2 fixation also affects the fluorescence signal, and (ii) at night, PAM fluorescence measurements of unicellular, N_2-fixing cyanobacteria such as *Cyanothece* can provide an experimentally easy method to monitor their actual N_2 fixation activity.

In conclusion, our results revealed a tight link between respiratory electron transport and nocturnal nitrogenase activity, and support the hypothesis that the day-night regime is an important determinant of the ecological niche and biogeographical distribution of unicellular diazotrophic cyanobacteria. Nitrogen acquisition in *Cyanothece* BG 043511 was maximized when the day-night regime provided balanced accumulation of both carbon and nitrogen It would be interesting to investigate whether the same 12:12 LD cycle would also be optimal for other *Cyanothece* strains with a different timing of their nitrogenase activity peak (Bandyopadhyay *et al.*, 2013). While the biogeographical distribution of unicellular diazotrophs is currently being revealed (Moisander *et al.*, 2010), the finding that a 12:12 light:dark cycle appears to yield the highest daily nitrogen fixation rate might offer an elegant explanation why unicellular diazotrophs seem particularly successful at (sub)tropical latitudes.

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Figure Legends

Figure 1: Dynamics of N_2 fixation (closed circles), maximum fluorescence F'_m in the light and F_m in the dark (open circles), and the quantum yield of photosystem II (crosses) in continuous cultures exposed to different light:dark regimes. a) 12L:12D regime (fluorescence data not available), b) 16L:8D regime, and c) 8L:16D regime. Shaded areas represent dark periods.

Figure 2: The estimated cross-correlation function between time series of the quantum yield of PSII and the nitrogen fixation rate. a) 16L:8D regime and b) 8L:16D regime. Shaded areas represent the 95% confidence interval based on a bootstrapping approach using the same sample size, mean, variance and lag-1 autocorrelation as in the measured time series. The vertical, dashed line locates the highest cross correlation and the corresponding lag time.

Figure 3: Schematic representation of electron flow within the thylakoid membrane, illustrating the role of the plastoquinone (PQ) pool. a) In the light, electron fluxes are essentially related to the light reactions of photosynthesis: a linear transport of electrons into the PQ pool originates from PSII, and are transported to the first stable terminal acceptor ferredoxin (Fd) by photosystem I (PSI). b) In the dark, light reactions of photosynthesis are inactive; electron fluxes are related to respiration only and reduction of the PQ pool occurs through oxidation of NADPH and succinate during the respiration of carbon reserves. hν: light energy; $C_{b/f}$: cytochrome b6f; NDH: NADPH dehydrogenase; NR: NADP reductase; PQ: oxidized plastoquinone pool; PQH2: reduced plastoquinone pool; PSI: photosystem I; PSII: photosystem II; SDH: succinate dehydrogenase.

Table 1. Characteristics of the N_2 fixation rate (±SD) under three different light:dark regimes.

LD regime (h:h)	Maximum N_2 fixation rate (pmol N_2 $cell^{-1}$ h^{-1})	Total N_2 fixed in the dark (pmol N_2 $cell^{-1}$)	Total N_2 fixed in 24 hours (pmol N_2 $cell^{-1}$)	Percentage of N_2 fixed in the light (%)
12:12	0.87 ± 0.20	6.09 ± 1.23	6.24 ± 1.25	2.4
16:8	0.81 ± 0.18	3.78 ± 0.50	4.39 ± 0.38	13.9
8:16	0.74 ± 0.04	3.14 ± 0.29	3.14 ± 0.29	0